The Maintenance of an Inversion Polymorphism in *Coelopa frigida*

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Abstract

The seaweed fly, Coelopa frigida, lives in piles of rotting seaweed deposited on beaches by tides and winds. In all populations studied it is polymorphic for two gene arrangements on Chromosome I. A polymorphism at the alcohol dehydrogenase locus is strongly associated with this inversion and can be used to estimate karyotype frequencies.

An extensive series of samples from natural populations has revealed a seasonal cycle in inversion frequencies but otherwise frequencies are remarkably constant both geographically and temporally. There is a consistent excess of heterokaryotypes in these samples.

Three selective forces influencing inversion frequencies have been investigated. 1) An association between karyotype and development time, previously observed in the laboratory, has been demonstrated in conditions close to those in natural populations. 2) Viability differences between karyotypes have been examined. In natural populations there is some evidence that the excess of heterokaryotypes increases with larval density. In the laboratory heterokaryotypes are shown to have higher viability than either homokaryotype but the strong density dependence reported previously has not been observed. Viability differences are concentrated in the first two days of larval life and are probably related to the rate of supply of nutrients. 3) An association is demonstrated between karyotype and adult size - especially in males. Adult size is shown to correlate with longevity and fecundity of both sexes. Several

experiments indicate that large males enjoy greater mating success than small males.

The relationship between larval density, development time and adult size is described. The possibility that the effect of the inversion varies between populations or between alcohol dehydrogenase genotypes has also been investigated.

A simulation has been used to study how these selective forces interact with one another, and with the changeable environment in which the flies live, and to examine the extent to which they can account for the observed karyotype frequencies.

The gods did not reveal, from the beginning,
All things to us, but in the course of time
Through seeking we may learn and know things better.
But as for certain truth, no man has known it,
Nor shall he know it, neither of the gods
Nor yet of all things of which I speak.
For even if by chance he were to utter
The final truth, he would himself not know it:
For all is but a woven web of guesses.

Xenophanes (translated by Karl Popper)

Chapter 1

Introduction

1.1 General

The modern view of the mechanism of organic evolution is enshrined in the "Synthetic Theory" developed from the union of Darwinism and Mendelism by Huxley, Fisher, Wright, Simpson, Mayr, Dobzhansky and others in the 1930s and 1940s (see Mayr and Provine 1980 for an overview of the synthesis). According to this view evolution consists of the accumulation of changes in gene frequencies. Gene frequencies can be altered by four forces: mutation, natural selection, genetic drift and gene flow. Of these mutation, which includes point mutations, duplications, chromosomal rearrangements, etc., holds a special place as the ultimate source of all new variation. Natural selection, on the other hand, is the only directional force, the only force capable of producing adaptation.

The Synthetic Theory has recently been challenged on the basis that its supposed prediction of phyletic gradualism is inconsistent with data from the fossil record (Eldredge and Gould 1972, Stanley 1979, Williamson 1981, but see Maynard Smith 1981,1982, Jones 1981). However it continues to provide the framework for the great majority of evolutionary studies, especially evolutionary genetics. This is not to say that there are no controversies or new ideas being explored in evolutionary genetics. The application of electrophoretic techniques in population studies and the consequent discovery of ubiquitous genetic polymorphism (Lewontin 1974, Nevo 1978) has led to an extensive debate between the "Neutralist" and "Selectionist" schools (Kimura 1977, Clarke 1979). This is essentially a debate

about the relative importance of natural selection and random processes - mutation, drift and gene flow - in maintaining genetic diversity. It has stimulated new interest in these processes - especially in the various ways in which selection can maintain genetic polymorphism. Heterosis no longer seems to be the predominant type of selection. Frequency dependence (Clarke 1979) and selection in heterogeneous environments (Maynard Smith & Hoekstra 1980) may be more important.

Another, related, area of debate is the unit on which natural selection can be said to act. Lewontin (1974) argued that it was unsatisfactory to consider the action of selection on individual loci in isolation and emphasised the importance of linkage and recombination. In this view epistasis is considered a common phenomenon to the extent that "every gene affects every character". Selection acts on interacting sets of genes rather than on individual loci and the gene pool of a population is a coherent, coadapted, entity resistant to change (Mayr 1963). From a different point of view Dawkins (1976, 1982) has made a strong case for the gene, rather than the individual or any higher unit, being the unit of selection. This idea arose mainly from the consideration of evolutionary explanations for animal behaviour patterns. It is not inconsistent with the view of Mayr or Lewontin because genes are selected, in part at least, for their favourable interactions with other genes in the same organism or gene pool. The study of animal behaviour, along with the question of the evolution of sex, has also helped to bring out the controversy over group selection (Maynard Smith 1976). A further debate about the level at which selection acts has arisen in molecular biology. To explain the existence of large amounts of

non-coding repetitive DNA in eukaryotes the "selfish DNA" theory has been advanced (Orgel & Crick 1980, Doolittle & Sapienza 1980) - selection increases the representation of DNA sequences which are good replicators within cells without their necessarily having beneficial effects on the organism's phenotype.

The process by which new species are formed, speciation, has always been a central problem in evolutionary biology. It remains an active area of research and debate as evidenced by White's (1978) extensive survey. It now seems likely that speciation does not always follow the strictly allopatric model of Mayr (1963) but may occur in a wide variety of ways ranging from fully allopatric through parapatric to sympatric and involving various degrees of genetic reorganisation.

From the earliest days of the Modern Synthesis one of the most important sources of information has been the study of chromosomal inversion polymorphism. The work of Dobzhansky and his colleagues on the third chromosome polymorphism of Drosophila pseudoobscura provided one of the first clear demonstrations of the action of selection in natural populations. Since then the study of inversion polymorphism has been important in the development of many areas of evolutionary genetics: the maintenance of polymorphism, the evolution of heterosis, the coadaptive integration of the genotype, the genetics of mating behaviour, the interaction of drift and natural selection and so on. The great majority of this work has been with species of Drosophila, D. pseudoobscura being the most important but with extensive investigations on D. persimilis, D. subobscura, D. funebris, D. pavani and others. The main advantages of working with Drosophila are the ease of their culture, at least of the well studied species,

in the laboratory, the excellent polytene chromosomes and the wealth of background knowledge of the cytology and genetics of the genus. Perhaps the most serious disadvantage is the lack of knowledge about the natural ecology of these flies (Dobzhansky 1971). Despite the intensive study of D. pseudoobscura little is known about the conditions in which it lives in nature, about its food, predators or parasites, or about the way in which it survives adverse conditions such as cold or drought. This means that although a variety of potentially important phenotypic effects of inversions have been identified in the laboratory it is often difficult to relate them to the field situation. For example it is known that in population cages larval crowding affects the relative viabilities of inversion homozygotes and heterozygotes of D. pseudoobscura (Birch 1955). However, in the absence of larval density estimates in the field, the relevance of this observation to the maintenance of the polymorphism is not clear. Indeed on the basis of adult sizes it seems unlikely that wild larvae do experience crowding (Roff 1981).

This thesis is a study of an inversion polymorphism in a species of fly which goes some way towards combining the advantages of Drosophila with a field ecology which is much more amenable to study. The species is Coelopa frigida, the seaweed fly or kelp fly. It is easy to culture in the laboratory, has good salivary gland polytene chromosomes and it lives in large numbers in a clearly defined and easily studied habitat. Its ecology has already received some study and it has several polymorphic inversions. One of these has been singled out for study by Dr.T.H.Day and others (Collins 1978, Day & Buckley 1980, Day et al 1980, Day et al 1982). The object of this work is to relate phenotypic effects of the inversion to the natural

ecology of the fly in order to understand the maintenance of the polymorphism and explain the pattern of frequencies observed.

In the remainder of this introduction I will first survey the information available about inversion polymorphisms in other species and the theories that have been proposed to explain their origin and maintenance. I will then describe the state of knowledge about the ecology of Coelopa frigida and the previous studies of its inversion polymorphism.

1.2 Inversion polymorphism

1) Background Very early on in the work of T.H.Morgan and his group on the genetics of Drosophila melanogaster suppressors of recombination were discovered which were called C-factors. Their effect was to reduce the recombination rate between loci on a particular chromosome. Sturtevant (1926) demonstrated (by laboriously mapping mutants on the affected chromosome) that the C-factor was, in fact, an inverted segment of chromosome. This conclusion was confirmed and the identification of many more inversions made possible by the introduction of techniques for examining salivary gland polytene chromosomes in the early 1930s. Studies on the frequencies of inversions in natural populations of Drosophila quickly followed (Dobzhansky & Sturtevant 1938, Dobzhansky & Epling 1944) and revealed an extraordinary amount of variation.

The suppression of recombination by inversions is, in some cases, a mechanical disruption of chiasma formation due to the pairing configuration in heterozygous individuals. In other cases, where chiasmata do form, the effect depends on whether the centromere is included in the inverted segment, a pericentric inversion, or excluded,

a paracentric inversion. In the former case a crossover produces chromosomes carrying duplications and deletions and thus the heterozygous individual, male or female, has a reduced fertility. A chiasma within a paracentric inversion generates one chromosome with two centromeres and one with no centromere in addition to two normal chromosomes. Again this might be expected to cause a reduction in fertility but in Drosophila there are no chiasmata in male meiosis and in oogenesis the acentric and dicentric fragments are regularly directed to the polar bodies (Sturtevant & Beadle 1936). There is no loss of fertility. This property of oogenesis occurs throughout the Diptera but the achiasmate male meiosis is more restricted.

Paracentric inversions are very common in the Drosophilidae, presumably because of this combination of features, and they have been extensively studied in many species of the genus Drosophila (Da Cunha 1955, Dobzhansky 1970, Wright 1978). There have also been studies of inversions in mosquitoes (White 1980) and in Chiromonids, Simuliids, Cecidomyids and others despite the fact that some of these have chiasmate male meioses (Dobzhansky 1970). Pericentric inversions are rare in the Diptera.

Outside the Diptera inversions are more difficult to detect because of the absence of polytene chromosomes. They can be detected through the production of chromosome "bridges" and "fragments" at meiosis (paracentric) or as "centric shifts" producing unequal bivalents (pericentric). In this way inversions have been detected in natural populations of grasshoppers, beetles, rodents and some plants (Dobzhansky 1970). It seems likely that the problem of reduction of fertility is overcome by localisation of chiasmata outside the inverted segments. If this happens many short inversions may have

gone undetected and the range of occurrence of inversions could be much wider.

Many pairs of closely related species differ by one or more inversions and it has been suggested that the inversion event might be important in speciation. This area was extensively reviewed by White (1978).

An inversion can be defined by its two break points. In animals with polytene chromosomes these points can be mapped to the nearest band. This means that in Drosophila there are about 5000 distinguishable break points or 25×10^6 possible inversions (or rather less if allowance is made for the separate chromosomes). Inversions occur spontaneously at a rate of about 1 in 500 individuals and, therefore, a particular inversion occurs at a rate of about 1 in 10^{10} individuals. Each inversion can be looked on as a unique event (White 1978) in contrast to a single gene mutation which is a recurrent event occurring at a frequency of about 10^{-5} or five orders of magnitude more often than a particular inversion. Clearly the establishment of a new inversion is a very different problem from the establishment of a new allele.

The calculation above assumed that break points were evenly distributed throughout the genome whereas if "hot spots" for chromosome breakage exist then the probability of a given inversion recurring would be greatly increased. Break points of polymorphic inversions appear to be distributed at random on the third chromosome of Drosophila pseudoobscura, probably the best studied species (Olvera et al 1979), but hot spots have been identified in D. subobscura the highly polymorphic European relative of pseudoobscura (Krimbas & Loukas 1979). Short inversions are rarer than predicted from random breaks in D. pseudoobscura and also in D. subobscura. This is probably for mechanical reasons.

Long inversions are less efficient suppressors of recombination than short ones because of the increased probability of double cross-overs which produce normal gametes. However this is not true of complex inversions where a number of inversions overlap. Polymorphic complex inversions might be expected to reach greater lengths than simple ones. There is some evidence that this is the case in D. pseudoobscura (Wallace 1953) but in D. subobscura the mean length of complex inversions is not greater than would be predicted from random combination of simple inversions (Krimbas & Loukas 1979).

The distribution of inversion polymorphism among chromosomes is often non-random. In D. subobscura inversions are found on all the large chromosomes but 24 inversions are known on the O chromosome and only 3 on the J chromosome (Krimbas & Loukas 1979). In D. pseudo-obscura and D. persimilis inversion polymorphism is largely restricted to the third chromosome, with the notable exception of the "sex-ratio" inversions on the X chromosome. The situation in other species was reviewed by Wright (1978). A possible explanation for the concentration of inversions in one chromosome was given by Sturtevant and Mather (1938). Once an inversion is established in one chromosome then further inversions within that chromosome will reduce recombination less than similar inversions in other chromosomes. If selection favours a balance between inversions and recombination the result might be the observed concentration. Haldane (1957) has shown that the conditions for establishment of an inversion are less stringent for the sex chromosomes than for the autosomes. In some groups inversions on the sex chromosomes are particularly common (Post 1982).

Where overlapping inversions occur it is possible to construct a phylogeny because only certain rearrangements are possible by single

step inversions (see Dobzhansky 1970). A detailed phylogeny has been constructed in this way for the third chromosome arrangements of D. pseudoobscura and D. persimilis (Dobzhansky & Sturtevant 1938, Olvera et al 1979). The technique has also been used extensively in the study of Hawaiian Drosophila species (Carson 1980).

2) Frequencies in natural populations When the study of inversion frequencies in natural populations began inversions were considered to be prime examples of selectively neutral variants and thus ideal for the study of random genetic drift. Instead they have become the classic paradigm for the study of the action of natural selection. Investigations of natural populations in Drosophila have been reviewed by Dobzhansky (1971) and by Wright (1978). Relatively little work has been done outside Drosophila lathough there have been important studies on grasshoppers (Lewontin & White 1960) and on medically important mosquito species (White 1980).

The first indication that selection was acting on inversions came from the observation of seasonal fluctuations in frequencies in D. pseudoobscura. Dobzhansky (1943, 1947a,b) studies the frequencies of third chromosome arrangements at three localities in California. At the Pinon Flats locality the common gene arrangements were Standard (ST), Arrowhead (AR) and Chiricahua (CH), described by Dobzhansky and Epling (1944). When flies became active in early spring ST was the most common arrangement but its frequency declined and that of AR and CH increased until mid summer. In the late summer and autumn the process was reversed. The magnitude and rapidity of these frequency changes (50% to 30% in one or two months for ST) required larger selection coefficients than seemed reasonable at the

time but the regular annual repeat of frequency changes made selection the only plausible explanation. The cycle at Pinon Flats has now been observed repeatedly from 1939 to 1970 (Dobzhansky 1971).

Two other sites in the same area, Mount San Jacinto, were also studied only about 25 km from Pinon Flats and from each other. At Andreas Canyon on the desert's edge flies were rare in the summer but common in winter. Nevertheless the same cycle of frequencies was observed. At Keen Camp on the wet side of the mountain but at a similar elevation to Pinon Flats no evidence for a cycle could be obtained. Further away at Berkeley a seasonal cycle has been observed but it is reversed - ST is common in the summer and CH in the winter with little change in AR frequencies (Strickberger & Wills 1966). Initially it seemed likely that ST was favoured at low temperatures and CH and AR at high temperatures. Strickberger and Wills suggested that, at Berkeley, rainfall was more important. However the ecology of D. pseudoobscura is complex and poorly understood and this sort of speculation is probably not justified on the basis of field observations alone.

Seasonal cycles have been observed in other Drosophila species including D. funebris (Dubinin & Tiniakov 1946, and see Dobzhansky 1965), D. subobscura (Burla & Gotz 1965), D. melanogaster (Zacharopoulou & Pelecanos 1980) and D. melanica (Tonzetich & Ward 1973a). In melanica seasonal fluctuations affect the inversions on chromosomes II and IV but not the X chromosome. It should be noted that several studies have failed to detect seasonal fluctuations in D. subobscura (see Krimbas &Loukas 1979) and there are only weak indications of seasonal cycles in D. persimilis (Dobzhansky 1956).

In addition to the observation of seasonal fluctuations

Dobzhansky (1943) found a decrease in ST frequency and an increase in CH with altitude on Mount San Jacinto. The observation was confirmed by a 60 mile transect of the Sierra Nevada range (Dobzhansky 1948). Here the frequency of AR increased much more markedly than CH with altitude. This tended to support the suggestion that the ST and AR arrangements were favoured by different climatic conditions. However, as with seasonal fluctuations, altitudinal clines were not found in all populations (Dobzhansky 1962).

In Drosophila funebris inversion frequencies show large scale north-south clines and also differences between urban and rural environments (Dubinin & Tiniakov 1946, Dobzhansky 1965). This pattern has been attributed to the greater cold tolerance of flies with the standard arrangements. There are also large scale north-south clines in D. melanogaster (Knibb et al 1981).

By contrast in Drosophila subobscura although there are conspicuous north-south clines in a number of gene arrangements, attempts to link these to environmental variables have been unsuccessful (Krimbas & Loukas 1979). On a smaller scale frequency differences between sites at different altitudes or with different ecological conditions have not been found but erratic differences between individual trap sites and trapping times have been observed.

Dobzhansky (1962) coined the terms "rigid" and "flexible" chromosomal polymorphisms to distinguish situations in which frequencies of arrangements change with season and altitude (flexible) from those in which frequencies do not change with environmental variables (rigid). The lack of altitudinal variation in the Chiricahua mountains is thus an example of a rigid polymorphism. Flexibility was supposed to indicate adaptation of different

arrangements to components of a complex environment whereas rigidity was the result of buffering of heterozygotes against environmental variation. Dobzhansky stressed that these types of selection were not mutually exclusive. It is thus quite possible to find the two responses within one species as in the different populations of D. pseudoobscura or in the rigid polymorphism of the X chromosome and the flexible polymorphism of the autosomes in D. melanica (Tonzetich & Ward 1973a). In D. pseudoobscura areas of rigid and flexible polymorphism of the third chromosome may meet at distinct boundaries (Crumpacker et al 1977) which can be related to ecological discontinuities. Here rigidity and flexibility seem to result from the "coadapted complexes" of genes contained by the chromosome arrangements of the different areas (see below). The polymorphism of D. pseudoobscura is generally considered flexible by comparison with that of D. subobscura (Anderson et al 1967) but Burla and Gotz (1965) presented evidence of flexibility in subobscura and Krimbas and Loukas (1979) have questioned the usefulness of the terms in making comparisons between the two species.

Inversion frequencies vary geographically on a wider scale than the local differences discussed so far although large scale clinal variations in D. funebris and D. subobscura have been mentioned. The geographic range of D. pseudoobscura can be divided into regions that differ in their overall chromosomal constitutions (Dobzhansky et al 1963, Wright 1978). For example in southern California ST is the most common arrangement with AR and CH also common, whereas in Texas Pikes Peak (PP) is the most common arrangement with AR also present but ST and CH absent or rare. In the isolated population of Bogota, Colombia only the Tree Line (TL) and Santa Cruz (SC) arrangements have been found. Adjacent regions tend to be similar

but within regions there can be large variations over short distances as in the Mount San Jacinto populations. The large scale pattern does not seem to be a simple extension of small scale variations but rather a reflection of the evolution of different coadapted gene complexes within the inversions of the different regions (see below).

In Drosophila willistoni (Da Cunha & Dobzhansky 1954) and D. robusta (Carson 1958) a pattern has been detected of high levels of inversion polymorphism in central populations and low levels in marginal populations. Here marginal is meant to imply the ecological rather than geographical extremes of the species range. Da Cunha and Dobzhansky suggested that this is because a wider range of niches is available to central than to marginal populations and they showed a significant correlation of the number of heterozygous inversions per individual with an index of environmental heterogeneity. Carson, on the other hand, has pointed out that the reduced inversion heterozygosity of marginal populations will be accompanied by a higher level of recombination and that this may be favoured in the stringent conditions at the edge of the species range. Lewontin (1974) has extended this idea by emphasising the temporal constancy of central populations and instability of marginal populations. In the central area inversion polymorphism is favoured because it allows adaptation to different niches but in peripheral populations high levels of genic heterozygosity and recombination are necessary to survive in an unpredictable environment. This explanation has the advantage of accounting for a lack of correlation between genic and chromosomal polymorphism (Lewontin 1974). Indeed in some cases genic heterozygosity is higher in marginal than in central populations (Prakash 1973, Zouros et al 1974).

By contrast to the species of Drosophila mentioned so far the chromosomal polymorphism of some other species is very uniform geographically. This is the case in most of the so called "domestic" species which live in association with man and have thus become very widespread. Drosophila melanogaster, ananassae, immigrans, hydei and busckii carry the same arrangements world wide, often in similar frequencies (Dobzhansky 1965). Three domestic species, D. simulans, virilis and repleta are everywhere chromosomally monomorphic.

Recently geographic differentiation of chromosomal polymorphism has been studied on a very fine scale in D. persimilis (Taylor & Powell 1977). Significant differences were found over very short distances (hundreds of metres) between inversion and allozyme frequencies in ecologically different patches. These differences were shown to be maintained by habitat choice (Taylor & Powell 1978). which could have important implications for the maintenance of the polymorphisms. Evidence for habitat choice was not forthcoming in similar studies on D. subobscura (Atkinson & Miller 1980).

Finally, in addition to seasonal fluctuations and geographical variation, observations on inversion frequencies in natural populations have revealed long term directional changes in the composition of populations. By far the most extensive observations have been on Drosophila pseudoobscura in the western United States beginning in 1939 and still continuing (Dobzhansky 1958, Dobzhansky et al 1966, Dobzhansky 1971, Anderson et al 1975). For example at Mather, California the frequency of PP rose from one chromosome in 336 in 1946 to 12% of chromosomes in 1954 and then fell again to 2% by 1969. Over the same period the frequency of CH declined steadily.

Parallel changes occurred about 500 km away on Mount San Jacinto but further east outside California there were few significant changes between 1940 and 1965. Inversion frequencies in D. persimilis have remained approximately constant from 1945 to 1969 at Mather (Dobzhansky 1971) but directional changes have been observed over shorter periods in other species. For example Inoue and Watanabe (1979) have observed directional changes in Japanese natural populations of D. melanogaster over a period of 10 years.

There are two types of explanation for these long term changes. Either they are in response to progressive changes in environmental conditions or they are the result of genetic changes within the inversions. There has been no real success in attempts to correlate the changes with environmental variables although the build up of DDT residues in the environment received some attention (Anderson et al 1968) and more recently DDT has been shown to affect the viability of inversion heterozygotes in D. melanogaster (Laurie-Ahlberg & Merrel 1979). The alternative possibility is that a new combination of genes arose within, say, the PP arrangement by mutation or recombination which increased the fitness of PP homozygotes. PP would then spread through the population until it reached a new equilibrium or until a similar change occurred in another, competing sequence. There is some evidence for genetic change from laboratory estimates of adaptive values. Different values were obtained for the same arrangements sampled at Mather in 1945-46 and again in the early 1960s (Pavlovsky & Dobzhansky 1966).

Many of these observations on natural populations are best interpreted in terms of selection. Compelling evidence for the action of selection and detailed investigations of its mode of

action have come from laboratory experiments with inversion polymorphisms. Some of these experiments will be described in the following sections.

3) Laboratory experiments The early demonstration of seasonal fluctuations in Drosophila pseudoobscura was rapidly followed by the classic population cage experiments of Wright and Dobzhansky (1946) and Dobzhansky (1947c). The flies used were derived from the Pinon Flats locality and carried the ST, CH and AR arrangements of the third chromosome. Laboratory populations were established with either pairwise combinations of arrangements or all three arrangements and at various initial frequencies. They were then maintained at either 25°C or 16.5°C and their chromosomal constitution was followed by regular sampling of eggs.

At 16.5°C the frequencies of the arrangements did not change significantly. But at 25°C the frequencies changed rapidly at first and then approached an equilibrium. For the ST and CH cages this equilibrium was in all cases close to 70% ST : 30% CH and in the cages with all three arrangements the equilibrium was at 54% ST : 30% AR : 16% CH. The data for the ST vs. CH cages were analysed by comparing the changes in gene frequency, over standard length intervals at different mean frequencies, with theoretical expectations. The expectations were generated from several models the most important being: (i) constant relative selective values of the genotypes with heterozygous advantage, (ii) linear frequency dependence of relative selective values, without heterosis, and (iii) constant relative selective values which differed between the sexes. The predictions from these models were actually very similar over most of the observed range and they provided equally good fits to the observations.

On model (i) the selection against ST/ST was 0.24 and against CH/CH was 0.62 relative to the ST/CH genotype. The three arrangement populations were also analysed on the constant selective value model. In this situation the selection coefficients relative to ST/CH were 0.57 for ST/ST, and 0.71 for CH/CH and ST/AR was the most fit genotype. The striking difference between these values and the values from the two arrangement populations indicates that the selective values are not constant but vary with the frequencies of the arrangements present. However this does not rule out heterosis - a wide range of mixed models being possible.

The importance of these experiments was that they demonstrated for the first time the existence of strong balancing selection maintaining a polymorphism. The strength of the selective pressures had been foreshadowed by the rapidity of frequency changes in natural populations. Different responses at the two temperatures offered a possible correlation with the seasonal fluctuations and selective values were shown to be variables by the three arrangement populations.

Dobzhansky (1947c) attempted a direct measure of heterosis. He compared the genotype frequencies of flies raised in optimal conditions with those of flies from the same cages raised in the normal crowded conditions of the cage populations. In optimal conditions there was a slight excess of heterozygotes in tests of both ST vs. CH and AR vs. CH but in crowded conditions the excess was much greater, especially for ST/CH. The possibility that these deviations from Hardy-Weinberg expectations were generated by dominance rather than overdominance could not be excluded but it was very unlikely as it would imply a much more rapid change in gene frequency than was observed.

The original population cage experiments were followed by many others that confirmed and extended the initial conclusions. When chromosomes were taken from within a locality the populations invariably established an equilibrium although this was often different from the natural equilibrium, presumably because of conditions in the laboratory cages (Anderson et al 1967). The relative selective values of the various heterozygous combinations were generally higher than the homozygotes with selective coefficients relative to the most fit genotype often as much as 0.5 or higher (Anderson et al 1968, Pavlovsky & Dobzhansky 1966). However selective values depended on the other arrangements present in the population suggesting some involvement of frequency dependent selection (Levine et al 1954, Spiess 1956). In experiments involving several arrangements the outcome also depended on the initial frequencies of the arrangements (Watanabe et al 1970). This was interpreted as evidence that there was variation within each arrangement, and that different versions became established if the starting frequencies were different enough. The selective values of particular genotypes within populations also seem to vary with time as mentioned above (Levine et al 1954, Pavlovsky & Dobzhansky 1966).

Environmental variables also affect the outcome of population cage experiments. The effect of temperature has already been mentioned. Larval density has received particular attention starting with the observations of Dobzhansky (1947c) as heterozygote advantage in optimum and crowded conditions. Birch (1955) maintained populations containing the ST and CH arrangements at all combinations of low and high larval and adult density. High larval density gave the expected 70% ST : 30% CH equilibrium but low larval density gave an equilibrium at

30% ST : 70% CH. Adult density had no effect. Birch related these observations to the annual cycle of frequencies suggesting that CH increased during the first half of the season while densities were low and then decreased when density increased later in the year. He postulated that ST/ST had an advantage in survival in competitive conditions over CH/CH, but CH/CH had an advantage in reproduction, while ST/CH was superior overall. However there is evidence that natural populations of D. pseudoobscura do not suffer from larval crowding (Sokoloff 1957, Roff 1981) and, using AR and CH, Druger and Nickerson (1972) were unable to detect viability differences between genotypes in crowded or uncrowded conditions after the population had been maintained in the laboratory for 10 years.

In Drosophila pavani heterozygotes for a chromosome IV inversion show higher viability than homozygotes in crowded, but not uncrowded conditions in the laboratory (Budnik et al 1971). This effect is believed to be due to the build up of "larval biotic residues" in the food medium rather than to shortage of nutrients (Brncic & Budnik 1976). Experiments with the Payne inversion of Drosophila melanogaster showed no viability differences between genotypes at low densities but evidence for frequency dependent selection in crowded cultures (Nassar et al 1973). At equilibrium frequency there was no heterozygote excess. However in D. ananassae Kojima and Tobari (1969) found frequency dependent selection acting on inversions on the second and third chromosomes coupled with heterosis at equilibrium frequencies.

Dependence of relative selective values on density and frequency are likely to be related phenomena (Clarke 1972, 1975 , 1979). If frequency dependent selection results from adaptation of different

genotypes to components of a heterogeneous environment then relative selective values will depend not only on the frequencies of the genotypes but also on the numbers of animals. Suppose, for example, that a patch of the environment (or a population cage) contains two different resources, A and B, each capable of supporting 50 larvae and that there are two types, a and b, of larvae adapted to the different resources. If there are only 50 larvae then there will be no selection irrespective of the frequencies of a and b but if there are 100 larvae there will be frequency dependent selection with an equilibrium at a frequency of 50% a : 50% b.

A corollary of this type of explanation for frequency and density dependence is that a polymorphic population of flies should exploit the environment more efficiently than a monomorphic population. This has been demonstrated a number of times using as measures of efficiency both the numbers of flies produced from a fixed amount of food (Beardmore 1963) and the intrinsic rate of natural increase (Dobzhansky et al 1964). But the strong selective pressures against homozygotes impose a high segregational load on populations polymorphic for inversions. This contradiction is probably apparent rather than real arising from the use of relative rather than absolute fitnesses in the calculation of load.

Population cage experiments, while demonstrating the action of natural selection, do not as a rule distinguish the ways in which inversions affect fitness. So far the examples used have involved differences in viability, especially under competitive conditions, but clearly there are many other characteristics that contribute to fitness. Viability might actually be a poor indicator of competitive success which may be more closely related to fitness

(Strickberger 1972) and it is certainly a complex character. For example in D. melanica inversion karyotype affects one component of viability, that is pupal survival (Tonzetich & Ward 1973b).

Moss (1955) and Nickerson and Druger (1973) have studied a variety of components of fitness in D. pseudoobscura using the ST and CH arrangements and the AR and CH arrangements respectively. Moss found differences in fecundity, longevity, viability and rate of development and was able to calculate overall "performance" values that corresponded well with estimates of fitness from population cage experiments. The variance in these characters was always lower for the heterozygotes than for the homozygotes suggesting better buffering against environmental variables. Nickerson and Druger found differences in longevity and fecundity but not viability. Selective effects may frequently vary with environmental conditions - for example tolerance to cold is probably important in some populations of D. pseudoobscura (Crumpacker et al 1977).

Differences in rate of development associated with inversions have been found in D. pavani (Brncic et al 1969) and in D. subobscura (Krimbas & Loukas 1979). In D. subobscura inversions also affect adult size (Prevosti 1966, Krimbas & Loukas 1979). Outside the genus Drosophila, a large number of characters potentially contributing to fitness have been related to inversion polymorphism in mosquitoes (White 1980) including effects on circadian rhythm, blood feeding rate, resistance to insecticides, egg length and environmental preference. In the grasshopper Keyacris (formerly Moraba) scurra pericentric inversions affect viability and adult size (White, Lewontin & Andrew 1963).

The sex-ratio inversions in the X chromosome of D. pseudoobscura

are apparently maintained in populations by meiotic drive in males against negative selection due to reduced male mating success and female fecundity (Wallace 1948). White (1978) considers meiotic drive a potentially important force in establishing newly arisen inversions.

Some cases are known where inversions on the same or even on different chromosomes interact in their effects on fitness. In Drosophila the best studied example is that of D. robusta where there are significant associations in natural populations between inversions in the left and right arms of the second chromosome (Levitan 1955). The rate of recombination between these linked inversions is less than expected and is affected by the presence of inversions in the X and third chromosomes (Levitan 1958). Similar associations are known in D. guaramunu (Levitan & Salzano 1959) and D. subobscura (Sperlich & Fueurbach-Mravlag 1974). An association between pericentric inversions in different chromosomes has been studied in detail in the grasshopper Keyacris scurra (Lewontin & White 1960, White et al 1963, Colgan & Cheney 1980, Wright 1978). Inversions on the CD and EF chromosomes interact in their effects on viability and adult size.

4) Inversions and Mating Behaviour An important component of fitness that has not previously been mentioned is the ability to obtain a mate. Generally speaking mating success is likely to be more variable in males than in females because each male is capable of inseminating many females and therefore all females are likely to be inseminated while some males will achieve several matings and others none.

Departures from random mating with respect to inversion karyotype in natural populations have rarely been observed - presumably because this is technically difficult. Borisov (1970) has obtained

evidence for departures from panimixis in D. funebris for the II-1 inversion and Stalker (1976) has found positive assortative mating for inversions in natural populations of D. melanogaster. However the majority of studies have been in the laboratory, especially with D. pseudoobscura and D. persimilis (reviewed by Spiess 1970 and Ehrman & Parsons 1976).

A contribution of unequal mating success to the maintenance of chromosomal polymorphism in D. persimilis was first suspected when a consistent increase in frequency of the Whitney (WT) arrangement was observed between the introduction of virgin flies and the collection of eggs (Spiess & Langar 1961). Comparisons in mating tests with Klamath (KL) homozygotes both alone and in competition for females, showed that WT homozygote males mated more than twice as often and that WT homozygote females also mated more often. This result was in agreement with the natural frequencies of the two sequences at their site of origin where WT was common and KL rare.

Later tests involving all possible genotype combinations were carried out (Spiess & Langar 1964a). These were "no choice" tests, that is with just one type of male and one type of female, and the results were expressed as the percentage of flies mating during one hour's observation. There were striking differences. In WT/WT male by WT/WT female crosses 84% of pairs mated, whereas in KL/KL male by KL/KL female crosses only 18% of pairs mated. WT/WT males by KL/KL females gave 30% mating and KL/KL males by WT/WT females gave 63% mating indicating that the females were the more important, although not the sole, determiners of mating speed. It seems that WT/WT males court more actively and WT/WT females are more receptive. WT/WT females also become receptive sooner after eclosion. Heterokaryotypes were intermediate when mated with themselves but when

mated with homozygotes the mating speed was largely determined by the homozygotes regardless of whether they were male or female.

Similar experiments with D. pseudoobscura (Spiess & Langar 1964b) showed a remarkable parallel between the frequencies of arrangements in the natural population from which they came and their mating frequency in homozygous combinations. Mating frequencies were: STxST 75%; ARxAR 81%; TLxTL 44%; CHxCH 45%; PPxPP 24% in one hour of observation with arrangements ordered in descending proportions in the natural population. When heterozygotes were examined as well (Spiess, Langar & Spiess 1966) heterozygous males were in all cases superior to either homozygote. This was not true of females. It was not clear whether heterozygous males were more sexually active, more persistent in courtship or were preferred by females.

Kaul and Parsons (1965) studied both mating speed and the duration of copulation in D. pseudoobscura for the ST and CH arrangements. They found both to be controlled by the males. ST/ST flies mated faster but CH/CH had a shorter duration of copulation giving an overall advantage to the heterozygotes. More recently Anderson and McGuire (1978) have studied mating "pattern" and mating "success" in large experimental populations of D. pseudoobscura with the ST, AR and TL arrangements. They define "pattern" as the tendency of flies to mate preferentially with flies of particular genotype, and "success" as the frequency of matings achieved by a genotype relative to its frequency in the population. Mating pattern did not deviate from the random expectation but there were significant differences in success between genotypes of both sexes.

The relationship between inversions and mating speed has been examined in several other species of Drosophila. In D. subobscura

inversion heterozygosity has an indirect effect on mating speed via its effect on adult size (Monclús & Prevosti 1971). Males of D. pavani court more actively if they are heterozygous for an inversion on chromosome IV (Brncic & Koref-Santibanez 1964). In D. robusta interactions between inversions on the X, second and third chromosomes affect mating speed in both males and females (Prakash 1969). Prakash (1967) has also shown, using D. robusta, that male mating speed is correlated with fertility and is therefore a good measure of reproductive fitness, at least in Drosophila species.

Components of mating behaviour associated with inversions are not independent of the environment, either external or genetic. Parsons (1977) reviewed evidence for differences in behaviour between strains with the same karyotype and concluded that the effect of the genetic background was considerable. The response of a particular karyotype is therefore unlikely to be constant either geographically or temporally. However Yu and Spiess (1978) found no significant effect of genetic background on female receptivity in D. persimilis. They did find a different order of the genotypes at 15°C (MD/MD > MD/KL > KL/KL) from the order at 25°C (MD/KL > MD/MD = KL/KL) where MD is the Mendocino arrangement. This may tie in with the annual cycle of these arrangements - MD is common in spring and KL in summer.

A variety of other environmental variables has been tested in D. persimilis (Spiess & Spiess 1967, 1969) including: 1) food types on which larvae were fed which had no effect on mating speed; 2) larval density - higher densities produced less active adults and slower mating; 3) adult density - higher densities produced faster mating especially of the less active genotypes and probably because of the extra stimulation experienced by females; 4) sex ratio -

little real difference, although males tended to waste less time courting other males when females were in excess; and 5) adult age - males became sexually active after 24 hrs and their activity increased for the next three days, females became receptive after 48 hrs and their receptivity increased for about 8 days (but note the differences between karyotypes above).

Probably the most significant environmental factor which has been investigated is the relative frequency of the competing karyotypes. Petit (1951,1958) was the first to observe the so called "rare male effect" or "minority advantage" in multiple choice matings involving Bar and wild type D. melanogaster. Since then extensive experiments have been carried out with gene arrangements of D. persimilis, D. pseudoobscura and members of the D. willistoni group. This work is well summarised by Spiess (1970,1982) and by Ehrman and Petit (1969) who have been the main workers in the field. Males have been found to mate more often when in a minority than when in equal proportions in almost all cases. This includes experiments where males differed in inversion karyotype or mutant genotype and also cases where they were of the same karyotype but from different populations or had been reared under different conditions. Variations in frequencies of females have rarely been shown to have any significant effect. Clearly the flies must be extremely sensitive to variations in courtship cues to be able to make these distinctions. Ehrman has used a double chamber technique in which the experimental flies are separated from other flies by a cheesecloth partition to investigate the nature of the stimuli involved. The rare male effect can be eliminated by enclosing males of the rare type in the other chamber to balance the ratio,

but enclosing females in this way has no effect. By generating an air flow between the chambers Ehrman has shown that the stimulus is air borne - presumably either olfactory or auditory. These results demonstrate that the minority advantage arises from the females' ability to detect the difference between the types of males and to alter their receptivity in favour of the minority type. A behavioural mechanism that has the effect of favouring rare males has recently been described in D. melanogaster (Spiess & Kruckeburg 1980) and D. silvestris (Spiess & Carson 1981). Females in both cases discriminate against males of the same type as the first male to court them. In D. melanogaster the discrimination is probably based on differences in courtship sequence between the males used, and in D. silvestris on the tibial bristles which are used in courtship.

The rare male effect is, of course, a type of frequency dependent selection and it is, therefore, potentially a very important force in maintaining inversion polymorphism in natural populations.

5) Coadaptation A wide variety of effects of inversions on the phenotypes of their carriers has been described. Many of the characters have been directly related to fitness and often the selective differentials involved have been surprisingly large (up to 0.5 and more). The laboratory estimates of selection might have seemed unreasonable had it not been for the evidence from natural populations - especially the regular seasonal cycles and steep clines. However the only proximal effects of inversions are the reduction in recombination in the length of chromosome covered and possible position effects caused by the change in gene order. How are the extensive and varied phenotypic effects to be explained?

Three types of explanation have been sugested:

(i) the movement of genes into a physically different position on the chromosome affects their expression (position effect) and this can sometimes confer an advantage on the heterozygote. This seems an unlikely combination of events and it is not generally considered important (see for example Dobzhansky 1970) but some evidence for such effects has been presented for Drosophila subobscura (Sperlich 1959, quoted in Charlesworth & Charlesworth 1973).

(ii) the suppression of recombination within the inverted segment allows the accumulation by chance of different mutations in the two sequences which are effectively evolving independently. If these mutations have recessive or partially recessive deleterious effects or are overdominant then structural homozygotes will on average be less fit than heterozygotes because they will be homozygous for more of these mutations. This hypothesis was due to Sturtevant and Mather (1938) but has since been elaborated by various authors (e.g. Ohta 1971).

(iii) the suppression of recombination holds together coadapted complexes of genes, that is genes which interact epistatically in their effects on fitness (Dobzhansky 1950). This idea derives from Fisher's (1930) observation that if the alleles at two polymorphic loci interact in such a way that the fitnesses of the combinations AB and ab are greater than Ab and aB then selection will favour reduction in recombination between the two loci. The lower the recombination rate the fewer unfavourable gametes will be produced by the double heterozygote AB/ab.

A fourth hypothesis has been derived from (iii) by Wasserman (1968). If each chromosomal arrangement harbours several coadapted complexes of genes then recombination in structural homozygotes which

carry two different complexes will produce some maladaptive recombinant gametes. Structural heterozygotes will not suffer this reduction in reproductive success. The advantage of this idea is that it does not require the assumption of any heterosis except that generated by the effect of recombination.

Two points need to be stressed. These hypotheses are in no way mutually exclusive, it is quite possible that all four mechanisms are involved in the origin and maintenance of chromosomal polymorphism. Once an inversion polymorphism is established it continues to evolve. It may accumulate deleterious recessives and the genic contents of the sequences are likely to evolve in such a way as to produce fit heterozygous combinations. The difficulty lies more in explaining the origin of the polymorphism than in its maintenance. The extreme rarity of a particular inversion event makes it very unlikely that a rearrangement could drift to any appreciable frequency unless the population goes through extreme bottlenecks. An explanation for the initial spread of an inversion, therefore, needs to involve an immediate selective advantage in the heterozygote in which the new arrangement first appears.

Experimental evidence relating to the way in which inversions affect fitness comes from two main sources. The first is the study of fitness parameters in interpopulation crosses and the second, more recent, method is the observation of linkage disequilibrium between inversions and allozymes.

Laboratory population cages of Drosophila pseudoobscura started with combinations of chromosomes from within a population invariably approach an equilibrium in which all the arrangements exist at stable frequencies. Often there is evidence of heterosis. In contrast when

Dobzhansky and his coworkers (Dobzhansky 1950, Dobzhansky & Pavlovsky 1953,1958, Dobzhansky 1957) used chromosomes extracted from different geographic populations the behaviour of the laboratory populations was unpredictable and they often went to fixation for one arrangement or the other. For example when populations were established with the ST and CH arrangements from the Pinon Flats population the frequencies always stabilised in the region of 70% ST : 30% CH but when the ST arrangement from Pinon Flats and the CH arrangement from a Mexican locality were used the result varied from replicate to replicate. However some of these inter-population crosses eventually produced stable equilibria in population cages (Dobzhansky & Levene 1951). Tests of these equilibrium populations showed evidence for heterosis in egg to adult viability which had been absent in the initial crosses.

These results demonstrate that heterosis is not a feature of the gene arrangement itself but develops through coevolution of the sequences present within a population. Those alleles are favoured by natural selection that function well in heterozygous combinations with other arrangements. By chance the allele combinations that predominate in one population will be different from those in other populations. The heterosis observed within populations is a result of the coadaptation of the sets of genes within the sequences present in that population. Interpopulation crosses bring together sets of genes which are not coadapted and the outcome is unpredictable. However heterosis can evolve in these new combinations as in Dobzhansky and Levene's experiments.

The observations on interpopulation crosses are not consistent with the hypotheses of heterosis generated by position effect or

accumulation of deleterious recessives. Position effects should be properties of the arrangements themselves and thus not affected by geographical origin. In interpopulation crosses of the type so far described, the heterokaryotypes have chromosomes from different populations and the homokaryotypes from the same populations. The heterokaryotypes should, therefore, have the same, or even a greater advantage from the masking of deleterious recessives. If this is the reason for the heterosis in intrapopulation crosses it should be equal or greater in interpopulation crosses. The evidence supports the coadaptation explanation for inversion polymorphism but, of course, does not preculde some contribution from the other effects.

The progress of population cages with strains derived from separate natural populations will depend not only on the genic contents of the inverted segments of chromosome but also on the genetic background. The backgrounds from separate populations might be expected to be differeatand may be coadapted in much the same way. The lack of heterosis in interpopulation inversion heterozygotes could be due to the mixed background rather than a breakdown of coadaptation within the inversion. Dobzhansky (1950) made an ingenious test of this possibility using the ST and AR arrangements from Chihuahua, Mexico (ST^M and AR^M) and from Pinon Flats, California (ST^P and AR^P). Crosses within either population gave heterosis in egg to adult viability. The between population crosses (ST^M/AR^P x ST^M/AR^P or AR^M/ST^P x AR^M/ST^P) did not show heterosis as expected but the cross ST^M/AR^P x ST^P/AR^M did. This last cross gave structural heterozygotes in which both chromosomes were from the same population (ST^M/AR^M and ST^P/AR^P) but in a mixed genetic background. It is therefore the loss of coadaptation within the inversion that results in the lack of heterosis, not the mixing of the genetic background. This result

also confirms that homozygosity for deleterious recessives in homokaryotypes is not important. The homokaryotypes in this experiment carried chromosomes derived from different populations. They should, therefore have suffered less from homozygosity and the crosses should have shown less heterosis than intrapopulation crosses.

Further evidence for coadaptation of the genic contents of inversions comes from a number of sources. Pavlovsky and Dobzhansky (1966) found that the selective values of third chromosome arrangements in Drosophila pseudoobscura from one population had changed between the 1940s and 1960s in a way that reflected the change in frequencies in natural populations. The coadapted complexes had apparently evolved new adaptive combinations. This type of change would be difficult to explain if the polymorphism was maintained by position effect or the accumulation of deleterious mutations.

The coadaptation of the genic contents of inversions is maintained by the suppression of recombination between arrangements in heterozygotes so that each arrangement is effectively isolated from others in the population. Wallace (1953,1959) pointed out that this isolation would break down if all three members of a triplet of overlapping inversions were present in a population together. Wallace called such groups "triads" and predicted that, if inversion polymorphism was maintained by coadaptation, they would be rare in natural populations. He gave evidence from D. pseudoobscura which fitted the prediction. However there are significant numbers of exceptions to the rule in D. robusta (Levitan, Carson & Stalker 1954) and in D. subobscura (Krimbas & Loukas 1979). Zapata et al (1982) have pointed out that the historical sequence in which a triad was formed will determine the effects of recombination on coadaptation. This might explain some exceptions to the rule. Clearly any localisation

of recombination would also influence the stability of triads.

Coadaptation, or "relational balance", is not restricted to inversions but is a feature of the whole genetic system of a population (Bodmer & Parsons 1962). Crosses between strains of Drosophila pseudoobscura from different populations but homozygous for the same third chromosome arrangement gave increased egg to adult viability in the F_1 but reduced viability in the F_2 and F_3 (Brncic 1954). The F_1 increase in viability is referred to in these crosses as luxuriance to distinguish it from heterosis because its relevance to fitness is not clear. Luxuriance was interpreted as the result of increased genic heterozygosity and the reduction in viability in the F_2 and F_3 as the result of break up by recombination of coadapted gene complexes. Brncic showed that the lowest viabilities were associated with the highest levels of recombination. Similar results - F_1 luxuriance and F_2 breakdown - were obtained for viability in D. willistoni and D. paulistorum (Vetukhiv 1954) and for other components of fitness in D. pseudo-obscura (fecundity - Vetukhiv 1956; longevity - Vetukhiv 1957; body size - Anderson 1968). In addition to these mean differences there was typically an increase in the F_2 variance which was taken to indicate a reduction in developmental homeostasis resulting from the mixing of the gene pools.

This type of experiment has since been repeated in many other species including other Drosophila species, butterflies, amphibians and mammals (reviewed by Endler 1977) and there are other indications of coadaptation such as the area effects in Cepaea (Clarke 1968). The results of interpopulation crosses have been very variable. For example McFarquhar and Robertson (1963) failed to find evidence for

coadaptation in body size or development time in D. subobscura and Singh (1972) found no evidence in D. ananassae. This variability of results should not be surprising because the extent of F_1 luxuriance and F_2 breakdown depends on the amount of divergence between the populations. Divergence depends on time since separation of the populations and on the population sizes and gene flow. Hedrick et al (1978) conclude that almost any combination of F_1 and F_2 means and variances can result. Gene flow is a particularly difficult component to estimate. In Drosophila pseudoobscura recent studies (Jones et al 1981, Coyne et al 1982) have shown regular long distance dispersal over unfavourable terrain between populations which were previously thought to be isolated. Coadaptation might itself impede gene flow if the progeny of immigrant flies suffer reduced fitness through the mixing of different gene pools.

Wasserman (1972, Wasserman & Koepfer 1975) has attempted to determine the relative contributions of what he calls genic, karyotypic and supergenic levels of selection to the maintenance of inversion polymorphism. These levels correspond essentially to hypotheses (ii), (iii) and (iv) above. In genic selection the heterosis of heterokaryotypes is due to either overdominance or dominance of individual loci whose alleles are limited to the different gene orders. Karyotypic selection is due to coadaptation of alleles within a gene arrangement which adapt carriers of that arrangement to a particular component of the environment. These may be heterosis because heterokaryotypic individuals can exploit a wider range of resources or the polymorphism might be maintained by frequency dependent selection without heterosis. Supergene selection arises from Wasserman's (1968) suggestion that within

populations each arrangement might harbour several alternative coadapted complexes or supergenes. Heterosis could then result from recombinational breakup of coadaptation in homozygotes but not heterozygotes. It is quite possible that all three effects contribute to any particular polymorphism.

The technique for distinguishing these levels of selection was by making crosses between inbred strains carrying the same or different arrangements and was applied to the U_{ST} and U_{1+2} arrangements of D. subobscura (Wasserman 1972) and the AR and PP arrangements of D. pseudoobscura (Wasserman & Koepfer 1975) using egg hatch and larva-to-adult viability. In neither case was there any evidence of genic dominance or overdominance. If genic heterosis was important crosses between strains should have shown increased fitness with the highest fitness values for heterokaryotypic F_1 progenies. No significant differences were observed but highly significant differences between karyotypes were found. In D. subobscura U_{ST} homokaryotypes were superior in egg hatch and U_{1+2} in larva to adult survival. This is clear evidence for selection at the karyotypic level.

To detect selection at the supergene level reciprocal crosses were made between homokaryotypic(HO) and heterokaryotypic(HE) F_1 flies. Where the cross was HO female by HE male the supergene effect should produce a reduction in fitness relative to the HE female by HO male cross in which there was no recombination. In D. subobscura a small but significant decrease in egg hatch was observed in recombinant offspring but not in non-recombinants. No consistent effect was observed in larva to adult viability or in D. pseudoobscura for either parameter. These experiments therefore confirm the preeminent role of karyotypic selection or coadaptation but with the proviso that at least in some cases a given arrangement may contain more than

one "supergene" within a population.

The second source of information about the action of selection on inversion polymorphisms is the observation of linkage disequilibrium between chromosome arrangements and electrophoretically detected alleles at enzyme loci. Once again the most detailed studies have been with Drosophila pseudoobscura. This species has a high level of allozyme heterozygosity throughout its range whether populations are central or marginal, with the exception of the isolated population around Bogota in Colombia (Lewontin 1974). At most loci allele frequencies show little variation between populations. However this is not true of three loci - pt-10, pt-12 (Major protein bands) and α-amylase - which show marked allele frequency differences between populations reminiscent of variation in inversion frequencies. These three loci are on chromosome three and show a very strong association with chromosomal arrangements (Prakash & Lewontin 1968,1971). For example the pt-10 allele 1.04 characterises the ST, AR and PP arrangements whereas the 1.06 allele is associated with the other arrangements. Subsequently associations have been found between inversions on the third chromosome and the acid phosphatase 3 locus (Prakash 1976) and between the sex ratio X chromosome and alleles at the esterase 5 and acid phosphatase 6 loci (Prakash & Merritt 1972). Similar associations have been reported in other species including D. subobscura (Loukas & Krimbas 1975, Loukas et al 1979,1980, Charlesworth et al 1979), D. melanogaster (Kojima et al 1970, Mukai et al 1971,1974, Langley et al 1974, Laurie-Ahlberg & Merrell 1979) and D. pavani (Nair & Brncic 1971). In D. melanogaster the enzyme loci involved extend outside the physical limits of the inversions but are probably within the areas of suppressed recombination.

Some general points have emerged. The observed associations are consistent over broad geographical areas but they are not usually absolute associations, that is "wrong" combinations of arrangements and enzyme alleles occur. In D. pavani the two fourth chromosome arrangements have at least two acid phosphatase alleles each so the association is not so much a rigid fixation in each arrangement as a separate evolution of the two arrangements. The observation that overall genic heterozygosity is not associated with levels of chromosomal polymorphism in D. pseudoobscura is supported by evidence from D. subobscura (Loukas & Krimbas 1980, Loukas et al 1981).

Recently Norman and Prakash (1980a,b,c) have reexamined the association between third chromosome inversions and the α-amylase locus in D. pseudoobscura and also in the closely related species D. persimilis and D. miranda. By studying activity variation and developmental variation of amylase between strains, new alleles were revealed which removed many of the "wrong" associations between allozymes and inversions. Where the same allele occurred on different arrangements there was variation in activity associated with the arrangements. Similar activity variation was found for acid phosphatase 3 among inversions of D. pseudoobscura (Norman & Prakash 1980d).

The associations between enzyme loci and inversions have been regarded as "direct evidence of coadaptation in gene arrangements" (Prakash & Lewontin 1968,1971) and as evidence that suppression of recombination in heterokaryotypes can result in extensive genetic divergence between inversions (Norman & Prakash 1980a). However associations of this type would be expected on either the genic or karyotypic models of inversion polymorphism and, where a number of alleles are associated with each arrangement, the supergene model

as well. There is also the possibility that chance linkage disequilibria between inversions and selectively neutral enzyme alleles might decay very slowly because of the reduced recombination. Such disequilibria could arise either when the inversion first appears - when it will be absolutely associated with the allele in the gamete in which it arose - by mutation or by founder effects. The half-life of such an association is expected to be approximately the reciprocal of the double recombination rate (Ishii & Charlesworth 1977) and so a neutral explanation for an association cannot be ruled out unless the age of the inversion and the rate of double recombination are known. Nei and Li (1980) found non-random associations arose frequently in their models due to drift and founder effects and decayed very slowly. They concluded that it was not necessary to invoke selection acting on enzyme loci to explain the associations which had been published up to that time. If the neutral explanation is correct then the associations are no help to explain the selection acting on the inversions.

Whilst these neutral explanations can apply to many situations it is difficult to apply them to the observations of Norman & Prakash (1980a,b,c,d). Here each of a series of different arrangements has a unique combination of an allele at the structural locus (α-amylase or acid phosphatase 3) and a cis-acting activity variant - probably a regulatory locus of some sort. An explanation in terms of coadaptation of loci within the inversion to increase fitness in some component of the environment seems much more plausible. It will clearly be difficult to make an experimental distinction between these possibilities.

6) Theory The experimental evidence reviewed above favours karyotypic selection, or selection acting on coadapted gene complexes held together by the suppression of recombination, as the explanation for inversion polymorphism. Is this conclusion supported by theoretical studies?

The coadaptation hypothesis depends on the assumption that where alleles at two or more loci interact epistatically in their effects on fitness there will be selection for increased linkage (reduced recombination) between the loci. One way of achieving the reduction in recombination would be the introduction of an inversion covering the loci under selection. The idea originated with Fisher (1930). It was applied to inversions by Haldane (1957) who showed that for coadaptation to evolve it was necessary for heterosis to be cumulative. In other words it was necessary that the fitness of a heterozygote at any given locus in the coadapted complex should be increased by heterozygosity at other loci.

Lewontin and Kojima (1960) studied the evolution of multi-locus systems in general. They confirmed that linkage would have no effect in the absence of epistasis and found a relationship between the extent of epistasis and the linkage required to alter the equilibrium state. The greater the "epistatic deviation" - the reduction in fitness of single heterozygotes relative to double heterozygotes and homozygotes - the looser the linkage required. As inversions can provide very tight linkage only slight epistasis may be necessary for them to be favoured.

The general development of the theory of multi-locus systems and the importance of linkage has been reviewed by Bodmer and Parsons (1962) and Hedrick et al (1978). A solution of all possible equilibria

when an inversion is introduced into a two locus system was obtained by Deakin (1972) by a generalisation of Lewontin and Kojima's (1960) model. However the most useful contribution has been the development of a theory for the conditions of establishment of a new inversion (Charlesworth & Charlesworth 1973). The principal conclusion from this theory is that there will be appreciable selection for a newly arisen inversion only if the population is close to an equilibrium with linkage disequilibrium. As epistasis is a necessary condition for stable linkage disequilibrium it is also a necessary condition for the establishment of an inversion. However it is not sufficient because some types of epistasis do not generate linkage disequilibrium. For example in the model of Fraser and Burnell (1967) using multi-locus simulation, stable inversion polymorphism was only established when the inversion was introduced at a high frequency. This system did not generate stable equilibria with linkage disequilibrium. While it is possible for drift to produce high frequencies it seems unlikely that this will be a satisfactory explanation for the numerous inversion polymorphisms in species such as D. subobscura and D. pseudo-obscura.

The selection in favour of a newly arisen inversion in the Charlesworth and Charlesworth model is approximately equal to the excess fitness of the gametic type in which it arises relative to the mean fitness of the equilibrium population. From a slightly different point of view the chance of survival of a newly arisen inversion depends on the recombinational load in the original population due to the segment of chromosome covered by the inversion. The levels of linkage disequilibrium required to generate reasonable probabilities of survival are quite low.

Theoretical considerations therefore support the idea that inversion polymorphism depends on the coadaptation of the genes within the area of suppressed recombination. It must be emphasised that the selection observed on established polymorphisms is not equivalent to the selection pressures responsible for their establishment. During the spread of a new inversion the genetic composition of the original population will change, for example in a two locus system the equilibrium population may contain only the AB(inversion) and ab types of gamete. Selection will also favour genetic changes that increase the fitness of inversion heterozygotes and the inverted arrangement will tend to accumulate differences from the standard arrangement.

The genic selection hypothesis is also workable theoretically (Franklin & Lewontin 1970). In a model with many loci all showing slight heterosis, a stable inversion polymorphism can evolve. This does not require any interaction between loci in their effects on fitness, that is it does not involve coadaptation. However there is very little evidence for heterosis of individual loci and the genic selection hypothesis is not supported by the experimental evidence.

7) Conclusions Thanks mainly to the work of Dobzhansky and his colleagues inversion polymorphisms have a prominent place in the study of the mechanism of evolution both in natural populations and in the laboratory. Indeed it has been the interplay between laboratory and field investigations that has made the work so important. When it was first observed the effect of natural selection on inversion frequencies in the field was startling and it remains impressive. In few other systems are selective pressures strong enough for the detailed study of their effects in natural populations

and the analysis of their phenotypic basis in the laboratory. In addition inversion polymorphisms have been central to the development of the understanding of interactions between genes and the cohesive nature of the gene pool.

Perhaps the main shortcoming of the available information about inversion polymorphism is the difficulty of relating it to the ecology of the species studied. Relatively little is known of the natural environment of important species such as D. pseudoobscura and D. subobscura (Dobzhansky 1971, Krimbas & Loukas 1979). It is in this area that significant advances may be made outside the genus Drosophila in organisms that are perhaps less amenable to genetic study but whose ecology is more accessible. The seaweed fly, Coelopa frigida, is a good candidate for this role.

1.3 The Seaweed Fly, Coelopa frigida

It is clear from the preceding section that the great majority of work on chromosomal polymorphisms has been carried out with members of the genus Drosophila, especially D. pseudoobscura, D. persimilis and D. subobscura. These flies are very amenable to laboratory study but are difficult to study in the field and remarkably little is known about their ecology. Differences between flies of different karyotype which can be identified in the laboratory cannot be extrapolated to flies in natural populations to explain the observed karyotype frequencies. The seaweed fly, Coelopa frigida, combines many of the advantages of Drosophila in the laboratory with a clearly defined natural habitat which is suitable for detailed ecological study. It is also polymorphic for a number of chromosomal inversions, one of which, the α/β polymorphism of Chromosome I, shows a particularly

interesting pattern of frequencies in natural populations and has now been studied in some depth.

I will now review the literature on the taxonomy, distribution and ecology of Coelopa frigida and then describe the present state of knowledge about the Chromosome I inversion polymorphism.

1) Taxonomy

Coelopa frigida is a member of the family Coelopidae (Phycodromidae). This small family is classified in the acalypterate Diptera (sub order Cyclorrapha), as is the family Drosophilidae. The Coelopidae are considered primitive acalypterates (Surver 1974). Hardy (1956) gives a world list of the genus Coelopa in which he includes 15 species. Two of these occur in Europe together with three other members of the family (Dobson 1976) (specific names in brackets are synonyms from Egglishaw 1960a,):-

Orygma luctuosa Meigen

Coelopa pilipes Haliday (frigida Meigen)

Coelopa (Fucomyia) frigida Fabricius (gravis, simplex, parvula Haliday, eximia Stenhammer)

Malacomyia (Phycodroma, Malacomyza) sciomyzina Haliday

Oedoparea (Heteromyza, Heterochila) buccata Fallen

A key to the genera and the diagnostic features of the family are given by Séguy (1934) and the key is reproduced in Dobson (1976). All the species are littoral, living in piles of rotting seaweed. Recent descriptions are given in Egglishaw (1960a) and Burnet (1960a,b).

Since Fabricius (1805) described the species Musca frigida there has been considerable confusion over the taxonomy of the Coelopidae as evidenced by the number of synonyms (see Walker 1853, Malloch 1933, Hennig 1937). Much of this confusion was due to the wide range

of adult size in C. frigida. In natural populations adults vary from 3 to 10 mm in overall length. This variation was shown by Mayhew (1939) and Remmert (1955) to be related to the degree of crowding of larvae during growth. Previously the "species" eximia, gravis and parvula had been separated from frigida largely on the basis of size differences. Remmert (1957) has suggested that distinct ecological races of C. frigida exist within Scandinavia which might warrant species status. This was based on their different mating and egg laying requirements which could be a barrier to interbreeding. However Dobson (1974a) has successfully interbred flies from various different parts of the United Kingdom and Day et al (in prep.) found no evidence of reproductive isolation in laboratory crosses between British and Danish flies.

2) Distribution

Hennig (1937) describes the distribution of Coelopa frigida as extending up the Atlantic coast of Europe from Brittany to Northern Norway and Spitsbergen, the Faroes, Iceland, the New England coast of the U.S.A. and Alaska, Russia and Northern China in the Pacific. Surver (1974) describes the North American distribution in more detail. He claimed that C. frigida does not occur on the Pacific Coast, where it is replaced by other Coelopa species (Aldrich 1929, Poinar 1977), but that the East Coast specimens are morphologically indistinguishable from European C. frigida. Unfortunately there has been no attempt to interbreed European and North American flies.

Mayhew (1939) described the European distribution of C. frigida as extending further south to Cape Finisterre in Spain and compares this with the altogether more southerly distribution of C. pilipes which extends from Morocco to Sweden. C. frigida has not been found

in Greenland (Madsen 1936,1940 quoted in Egglishaw 1958) but definitely occurs in Spitsbergen (Summerhayes & Elton 1923 quoted in Egglishaw 1958, Remmert 1965, Mr.A.Newton, Cambridge Spitsbergen Expedition 1980 pers.comm.). Backlund (1945) reported that C. frigida extends into the Baltic as far as Nyland.

Dobson (1974a) has described the distribution around the coast of the British Isles in general and along the coast of Northumberland and Durham in particular. C. frigida is restricted to areas of rocky coast or offshore rocky outcrops where the large brown seaweeds grow. It is also restricted to areas where there are sheltered beaches on which seaweed can be deposited. The distribution found by Dobson is largely predicted by these requirements.

3) Ecology

There are several mentions of Coelopa frigida in the early years of this century (Carpenter 1901, Miall 1903, Elwes 1915, Yerbury 1919). All these authors described its association with piles of rotting seaweed around the high tide mark of beaches and noted that it can reach large numbers. More recently there have been systematic studies of the ecology of these accumulations of seaweed by Backlund (1945) in Scandinavia and Egglishaw (1958) in England both of which contain much information about Coelopa frigida. Dobson (1973, 1974a,b) has undertaken a detailed study of C. frigida and C. pilipes in North-East England.

Coelopa frigida breeds throughout the year in piles of rotting seaweed cast up on beaches by the action of tides and winds. These seaweed accumulations have been called "wrack beds" by Backlund (1945) who classified them under three headings:-

Wrack Strings - small accumulations less than 15 cm wide and less than 15 cm deep which are usually dry and decaying very slowly

Wrack Flakes - carpet like accumulations which may be extensive but shallow and usually decay slowly

Wrack Banks - larger accumulations, which are always moist, warm and rapidly decaying, at least in the deeper layers.

C. frigida is largely restricted to the wrack banks although Dobson (1974a) has observed larvae in smaller beds particularly in warm weather. Banks may reach very large sizes. Dobson reported one bed 90 m long, 10-15 m wide and 1 m deep, approximately 1000 m^3 of weed. Even larger beds occur in Scandinavia (Backlund 1945, Day, pers.comm.). However in these larger beds a distinct layering can be observed. The outermost layer is dry and brittle; there is then a layer apparently undergoing aerobic decay which is warm and moist; and finally the interior of the bank undergoes anaerobic decay and is hot and foul smelling. In these conditions, which are rare in British wrack beds, C. frigida is restricted to the middle, aerobic layer. The larvae of C. frigida are associated with a distinct type of decay in a wrack bed. Where they are present decomposition is rapid and the seaweed quickly disintegrates into a wet, slimy mass; where they are absent decay is slow and the wrack bed tends to dry out.

The decomposition of seaweed by microorganisms generates heat and wrack banks are almost always above ambient temperature, indeed a typical wrack bank containing larvae has a temperature of 20-25°C (Burnet 1961, Dobson 1974a) and the anaerobic layers of large banks

may reach temperatures in excess of 50°C (Day, pers.comm.). Burnet (1961) found that females laid more egg batches at 25°C than at 15°C when given a choice of laying sites in the laboratory. He also found in natural wrack banks that the density of larvae was strongly correlated with temperature up to a plateau at about 20°C. When a new wrack bed is deposited it probably starts to warm up unevenly due to variations in depth and composition. Females preferentially lay their eggs in "hot spots" and colonisation of the wrack bank spreads out from these points.

Coelopa frigida females lay their eggs in more or less discrete batches. Thompson (1951) found the average number of eggs per batch to be 72 and that a single female laid 3 or 4 batches at about 24 hr intervals when given seaweed and kept at 24°C. Burnet (1960c) observed batches ranging in size from 20 to 160 eggs per batch with the modal size range being 60-69 eggs per batch. Both Thompson (1951) and Collins (1978) found mean hatch rates of about 98%. This type of egg laying behaviour might be expected to lead to a patchiness in the distribution of larvae within a wrack bed. Burnet (1961) estimated larval densities using a soil corer and found a highly clumped distribution of larvae at low mean densities, but a more even distribution at high mean densities. He attributed the clumping to a tendency for larvae from a single batch to stay together in "family aggregates" and to the preference of females for "hot spots" at which to lay eggs. The more even distribution of larvae observed at higher temperatures and higher mean densities may represent the overlap of many, spreading aggregations. There may also be an effect due to greater activity of larvae at higher temperatures resulting in greater dispersion of individual aggregates.

Coelopa frigida has three larval instars, a prepupal instar, a pupa and adult. The pupa is enclosed within a puparium and for simplicity the prepupal and pupal instars will be considered together. The morphology of the preadult and adult stages has been described by Mayhew (1939), Egglishaw (1960) and Burnet (1960a,b). Rowell (1969) gave details of the morphology of the digestive system. Surver (1974) compared North American specimens with these descriptions and found no differences.

Thompson (1951) measured the average durations of the stages at 24°C in her standard laboratory conditions:-

	Larval Instars				
Egg	1st	2nd	3rd	Pupa	Total Egg to Adult
20.6 hrs	22.3 hrs	23.8 hrs	96.6 hrs	94.9 hrs	11.47 days

Aziz (1975) reported similar values although he found the length of the third instar to be very variable (96-227 hrs). This is more in accord with the work of Collins (1978) and Day et al (1980) which is discussed in detail below. Dobson (1974a) suggested that the life cycle in natural conditions probably takes about 3 weeks. Towards the end of the 3rd instar larvae become unusually active and tend to move to drier areas of the wrack bed. Pupation occurs at the outer edges of the bed where large "rafts" of pupae may form.

Surver (1974) found similar values to those of Thompson for the development time of North American C. frigida but the development was highly synchronised throughout the life cycle. Thus the mean time span over which flies from a single egg batch eclosed, averaged over ten batches, was 87 mins (standard deviation 3 mins). By contrast eclosion of British flies in laboratory culture might span several days.

Thompson (1951) found that, at 24°C, males first mated at 14 hrs and females at 16 hrs after eclosion but matings did not produce fertile eggs until the males were at least 20 hrs and the females 18 hrs from eclosion. (Females do not lay their first egg batch until at least 25 hrs after eclosion.) Females mate repeatedly before laying an egg batch but the last male to mate is apparently the successful one. Thompson mated 20 females homozygous for a recessive white eyed mutant with white eyed males and then with wild type males. In each case all the offspring from the first egg batch were wild type. Burnet (1960c) confirmed this observation using other visible mutations.

Evans and Philip (1964) found that the variances of sex ratios and lethal segregation ratios in C. frigida were lower than expected on a binomial model. They suggest that this observation could be explained if batches of eggs were fertilised by batches of sperm rather than individual sperm chosen at random.

Thompson (1951) observed no well defined courtship behaviour. Males may attempt to mount any fly, male or female, with which they come into contact in the course of normal activity. Females respond in one of two ways: by extending the ovipositor to allow copulation, or by bending the tip of the abdomen down to avoid copulation in which case the male dismounts. Matings were observed at all times of day.

Coelopa frigida is apparently completely restricted to wrack beds. Reports (Oldroyd 1954) that it will breed in compost heaps have not been substantiated, neither have observations that adults visit flowers for nectar.

In this country most of the wrack beds in which Coelopa larvae

feed are composed of brown algae of the genera Laminaria and Fucus (the term "wrack bed" is perhaps rather confusing as Fucus is commonly known as wrack and Laminaria as kelp). The proportions of these two types of weeds vary greatly between sites and also between wrack deposits at any given site. C. frigida will breed successfully in the laboratory on Laminaria alone but not on Fucus alone and on mixtures of the two its success is similar to Laminaria alone (Thompson 1951).

The chemical composition of a variety of seaweeds has been studied by Black (1948,1951a,b). In several species of Laminaria he found a marked seasonal cycle in a number of components including cellulose, laminarin and mannitol. The cycles are related to the periods of rapid growth in the spring and autumn when nutrient availability is high. Cycles were also found in some species of Fucaceae but not in others (Black 1951b). The impact of these changes on Coelopa populations is unknown.

The salinity of wrack beds may also be an important variable (Backlund 1945, Day et al, in prep.). C. frigida is known to be able to excrete a hyperosmotic fluid in the rectum (Sutcliffe 1960) as is commonly found in other salt tolerant insects.

Burnet and Thompson (1960, Thompson 1951) investigated the requirements of larvae in laboratory culture in a factorial experiment. Factors which were found to improve larval survival were the presence of Laminaria, but not of Fucus, the addition of fresh yeast and the addition of cellosene wadding soaked in seawater. The cellosene probably functions by increasing humidity in the early stages and providing drainage later in development.

Using a standard culture regime in half-pint milk bottles they

studied the relationship between viability and larval density with different amounts of Laminaria. They found that viability increased with density to a peak of about 90% at approximately 1 larva per gram of Laminaria. After a short plateau the viability fell rapidly with further increase in larval density. Development time increases and adult size decreases with increased larval density (Mayhew 1939, Collins 1978).

Rowell (1969) repeated Thompson's experiments with similar results and attempted, unsuccessfully, to rear Coelopa on seaweed-free media. He found that the larvae would not survive on sterile seaweed but required some component of the microflora of normal decaying seaweed for growth. Larvae have a well developed gut microflora within two days of hatching which is apparently essential for survival and growth beyond this point. Rowell was unable to isolate specific microorganisms capable of supporting growth of C. frigida or to specify what was produced from seaweed by micro-organisms which was essential for growth. However Surver and Bender (1973, Surver 1974) have developed a kelp free culture medium which gives good results with North American flies.

Adult flies also have a complex gut microflora. They can frequently be seen imbibing liquids from the surface of rotting seaweeds or culture media. Their longevity in the laboratory can be extended by providing any of a variety of sugar solutions of which mannitol is apparently the best (Burnet 1960c, Burnet & Thompson 1960). Mannitol is known to be an abundant constituent of brown algae.

Wrack beds are temporary deposits. From time to time they are washed away by the sea. This imposes important constraints on animals

that depend upon the beds for food, including Coelopa frigida.

The typical cycle is for a wrack bed to be deposited around the time of a spring tide and then to be removed by the next spring tide 28 days later. However the wrack bed may be removed after only 14 days by the inter-spring high tide or it may stay for 42 or even 60 days or more if subsequent spring tides fail to remove it. In addition to this periodicity there is a random effect of winds. These affect not only the amount of seaweed available (strong winds dislodge more weed) but also the chances of removal or deposition of wrack beds, because onshore winds give higher tides and vice versa. Dobson (1973,1974a) observed durations of individual wrack beds ranging from a few hours to 82 days. In the Baltic, which is not tidal, some wrack beds are permanent (Backlund 1945).

Typically the removal of an old wrack bed is followed immediately by the deposition of a new one (Dobson 1974a). However, there is sometimes a gap when there is no weed available on the beach. This gap may be several days or weeks, and at some sites even several months (Dobson 1973).

The development of C. frigida appears to be closely attuned to this cycle of food availability. In the laboratory egg to adult development takes about 2 weeks. In natural conditions development probably takes nearer 3 weeks (Dobson 1974a) and there is probably a delay of a few days between deposition of the seaweed and egg laying during which the temperature of the bed rises. Therefore, if the wrack bed duration is 28 days, most flies will have emerged before it is removed. When a bed is removed adults are able to retreat up the shore or into the vegetation immediately behind the beach. In contrast, the larvae and pupae will be washed out to sea

with the seaweed. Dobson (1974a) found that larvae could survive up to six days immersion in aerated seawater and often observed larvae redeposited on the beach after a wrack bed had been swept away. Ardö (1957) also observed redeposition of viable pupae. A large proportion of these larvae and pupae are probably eaten by birds and many must die through desiccation or starvation. Occasionally they may end up under stones or in a wrack string where they may survive several weeks. In this case they could contribute to the Coelopa population of a subsequent wrack bed.

The bulk of the colonization of a new wrack bed is by adults. Adult longevity has been estimated by Burnet (1960c), Dobson (1974a) and Collins (1978). They agree that in natural conditions a longevity of 3-4 weeks is likely to be typical. Only very rarely does the interval between wrack beds exceed this.

It is possible in some cases for Coelopa populations to complete two generations in a single wrack bed. However, given that a generation probably takes about 3 weeks in nature, a second generation is most unlikely in a typical bed of 4 weeks duration. By the time adults of the first generation start to emerge from a wrack bed decomposition is usually advanced and the bed has cooled down to only a few degrees above the ambient temperature. In these conditions flies are reluctant to lay eggs, making a second generation unlikely in any but the largest and longest lived of wrack beds (Dobson 1974a). When a second generation does occur the adults produced are usually small and the development time is long - probably the result of a combination of low temperature and poor quality of food.

A further complication is that occasionally a new wrack bed may be deposited without removal of the old one. This is not common in Britain but does occur in the Baltic (Backlund 1945). Clearly, in

this case, the new weed will be colonized by both larvae and adults from the old bed with little mortality.

The intervals between wrack beds, the durations of beds and the amounts of weed deposited must have major effects on the densities and absolute sizes of populations of Coelopa frigida. A succession of small beds or beds of short duration would drastically reduce the population size as would a long interval between wrack beds. Conversely large beds of long duration in quick succession could give a massive build up in numbers. Density of larvae in a wrack bed depends on a combination of the size of the bed and the size of the colonizing population.

At present only the work of Dobson (1973,1974a) gives a record of the actual succession of wrack beds at various sites. More information of this type would greatly increase our understanding of the population dynamics of C. frigida.

Population sizes of C. frigida can be very large. Indeed they can be so large as to cause a public nuisance (Taylor 1955, Oldroyd 1954).

Both Burnet (1960c,1961) and Dobson (1973,1974a) have attempted to estimate adult population sizes by mark-release-recapture techniques. As wrack beds only occur at specific sites and these sites are clearly separated, it is possible to consider each colony as a distinct population. This would seem to be an ideal situation for estimating population sizes and the degree of movement between populations.

Burnet obtained two estimates of the population size at the time of removal and deposition of wrack beds at St.Mary's Island, Northumberland:-

	Male	Female	Total
February 1959	1813	1166	2979
March 1959	696	672	1368

Dobson considered these figures to be surprisingly low and, as a preliminary to a mark-release-recapture experiment, he estimated movement of flies within the St.Mary's Island colony. With the exception of a few individuals which moved about 200 m, all recaptures of marked flies were made within 50 m of the release points and most within 5 m even after 48 hours. This highly skewed distribution of movements is most unsuitable for population estimates by release-recapture methods and so no further attempts using this method were made.

Burnet's larval density estimates (Burnet 1960c,1961) give some idea of the size of the larval population. In cores 5 cm diameter and 10 cm long taken with a soil auger he found up to 184 Coelopa frigida larvae. However the distribution of larvae was very patchy and even at the peak of larval population growth some cores contained no larvae. This makes estimates of population size much less accurate but one can see that if a moderate sized wrack bed of 100 m^3 contained an average of 10 larvae per core (about 0.001 m^3) the larval population would be of the order of 10^6.

Burnet has drawn attention to the fact that the effective population size is almost certainly very different from the observed population size. In particular, the effective population size is dependent on sizes of ancestral populations giving rise to the present population. Population bottlenecks in previous generations can have a major effect (Wright 1938, Crow 1954). Although Coelopa populations can be very large they probably suffer fairly frequent reductions

following rhe removal of wrack beds. The effective population size is, therefore, likely to be small, perhaps only a few hundred, and is best estimated at the minimum point in the cycle.

Egglishaw (1958), Burnet (1960c,1961) and Dobson (1973,1974a,b) all refer to a seasonal cycle in the abundance of Coelopa frigida. The peak of abundance occurs in October with sometimes a secondary peak in the spring or early summer.

From the point of view of ecological genetics it is important to know how much movement of flies occurs between the individual Coelopa colonies spaced out along the coastline. This is very difficult to measure and so far no attempts have been made at experimental measurement. There are, however, some relevant observations:

1) As a rule C. frigida is entirely restricted to the beach and the vegetation immediately above the beach. It is strongly attracted to seaweed and when wrack deposits are present is rarely seen more than a few yards away from them. When wrack beds are absent the flies are usually found under stones, in crevices or in amongst the roots of vegetation. They seem reluctant to fly, especially in cold weather, and when disturbed usually crawl down into the wrack rather than take off. These observations fit with Dobson's (1973) data on release/recapture noted above and suggest that Coelopa is unlikely to cross the seaweed-free stretches of coastline that separate regular colonies.

2) On the other hand Dobson (1973) observed a number of "freak" wrack beds occurring away from the normal sites in North East England. Wrack beds that were not colonised were observed as little as 1.5 km from a large Coelopa population, while other were colonised that were 16 km from the nearest regular site.

3) Adjacent sites may have very different proportions of Coelopa frigida and Coelopa pilipes even when less than 2 km apart (Dobson 1973). It seems unlikely that these differences would be maintained if there was free flow of individuals between colonies.

4) Occasional "mass migrational flights" of Coelopa have been observed (Egglishaw 1961, Dobson 1974a). On some occasions large numbers of flies were in flight above the wrack bed but with no obvious net movement. On other occasions dense clouds of flies have been seen to move concertedly in one direction, not necessarily down wind, and some distance from the wrack beds. Egglishaw thought these flights might have been a response to overcrowding. Clearly such mass movements could affect the isolation of neighbouring colonies but their effect would be difficult to estimate.

In the autumn of 1953 there was a "plague" of seaweed flies on the South Coast (Oldroyd 1954) probably caused by a combination of unusually large seaweed deposits and mild weather. Shortly after this "plague" had started, flies appeared in London and even as far inland as Oxford. There was a persistent southerly wind during this period but, whether the flies flew or were blown, Coelopa adults undoubtedly travelled up to 130 km in the space of about 2 weeks. Another plague of seaweed flies was reported by Taylor (1955).

The degree of isolation of Coelopa colonies thus remains an open question. It seems unlikely that there is any regular exchange between colonies more than 1 or 2 km apart but that individuals or "swarms" of flies occasionally move much larger distances.

Backlund (1945) gave a comprehensive list of animal species found in wrack beds in the Baltic, and Egglishaw (1958) listed the species he found in North East England, mainly at Whitburn, Co.Durham.

Coelopa frigida is itself a very important species being, almost invariably, the most numerous of the large Diptera. Coelopa pilipes can sometimes become more numerous than C. frigida in the summer months. Other common large flies are Helcomyza ustulata and Fucellia maritima (Egglishaw 1960b,c). The small Borborid Thoracochaeta zosterae is also very common. Outside the Diptera the most conspicuous wrack bed inhabitants are the amphipod, Orchestia gammarella, annelids of the family Enchytraeidae, nematodes (unidentified), and several species of Staphylinid beetle.

C. frigida probably suffers little predation. Some of the Staphylinids of wrack beds are predatory on dipteran larvae (Backlund 1945) and several species of birds have been observed feeding in the surface layers of wrack beds (Backlund 1945, Ardö 1957, Egglishaw 1958, Dobson 1976). Small mammals may also feed on larvae in wrack beds (Dobson 1974a,1976).

Scott (1920) reported that C. frigida may be used as host by the parasitic staphylinid Aleochara algarum. Backlund (1945) and Egglishaw (1958) both found a variety of parasitic hymenoptera in the wrack fauna. Backlund recovered dipteran pupae from wrack beds to assess the amount of parasitisation and found high levels of infestation in some species but not in C. frigida. On the other hand Ardö (1957) collected C. frigida pupae washed up on the beach after removal of an old wrack bed and found a high frequency of parasites. He attributed this to the fact that several generations of C. frigida had grown in the same wrack bed allowing the parasite population to build up.

Egglishaw (1958) also found a mite Thinoseius fucicola to be

common in wrack beds. It is phoretic on C. frigida and C. pilipes but whether its effect is harmful or not is unknown. Mites were frequently encountered in my collections from natural populations and when they infested laboratory cultures they had a detrimental effect. I observed very few parasites in pupae from these collections.

In general it seems unlikely that predation and parasitism are major causes of mortality in Coelopa populations.

An interesting aspect of the ecology of C. frigida is the fact that it occurs in close association with the other European Coelopa species C. pilipes. Adults of the two species are easily distinguished - C. pilipes is darker in colour and hairy rather than bristly - but the larvae are difficult to distinguish (Egglishaw 1958,1960a, Burnet 1960b). The two species are almost always found together but C. frigida tends to be more common in the winter months and C. pilipes in the summer, possibly reflecting the more southerly geographic range of C. pilipes. Egglishaw (1958) observed a rapid switch in abundance in spring and autumn but this does not appear to have occurred in more recent years (Dobson 1974b). The pattern of frequencies is probably more complex than was previously thought. The larvae occur together (Burnet 1960b,c,1961) but C. pilipes larvae are less clumped than C. frigida, probably because C. pilipes lays its eggs singly. Much of what has been said about C. frigida applies also to C. pilipes but Dobson (1973,1974b) has identified three areas of difference:-

1) C. pilipes develops more slowly than C. frigida taking about 4 days longer from egg to adult in laboratory culture at 22°C.

2) C. pilipes is reluctant to lay eggs in the absence of Fucus unlike C. frigida which requires Laminaria alone.

3) C. frigida is more willing to take flight and may be a better coloniser of new wrack beds.

Dobson doubts whether these factors alone can explain the observed proportions of the two species in natural populations and so the ecological relationship between the two species remains an open question.

4) Genetics

The first genetical studies on Coelopa frigida were made by Thompson (1951). She isolated mutations by repeated sib matings of flies from natural populations including eye colour, bristle and wing morphology mutants. One of the latter, "Delta", was a recessive lethal and its effects were further investigated by Bawady (1954). 9.3% of wild flies carried a recessive mutation - a low frequency by comparison with Drosophila - which might be a consequence of a small effective population size.

Burnet (1960c,1961) isolated recessive embryonic lethals from a natural population. The gross frequencies on three separate occasions were 2%, 3.7% and 6.5%. He tested these lethals for allelism and estimated the frequencies of those which occurred more than once. These were higher than expected on the basis of his estimates of the population size, assuming a mutation rate similar to Drosophila and no selection acting on heterozygotes. The most plausible explanation for the discrepancy is probably that the effective population size is smaller than the census size. The observed gene frequencies might then be in the upper tail of the wide frequency distribution expected in a small population. A small effective population size could result from non-random mating, a fluctuating population size, unequal numbers of males and females, or a non-Poisson distribution of progeny number (Crow 1954), all of which may well occur in Coelopa. Burnet (1960c) estimated the ratio

of effective to census population size as 0.15.

Burnet (1960c,1962) examined the embryological development of homozygotes carrying the recessive lethal mutations mentioned above. The fact that Coelopa frigida lays eggs in batches which develop synchronously makes it a particularly suitable subject for embryological work and the early studies of Bawady (1954) and Burnet (1962) have been followed by several others (Schwalm, Simpson & Bender 1971, Kruse 1972, Schwalm & Bender 1973, Muhlach & Schwalm 1977).

C. frigida has 5 pairs of metacentric chromosomes and 1 pair of dot shaped chromosomes (Aziz 1975). There is no detectable difference in karyotype between the sexes. Philip (1958,1966) found three populations on the north east coast of England to be polymorphic for a number of inversions. Two sequences of Chromosome I were observed which differed in three overlapping inversions. The chromosome I heterokaryotypes were more common than expected. 8 sequences of Chromosome III were present in approximately equal proportions - they resulted from 3 non-overlapping inversions combined at random. Philip observed no association between the Chromosome I and III polymorphisms.

Polyteme chromosome maps have been prepared by Aziz (1975) for chromosomes I, II and III of C. frigida. All three were polymorphic for inversions in natural populations.

Chromosome	No. of Bands (including double bands)	No. of sequences observed
I	355	2 (differing in 3 overlapping inversions)
II	260	2 (differing in 1 inversion)
III	342	8 (all combinations of 3 non-overlapping inversions)

No inversions were observed in Chromosomes IV and V. The two sequences of Chromosome I are designated α and β. The three overlapping inversions by which they differ span a total length of 127 named bands (about 180 bands when multiple bands are taken into account). This is about a third of the length of Chromosome I, the largest, and therefore may be up to 10% of the entire genome. Fig.1.1 shows the normal configuration of the heterokaryotype which has two loops of unequal size separated by a paired section representing 63 bands or about half of the total length. Aziz (1975) did observe occasional variations on this pattern but these were rare. The two hypothetical sequences via which one sequence was probably derived from the other, Fig.1.2, have never been observed. Aziz claimed that the α sequence was the ancestral one but there seem to be no good grounds for this conclusion.

If the homologous chromosomes pair during meiosis in the same configuration as in the salivary gland polytenes then recombination would not be expected to occur in the two looped sections. Single recombination events in the paired region will produce duplications and deletions but double recombinations in this region should be viable. The length of chromosome over which recombination is suppressed is not known but it probably has two peaks in the loops and may extend beyond the break points at the outer ends as well as into the central region (Day et al 1982).

Aziz (1975) also studied the pattern of activity of some chromosome puffs including those in the 3B and 5C sections of Chromosome I, that is within the inversion. The 5C puff reached its peak of activity slightly later in development when on the α sequence than when on the β sequence. Puff 3B showed no difference between sequences.

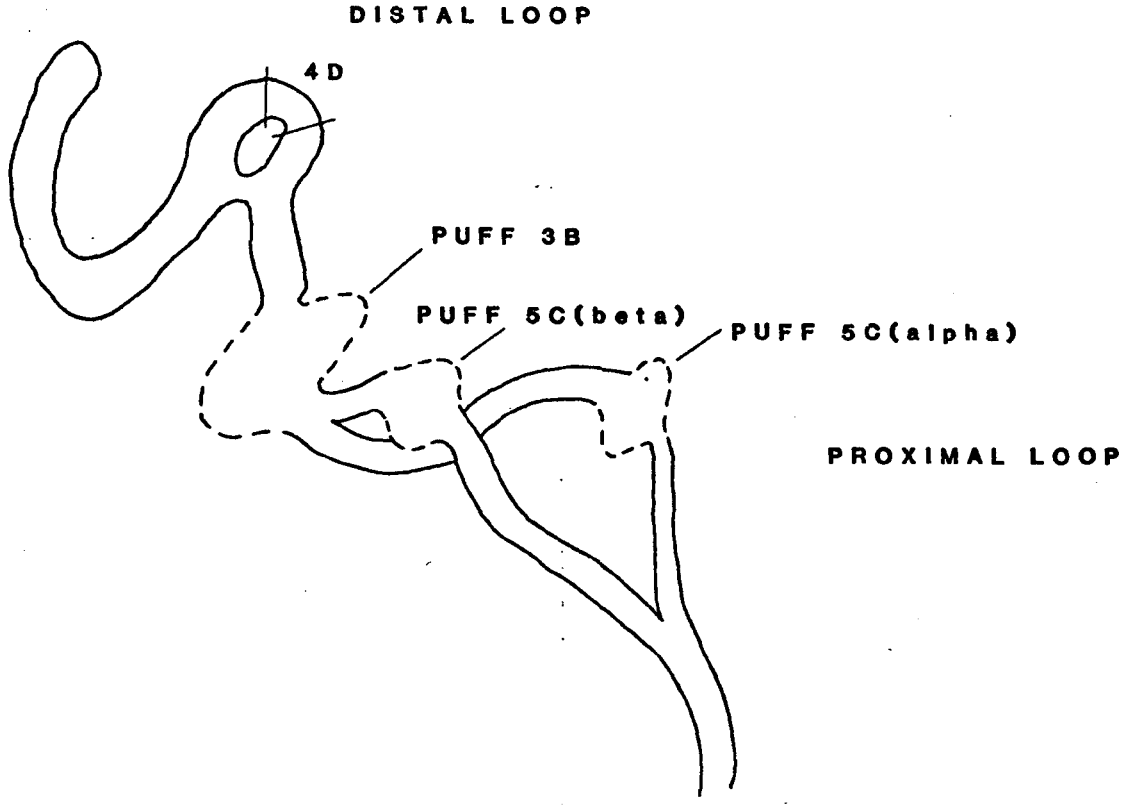


Fig.1.1 Typical appearance of chromosome arm IL in an αβ heterokaryotype

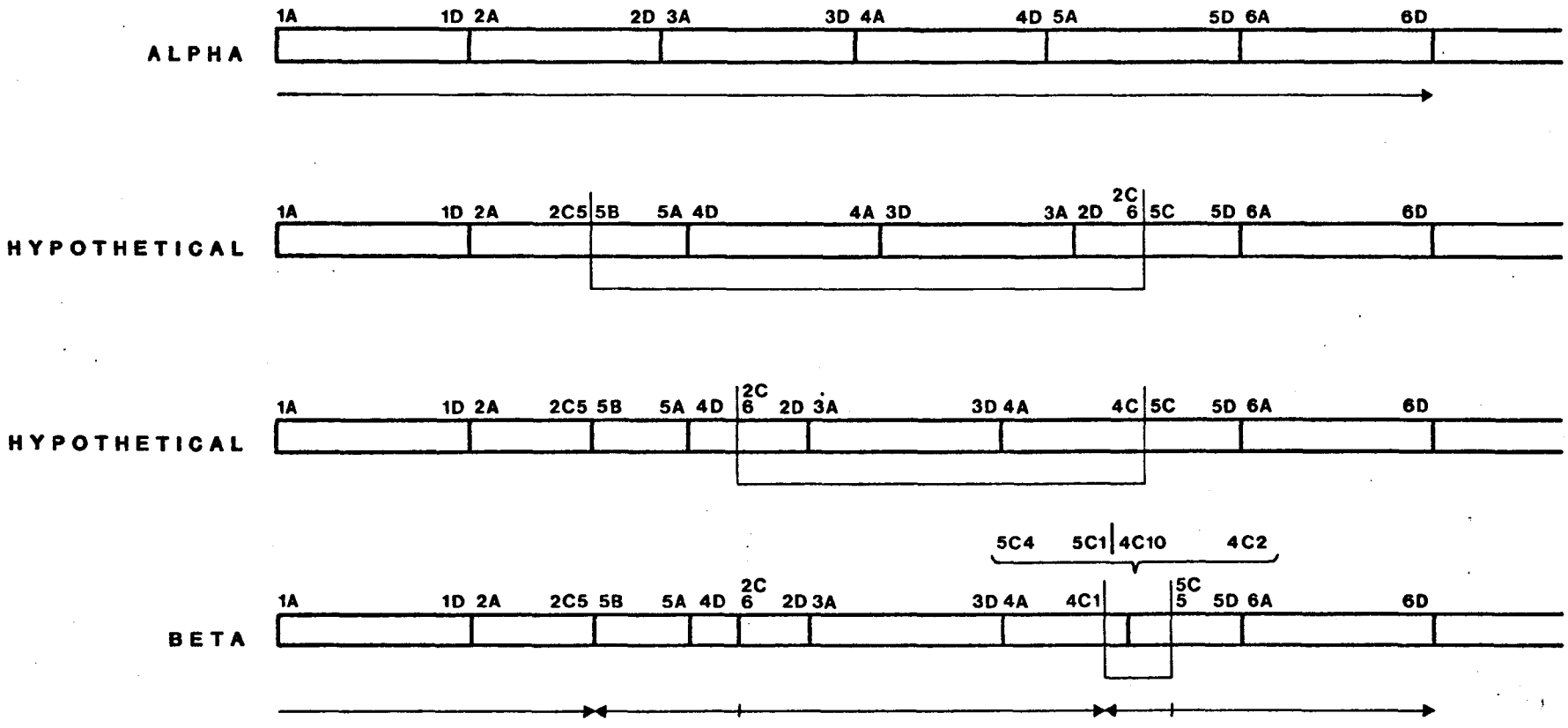


Fig.1.2 Arrangement of the principal bands in the observed and hypothetical sequences of the distal part of chromosome arm IL (after Aziz, 1975).

A number of enzyme loci in Coelopa frigida have been investigated using starch gel electrophoresis. Kelsey (1969) found a Scottish population to be polymorphic at the alcohol dehydrogenase locus. The isozyme patterns and their genetic and biochemical interpretation were reported by Day and Buckley (1980). 5 alleles have been distinguished, designated Adh-A to E, of which three B, C, and D are common in natural populations. The homozygotes have three isozymes which Day and Buckley conclude result from binding of NAD molecules on the basis of affinity for 5'adenosine monophosphate and by analogy with alcohol dehydrogenase in Drosophila (Day & Needham 1974; Day et al 1974a,b; Schwartz et al 1975). The most cathodal band has no NAD, the next one molecule of NAD and the most anodal band, usually with very weak activity has two molecules of NAD bound. The enzyme is dimeric (Day & Buckley 1980) and so the heterozygotes have basically 9 bands. However the third isozyme (+2NAD) of each allozyme stains only very weakly in most cases and some of the other bands may coincide on zymograms. A typical zymogram obtained during the present study is shown in Plate 2.2.

Day and Buckley (1980) estimated the subunit molecular weight of Coelopa ADH to be 27,000 to 28,000. They compared specific activities and thermal stabilities of six alleles extracted from natural populations. They found significant differences in both properties with a suggestion of an inverse correlation between activity and stability. A laboratory selection experiment in which larvae were stressed with ethanol did not produce any change in allele frequencies.

Collins (1978) surveyed 32 enzyme loci and found 11 of these to be polymorphic (34.4%). He selected one of the polymorphic loci, peptidase-1,a dipeptidase, for further study. There was strong

linkage disequilibrium between this locus and the Alcohol dehydrogenase locus in all populations studied. Adh-BB individuals are generally Pep1-FF, DD individuals are SS and BDs are FS. Exceptions, that is DF and BS chromosomes, amount to about 7% of chromosomes sampled. The other common Adh allele C occurs with both F and S but Collins' data suggest that the CF combination is more common than would be expected on the basis of the overall F frequency.

Linkage disequilibrium has also been detected between the esterase-2 locus and the Adh locus (Day et al 1982, Day et al, in prep.). The association is weaker than in the case of peptidase-1 but is still significantly non-random.

The linkage disequilibrium between the alcohol dehydrogenase, peptidase-1 and esterase-2 loci might be explained if these loci are within the area of suppressed recombination caused by one of the inversions described by Philip (1966) and Aziz (1975). In fact the Adh and Esterase-2 loci have been shown to be associated with the α/β inversion system of chromosome I (Day et al 1982). In the case of Adh the association is very strong indeed. The Adh-B allele is found with the α sequence and the D allele with the β sequence. In 622 chromosomes studied from natural populations only 7 exceptions (1.1%) were recorded and it is possible that these represent errors in scoring especially as they all occur in the earlier set of samples taken by Day and his coworkers. The Adh-C allele was found with both sequences but the Cα combination was more common than expected from the overall α frequency. The strong linkage disequilibrium between peptidase-1 and Adh suggests that the peptidase-1 locus is also associated with the inversion (Collins 1978). It is interesting to note that the excess of the CF combination corresponds with an excess of the Cα combination.

Day et al (1982) found a weaker association between the esterase-2 locus and the α/β inversion as expected from the weaker linkage disequilibrium between the Adh and esterase-2 loci.

The strong, if not complete, association of the Adh-B and D alleles with the α and β sequences of chromosome I respectively offers an excellent opportunity to study the maintenance of the α/β inversion polymorphism. Karyotypes can be inferred from Adh genotypes determined by starch gel electrophoresis which can be carried out at any developmental stage (larva, pupa or adult) and which allows much larger samples to be studied. Using this technique a number of important observations have been made.

Inversion frequencies in all natural populations so far studied in the U.K. are close to 0.4α and 0.6β (Collins 1978). There is also a consistent excess of heterokaryotypes and deficiency of homokaryotypes. These observations are discussed in Chapter 3 along with additional data obtained during the present study.

Day et al (in prep.) have found that α frequencies can be considerably lower in Baltic populations. Some possible reasons for this were discussed by Collins (1978) and will be reviewed in Chapter 10.

Seaweed flies of different karyotypes develop from egg to adult at different rates (Collins 1978, Day et al 1980). ββ flies develop more rapidly than αα flies and the heterokaryotypes are intermediate. This is true of both males and females but is much more pronounced in males. Day et al (1980) obtained the following results in laboratory culture at 27°C:-

Karyotype	Mean Development Time (± standard error) in days		Number of animals	
	Males	Females	Male	Female
αα	20.23±0.33	17.87±0.49	22	23
αβ	19.43±0.16	17.61±0.19	129	179
ββ	17.07±0.13	17.49±0.22	151	139

The bulk of the difference probably accumulates before pupation. Day et al (1980) have found evidence for a similar difference in development time in natural populations.

Bearing in mind the variability of duration of wrack beds (see above) these differences in development time are potentially very important in determining the frequencies of the alternative sequences. A succession of short lived wrack beds would be expected to favour the β sequence and a succession of long lived wrack beds might favour the α sequence. It is possible that, on balance, the heterozygote has an advantage. The effects of differences in development are discussed further in Chapters 4 and 10.

Collins (1978) found no differences between karyotypes in fecundity or fertility although he did note that larger females tend to lay more eggs. The karyotypes do, however, differ in longevity at 27°C. ββ flies live longer than αα flies (by 1.0 to 1.5 days on average) and the heterokaryotypes are intermediate. This would appear to be an advantage to the ββ flies which to some extent counteracts any disadvantage they might suffer from a short development time when wrack beds are long lived. Some further studies on fecundity and longevity are included in the present thesis (see Chapter 8).

There are also viability differences between the karyotypes. Collins (1978) cultured the progeny of αβ by αβ crosses at three

different larval densities. The experiments were carried out in the laboratory at 27°C on a "Marinure" based culture medium (see Materials and Methods section) using as parents flies caught in the field. Collins obtained the following estimates of relative viability -

	Inversion karyotype		
Density	αα	αβ	ββ
LOW	0.85	1	0.91
MEDIUM	0.46	1	0.48
HIGH	0.44	1	0.33

There is marked heterosis which increases with larval density. This result fits very well with the observation of a consistent excess of αβ individuals over Hardy-Weinberg expectations in natural populations. It seems likely that heterosis in viability is the major selective force maintaining this inversion polymorphism. Frequencies may, however, be modified by the interaction of development time and longevity differences (and probably other differences) with the environment. Viability differences will be discussed further in Chapters 5, 6 and 10.

Collins (1978) noted in his density experiments that there was a decrease in adult size with increasing density (as previously recorded by Mayhew 1939 and Thomson 1951) and an increase in development time. These effects are described in detail in Chapter 5.

Two further studies by Collins (1978) should be mentioned. The first is a series of laboratory population cages which he maintained, one set at high density (8 replicates) and one at low density (12 replicates) and with starting frequencies of 0.25α and 0.75α. Over 10 generations all populations moved towards an equilibrium point in the 0.4-0.5α region. Movement was slow in low density replicates

but more rapid at high density as predicted from the observed viabilities.

Secondly, Collins devised a computer simulation of the α/β inversion polymorphism in natural populations of seaweed flies. The simulation took into account heterozygote advantage, differences in development time and longevity, and seasonal changes in population size together with a probability distribution for wash-away/deposition of wrack beds based on tides and weather. Using starting frequencies of 0.1, 0.5 and 0.9α and various levels of heterozygote advantage the simulation gave the following results:-

$s_1 = s_2 = 0$ α tended slowly to be eliminated

$s_1 = s_2 = 0.1$ slow convergence to about 0.3α with wide variation

$s_1 = s_2 = 0.2$ rapid convergence to about 0.4α with less variation

(where s_1 and s_2 are the viability selection coefficients for $\alpha\alpha$ and $\beta\beta$ relative to $\alpha\beta$).

With the higher level of heterosis the resulting α frequency is strikingly similar to that observed in natural populations in this country. Furthermore removing the systematic effect of tides so that wash-away and deposition of wrack beds occurred at random produced an equilibrium α frequency of 0.34. This is very similar to the frequencies observed in natural populations in the Baltic (Day et al, in prep.).

Collins described his simulation as "simple" and admitted that it left out a number of variables. He took the surprisingly close correspondence with natural frequencies as support for the conclusion that heterosis is the main selective pressure maintaining the polymorphism. Inversion frequencies are modified by the interaction of development time and longevity differences with the variations in the wrack bed cycle.

1.4 Outline of the present thesis

I took up the study of the α/β inversion system from the point reached by Collins (1978). My initial intention was to concentrate on the viability differences between the karyotypes. The fact that these differences are dependent on larval density offered a starting point for the study which set out to answer two questions: (i) are there density dependent differences in viability in natural populations? and (ii) are the viability differences the result of depletion of limited resources in the food, or the result of build up of toxic residues, or do they have some other cause? Because progress in answering these questions was slow, and because other areas of study were suggested by observations made during laboratory culture and field sampling, I later widened the scope of my work. In particular this thesis includes results on size variation in adult seaweed flies and the effect of adult size on longevity, mating and fecundity.

The thesis is arranged in what, I hope, is a logical progression rather than in the order in which experiments were conducted. Following this introduction some general materials and methods are described together with details of the derivation of stock lines used in laboratory experiments. Methods specific to individual experiments are described in the relevant chapters. I then discuss (Chapter 3) the inversion and karyotype frequencies observed in field samples collected during this study.

There is then a short chapter giving some results on development time which extend previous observations.

In Chapters 5 and 6 I describe attempts to analyse viability differences between karyotypes and the effects of larval density. Both

viability and development time differences are used in the experiments reported in Chapter 7. These experiments examine the possibility that the inversion holds together a coadapted complex of genes and ask whether this coadapted complex is the same in two widely spaced natural populations.

Chapters 8 and 9 look at variation in adult size and its effects on longevity in males and females, on male mating success and on fecundity.

Finally I attempt to bring together all the observed differences between the α/β inversion karyotypes and discuss to what extent they account for the frequencies found in natural populations.

Chapter 2

Materials and Methods

2.1 Introduction

This chapter describes the experimental procedures that were used routinely. Variations from the general techniques will be emphasised in later chapters where appropriate. The derivation of all stock lines is also given here.

2.2 Laboratory culture

Flies were cultured in the laboratory on an artificial medium based on the seaweed fertiliser "Marinure" (Wilfred Smith (Horticultural) Ltd., Edgware, Middx.). This is produced by drying and coarsely powdering the brown seaweed Ascophyllum nodosum. The culture medium was similar to that described by Collins (1978) and by Day and Buckley (1980) but with the omission of powdered milk and of strips of Laminaria stipe on the medium surface. The constituents were as follows:-

750 ml Marinure wetted with 1200 ml of tap water
45 ml New Zealand Agar (Sigma Chemical Co., Poole, Dorset)
200 ml maize meal (Harrison Ltd., Radcliffe, Nottingham)
1650 ml tap water

The medium was prepared by bringing 1250 ml of tap water to the boil in a steel bowl and then adding the agar and maize meal, thoroughly mixed with the remaining 400 ml of water. This mixture was brought back to the boil and then added to the wetted Marinure. The complete medium was stirred vigorously to ensure homogeneity and then poured into the required containers (see below). This

recipe was followed precisely throughout the work (with the exception of some experiments described in Chapter 6) to maximise comparability of results. However the Marinure itself did show some variation, particularly in the way in which it absorbed water. Wherever possible all replicate containers for any particular experiment were made from a single batch of medium.

The sizes of containers are given in Table 2.1 with an indication of their use. All containers were of clear plastic. The tanks had

Table 2.1 Containers used in laboratory culture of seaweed flies

Name	Dimensions	Usage	Source
Tank	30x20x20 cm	Mass mated populations	North West Plastics Ltd Manchester
Large Canister	12x12x18 cm	Inbred lines	Stewart Plastics Ltd. Croydon
Small Canister	9x9x13 cm	Inbred lines	"
Round Canister	7.5 cm diameter 6.5 cm deep	Pair matings	"
Mating pots	4 cm diameter 3 cm deep	Pair matings	"

hardboard lids sealed in place with sellotape and with a central hole fitted with a nylon gauze sleeve. The large canisters, small canisters and mating pots had plastic snap-on lids and were sealed with masking tape. The round canisters had screw on lids. Sealing of lids was important because Coelopa are able to escape through very narrow openings and may contaminate other cultures. All these lids had nylon gauze covered air-vents. The vents in all lids, except the mating pots, were covered with tissues, or filter papers for the round canister, while in use. This helped to maintain a high humidity in the containers, to reduce fungal infection of the

medium, and to reduce the chances of escaped flies contaminating cultures by copulating through the holes in the nylon gauze.

All containers were filled with medium to a depth of about 3.0 cm except the mating pots which required only 1.0 cm of medium. The medium was allowed to set and then thoroughly forked over. The forking appeared to make the medium both more attractive for egg laying and more easily penetrated by young larvae. Where comparability between experimental replicates was required the forking was followed by levelling out the medium to give a more standard surface area.

Unless otherwise stated all stocks were maintained in a constant temperature room at 26°C in constant darkness. Under these conditions the generation time was usually between 14 and 21 days.

Mass cultures maintained in tanks using 100-200 pairs of flies to start each generation were rarely unsuccessful. Such cultures can produce more than 3000 adult progeny. Tank cultures started with smaller numbers of flies were less reliable as were cultures in smaller containers. However a good success rate was obtained by paying careful attention to stock maintenance. Medium was normally used on the day on which it was made but exceptionally it was stored overnight at 4°C. Cultures were never kept in the same container for more than one generation and old or unsuccessful cultures were removed from the constant temperature room.

A common problem with cultures, especially in smaller containers was drying out of the medium. Regular inspection of cultures and addition of tap water where necessary was generally sufficient to overcome this problem. A more serious danger was the infection of

cultures with a variety of microorganisms. Day and Buckley (1980) mention the fungus, Geotrichum, and a type of Streptomyces as the major contaminants but the Geotrichum was probably associated with the now discontinued use of powdered milk in the medium. Microbial infections were most common in cultures with low densities of larvae - the churning effect of larvae in dense cultures possibly prevents growth of detrimental microorganisms. Coelopa larvae apparently depend on the action of some microorganisms in the medium for their nutrition (Rowell 1969) and so the medium cannot be sterilized or have antibiotics added to remove the unwanted organisms without adversely affecting the survival of the larvae. Mites introduced with samples from natural populations can also be a problem but they do not survive in regularly changed laboratory stocks.

2.3 Handling techniques

Adult flies were removed from tanks using a standard insect aspirator and then anaesthetised with carbon dioxide (Distillers Co. (Carbon dioxide) Ltd., Reigate, Surrey) for handling. In the other types of containers flies were first anaesthetised by flooding the container with carbon dioxide through its air vent and then brushing them out. In both cases the positive phototaxis displayed by seaweed flies was used to concentrate the flies in one part of the container which greatly facilitated their removal. Separation of sexes, measurement and other operations were carried out on a porous pad (Strand Scientific, Sandiacre, Nottingham) through which carbon dioxide was passed continuously.

The sexes were very easily separated by examination of the underside of the abdomen without magnification. The male has

prominent external genitalia which are often dark in colour. The abdomen is parallel sided and rounded at the posterior end. The female has convex sides to the abdomen which is pointed at the posterior end where there are two short cerci(Plate 2.1).

When necessary flies were stored in 1/3 pint milk bottles with foam bungs at 4°C. They were supplied with 0.5% w/v Mannitol solution (BDH Chemicals, Poole, Dorset) absorbed in "Cellosene" cellulose wadding (Robinsons Ltd., Chesterfield). Burnet (1960c) found mannitol to be the best of a range of sugars on which to maintain adult Coelopa. In the course of this work adults have been stored for up to 4 weeks and still been found capable of mating and laying viable eggs.

Flies required only for electrophoresis or measurement were stored at -20°C in plastic snap-lid pots (Azlon Products Ltd., Glyn St., London).

2.4 Techniques for single pair matings

Two methods have been employed for the isolation of virgin females. The first was to remove large third instar larvae from a tank culture at about the time the first pupae appeared. These larvae were placed two at a time in mating pots with medium and returned to the constant temperature room. The pots were inspected daily and any adults which had emerged were removed. Only rarely did both adults from any one pot emerge on the same day and in some cases these were of the same sex anyway and so they were known to be virgins. If a male and a female emerged in one pot on the same day these were discarded. Where only one fly emerged on any one day from a pot it was, of course, known to be virgin.

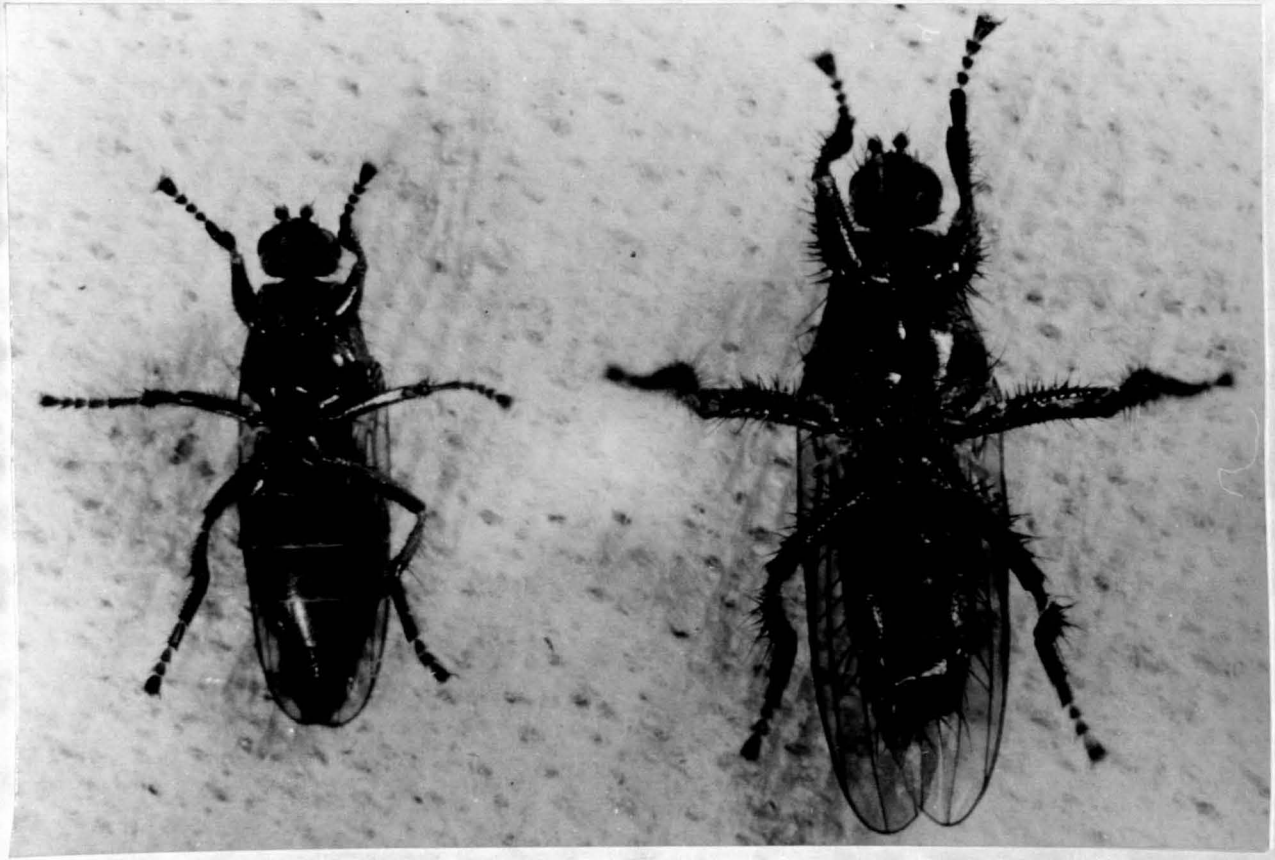


Plate 2.1 Underside of male (right) and female (left)

Coelopa frigida

This technique produced flies whose virginity was in no doubt, but the process was very time consuming if large numbers of virgin flies were required. About 75% of larvae produced adults but the adults appeared over a period of several days.

The alternative technique was to collect all flies emerging in a culture either twice daily or once daily and then separate the sexes immediately. This technique relies on the observation by Thompson (1951) that females will not mate until 18 hours, and males 20 hours, after eclosion at 26°C. A high proportion of virgins was, therefore, expected and this was confirmed by the results reported in Chapter 8. The method was particularly useful for collecting large numbers of virgins from tank cultures and for breeding programmes where individual egg batches were reared in round canisters and virgins were required in each generation. It should be noted that the reliability of this technique was dependent on the efficiency of collection of all flies each day.

Single pair matings were generally carried out in mating pots with the usual amount of medium. Pairs were left in the pots in the constant temperature room and observed daily until the first egg batch appeared, usually between 1 and 4 days after eclosion of the female. Adults were then discarded or stored for electrophoresis if required. After a further 1 or 2 days the pots were examined for the presence of larvae and if they were present the whole mating pot was placed in a round canister with medium in such a way that the medium in the pot was in contact with the medium in the canister. This was essential because the amount of food available in a mating pot was insufficient to support the larvae from a single egg batch. On the other hand if pairs were placed straight into round canisters

to lay eggs microbial infection was often advanced by the time eggs had been laid and had hatched and the success rate was low. The mating pot and canister technique was more successful but even so only about 60-70% of pairs produced adult offspring.

2.5 Electrophoresis

The method of electrophoresis was modified from Collins (1978). Individual animals (third instar larvae, pupae or adults) were homogenized in 1 to 2 drops of distilled water with a little powdered glass. The amount of water was varied according to the size of fly, to give a more uniform concentration of enzyme. The homogenates were absorbed first into squares of Whatman 3MM Chromatography paper (1 cm x 1 cm) and then into smaller squares (about 3 mm x 3 mm) which were inserted into starch slab gels. Using the larger squares first gave clearer banding patterns on the zymograms, probably because they prevented pieces of glass and solid insect remains from being introduced into the gel with the soluble extract. Preparation of the homogenates was carried out on ice and the starch gels were cooled to 4°C before use.

Horizontal slab gel electrophoresis was performed in 12% w/v starch gels (Connaught Laboratories Ltd., Ontario, Canada). The electrode buffer was 0.17 M Tris(hydroxymethyl)aminomethane (tris) (Sigma Chemical Co., Poole, Dorset), 0.05 M Boric acid (BDH Chemicals Ltd., Poole, Dorset), and 0.002 M Ethylenediaminetetra-acetic acid disodium salt (BDH Chemicals Ltd.) adjusted to pH 8.7 with Boric acid. Gels were made up in the same buffer diluted 1:1 with distilled water. Electrophoresis was carried out at 350V and 50mA for 1.5 hours at about 4°C. The inserts were then removed

and gels sliced before staining for alcohol dehydrogenase with agar overlay on the cut surface. The agar overlay for one gel slice contained the following:-

8 ml Tris-HCl buffer pH 8.6 (0.1 M tris brought to pH 8.6 with 10 N HCl)

2 ml Propan-2-ol (Fisons Scientific Apparatus, Loughborough)

1 ml 15 mg/ml Nicotinamide-adenine dinucleotide (NAD) in distilled water (BDH Chemicals Ltd., Poole, Dorset)

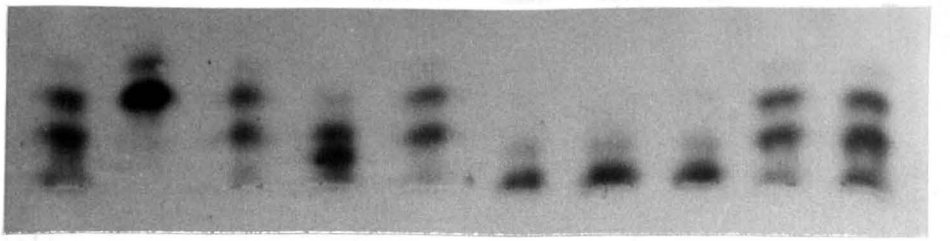
1 ml 10 mg/ml 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) in distilled water (Sigma Chemical Co., Poole, Dorset)

0.5 ml 2 mg/ml phenazine methosulphate (PMS) in distilled water (Sigma Chemical Co.,)

12.5 ml 2% w/v agar (Oxoid, bacteriological No.1) (Oxoid Ltd., Basingstoke, Hants) at 70°C

NAD, MTT and PMS were stored in stock solutions at 4°C in the dark. Gels were incubated in the dark at 37°C for about 30 minutes - or longer for smaller or older flies which had less enzyme activity. A typical zymogram is shown in Plate 2.2 with the interpretation of the Adh genotypes.

A total of 47421 animals was gelled in the course of this project (about 1000 gels). Only 4 gels failed completely and in all cases this was due to lack of proper cooling during electrophoresis. 680 individual banding patterns were unscoreable, that is 1.43% of animals gelled. Most unscoreable individuals were either old, for example when they had been used in pair matings before electrophoresis, or small, as from very high density cultures. Overall the results are unlikely to be biassed by this very low failure rate.



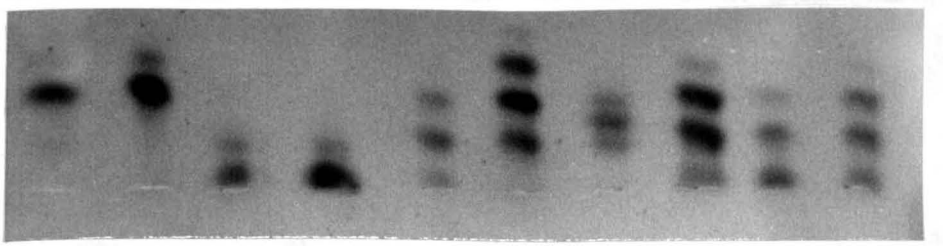


Plate 2.2 Portions of some typical Adh zymograms with their interpretations.

2.6 Polytene chromosome preparation

The chromosome 1 karyotypes of flies were determined by examination of polytene chromosomes from the salivary glands of large third instar larvae. Glands were removed in 45% acetic acid fixative in a watch glass and then transferred to a microscope slide in one drop of a 2% w/v solution of synthetic orcein (Searle Diagnostic, High Wycombe, Bucks) in 50% acetic acid. It was important to filter the stain before use. Glands were macerated in the stain with a glass rod and then left for about 5 minutes. A cover slip was placed over the drop of stain and the preparation was squashed. Slides could be stored for several days if the cover slip was ringed with a saturated solution of gelatine in glacial acetic acid.

Chromosomes were examined under a light microscope at 400x or 1000x magnification with bright field illumination. A green filter sometimes aided resolution.

2.7 Measurement of wing lengths

Wing length was chosen as a measure of the size of adult flies because it can be measured easily and accurately, because it is highly correlated with other bodily dimensions (H.S.Berry, pers.comm.), and because wing area itself may be an important aspect of size from the point of view of mating success (see Chapter 9).

Wing lengths were measured under a binocular microscope using x6 magnification with an eyepiece graticule. Lengths are quoted in graticule divisions, unless otherwise stated, where 1 division = 1.45 mm. The length recorded was from a prominent bristle at the base of the wing (arrowed in Plate 2.3) to the

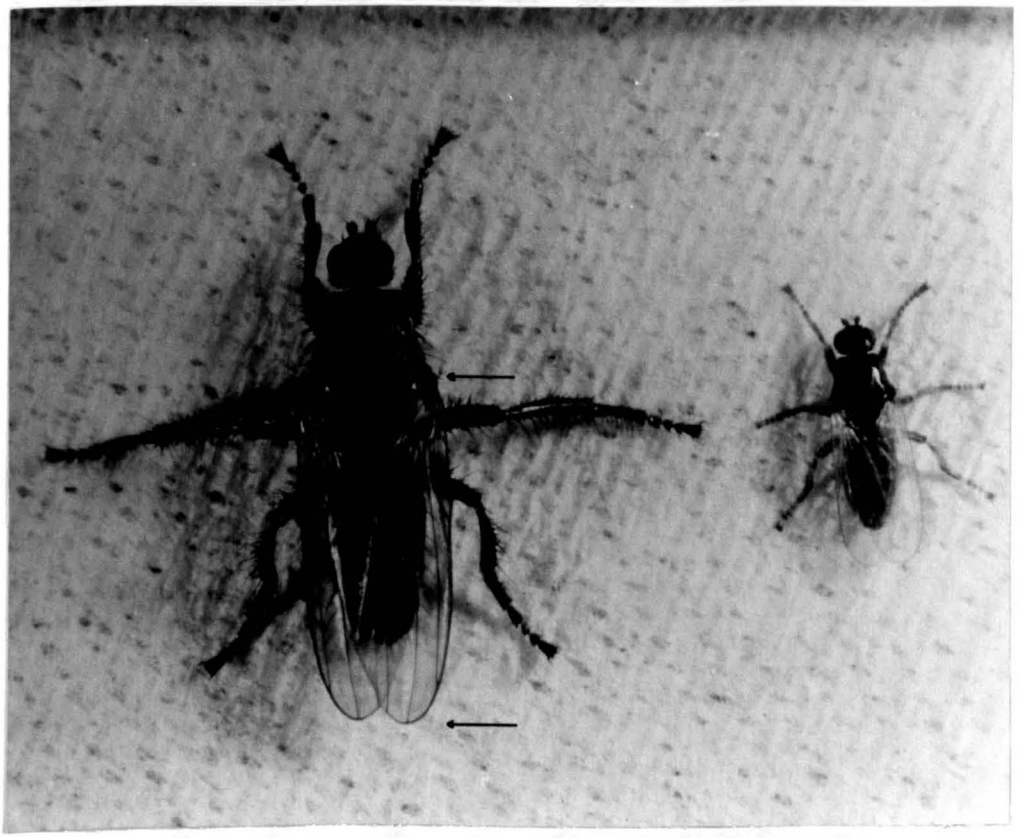


Plate 2.3 Male Coelopa frigida showing the points between which wing length was measured and the range of sizes encountered. The wing length of the male on the left was 2.2 and the right 4.5 divisions.

furthest margin of the longer wing in the folded position. The two wings were never observed to differ by more than 0.1 divisions. Lengths are quoted to the nearest tenth of a division, at this level of accuracy the results are highly repeatable.

2.8 Derivation of stock lines

The following stock lines were used in the experiments reported in this thesis and will be referred to by the names given in this section.

BB(lab) The exact history of this line is uncertain. It was originally derived from the progeny of two animals collected in 1973 from a natural population at St.Mary's Island in Northumberland. The progeny were sib-mated until homozygous for the Adh-B allele (see Day & Buckley, 1980). However at least twice during its subsequent laboratory culture the line has become contaminated with flies carrying other Adh alleles. On each occasion flies were sib-mated until the culture was homozygous again at the Adh locus, and the α-arrangement. The line has therefore been through a number of genetic 'bottle-necks' between which it has been maintained by mass transfer. It has also been outbred on at least two occasions.

DD(lab) As above, but fixed for the Adh-D allele and the β arrangement.

ST.MARY'S A sample of larvae was taken from a natural population at St.Mary's Island, Northumberland on 6 April 1980 (for location of sites and collecting techniques see Chapter 3). Flies emerging in the

laboratory from 12 April to 22 April were collected daily and pooled. About 500 flies were used to start a tank culture which was then maintained by transfer of about 500 flies each generation.

PORTLAND As above but from a natural population at Portland Bill, Dorset sampled in August 1979.

B + D LINES The following three lines were established from samples of larvae taken from natural populations. The objective was to establish broadly based lines which contained only the B and D alleles at the Adh locus. The advantage of removing the rare A and E alleles and the rather more common C allele lies in the fact that the B and D alleles are completely associated with the α and β sequences of chromosome I respectively (see Chapter 1), whereas the C allele occurs with both sequences and the association of the rare alleles is not known. A broadly based line containing B and D alleles alone could be used for laboratory experiments and karyotype frequencies could be followed by electrophoresis without the ambiguity caused by the other alleles, and without using highly inbred lines.

Virgin adults were obtained from the field collections of larvae and pair mated in mating pots. As soon as an egg batch appeared the adults were removed and stored at $-20^{\circ}C$ for subsequent electrophoresis. Batches laid by parents with Adh-B and Adh-D alleles alone were transferred into round canisters. Adult progeny were collected and mixed. Batches laid by parents with other alleles were either used to derive different stock lines (see below) or discarded. The mixed progeny were used to establish at least three tank cultures started with about 500 flies each and the lines

were then maintained by starting each generation in this way.

The three lines were:-

PORTLAND B + D Based on 160 individuals collected as larvae from Portland Bill, Dorset in August 1979

SM80 B + D Based on 336 individuals collected as larvae from St.Mary's Island, Northumberland in September 1980

MN80 B + D Based on 228 individuals collected as larvae from Morfa Nefyn, Gwynedd on 4 December 1980.

Details of the Adh genotype frequencies in the base populations and of the mating types on which the B + D lines were based are given in Table 2.2. Both the Portland B + D and the MN80 B + D lines were subsequently found to contain very low frequencies of the Adh-C allele. This must have been due to either non-virginity of one or more of the females used in the founding pairs or to misscoring of a zymogram. The latter possibility is the more likely. Flies that have been used to produce egg batches are frequently several days old when they are used for electrophoresis. They therefore produce weakly stained gels which can be difficult to score. A third possibility - that the lines were contaminated after being established - is very unlikely as very few Adh-C carrying flies were kept in the stock room at that time.

ISOGENIC LINES This set of stock lines was derived from field collected animals such that all Adh alleles in any one line were identical by descent. The starting pairs were chosen from the sets of pair matings used to establish the B + D lines, except in the case of the SMEE line which was from a set of matings of ST.MARY'S flies from the first laboratory generation of that line. The

Table 2.2 Flies used to establish the B + D stock lines

PORTLAND B + D

Base population:	Adh genotype	AD	BB	BC	BD	CC	CD	DD	BE	TOTAL
	No.	1	33	15	119	0	28	58	1	255

Frequencies: Adh-B = 0.39
Adh-C = 0.08
Adh-D = 0.52

Founder pairs:	Adh genotypes	BBxBB	BBxBD	BBxDD	BDxBD	BDxDD	DDxDD	TOTAL PAIRS
	No.	1	18	6	23	29	3	80

Frequencies: Adh-B = 0.45
Adh-D = 0.55

SM80 B + D

Base population:	Adh genotypes	AB	AC	AD	BB	BC	BD	CC	CD	DD	CE	DE	TOTAL
	No.	4	2	4	81	54	311	15	80	171	1	2	725

Frequencies: Adh-B = 0.37
Adh-C = 0.12
Adh-D = 0.51

Founder pairs:	Adh genotypes	BBxBB	BBxBD	BBxDD	BDxBD	BDxDD	DDxDD	TOTAL PAIRS
	No.	1	26	16	56	55	14	168

Frequencies: Adh-B = 0.42
Adh-D = 0.58

MN80 B + D

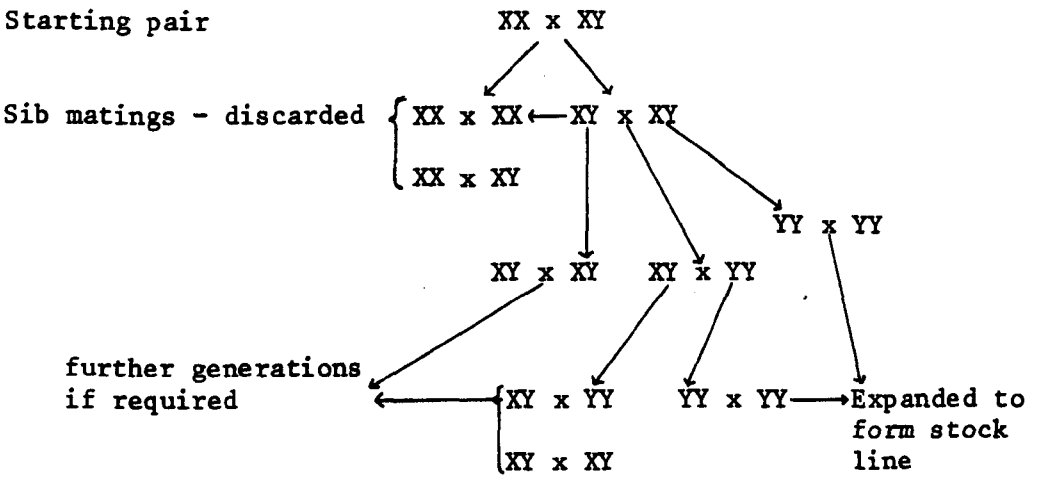
Base population:	Adh genotypes	AD	BB	BC	BD	CC	CD	DD	BE	TOTAL
	No.	2	52	38	201	4	61	102	1	461

Frequencies: Adh-B = 0.37
Adh-C = 0.12
Adh-D = 0.51

Founder pairs:	Adh genotypes	BBxBB	BBxBD	BBxDD	BDxBD	BDxDD	DDxDD	TOTAL PAIRS
	No.	3	17	10	33	38	13	114

Frequencies: Adh-B = 0.41
Adh-D = 0.59

programme of pair matings given below was then conducted for each line, selecting the most suitable progeny in each generation after electrophoresis of the parents, until the required allele was fixed. The line was then maintained in small canisters transferring about 20 pairs each generation.



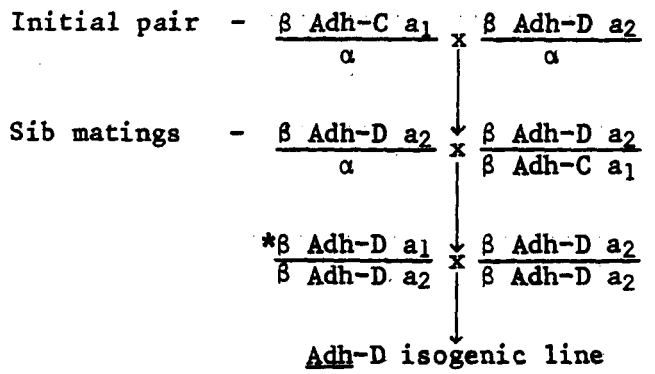
Y is the required allele at the Adh locus

X is any Adh allele or alleles other than Y

A minimum of 8 pairs was used in each generation of pair mating - the number of generations required to obtain fixation is shown in Table 2.3 for each line, together with the genotypes of the starting pair.

It is important to note that these lines were also expected to be fixed for the chromosome I inversion because the Adh locus is either within or very close to the inversion (Day et al 1982). Once the lines were established the salivary gland polytene chromosomes of two or three larvae from each line were examined to establish which sequence had been fixed. This information is also given in Table 2.3 for each line. However this does not necessarily mean that all other loci within the inversion were fixed in these lines. Let us assume for example, that there is another

locus, a, within the inversion at which two alleles are segregating, a_1 and a_2. The following sequence of matings is then possible:-



The chromosome marked with an asterisk results from recombination in the ββ individual in the first generation and the Adh-D isogenic line produced is segregating for the two alleles at the a locus. Only alleles occurring in the natural population in association with the β chromosomal sequence can be incorporated in a β line and similarly for α lines. Had a β line been fixed from a starting pair which was αα x αβ (or an α line from an αβ x ββ pair) then all loci within the inversion would have been fixed (assuming little or no recombination in the heterokaryotype, Day et al 1982). In none of the lines produced was this starting combination known to have existed because of the presence of the Adh-C allele. The only pairs known to have been of this type were BB x BD and BD x DD pairs which were used in establishing the B + D lines.

The line names were constructed as follows:-

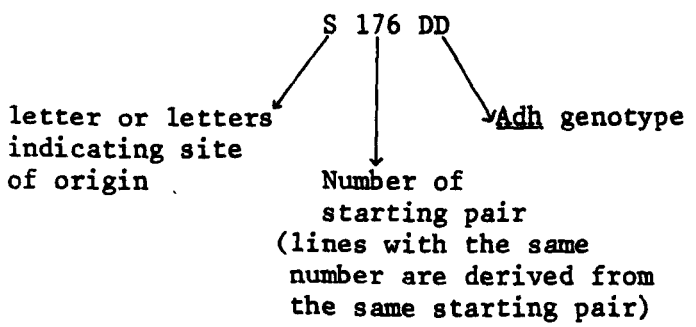


Table 2.3 Derivation of isogenic lines

Collection Site	Line name	Starting pair	Generations of sib mating	Inversion sequence	Adh allele
St.Mary's Island, Northumberland September 1980	S8DD	BCxCD	4	β	D
	S9DD	BCxAD	3	β	D
	S11DD	BCxCD	4	β	D
	S15DD	BCxBD	4	β	D
	S82DD	BCxBD	3	β	D
	S103DD	BDxBC	3	β	D
	S176DD	BCxDE	4	β	D
	S2CC	BCxAB	4	β	C
	S15CC	BCxBD	3	β	C
	S176CC	BCxDE	4	β	C
	S39CC	BCxDD	5	α	C
	S220BB	BDxCD	6	α	B
	S249BB	BDxCD	4	α	B
	S39BB	BCxDD	8	α	B
	S111AA	ADxCC	4	α	A
St.Mary's Island, Northumberland April 1980	SMEE	BDxDE	4	β	E
Morfa Nefyn Gwynedd December 1980	M122DD	BDxBC	3	β	D
	M114CC	BDxCD	3	β	C
	M115BB	BDxCD	3	α	B
	M179BB	BCxCD	3	α	B
Portland Bill, Dorset August 1979	PORTEE	BExBD	4	β	E

MIXED LINES These lines were derived by mixing the isogenic lines to produce stocks in which all flies carried the same chromosome I sequence and had electrophoretically indistinguishable Adh alleles but which were not highly inbred like the isogenic lines themselves. They were maintained as tank cultures.

SM DD MIXED Started with equal numbers of flies from the seven S-DD lines

SM/MN CC MIXED Started with equal numbers of flies from the S2CC, S15CC, S176CC and M114CC lines

SM/MN BB MIXED Started with equal numbers of flies from the S220BB, S249BB, M115BB and M179BB lines.

2.9 Statistical techniques

A statistical problem which has been encountered repeatedly in this study is the analysis of binomial proportions, for example the frequencies of alleles or genotypes amongst samples from natural populations, or the proportions of individuals surviving in viability experiments. A common practice with data of this type is to transform the results to angles to give an approximation to a linear scale with normal error distribution. However this transformation is not always reliable especially where a wide range of proportions and sample sizes is involved. An alternative is to use a "generalised linear model" (Nelder & Wedderburn 1972) in which a scale and error distribution can be specified independently and estimation of model parameters is by maximum likelihood. This analysis can be carried out using the "Genstat" computer package (copyright owned by Rothamsted Experimental Station, Harpenden, Herts.).

For binomial data I have used a model with a logit scale (logit $p = \ln|p/(1-p)|$) and binomial error distribution. The program calculates a value called the "deviance" which is equal to twice the log likelihood ratio between the current specified model and a full model. This is directly equivalent to the mean square in a conventional analysis of variance. The reduction in deviance obtained on adding a term to the model can be compared with the residual deviance by testing the "deviance ratio" against the F-distribution on an exact analogy with the variance ratio test. This test should be used with caution as the deviance ratio does not follow the F-distribution precisely but it is likely to be at least as reliable as the use of the arcsin transformation in a standard analysis of variance.

Chapter 3

Observations on Natural Populations

3.1 Introduction

The first step in the study of any polymorphism must be to obtain reliable data on the frequencies of genes and genotypes in natural populations. These frequencies are, in effect, the phenomena which one sets out to explain. I will, therefore, bring together all the results of field samples taken during this project in this chapter and I will also quote the results of field samples taken by Collins (1978). Together these samples provide by far the most comprehensive picture available of geographical and temporal variation in gene frequencies and of departures from Hardy-Weinberg equilibria. The questions raised by these observations are discussed at the end of the chapter and the remainder of the thesis can be looked on as an attempt to answer these questions. Some of the results have been published elsewhere (Butlin, Collins, Skevington & Day, 1982).

3.2 Field sampling methods

If samples taken from different sites and at different times are to be compared it is necessary to use a standardised sampling method. The method used here was devised by Dobson (pers.comm.) and is the same as that used by Collins (1978).

The sampling method needed to take into account the differences in development time between chromosome I karyotypes (Day et al 1980) and the clumping of larvae in family aggregates (Burnet 1961). Samples were only taken from wrack beds in which pupation of Coelopa frigida had not yet begun. There could, therefore, be no bias due to early emergence of some karyotypes. About 20 handfuls of seaweed,

containing larvae, were taken from widely spaced points in the wrack bed and placed together in a culture tank. This avoided any over-representation of individual family groups. The culture tank was closed with a normal lid and returned to the laboratory.

Larvae collected in this way were allowed to develop at room temperature, normally about 18°C. Collections from natural populations were not kept in the constant temperature room with laboratory stocks because they always contained large numbers of mites and other wrack bed animals which might have contaminated valuable stocks. Flies emerging from the samples were collected daily to minimise the chances of their laying eggs in the collection tanks. Daily collections were stored at -20°C until all flies had been collected. Emergence was considered complete when no flies emerged on three consecutive days. All flies from the sample were then pooled and a random sub-sample used for electrophoresis. Where possible a sample size of at least 144 flies was used but in some of the less dense collections all the flies were used.

This technique suffered from several disadvantages. It relied on efficient daily collection of all flies in the tank. Failure to do this could lead to the development of a second generation of flies in the tank which may bias the results. Also failure to collect the later emerging flies because collection was terminated too soon could distort the observed frequencies. The larval density in the collection tanks was sometimes very high and there was certainly heavy mortality in some cases between field sampling and collection of adults. If this mortality was selective then again the results could have been biased.

Because of these shortcomings a slightly different technique was applied to some of the samples taken in 1979. Larvae were collected in the field in exactly the same way but on return to the laboratory they were only allowed to develop until the first pupae appeared. At this stage a handful of weed was removed from the tank and all flies, regardless of size and developmental stage, were collected from it. This sample was stored at -20°C until required for electrophoresis. I will call this the "preadult" as opposed to the "adult" method.

The preadult method also suffered some disadvantages. It was laborious to collect a sufficient number of animals, especially from low density samples. Pupae were very difficult to find in the decaying seaweed and so the sampling had to be carried out as soon as the first pupae were observed. At this stage some larvae were still very small and gave unscoreable zymograms. The problem of selective mortality was not entirely avoided as the larvae were still maintained at high density for several days. For these reasons I returned to the adult method and took great care over the collection of flies from sample cages. In collecting larvae in the field I attempted to make the density in the collection tank representative of the larval density in the wrack bed. With these precautions I believe the adult method to be the more reliable of the two.

Three collections made in 1980 were sub-sampled by both the adult and preadult methods in order to compare the results obtained.

3.3 The samples

The samples fall into four groups:-

1) Six samples taken by Collins (assisted by Dr.D.T.Parkin and

P.C.Hillier) in the period October 1974 to January 1976.

2) Six samples collected by myself and Dr.T.H.Day in 1979 and analysed by Miss S.J.Skevington, an undergraduate project student under my supervision.

3) Six samples collected by myself and Dr.T.H.Day in 1980 for the comparison of adult and preadult methods and for establishing stocks. These I analysed myself with some help in the electrophoresis from Miss P.M.Goody.

4) 29 samples collected in 1981. I collected the majority of these samples myself but some were collected by Dr.T.H.Day and one by Miss C. Buckman. I have conducted all of the analysis myself. In this long series of samples extra observations have been made wherever possible including:

a) The proportion of Coelopa pilipes in the sample. To assess this collection of adults was continued until all C. pilipes had emerged - usually several days after the end of C. frigida emergence. All flies from a sample, irrespective of species, were mixed. Flies were then selected from the total sample at random and the C. frigida individuals used for electrophoresis. When the required number of C. frigida had been reached, the number of C. pilipes encountered was recorded.

b) The sex ratio. The randomly selected C. frigida were sexed before electrophoresis. This information is also available for some of the previous samples.

c) The air temperature and wrack bed temperature at the time of collection.

d) Subjective assessments of features of the wrack bed such as the proportions of Fucus and Laminaria, the size and age of the bed, and the larval density.

I would like to take this opportunity to thank all those who have helped in the collection and analysis of these samples and Dr.P.M.Collins for allowing me to use his data.

3.4 Results

The results from these samples will be presented as they were obtained, that is as alcohol dehydrogenase genotype and gene frequencies. However it is important to bear in mind the association between the *Adh* locus and the α/β inversion system. The *Adh*-B allele is associated with the α sequence, the D allele with the β sequence, and the C allele with either sequence. So far as the rare alleles are concerned Day *et al* (1982) report finding the A allele with both sequences but give no information about the E allele.

The complete results, in terms of numbers of each genotype with the sexes separated (where this information is available) are given in Appendix 1 and they are summarised in Tables 3.1 to 3.5. A considerable amount of information can be extracted from this large collection of data. I will look first at the frequencies of the three common *Adh* alleles (B,C and D), then at the frequencies of the two rare alleles (A and E), some characteristics of wrack beds, the differences between the sexes, and deviations from Hardy-Weinberg expectation.

The set of samples taken in 1981 is by far the most comprehensive set of observations available on the *Adh*, and consequently the α/β inversion polymorphism of *Coelopa frigida*. It is unfortunate that the samples do not yet cover a whole year. A sampling programme of

Table 3.1 Allele frequencies at the alcohol dehydrogenase locus - 1974-76 samples

Sampling Site*	Date	Adh Allele B	Adh Allele C	Adh Allele D	Sample Size†
Beer, Devon (SY229890)	October 1974	0.33(0.03)	0.15(0.02)	0.52(0.03)	146
Portland, Dorset (SY683687)	November 1974	0.33(0.03)	0.12(0.02)	0.55(0.03)	115
Flamborough, Humberside (TA255708)	February 1975	0.35(0.03)	0.10(0.02)	0.55(0.03)	177
Robin Hood's Bay, North Yorkshire (NZ954048)	March 1975	0.37(0.02)	0.10(0.01)	0.54(0.02)	216
St.Mary's Island, Tyne & Wear (NZ350753)	October 1975	0.40(0.02)	0.06(0.01)	0.53(0.02)	354
Rustington, West Sussex (TV049014)	January 1976	0.36(0.02)	0.07(0.01)	0.57(0.02)	429

*The exact location of each collecting site is given as the Ordnance Survey map reference.

†The sample size is the total number of individuals subject to electrophoresis.

The figure in parenthesis following each allele frequency is the standard error of that frequency (given by $\sqrt{p(1-p)/n}$ where p is the allele frequency and n is the total number of alleles sampled)

Table 3.2 Allele frequencies at the alcohol dehydrogenase locus - 1979 samples

Sampling site*	Date	Adh Allele B	C	D	Sample Size
Beer	October 1979	0.40(0.02)	0.07(0.01)	0.53(0.02)	285
Portland	August 1979	0.40(0.02)	0.09(0.01)	0.52(0.02)	254
Flamborough	August 1979	0.40(0.02)	0.16(0.02)	0.44(0.02)	268
Robin Hood's Bay	October 1979	0.43(0.02)	0.16(0.02)	0.41(0.02)	275
St.Mary's Island	November 1979	0.33(0.02)	0.14(0.01)	0.53(0.02)	268
Rustington	October 1979	0.42(0.02)	0.08(0.01)	0.51(0.02)	282

*Exact site locations are given in Table 3.1. Allele frequencies and sample sizes are quoted as in Table 3.1. Note that all samples in this set were taken using the preadult method, except the Portland sample where the adult method was used.

Table 3.3 Allele frequencies at the alcohol dehydrogenase locus - 1980 samples

Sampling site	Date	Adh Allele B	C	D	Sample Size
Barn's Ness, Lothian (NT722773)	April 1980	0.46(0.02)	0.09(0.01)	0.45(0.02)	519
St.Mary's Island	6 April 1980	0.41(0.01)	0.13(0.01)	0.46(0.01)	1049
Portland	July 1980	0.45(0.01)	0.09(0.01)	0.47(0.01)	647
St.Mary's Island	September 1980	0.37(0.01)	0.12(0.01)	0.51(0.01)	725
Morfa Nefyn, Gwynedd (SH296408)	26 October 1980	0.40(0.02)	0.11(0.01)	0.50(0.02)	411
Morfa Nefyn	4 December 1980	0.37(0.02)	0.12(0.01)	0.51(0.02)	461

(i) Exact locations are given in Table 3.1 for those sites mentioned previously

(ii) The Barn's Ness, St.Mary's Island (April), and Portland samples were subsampled by both the preadult and adult methods. The results from the adult method are given here.

(iii) Allele frequencies and sample sizes are quoted as in Table 3.1.

Table 3.4 Allele frequencies at the alcohol dehydrogenase locus - 1981 samples (arranged in chronological order)

Sampling site	Date	Adh Allele B	C	D	Sample Size
Whitburn, Tyne & Wear (NZ408614)	18 March	0.47(0.02)	0.12(0.01)	0.41(0.02)	287
St.Mary's Island	18 March	0.34(0.02)	0.11(0.01)	0.55(0.02)	288
St.Mary's Island	15 April	0.29(0.02)	0.11(0.01)	0.59(0.02)	285
Barn's Ness	15 April	0.30(0.04)	0.10(0.03)	0.59(0.05)	52
Rustington	20 April	0.34(0.02)	0.11(0.01)	0.55(0.02)	287
St.Mary's Island (North)	14 May	0.36(0.02)	0.12(0.01)	0.51(0.02)	273
St.Mary's Island (South)	14 May	0.37(0.02)	0.09(0.01)	0.54(0.02)	280
Whitburn	14 May	0.49(0.06)	0.11(0.03)	0.39(0.05)	41
Rustington	17 May	0.34(0.07)	0.06(0.03)	0.60(0.07)	25
St.Mary's Island	18 June	0.33(0.02)	0.10(0.01)	0.57(0.02)	217
Whitburn	18 June	0.33(0.03)	0.10(0.02)	0.57(0.03)	144
Lulworth, Dorset (SY825799)	24 June	0.41(0.03)	0.09(0.02)	0.49(0.03)	138
Portland	26 June	0.39(0.02)	0.09(0.01)	0.52(0.02)	309
St.Mary's Island	8 July	0.38(0.02)	0.08(0.01)	0.53(0.02)	277
Whitburn	8 July	0.44(0.03)	0.11(0.02)	0.43(0.03)	144
Robin Hood's Bay	8 July	0.41(0.03)	0.15(0.02)	0.42(0.03)	126
Rustington	13 July	0.36(0.03)	0.09(0.02)	0.55(0.03)	141
St.Mary's Island	30 July	0.40(0.02)	0.11(0.01)	0.49(0.02)	319
Whitburn	30 July	0.34(0.03)	0.11(0.02)	0.54(0.03)	144
Portland	19 August	0.40(0.03)	0.09(0.02)	0.50(0.03)	141
St.Mary's Island	27 August	0.39(0.02)	0.09(0.01)	0.52(0.02)	283
Whitburn	27 August	0.43(0.03)	0.09(0.02)	0.46(0.03)	143
Beer	9 September	0.41(0.03)	0.07(0.02)	0.51(0.03)	141
St.Mary's Island	25 September	0.41(0.02)	0.13(0.01)	0.46(0.02)	287
Whitburn	25 September	0.50(0.03)	0.09(0.02)	0.40(0.03)	146
Whitburn	11 October	0.51(0.03)	0.10(0.02)	0.37(0.02)	189
Flamborough	25 October	0.44(0.03)	0.15(0.02)	0.41(0.03)	141
St.Mary's Island	4 November	0.48(0.02)	0.10(0.01)	0.41(0.02)	288
Whitburn	4 November	0.41(0.03)	0.11(0.02)	0.47(0.03)	135

(i) Exact locations are given in Tables 3.1 and 3.3 for those sites mentioned previously

(ii) Allele frequencies and sample sizes are quoted as in Table 3.1

(iii) Samples were normally taken from the North Bay at St.Mary's Island. On 14 May a wrack bed was also present in the South Bay and independent samples were collected from the two bays.

Table 3.5 Allele frequencies at the alcohol dehydrogenase locus - 1981 samples (arranged by sampling site)

	Date	Adh Allele B	C	D	Sample Size
A. North East Coast					
Whitburn	18 March	0.47	0.12	0.41	287
	14 May	0.49	0.11	0.39	41
	18 June	0.33	0.10	0.57	144
	8 July	0.44	0.11	0.43	144
	30 July	0.34	0.11	0.54	144
	27 August	0.43	0.09	0.46	143
	25 September	0.50	0.09	0.40	146
	11 October	0.51	0.10	0.37	189
	4 November	0.41	0.11	0.47	135
St.Mary's Island	18 March	0.34	0.11	0.55	288
	15 April	0.29	0.11	0.59	285
(North)	14 May	0.36	0.12	0.51	273
(South)	14 May	0.37	0.09	0.54	280
	18 June	0.33	0.10	0.57	217
	8 July	0.38	0.08	0.53	277
	30 July	0.40	0.11	0.49	319
	27 August	0.39	0.09	0.52	283
	25 September	0.41	0.13	0.46	287
	4 November	0.48	0.10	0.41	288
Barn's Ness	15 April	0.30	0.10	0.59	52
Robin Hood's Bay	8 July	0.41	0.15	0.42	126
Flamborough	25 October	0.44	0.15	0.41	141
B. South Coast					
Rustington	20 April	0.34	0.11	0.55	287
	17 May	0.34	0.06	0.60	25
	13 July	0.36	0.09	0.55	141
Portland	26 June	0.39	0.09	0.52	309
	19 August	0.40	0.09	0.50	141
Lulworth	24 June	0.41	0.09	0.49	138
Beer	9 September	0.41	0.07	0.51	141

(i) See Table 3.4 for standard errors of these ellele frequencies

this sort is expensive and time consuming and so it was not started until analysis of previous samples suggested that it was necessary and likely to be rewarding (Butlin et al 1982a). The sampling programme is being continued by Dr.T.H.Day.

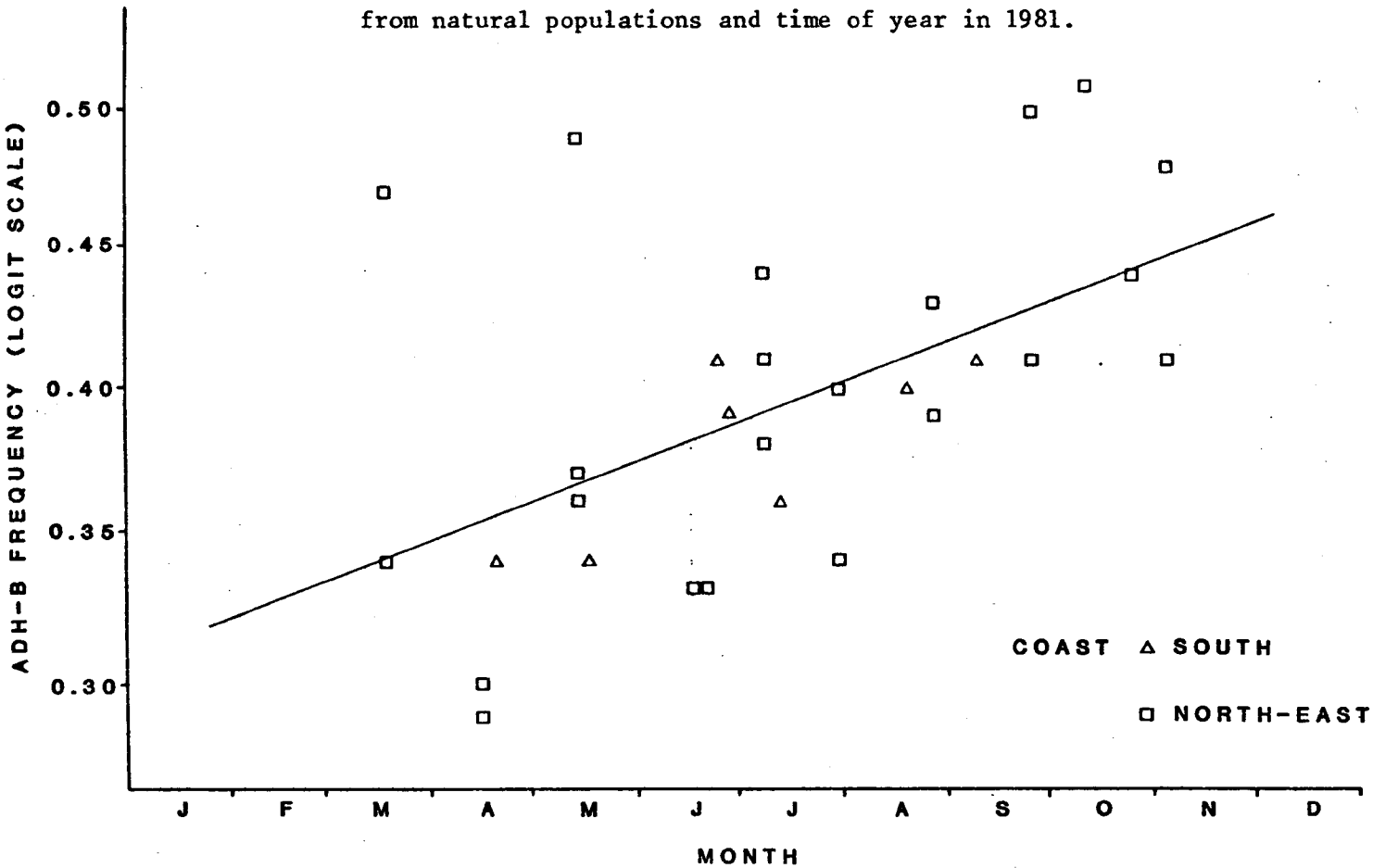
Because the 1981 samples are the most comprehensive I will discuss them first in these sections and then compare them with samples from previous years.

a) Variation in gene frequencies with time

In the 1981 set of samples the frequency of the Adh-B allele increases and that of Adh-D decreases steadily from April to October. After logit transformation and assuming a binomial error distribution (see Chapter 2) the regression of Adh-B frequency on the day of the year is statistically significant ($b = 1.9 \times 10^{-3} \pm 2.8 \times 10^{-4}$, $P < 0.001$). The regression for D frequency is also significant ($b = -1.8 \times 10^{-3} \pm 2.7 \times 10^{-4}$, $P < 0.001$) but there is no significant change in Adh-C frequency ($b = -1.3 \times 10^{-4} \pm 4.4 \times 10^{-4}$, $P > .20$). Autocorrelation may contribute to the significance of these regressions. Where samples were taken repeatedly from the same sites the sample in one month is clearly not independent of the previous sample because the larvae are the offspring of adults from the population sampled in the previous month. This is only likely to be a problem in the cases of Whitburn and St.Mary's Island and the overall trend is so strong that it is unlikely to influence the conclusions. The sample B frequencies and the regression line are plotted Figure 3.1.

Two samples had exceptionally high frequencies of Adh-B compared with the regression line. These were the samples from Whitburn taken on 18 March and 14 May. Both of these samples had high frequencies of Coelopa pilipes (67% and 95% of the total

Fig.3.1 The relationship between Adh-B allele frequency in samples from natural populations and time of year in 1981.

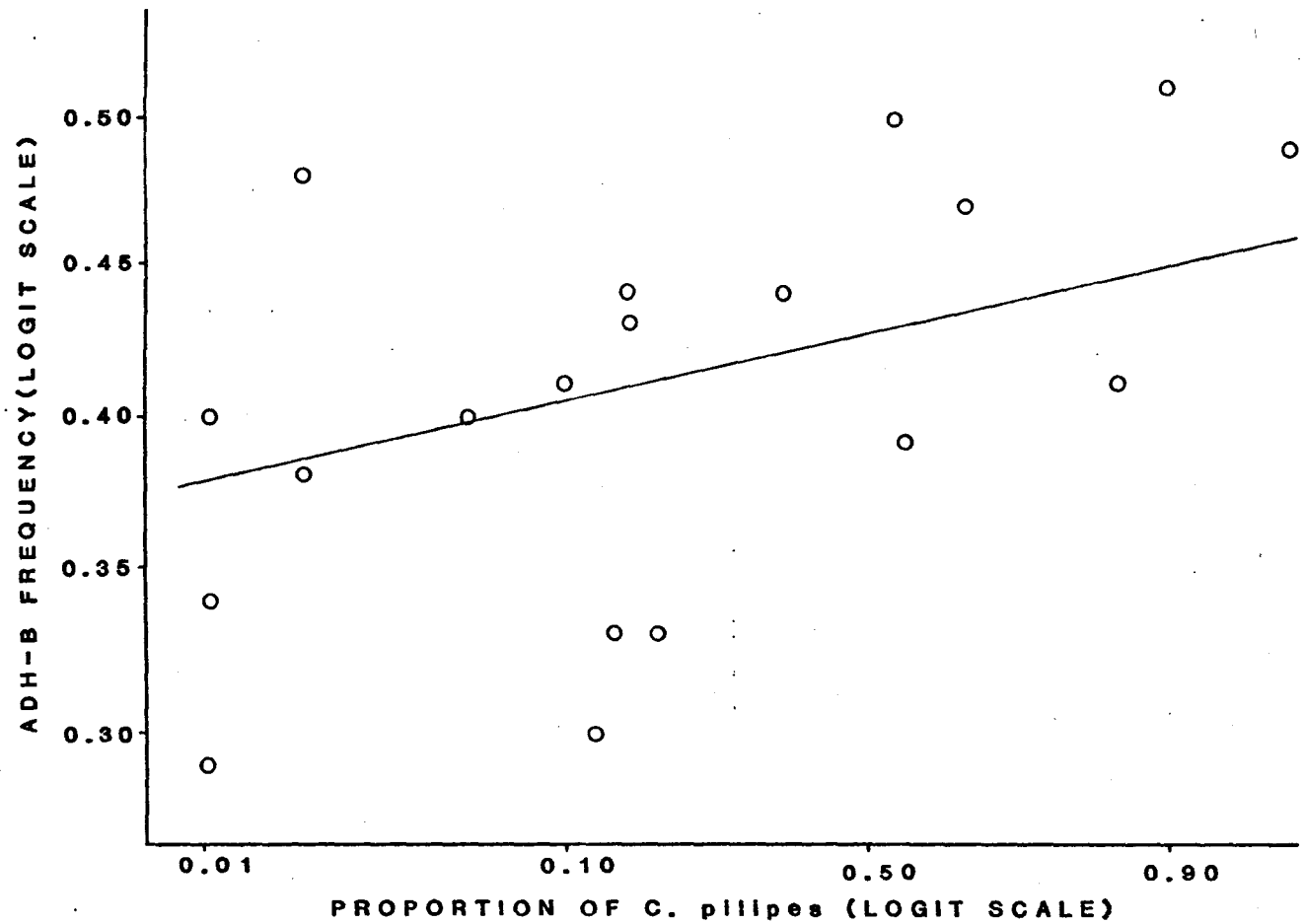


Coelopa adults respectively). This suggested that there might be a correlation between frequency of Coelopa pilipes in a population and the frequencies of the Adh alleles. The regression of B frequency on frequency of C. pilipes (Fig.3.2), using logit transformation and binomial errors as above, is statistically significant ($b = 0.0447 \pm 0.0068$, $P < 0.001$) and remains so if the effect of time of year is removed first ($b = 0.0303 \pm 0.0075$, $P < 0.001$). This is also true for the Adh-D allele - fitting frequency of C. pilipes alone $b = -0.0474 \pm 0.0067$, $P < 0.001$, fitting frequency of C. pilipes after time of year $b = -0.0346 \pm 0.0073$, $P < 0.001$ - but it is not true for the C allele ($b = 0.0052 \pm 0.011$, $P > 0.20$). There is no significant correlation between time of year and the frequency of Coelopa pilipes but there is a tendency for it to be more common at some sites, particularly Whitburn, and it is generally more common on the north-east coast than on the south coast. Some workers have reported that C. pilipes is more common than C. frigida in the summer months and that there is a sudden reversal of frequencies in the spring and autumn (Egglishaw 1958, Burnet 1960c) but Dobson (1974b) did not find this pattern. There is no clear difference between seasons in these 1981 samples but in some cases there are very rapid changes in proportions of the two species, for example at Whitburn the proportion of C. pilipes fell from 0.89 to zero between 11 October and 4 November and at St.Mary's Island it rose from 0.06 to 0.57 from 30 July to 27 August.

It seems likely that the rise in Adh-B frequency and fall in Adh-D frequency in this set of samples from 1981 represents part of an annual cycle in gene frequencies. The alternative possibilities would be that Adh-B frequency is rising steadily and continuously with

Fig.3.2 The relationship between Adh-B allele frequency and the proportion of Coelopa pilipes in samples taken from natural populations in 1981.

Samples with no Coelopa pilipes have been omitted from the graph, but have been replaced in the calculation of the regression line with a value given by $\log_e \left| \frac{0.5}{n + 0.5} \right|$, where n is the number of flies in the sample


ADH-B FREQUENCY(LOGIT SCALE)
0.50
0.45
0.40
0.35
0.30
0.01
0.10
0.50
0.90
PROPORTION OF C. pilipes (LOGIT SCALE)

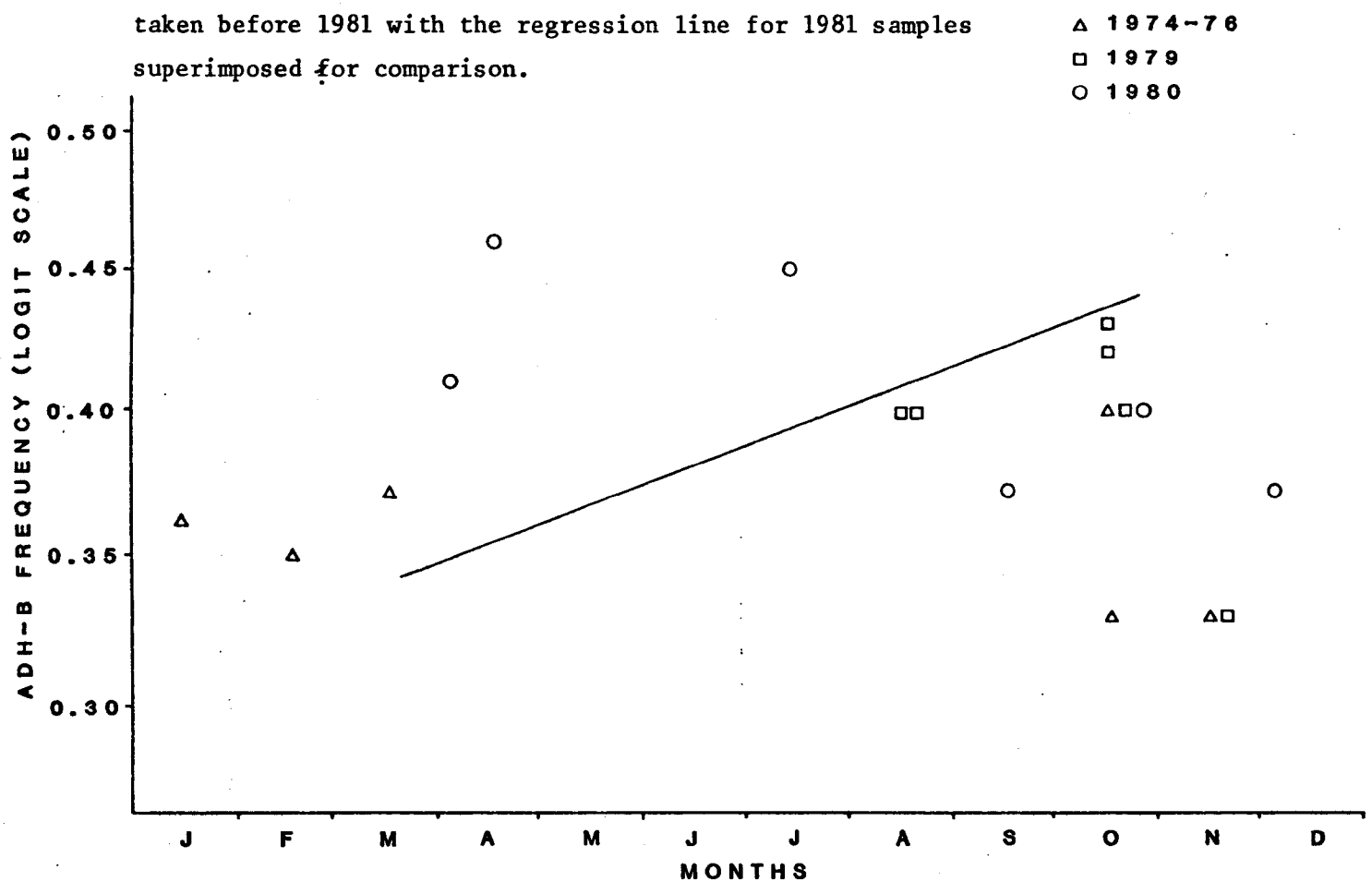
time or that this rise in 1981 was simply a "freak" fluctuation in frequencies due either to chance or to unusual climatic, or other environmental, conditions. The last possibility is unlikely where the trend is consistent over a number of widely spaced sites and continues for at least 6 generations of flies. The other possibilities can be distinguished by closer examination of the data for 1981 and comparison with the earlier samples.

The two samples taken in March 1981 gave higher Adh-B frequencies than the samples taken in April. One of these was the Whitburn sample of 18 March which had a high proportion of C. pilipes but after allowing for this the frequency is still higher than in any of the three April samples (adjusted frequency of B = 0.40). The Whitburn sample for November has a much lower frequency of B than the two preceding Whitburn samples after adjustment for the proportions of C. pilipes. However the St.Mary's Island sample for 4 November has an unusually high frequency of Adh-B.

It appears that in 1981 at least the frequency of B fell to a minimum in April and then rose through the summer to October. It is possible that the low frequency of B at Whitburn in November represents the beginning of a decline in B frequency over the winter.

Do the data from previous years support the hypothesis of an annual cycle? Unfortunately the frequencies of Coelopa pilipes in these earlier samples were not recorded and, therefore, this complicating factor cannot be removed from the observations. It might explain unexpectedly high frequencies of the B allele. The data in the form of B frequencies, after logit transformation, are plotted in Figure 3.3 with the regression line for the 1981 data superimposed. There is evidence for a sharp drop in B frequency in

Fig.3.3 Adh-B allele frequencies in samples from natural populations taken before 1981 with the regression line for 1981 samples superimposed for comparison.



October and November from about 40% to about 36%. The samples for January, February and March also have low frequencies of B. The two April samples have high frequencies, especially the one from Barn's Ness in Lothian (46%). This site often has a high proportion of C. pilipes and this might explain the high B frequency in the April 1980 sample.

The monthly mean allele frequencies over all 47 samples are given in Table 3.6 and plotted in Figure 3.4. Adh-C frequencies show little variation from around 0.10. Adh-B frequencies rise gradually from a minimum in January/February to a maximum in September and then fall relatively sharply in the autumn months. The frequencies of Adh-D change in a complementary way.

The full results have been analysed for differences between months of the year and between years by an analysis of deviance as described in Chapter 2 and similar to that used by Butlin et al (1982a). The samples were grouped into months, because the precise dates of some of the earlier samples are not known, and into year groups as in Tables 3.1 to 3.5.

The differences between months were not significant for any of the three alleles (for B, $F_{11,23} = 0.59$; for C, $F_{11,23} = 1.22$, $P > 0.20$;for D, $F_{11,23} = 1.11$, $P > 0.20$) nor were there differences between years (for B, $F_{3,23} = 1.93$, $P > 0.20$; for C, $F_{3,23} = 0.66$; for D, $F_{3,23} = 2.89$, $P > 0.05$). No level of probability can be attached to F values of less than 1.0 in a one-tailed test but such values are clearly not significant.

The mean allele frequencies for the year groups are given in Table 3.6. The difference in Adh-D frequency between years comes close to the conventional 5% level of significance. This is due

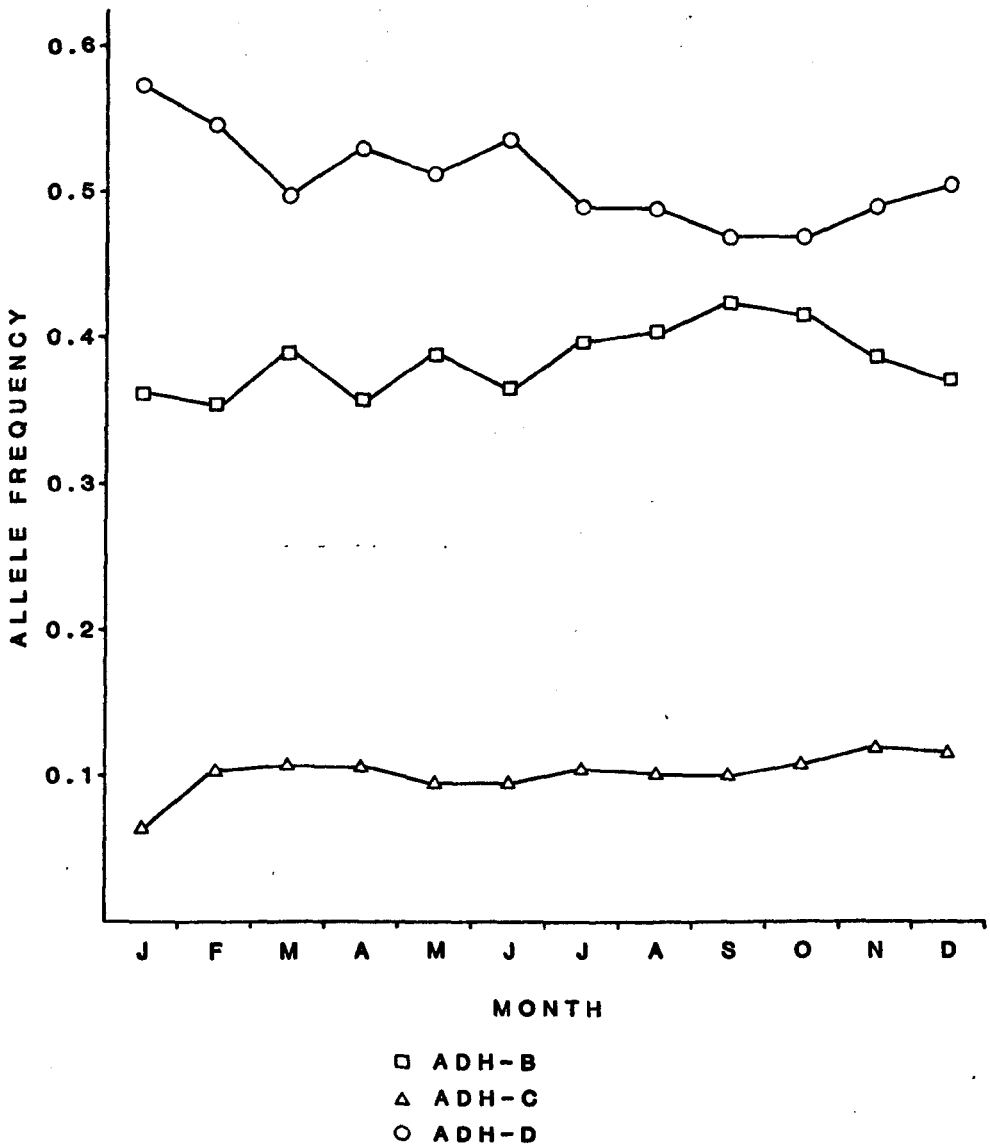


Fig.3.4 Mean allele frequencies over all 47 samples from natural populations.

Table 3.6 Mean *Adh* allele frequencies in samples from natural populations

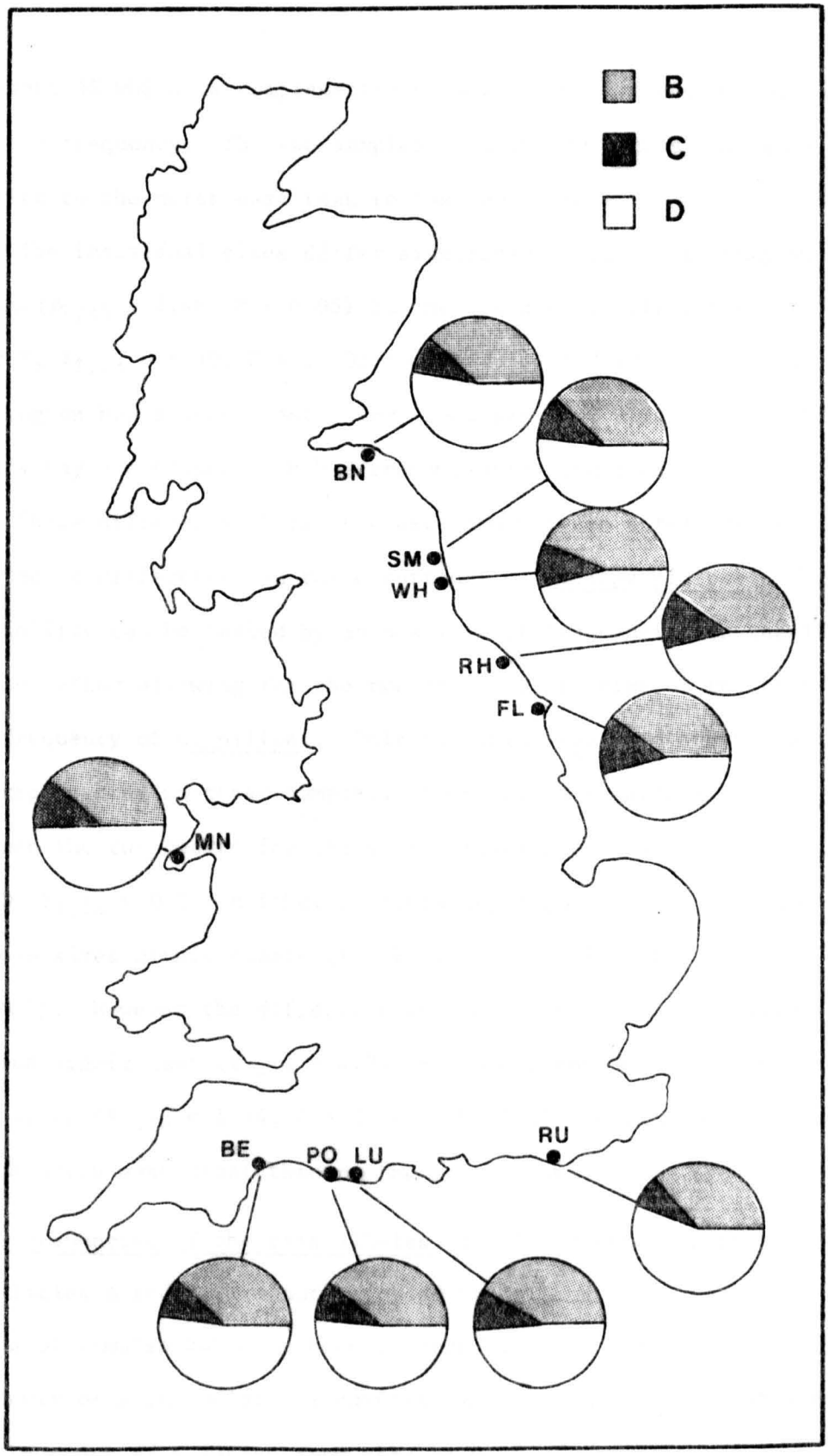
		Adh-B	Adh-C	Adh-D	Number of Samples
MONTHS	1	.362	.065	.572	1
	2	.353	.102	.545	1
	3	.389	.109	.497	3
	4	.357	.107	.529	5
	5	.388	.095	.511	4
	6	.365	.095	.534	4
	7	.396	.105	.489	7
	8	.403	.103	.487	5
	9	.423	.101	.468	4
	10	.415	.109	.469	8
	11	.388	.119	.490	4
	12	.371	.116	.505	1
YEAR	'74-76	.357	.101	.542	6
	'79	.396	.114	.487	6
	'80	.406	.107	.480	6
	'81	.395	.102	.494	29
COAST	NE	.397	.110	.485	31
	S	.381	.090	.526	14
	W	.384	.111	.497	2
SITE	WH	.435	.103	.450	9
	SM	.374	.106	.512	14
	BN	.376	.094	.523	2
	RH	.401	.135	.458	3
	FL	.397	.138	.462	3
	RU	.364	.079	.555	5
	L,U	.413	.091	.489	1
	PO	.392	.095	.511	5
	BE	.381	.098	.516	3
	MN	.384	.111	.497	2
OVERALL		.387	.104	.503	47

mainly to an unusually high frequency of D in the 1974-76 year group compared with the other three years. A difference between the 1974-76 year group and the 1979 samples was reported by Butlin et al (1982a) but they were unable to distinguish it from the possibility of a seasonal cycle because of the timing of the samples in the different years. In the present analysis the effect of time of year has been removed before assessing the difference between years. Butlin et al also considered the possibility that the use of the preadult sampling method in five of the six 1979 samples could account for the difference but they found that if anything the preadult method gave higher estimates of D frequency and therefore could not account for the change. The 1980 and 1981 samples were taken by the adult method and are much closer to the frequencies of the 1979 samples than the 1974-76 samples.

b) Geographical Variation Two further factors were included in the analysis of deviance described above after the effects of months and years. First the data were divided into north-east, south and west coasts and then into individual sites. The north-east coast group includes all sites from Flamborough to Barn's Ness, the south coast all sites from Rustington to Beer, and the west coast group includes only the two samples from Morfa Nefyn. Each of these three groups is separated by more than 500 km of coastline from the others. The mean allele frequencies for the coasts and the individual sites are given in Table 3.6 and Fig.3.5.

The difference between coasts is not significant for the Adh-B allele ($F_{2,23} = 2.56$, $P \simeq 0.10$) but is significant for the C allele ($F_{2,23} = 3.81$, $P < 0.05$) and for the D allele ($F_{2,23} = 3.54$, $P < 0.05$). The south coast has a higher D frequency than the north east coast

Fig.3.5 Mean allele frequencies at all sites sampled.



by about 4% which is complemented by a 2% lower C frequency and 2% lower B frequency. The two samples from the west coast are more similar to the north east than to the south coast.

The individual sites differ significantly in C frequency within coasts ($F_{7,23} = 2.46$, $P < 0.05$) but not in B or D allele frequencies (for B, $F_{7,23} = 1.50$, $P > 0.10$; for D, $F_{7,23} = 2.22$, $P > 0.05$). Rustington had a consistently low frequency of C alleles and Robin Hood's Bay and Flamborough had consistently high frequencies.

These differences between coasts and between sites may be related to differences in the occurrence of Coelopa pilipes. This possibility can be tested by an analysis of frequencies in the 1981 samples after allowing for the two covariates: time of the year and frequency of C. pilipes. Only the north east and south coasts are represented in these samples. There is no significant difference between the two coasts for the B or D alleles (for B, $F_{1,18} = 0.31$; for D, $F_{1,18} = 0.16$) neither is there any significant difference between sites within coasts (for B, $F_{7,18} = 0.88$; for D, $F_{7,18} = 1.10$, $P > 0.2$). However the difference between coasts for the C allele remains significant ($F_{1,18} = 4.75$, $P < 0.05$) and this is consistent over sites ($F_{7,18} = 1.94$, $P > 0.1$). The C allele is more frequent on the north east coast than on the south coast.

c) Frequencies of the rare alleles The frequencies of the rare Adh alleles A and E were not recorded by Collins in his 1974-76 series of samples but have been recorded in all other samples. The frequency of A in individual samples ranges from zero to 2.4% and the frequency of E ranges from zero to 0.6%.

In the 1981 set of samples there is a striking difference in the occurrence of the A allele between the north east and south coasts:

Coast	North East	South
A alleles observed	65	0
All other alleles	8921	2364

The heterogeneity χ_1^2 is 17.2 indicating a probability of less than 0.1% that this result was obtained by chance. The E allele shows no difference between coasts:-

Coast	North East	South
E alleles observed	23	8
All other alleles	8963	2356

In this case $\chi_1^2 = 0.47$, $P > 20\%$

In previous years A alleles have been observed in south coast samples from Portland and Beer but not from Rustington. This removes the possible explanation that the A allele has recently arisen in the north east and has not yet spread to the south coast.

Combining the results for 1979 and 1980 the distribution of A alleles between the coasts is similar to the 1981 data:-

Coast	North East	South
A alleles	51	7
All other alleles	8702	4845

$\chi_1^2 = 14.2$, $P < 0.1\%$

A and E alleles were present in the two Morfa Nefyn samples. There is no suggestion in the data of consistent variation in A or E frequency between sites within coasts nor is there any evidence for a seasonal cycle in A or E frequency.

d) Characteristics of wrack beds which might affect allele frequencies

During 1981 notes were made about various characteristics of the wrack beds from which samples were taken. In some cases where other

people collected samples for me this information is not available. The observations are summarised in Table 3.7 with the Adh-B frequencies and the proportions of C. pilipes for comparison.

Large wrack beds occurred more frequently in the spring and autumn than in the summer probably because there were more storms to dislodge seaweed. None of the other characteristics show seasonal changes and there does not seem to be any direct relationship between size of the wrack bed and Adh-B frequency or proportion of C. pilipes.

A comparison of the seaweed composition of the wrack beds and the presence or absence of C. pilipes in the sample shows more Fucus in wrack beds where C. pilipes is present but this difference is not statistically significant (Mann Whitney test; $U = 55$, $P > 0.05$). The proportion of Fucus in the bed is used rather than that of Laminaria to allow for the Rustington sample of 13 July which contained only 50% Laminaria but no Fucus whereas the two normally make up most of the bulk of the wrack beds.

Wrack beds at Rustington rarely contain any Fucus because of the lack of intertidal rocks and in none of the 1981 samples were there any C. pilipes. By contrast wrack beds at St.Mary's Island and Whitburn were never observed to have less than 50% Fucus and in 15 out of 19 samples from these sites C. pilipes was present.

In samples which contained C. pilipes there is no correlation between the proportion of Fucus and the proportion of C. pilipes. Adh-B frequency does not correlate with proportion of Fucus.

There is no clear pattern in wrack bed age, larval density or wrack bed temperature but it is interesting to note that temperatures are not necessarily higher in the summer than in the spring and autumn.

Table 3.7 Subjective estimates of characteristics of some wrack beds sampled in 1981

SITE	DATE	TEMP°C	PROPORTION OF LAMINARIA	AGE	SIZE	LARVAL DENSITY	FREQUENCY ADH-B	FREQUENCY C.pilipes
St.Marys	18.3	23	40	O	S	M	.34	0
Whitburn	18.3	24	50	O	L	L	.47	.67
St.Marys	15.4	-	?	O	L	H	.29	.01
Rustington	20.4	9	90	O	S	L	.34	0
St.Marys North	14.5	19	20	I	M	M	.36	0
St.Marys South	14.5	17	50	I	S	H	.37	0
Whitburn	14.5	21	50	O	M	L	.49	.95
Rustington	17.5	11	100	O	S	L	.34	0
St.Marys	18.6	15	40	O	S	L	.33	.15
Whitburn	18.6	16	40	Y	S	M	.33	.19
St.Marys	8.7	-	-	I	S	M	.38	.02
Whitburn	8.7	-	-	O	S	L	.44	.16
Robin Hoods Bay	8.7	-	-*	O	M	H	.41	0
Rustington	13.7	20	50*	I	S	M	.36	0
Whitburn	30.7	22	40	Y	S	L	.34	.01
St.Marys	30.7	20	40	Y	S	L	.40	.06
Portland	19.8	-	-	I	S	M	.40	.01
St.Marys	27.8	"warm"	10	Y	M	M	.39	.57
Whitburn	27.8	"hot"	40	Y	S	L	.43	.16
Beer	9.9	24	70	I	L	H	.41	.10
Whitburn	11.1	16	50	O	L	M	.51	.89
Whitburn	4.11	14	30	Y	S	M	.41	0
St.Marys	4.11	17	15	O	L	M	.48	.02

Temperature: mean of five points

Proportion of Laminaria: subjective estimate - remainder usually Fucus except at * where the remainder was of red seaweeds, no Fucus
? too decayed to make estimate

Age: O = Old, I = Intermediate, Y = Young

Size: S = Small, M = Medium, L = Large

Larval density: L = Low, M = Medium, H = High

e) Sex ratio and comparison of male and female genotype frequencies

In the samples taken during 1981 males and females were kept separate in order to estimate the sex ratio and to compare the distributions of genotypes in the two sexes. The sex ratio varied from 0.31 to 0.71 (number of males divided by number of males and females, see Appendix 1). The mean of the 29 samples was 0.497 (with variance 0.0054) which is very close to 0.5. There is, however, significant heterogeneity between the ratios ($\chi_{28}^2 = 85.85$, $P < 0.001$). More than two thirds of this χ^2 value is due to just five samples, all from Whitburn: 18 March - 0.61, 14 May - 0.71, 30 July - 0.31, 27 August - 0.36, and 11 October - 0.63. All the remaining samples fall in the range 0.44 to 0.56.

It is possible that these discordant sex ratios are due to poor sampling technique but this should be detectable by comparison of male and female genotype frequencies. If a sample was collected in the field after adults had started to emerge the sex ratio would be biassed towards males because, on average, their development time is longer, but the males would also have a higher Adh-B frequency than the females because DD males emerge first. Conversely if the last flies to emerge from a sample were not collected the sex ratio would be biassed towards females and males would have a lower Adh-B frequency than females.

In only two of the 29 samples do the male and female genotype distributions differ significantly (see Appendix 1). These are the St.Mary's Island sample of 8 July ($\chi_5^2 = 17.5$, $P < 0.01$) and the Whitburn sample of 30 July ($\chi_5^2 = 17.1$, $P < 0.01$). The St.Mary's sample had less BB males than expected and a female biassed sex ratio (0.46) so failure to collect the last flies to emerge is a possible

explanation. This is also true of the Whitburn sample in which both BB and BD males are below expectation.

While the differences between males and females were not statistically significant in the other Whitburn samples they were in the right direction to explain the aberrant sex ratios of the 18 March, 11 October and 27 August samples. The 14 May sample was very small (41 C. frigida individuals) but the genotype frequencies in males and females were very similar.

The 18 March, 14 May and 11 October wrack beds at Whitburn were old (Table 3.7) and it is quite possible that some flies had emerged before the samples were taken. On the other hand it seems unlikely that late emerging adults were missed in any of the samples. Collection was continued until no adult C. frigida or C. pilipes had emerged for three consecutive days. As C. pilipes take longer to develop than C. frigida, and they were present in both of these samples, it seems very unlikely that any late emerging C. frigida could have been missed.

An alternative explanation could be that conditions in the collection tank became unsuitable for Coelopa larvae and slow developing individuals died. It sometimes happens that an over-enthusiastic collector puts too much seaweed in a collection tank. After some time in the laboratory the weed in an overfull tank will often become semi-liquid, putrid and foul smelling. Larvae cannot survive in these conditions. I also noticed that in some collection tanks large numbers of staphylinid beetles were present towards the end of the emergence span of Coelopa adults. Some species of staphylinid are predatory on dipteran larvae and some are parasitic on dipteran pupae. Their build up in a tank may bias the collection towards the faster developing genotypes. This last explanation may

help to explain why Whitburn is particularly prone to producing "unusual" samples. There is a small area of the site where there is an almost permanent wrack bed. This bed was noted by Egglishaw in 1958 and still occurs in the same position. It seems to make possible a richer wrack fauna (in terms of taxonomic diversity) than is present at other sites. Certainly large numbers of staphylinids are more common in Whitburn samples than in samples from other sites (although I have only visited it twice Barn's Ness also seems to have a very rich fauna).

With the exception of the samples mentioned above the genotype frequencies are always very similar in males and females and the sex ratio is close to 0.5. Evans and Philip (1964) found a trend towards excess of females with increasing larval density in C. frigida. Using the subjective assessments of density in Table 3.7 and omitting the exceptional samples the High and Medium density samples have a mean sex ratio of 0.48 and the Low density samples have a mean ratio of 0.51 indicating a trend in the same direction. This may be related to Collins' (1978) observation that males showed slightly greater differences in viability between genotypes than females. In these field samples the deviations from Hardy-Weinberg expectations are generally very similar in males and females.

Evans and Philip (1964) also observed that the variation in sex ratio between individual egg batches was lower than expected from random union of sperm and eggs. They suggested that it might be due to fertilisation of eggs by "batches" of sperm which would be more similar than random samples of sperm. This sort of effect might contribute to the narrow distribution of sex ratios in these samples.

f) The adult and preadult collection methods The great majority of samples were collected by the adult method but, for reasons mentioned above, five of the six 1979 samples were taken by the preadult method. Three of the 1980 samples were subsampled by both methods to test for any differences in result.

The results are compared in Appendix 1 for the three sites - Barn's Ness, St.Mary's Island (6/4/80) and Portland. The genotype frequencies obtained by the two methods did not differ significantly for Barn's Ness ($\chi_5^2 = 3.1$, $P > 0.5$) or for St.Mary's Island ($\chi_5^2 = 5.1$, $P > 0.2$) but they did for the Portland sample ($\chi_5^2 = 13.5$, $P < 0.05$). The difference in the case of the Portland sample was due to a higher frequency of BB and BD individuals in the adult sample giving an increase in the estimate of the Adh-B allele frequency from 0.38 by the preadult method to 0.45 by the adult method.

The density of this Portland sample was high and the most likely reason for the difference is selective mortality in the collection tank.

It is possible, then, that some of the 1979 samples underestimate the Adh-B frequencies but this would probably only happen where the density was high and it has not resulted in a significant difference between year groups (see above). Where samples were subsampled by both methods the results of the adult method have been used in the analyses reported in this chapter to give maximum comparability with the majority of samples.

g) Deviations from Hardy-Weinberg Expectations The χ^2 values for deviations from Hardy-Weinberg expectations for the six most common genotypes (BB, BC, BD, CC, CD, DD) are given with the raw data in

Appendix 1. The contribution of the CC genotype to the χ^2 is omitted where the expectation falls below 1.0 because the assumptions of the χ^2 test are unreliable with small numbers. In 25 of the 47 samples the deviations from expectation were significant at at least the 5% probability level. However the most striking feature of the results is the consistent nature of the deviations. In all 47 samples the observed number of BD individuals was greater than the expected number. The proportions of samples with excesses over Hardy-Weinberg expectations are given for the other genotypes in Table 3.8. There is clearly a consistent deficiency of both BB and DD homozygotes but there is also a tendency for the BC and CD genotypes to be below expectation and the CC genotype above. This last difference is really quite striking, with an average excess of CC individuals of more than 50% (Table 3.8).

Table 3.8 Summary of deviations from Hardy-Weinberg expectations in samples from 47 natural populations

	Genotype					
	BB	BC	BD	CC	CD	DD
Proportion of samples with excess of each genotype over Hardy-Weinberg expectation	0.06	0.28	1.00	0.60	0.47	0.06
χ_1^2 (expected proportion 0.45)	28.3^{***}	5.7^{*}	57.4^{***}	4.0^{*}	0.1	28.3^{***}
χ_1^2 (expected proportion 0.55)	44.9^{***}	14.2^{**}	38.5^{***}	0.4	1.3	44.9^{***}
Average deviation from Hardy-Weinberg expectation (%)	-23.7	-6.7	+18.4	+58.6	-5.1	-14.1

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

A problem arises in testing the significance of these apparent regularities. With large samples one might expect 50% of samples to show excesses over Hardy-Weinberg predictions for each genotype due to chance alone. However with small sample sizes this expectation does not hold (Majumder & Chakraborty 1981) and the critical size of sample depends on the expected frequency of the genotype in question. Only three of the 47 samples of Coelopa fall below 100 individuals, the mean sample size being 264, but the expected frequency of the CC genotype is generally about 0.01 and so, for this genotype at least, the assumption of a null hypothesis of 0.5 may be inadequate.

Majumder and Chakraborty (1981) give a method for determining the expected proportion of samples with an excess of heterozygotes in a two allele case from the sample sizes and population gene frequencies. This involves scanning all possible combinations of genotypes for each sample size and summing the probabilities of occurrence of those combinations in which the heterozygotes are in excess of the Hardy-Weinberg expectation as calculated from those "observations". The probability of occurrence of each combination is given by:-

$$P = \frac{N!}{n_1!n_2! \ldots\ldots n_i!} . p_1^{n_1} . p_2^{n_2} . \ldots\ldots p_i^{n_i}$$

where N is the sample size, n_1 to n_i are the numbers of each genotype ($\sum n = N$) and p_1 to p_i are the expected proportions of the genotypes.

I have extended this procedure to the three allele case and calculated the proportion of samples in which, by chance, each genotype would be in excess of expectation. The population gene frequencies were taken as 0.4 B, 0.1 C, 0.5 D and the expectations

were calculated by computer for sample sizes of 10, 20, 25, 30 and 40 (Table 3.9). Unfortunately it was not possible to go beyond 40 because of the amount of computer time required (over 500 seconds for N = 40 and rising exponentially) to scan all combinations of six numbers which add up to the sample size.

All of the expectations approach 0.5 as the sample size increases (Table 3.9) but this approach is not always smooth. This is because the observed numbers of a given genotype are constrained to be integers whereas the expectations are not. It is clear that with the sample sizes used here the expectations for BB, BC, BD, CD and DD will all be close to 0.5 and certainly in the range 0.45 to 0.55.

Table 3.9 Expected proportion of samples in which the observed number of individuals of a given genotype exceeds the Hardy-Weinberg expectation (assuming population gene frequencies B = 0.4, C = 0.1, D = 0.5).

	Sample size				
Genotypes	10	20	25	30	40
BB	0.389	0.417	0.489	0.425	0.447
BC	0.458	0.513	0.509	0.513	0.508
BD	0.584	0.546	0.541	0.537	0.528
CC	0.094	0.175	0.218	0.245	0.307
CD	0.490	0.528	0.527	0.525	0.518
DD	0.368	0.400	0.470	0.415	0.426

At sample size 40 the expectation for CC has only risen to 0.3. A prediction for sample size 100 can be made because the departure of the expected proportion from 0.5 is approximately linearly related on a logarithmic scale to the sample size. The value obtained is 0.45.

The observed proportions of samples showing an excess over Hardy-Weinberg frequencies for each genotype have been compared with the limiting expectations of 0.45 and 0.55 (Table 3.8). As expected highly significant deviations are revealed for the BB, BD and DD genotypes. The BC genotype shows a significant tendency to be below expectation but the CD genotype does not. The tendency for the CC genotype to be in excess is on the borderline of conventional significance. A more precise determination of the expectation for this genotype is really needed taking into account the individual sample sizes and allele frequencies. Unfortunately the computer time required makes this impractical at present.

The results do not suggest any consistent differences in genotype frequencies between times of the year, between years or between coasts. Some sites tend to give larger or smaller deviations from Hardy-Weinberg expectations than the majority, for example none of the three samples from Flamborough show significant deviations from expectation whereas four of the five Portland samples do.

The subjective assessments of larval density made during 1981 suggest an effect on deviation from Hardy-Weinberg expectation. The samples assessed as low density had a mean excess of BD individuals of 14% (8 samples) while those assessed as medium density had an excess of 20% (10 samples). This trend is not continued in the four high density samples where the excess was only 16%. The question of larval density is examined further in Chapter 5. The density effect suggests that the deviations from expectation are at least in part due to differential viability and this conclusion is supported by the laboratory experiments of Collins (1978).

h) Summary Before proceeding to the discussion of these results it may be useful to summarise the principal points which have emerged:

(i) There is an annual cycle of Adh-B and D frequencies with B at low frequency in the winter and rising to a peak in the late summer. The Adh-C allele does not show an annual cycle.

(ii) There are no significant differences between years for any of the Adh alleles.

(iii) There is a correlation between the proportion of C. pilipes in a sample and the frequencies of the B and D alleles but not the C allele. High B and low D frequencies are associated with high proportions of C. pilipes.

(iv) There are significant differences between the north east and the south coast for both the C and D alleles. The D allele has a higher frequency on the south coast and this is correlated with a lower proportion of C. pilipes in south coast samples. The C allele has a lower frequency on the south coast and this is independent of the proportion of C. pilipes.

(v) The frequencies of the B and D alleles do not differ significantly between sites within coasts but the C allele frequencies do.

(vi) The Adh-E allele shows no consistent pattern of frequencies but the Adh-A allele is very much more common on the north east coast than on the south coast.

(vii) The presence of C. pilipes at a site may be associated with the proportion of Fucus in the wrack beds.

(viii) Genotype frequencies consistently depart from Hardy-Weinberg expectations. There is an average 18% excess of BD individuals and

a corresponding deficiency in the BB and DD genotypes. The proportions of the genotypes involving the C allele are more variable but there is a tendency for there to be a deficiency of BC and an excess of CC while CD is close to expectation.

3.5 Discussion

The results in this chapter have been presented entirely in terms of the alcohol dehydrogenase alleles and genotypes because this is the way in which they were obtained. But there is known to be a strong, if not complete association of the Adh-B and D alleles with the α/β inversion system (Day et al 1982) and this must be taken into account in interpreting the data.

The first, and most important, question to ask is whether these polymorphisms are maintained in natural populations by random drift or by some form of natural selection. If random effects are important then one expects to find variation in morph frequencies between geographically isolated populations and also variation between temporally separated samples from the same population. Uniformity of frequencies, on the other hand, provides evidence for natural selection. However selection can produce differences between populations if there are differences in the environment or it can produce changes in frequencies with time if environmental conditions fluctuate with time. Thus the distinction between random effects and selection is not always easy to make.

Perhaps the best case for natural selection can be made when several populations change in a predictable way over many generations. This is the type of situation which has been described in Coelopa frigida. The frequencies of the Adh-B and D alleles undergo an

annual cycle with no significant differences between years or between widely spaced sites. These two alleles are associated with the α and β inversion sequences which therefore, probably undergo the same consistent annual cycle, (although some variation in B and D frequencies could occur without change in α and β frequencies because of the presence of the C allele). Selection might be acting on the inversion as a whole, on the Adh locus itself, on some other locus within the inversion, or on some combination of these. There are many examples of selection acting on inversion polymorphisms but rather few examples of selection acting on individual enzyme loci (see Introduction) and so, without further evidence, the hypothesis of selection acting on the inversion should be favoured.

The Adh-C allele does not show the seasonal cycle of the B and D alleles and does show significant variation between coasts and, less markedly, between sites. This pattern of frequencies is to be expected from random drift. The degree of similarity between sites for an allele which is not subject to selection depends on the gene flow between the sites. The movement of Coelopa individuals between populations has not been measured but a number of observations (see Introduction) suggest that, while most individuals move only a few metres in their lifetime, a few individuals might move several kilometres or even tens of kilometres. The two regions studied in this case, the north east coast and the south coast, are separated by more than 500 km, at least 350 km of which is unsuitable for Coelopa colonies. There is unlikely to be any appreciable gene flow between these regions. Within each region sites can be as close as 15-20 km apart (St.Mary's Island and Whitburn, Lulworth and Portland)

or as much as 150 km from their nearest neighbour (Barn's Ness and St.Mary's Island, Rustington and Lulworth). Migration is quite likely between sites like St.Mary's Island and Whitburn but is much less likely between, for example, these two sites and either Barn's Ness or Robin Hood's Bay and Flamborough. The distribution of Adh-C allele frequencies fits very closely to this pattern:

Approx. Distance	Site	Adh-C frequency
	Barn's Ness	.094
125 km		
	St.Mary's Island	.106
15 km		
	Whitburn	.103
75 km		
	Robin Hood's Bay	.135
50 km		
	Flamborough	.138
>500 km		
	Rustington	.079
150 km		
	Lulworth	.091
20 km		
	Portland	.095
50 km		
	Beer	.098
>500 km		
	Morfa Nefyn	.111

Sites which are close together have similar frequencies while sites which are a long way apart have rather different frequencies.

While this pattern is consistent with random drift it is not inconsistent with selection. Sites which are close together may be more similar ecologically and this might explain the similarities between adjacent sites. The fact that there are no significant differences between months or years for the Adh-C allele suggests that the frequencies do not vary much with time. Temporal variation

would be expected in the absence of selection but the magnitude of the variation would depend on the population sizes for which there are no reliable estimates in Coelopa.

The Adh-C allele occurs on both the α and β inversion sequences. There is a tendency for the Cα combination to be more common than expected from the overall α and β frequencies (Introduction, Day et al 1982). In the absence of selection on the Adh locus itself (or neighbouring loci) the frequency of the C allele, and the proportions of Cα and Cβ, could vary without affecting the inversion frequencies whereas the Adh-B and D frequencies are limited by the α and β frequencies. Nevertheless the Adh-C frequency only varies within a narrow range (6-15%) and the available evidence suggests that the proportions of Cα and Cβ are fairly constant.

The Adh-A allele has a much higher frequency in samples from the north east coast than from the south coast. This is true in all the years in which samples have been taken. The difference tends to confirm the suggestion that populations on these two coasts are genetically isolated from one another. However the allele does occur on both coasts and if it is not subject to selection it is surprising that the difference in frequency has been maintained over at least 30 generations. This observation is more easily explained if there is selection against the A allele on the south coast which is weak or absent on the north east coast.

The observed gene frequencies, therefore, provide strong evidence for the action of selection. It seems likely that the consistent variations in B and D frequencies reflect the action of selection on the α/β inversion system but the possibility of

selection acting on the Adh locus remains and this may help to explain the variation observed in the A and C allele frequencies.

Further evidence for the action of natural selection is provided by the comparison of genotype frequencies with Hardy-Weinberg expectations. The two most likely explanations for the excess of heterozygotes and deficiency of homozygotes are differences in viability and non-random mating. The latter has not previously been observed but will be discussed in Chapters 8 and 9. Viability differences of two kinds could result in an excess of heterozygotes - heterosis or selection against one homozygote. The latter seems most unlikely as it would be necessary to postulate a fitness of less than 0.5 for, say, the BB genotype to explain the observed excess. If this were the case viability selection would have to be opposed by very strong selection at some other stage to give the stable gene frequencies observed.

Collins (1978) found that in the laboratory BD heterozygotes have a higher viability than BB or DD homozygotes expecially at high larval density. He attributed this to a heterotic effect of the inversion. Heterosis in viability appears to be the most likely explanation of the observed BB, BD and DD genotype frequencies but can it explain the observed frequencies of the other genotypes? Assuming the deviations from Hardy-Weinberg proportions to be entirely due to differences in viability then the mean viabilities relative to the BD genotype are:-

BB	BC	BD	CC	CD	DD
0.64	0.79	1	1.34	0.80	0.73
$\alpha\alpha$	$\alpha\alpha+\alpha\beta$	$\alpha\beta$	$\alpha\alpha+\alpha\beta+\beta\beta$	$\alpha\beta+\beta\beta$	$\beta\beta$

The karyotypes represented in each genotype are also shown. The relative viabilities for the BB and DD genotypes are intermediate between those obtained by Collins in his "Low" and "Medium" density replicates. The BC and CD genotypes are mixtures of heterokaryotypes and αα or ββ homokaryotypes respectively. If the viabilities observed are due solely to heterotic effects of the inversion then the viability of BC will be intermediate between that of BB and BD and similarly CD will fall between BD and DD. This is indeed the case, but the observed viabilities of the BC and CD genotypes, if they are the result of a mixture of αα (or ββ) individuals and αβ individuals with the same relative viabilities as the BB (or DD) and BD genotypes, cannot be accounted for by the same proportions of Cα and Cβ. The BC viability is consistent with a proportion of Cα of 58% whereas the CD viability is consistent with a proportion of 26%. The viability of the CC homozygotes should, on this hypothesis, fall below that of BD and close to that of BC and CD. Instead it is considerably higher than BD. If this is a real effect rather than an artefact resulting from the very small numbers of this genotype then it cannot be explained by selection acting on the inversion. The viabilities are more consistent with selection acting on the Adh locus itself (or a closely linked locus in linkage disequilibrium), as well as on the inversion.

Viability differences have been the subject of experiments to be described in Chapters 5, 6 and 7. Chapter 7, especially, addresses the question of selection acting on the Adh locus as well as on the inversion.

Viability heterosis could explain the observed seasonal cycle in gene frequencies if the relative viabilities of the homozygotes

varied with a seasonal factor such as temperature, salinity, humidity, or composition of the food. The chemical constituents of the brown seaweeds are known to vary seasonally (Black 1948, 1951a,b, Chapman & Chapman 1980) and these variations could affect larval survival. However the polymorphism is subject to other selection pressures which may also vary seasonally.

There is an association between Adh genotype and rate of development in Coelopa frigida (Day et al 1980 and see Introduction). BB individuals develop slowly and DD individuals rapidly while BD individuals are intermediate and individuals with the C allele are rather more variable. These effects are pronounced in males and only slight in females. The differences have been interpreted as effects of the inversion but, as with viability, the observations on BC and CD individuals are not fully consistent with this view.

Variation in rate of development might interact with variations in the lifespan of wrack beds to produce changes in gene frequencies. Short lived wrack beds might favour fast developing DD individuals and long lived wrack beds BB individuals. If wrack bed life times tend to vary with the time of year this could explain the seasonal cycle. Perhaps the higher frequency of storms in the winter months leads to a shorter mean wrack bed life time. In the summer months, with longer lived wrack beds, the frequency of B increases gradually - gaining a little with each long lived bed and perhaps dropping with the occasional short lived bed. A series of short lived beds in the autumn brings a rapid decline in B frequency. This hypothesis fits the observations well but has a number of problems. (1) The differences in development time were measured in the laboratory with

synchronised populations. Are there differences in natural populations where eggs are laid over at least a few days and if so are these large enough to explain the changes in gene frequencies? Some observations relevant to this question are described in Chapter 4. (2) What is the advantage to the slow developers, if any, in long lived wrack beds? They will clearly have an advantage if fast developers lay eggs in the old weed which are then washed out to sea, or if the fast developers die before a new wrack bed is deposited. However the difference is mainly in males, females are reluctant to lay eggs in old, cool wrack beds, and longevity in natural conditions is thought to be two to four weeks (Dobson 1974a). A possible advantage to slow developing males is larger size which confers a higher mating success - this idea is examined later in this thesis (Chapters 8 and 9). (3) Very little data is available on lengths of life of wrack beds. Dobson (1974a) reports some observations made on the north east coast but these are based on weekly visits whereas a few days difference in duration could be crucial. This lack of information remains an important obstacle to understanding Coelopa populations.

The Adh-C allele does not show a seasonal cycle in frequency. This would be expected if the cycle resulted from an effect of the inversion on development time which was not influenced by the Adh locus. Development times of genotypes involving the C allele have been investigated and are reported in Chapters 4 and 7.

Development rate may also explain the correlation between Adh-B and D frequencies and the proportion of C. pilipes in a sample. C. pilipes develops more slowly than C. frigida (Dobson

1974b), emerging about 4 days later in the laboratory. The positive correlation with the slow developing Adh-B carrying genotypes may reflect longer lived wrack beds. On the other hand this would predict that C. pilipes should become progressively more common through the summer months, as does the B allele. This is not observed in these results. Egglishaw (1958) reported an annual cycle in C. pilipes frequency but Dobson (1974b) was unable to confirm this.

The presence of C. pilipes in a sample also shows a weak connection with the regular occurrence of Fucus seaweeds in wrack beds at a site. This association is not surprising as C. pilipes is very reluctant to lay eggs in the absence of Fucus (Dobson 1974b). It seems likely that the proportion of C. pilipes in a sample depends on a combination of the availability of Fucus in wrack beds and the duration of the beds at a site. On the other hand Adh-B frequency does not seem to be connected with the presence of Fucus but may well be affected by wrack bed duration. The combination of these factors can explain the observed correlation and the lack of consistent seasonal variation in C. pilipes. The remaining possibility that the presence of C. pilipes itself affects Adh-B and D frequencies more or less directly, for example by competition for a food resource, cannot be ruled out at present but is not necessary to explain the observations.

The frequency of the Adh-C allele does not correlate with the proportion of C. pilipes in individual samples. However the frequency of the C allele is lower on the south coast than in the north east, as is the proportion of C. pilipes. If this difference in C frequency is due to selection it may be a response to the same environmental variable as affects C. pilipes.

The observations on natural populations reported in this chapter provide strong evidence for the action of natural selection - principally the existence of a seasonal cycle in frequencies which is uniform over several sites and for a number of years. This raises two questions: is selection acting on the inversion polymorphism alone, or do the alleles at the Adh locus have some effect and what selective forces are important? Two selective effects are known - viability heterosis and differences in development rate. Can these effects explain the observed frequencies in natural populations? How do they interact? Are other selective forces involved? Are these effects of the inversion or of the Adh locus? What is their physiological basis? Do the inversion sequences in the different, and apparently isolated, populations contain the same coadapted sets of genes and have the same phenotypic effects?

In the remaining chapters of this thesis I describe attempts to answer some of these questions by experimental work both in the field and in the laboratory.

Chapter 4

Development times in natural populations

4.1 Introduction

Different rates of development are associated with the alcohol dehydrogenase locus, and presumably therefore with the α/β inversion system (Collins 1978, Day et al 1980). This association had only been observed in synchronised laboratory populations but I report here observations on a field population which confirm its importance under natural conditions.

Day et al used flies from a natural population which had been maintained by mass culture in the laboratory for several generations. 50 pairs were allowed to lay eggs on laboratory medium in a culture tank for 24 hours. The offspring developed at a constant 27°C. Adults emerged over a period of 12 days, the order of mean development times being:-

Fastest ⟶ Slowest					
Males	DD	CD	BC	BD	BB
Females	BC	DD	BD	BB	CD

Differences between genotypes were significant in both sexes but much more marked in males. These results are summarised in Table 4.1.

Collins (1978) also carried out his experiments at 27°C on "Marinure" based culture medium. He used the progeny of matings between wild caught BD flies synchronised by allowing 24 hours for egg laying. The development time order was the same for both sexes, DD fastest, BB slowest, BD intermediate, and was the same as that observed by Day et al for these three genotypes. Again the differences were much more marked in males.

Table 4.1

Mean development times (± standard errors) of each genotype in days (from Day *et al* 1980)

Genotype	Males	Females	Total	Number of Animals Male	Female
BB	20.23±0.33	17.87±0.49	19.02±0.34	22	23
BC	18.89±0.42	16.75±0.54	17.52±0.43	9	16
BD	19.43±0.16	17.61±0.19	18.37±0.14	129	179
CD	17.56±0.61	19.21±0.60	18.46±0.45	16	19
DD	17.07±0.22	17.49±0.22	17.27±0.16	151	139
TOTAL	18.29±0.14	17.63±0.13	17.93±0.10	327	376

For males $F_{4,322} = 23.4$ $p < 0.001$

For females $F_{4,371} = 2.41$ $p = 0.05$

Both Day and Collins suggested that if these development time differences occur in natural populations they might interact with the wrack bed cycle to influence the allele frequencies at the *Adh* locus (and the inversion frequencies). Day *et al* (1980) sampled larvae from five natural populations and allowed them to develop in the laboratory in the seaweed with which they were collected. *Adh* genotype frequencies of adults eclosing early from each sample were compared with those eclosing late. There were consistently more BB and fewer DD flies in the late emergers than in the early emergers. This confirms that development time differences similar to those found in the laboratory do occur in natural populations. However before the effect on *Adh* and inversion frequencies can be assessed it is necessary to measure the time from deposition of a wrack bed to emergence of adults for the different genotypes. There is likely

to be a lag between deposition and egg laying (Dobson 1974a) and then egg laying is probably spread over several days. Development rate is probably temperature and larval density dependent, either of these factors might vary in the life of a natural wrack bed. Are there differences in emergence times of adults despite these complications?

4.2 The experimental wrack bed at St.Mary's Island

To answer questions about development rates, and other properties, of seaweed flies in natural populations ideally requires daily visits to a suitable site over an extended period of time. In this way one could observe the deposition and colonisation of natural wrack beds, the emergence of adult flies and the eventual removal of the old beds. Unfortunately this was impracticable from Nottingham so I attempted to observe the "life history" of a single wrack bed at St.Mary's Island in Tyne & Wear.

Unusually high spring tides on 17 and 18 March 1980 seemed to offer a good chance of witnessing the deposition of a new wrack bed which would be likely to last a long time.

A plan of the site is given in Fig.4.1. No natural wrack bank was formed although for several days a large amount of fresh seaweed was present in the water and between the tide marks in the South Bay. Some of this weed was collected on 24 March to form an artificial wrack bed at the very top of the beach (see Fig.4.1). When fresh this bed was about 3 m long, 1 m wide and 0.5 m deep. Unfortunately it only lasted 26 days but during this time detailed field notes were made (see Table 4.2) and four sets of samples were taken from the bed using a soil corer, and one mass sample was taken. The method of sampling with the corer and the results obtained are

Fig.4.1 Plan of the site at St.Mary's Island, Tyne & Wear

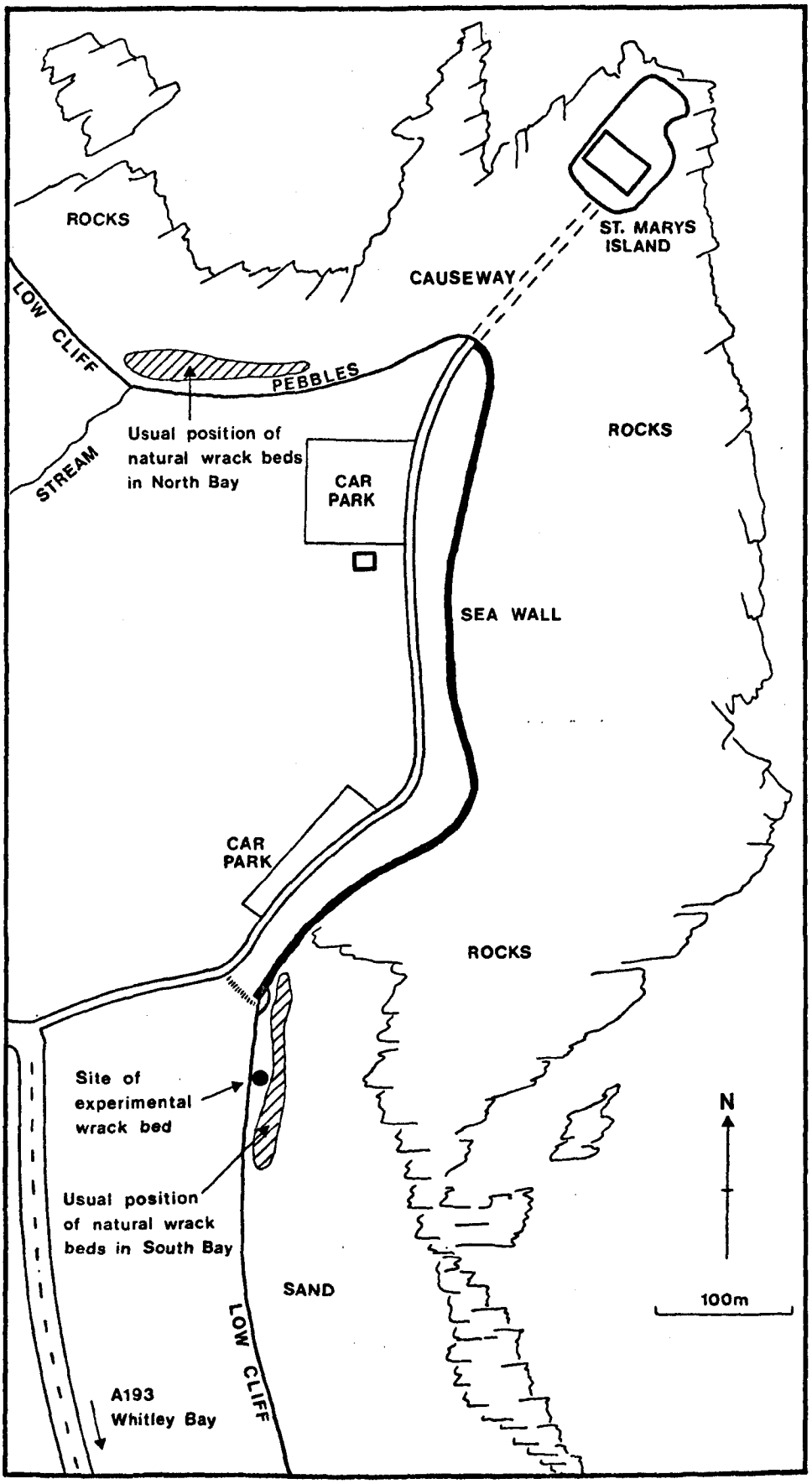


Table 4.2

Observations made at St.Mary's Island, Tyne & Wear, March-April 1980

Date	Notes
17 March	Air temperature 3°C. No wrack beds in North Bay, only wrack strings in South Bay. Large amount of fresh weed between tide marks in North Bay and in sea.
18 March	More fresh weed between tides. Extremely high tide in afternoon backed by gale force onshore winds.
19 March	Beaches completely clear of seaweed in both Bays and at least 3 kilometres north and south of St.Mary's Island. Unable to collect any Coelopa frigida.
20 March	North Bay - no seaweed. South Bay - large amount of fresh seaweed in sea under sea wall.
21 March	Seaweed in South Bay now piled up under sea wall but still in the water at high tide.
24 March	No change in position of fresh weed in South Bay, still no weed in North Bay. 30 plastic dustbin sacks of weed moved from pile under sea wall to experimental bed (see Fig.4.1). Bed about 3m x 1m x 0.5m (1-1.5 m^3). The weed was estimated to be about 60% Laminaria, 40% Fucus broken up into fairly small pieces.
25 March	Air temperature 6.5°C. Wrack bed temperatures 8, 7, 7, 6.5°C. Wrack string being deposited by high tides but bulk of weed still in the water. During the sunny morning flies were active around the experimental wrack bed. About 80 C. frigida adults were collected. (The majority of these gave unscoreable results on electrophoresis. They were not particularly small flies so this probably indicates that they were old.)

Table 4.2 continued

31 March Air temperature 6.5°C
Wrack bed temperatures 10, 14, 16, 21°C
All fresh seaweed from under sea wall had gone. Apart from experimental bed only wrack string present in either bay.
Experimental bed had sagged slightly to a depth of 30-40 cm. The surface had started to dry out and the interior was becoming slimy. The Laminaria fronds had turned green. C. frigida, Heterochila buccata and Fucellia maritima were flying in the vicinity of the bed. Inside the bed adult C. frigida were present and also Thoracochaeta zosterae. Enchytracids, nematodes and mites were also present but not Orchestia spp. or any Staphylinidae. No dipteran eggs or larvae could be found.

1 April Air temperature 12°C
Wrack bed temperatures 18, 15, 16, 21°C
Warm sunny day. Many flies active around experimental bed. C. frigida mainly within the bed. 4 batches of eggs, probably C. frigida, found close together deep in the warmest part of the bed (towards North end) laid on Laminaria frond. Also some eggs found singly on Fucus in shallower cooler parts of the bed - these could have been C. pilipes but no adults had been seen. No dipteran larvae were found.

6 April Air temperature 12°C
Wrack bed temperatures 16, 16, 15, 14°C
The experimental wrack bed had sagged further to a depth of about 15 cm. There was a thin surface layer which was dry and brittle. The rest of the bed was dark in colour, slimy and smelly. Dipteran larvae were present throughout this layer ranging in size from that of a newly hatched C. frigida to the size of a full grown C. frigida larva. A few pupae were found. Adult flies of various species were present, only a few C. frigida. Mass sample and 10 core samples taken. Core samples numbered 1 to 10 from South to North along bed at 25-30 cm intervals.

Table 4.2 continued

10 April	Air temperature 12°C Wrack bed temperatures 12, 12, 12, 12°C Dry layer thicker, some sand piled against seaward side of bed, otherwise no change from 6 April. 10 core samples taken.
14 April	Air temperature 9°C Wrack bed temperatures 12, 13, 11.5, 11°C Bed now only 10-15 cm deep, dry surface layer 2-3 cm deep. North end of bed wettest and smelliest. Larvae still present throughout depth and length in wide variety of sizes but many pupae now present too, some empty. Large numbers of Thoraeochaeta zosterae but few larger flies possibly due to overcast weather. Concerted search for C. frigida adults yielded only 6. 10 core samples taken. Approaching next spring tide, some fresh weed in the sea.
17 April	Air temperature 9°C Wrack bed temperatures 10, 11, 10, 10°C New wrack string probably deposited by spring tide about 2 m from experimental bed. Bulk of fresh weed had disappeared. Experimental bed largely unchanged except rather more compressed and sanded up. Larvae of all sizes still present throughout, as are pupae. 10 core samples taken.
21 April	Experimental wrack bed had been completely removed, probably by onshore gales on 19-20 April. Beach also clear of all natural weed in both bays. Extensive search for adult C. frigida produced only 2.

described in Chapter 5. The mass sample was taken by the normal field sampling method described in Chapter 3, taking particular care to take handfuls of weed from all parts of the bed.

As usual this mass sample was returned to the laboratory and the larvae were allowed to develop in the natural decomposing weed. The laboratory temperature was usually in the range 16-19^{o}C which is broadly similar to the temperature in the wrack bed early in April but higher than the natural temperature in the later stages of decay of the bed. The experimental bed was smaller than most natural beds and so may have cooled down unusually quickly. The laboratory temperature was probably not unlike the temperature of natural wrack beds during larval development.

As far as possible all flies were collected daily from this sample cage. Instead of being pooled these daily samples were electrophoresed separately, with males and females separate.

4.3 Development time results

The male and female genotype frequencies for each collection are given in Appendix 2 and are shown diagrammatically in Figs.4.2 and 4.3 for the 6 most common genotypes. Development times are quoted as days from the establishment of the experimental wrack bed on 24 March.

It is clear from these histograms that the distribution of development times is highly skewed. In all cases there is a peak of emergence at the beginning and a long tail. The means and standard errors of development time are given in Table 4.3 for the five common genotypes. The genotypes have been compared using a Kruskal-Wallace one way analysis of variance on ranks because of the non-normal distributions of development times. Differences

Fig.4.2 Emergence times of males classified by Adh genotype

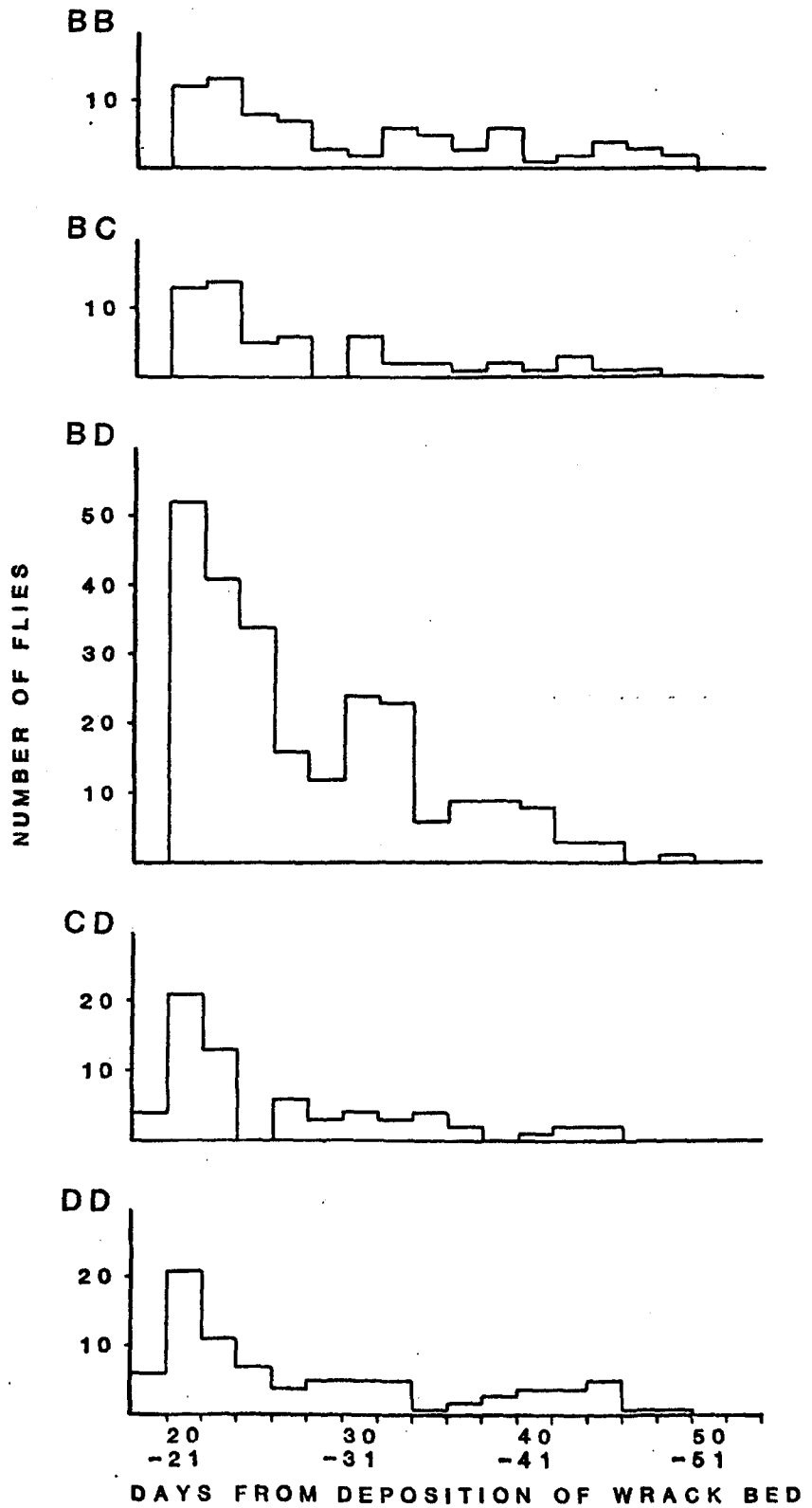
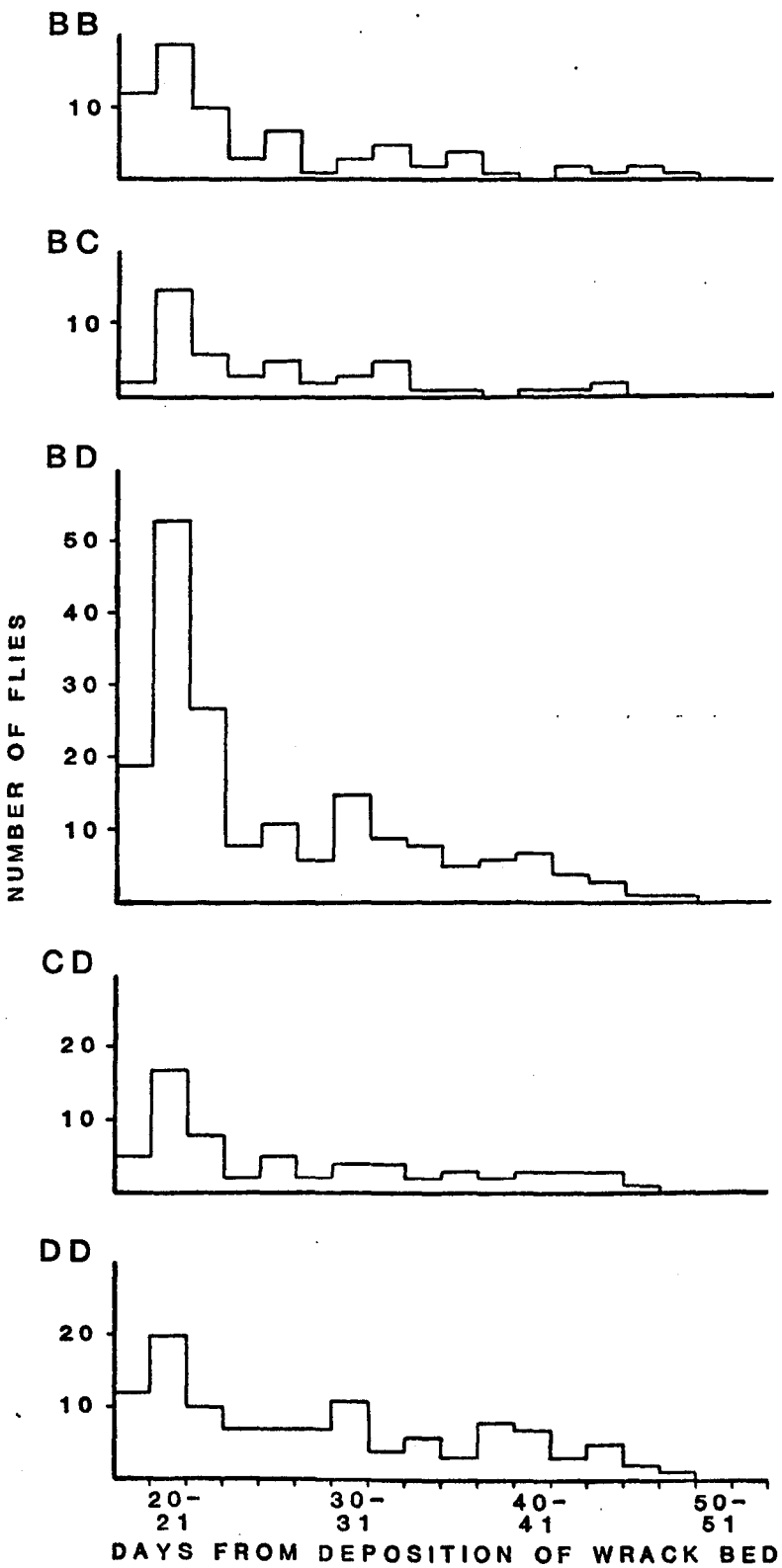


Fig.4.3 Emergence times of females classified by Adh genotype



between genotypes in males are highly significant. In females the differences approach the conventional level of significance but this is mainly due to the high values for the CD and DD genotypes. These high values probably represent the effect of a few second generation individuals and there is unlikely to be any real difference between genotypes in development time of females.

The order of emergence of the common genotypes is:-

Fastest ⟶ Slowest

Male	CC	CD	BC	BD	DD	BB
Female	BB	BC	CC	BD	CD	DD

In males the order is the same as that observed by Day et al (1980) except that DDs are much slower than expected. In females the orders appear very different at first sight but the first 4 genotypes to emerge (BB, BC, CC and BD) have closely similar development times and CD is slow in both sets of results. Once again the major difference is the late emergence of the DDs in this case.

The mean development times for all the common genotypes in both sexes fall in the range 26 to 30 days from the deposition of the wrack bed. This range spans the most probable time of wrack bed removal and deposition at about 28 days.

The first egg batches were observed in the experimental wrack bed on 1 April (Table 4.2), that is on day 8. Mean egg to adult development times must, therefore, be in the range 18 to 22 days or less. Day et al (1980) observed egg to adult development times of 16.5 to 20.5 days at 27°C in the laboratory. In their experiments with synchronised cultures adult emergence spanned 12 days. In this case emergence spanned 32 days.

Table 4.3

Development times of flies from the experimental wrack bed

Males

Genotype	Number	Development time mean ± standard error
BB	77	30.00±8.53
BC	57	27.18±7.15
BD	241	27.24±2.75
CD	65	25.87±6.31
DD	85	28.16±8.35

Kruskal-Wallace H = 13.55 P = 0.009

Females

Genotype	Number	Development time mean ± standard error
BB	73	25.97±7.32
BC	47	26.16±7.38
BD	183	26.35±4.26
CD	64	28.05±9.27
DD	113	28.97±6.93

Kruskal-Wallace H = 9.20 P = 0.056

4.4 Discussion

How representative are these results of development times in natural populations of Coelopa frigida? Several features of the experimental bed and the tank collection are relevant:-

(i) The deposition of the experimental bed was, of course, artificial. However it was in a position where natural wrack beds have been observed and it was deposited shortly after a spring tide and storm. A large amount of seaweed had been dislodged by the storm and brought inshore by the wind and tide. This is surely the sort of combination that brings about the deposition of natural wrack beds. I have quoted development times from the day on which the bed was deposited clear of the sea. No Coelopa adults were seen around the natural banks of seaweed while they were in the sea water but within 24 hrs of moving weed to the top of the beach it was possible to collect 80 C. frigida in the immediate vicinity of the new bed. I suspect that seaweed is only attractive to seaweed flies when it begins to decompose and warm up and that this does not happen until the weed is clear of the sea water. The time of deposition at the top of the beach therefore seems the most reasonable starting point. One should note that there may be several days lag between the removal of an old bed by spring tides and the deposition of a new bed in a position clear of the water in natural conditions.

(ii) The composition of the experimental bed was not noticeably different from that of natural wrack beds at St.Mary's Island. The proportions of Laminaria and Fucus were typical of this site and the fronds were broken up while the stipes were largely intact. The appearance of the bed in later stages of decay was also very similar to natural beds. The food available to the developing larvae

was thus typical of natural conditions. When the mass sample was transferred to the laboratory the larvae remained in the seaweed in which they were collected.

(iii) The most unnatural feature of the experimental bed was its small size. Natural wrack beds are generally much more extensive although they are not usually much deeper than this bed. The progress of decay and thus the temperature of a bed probably depend largely on its proportions. A large bed, especially a deep bed, will decay rapidly reaching high temperatures and will cool down slowly. Certainly temperatures much higher than those observed in this bed (Table 4.2) have been observed (Table 3.7 and Introduction) but older beds are often only just above air temperature as in this case. Decay certainly proceeded rapidly in the experimental bed and it had the expected dark, slimy appearance and smell. Larvae occurred throughout the experimental bed at a fairly even, high density. A larger bed might have had a more typical, patchy distribution of larvae and, effectively, a lower density. Development time tends to increase with increasing larval density (Collins 1978, and Chapter 5). However the density in the experimental bed, whilst high, did not appear to be exceptionally so compared with some other wrack beds sampled during this project. Larval density in the experimental bed is discussed further in Chapter 5.

(iv) Conditions of temperature and larval density were different in the collection cage. Temperature was fairly steady in the range 16-19^{o}C. This may have speeded up development slightly compared with the experimental wrack bed but, as mentioned above, these temperatures are not unnatural. The initial density in the collection cage was representative of the density in the experimental bed.

Larval dispersion was restricted to the confines of the tank whereas in natural populations larvae may respond to high density by moving to other parts of the wrack bed. This possibility is discussed in Chapter 5. Certainly larvae are sufficiently mobile for it to be a real possibility. In the laboratory they can frequently be observed crawling round and round the culture tanks. However in the case of the experimental bed dispersion is unlikely to have been important as the whole bed was already colonised when the mass sample was taken on 6 April.

Development time in natural populations is likely to be variable between wrack beds, influenced mainly by temperature and larval density. The effect of temperature on development time has not been investigated but the effect of larval density in laboratory culture is examined in Chapter 5. The results obtained from the mass sample taken from the experimental bed can reasonably be expected to fall within the range of development times occurring in natural populations. They are certainly the best estimates of natural development times currently available for Coelopa frigida. It is therefore, worthwhile to examine them in some detail.

When were eggs laid in the experimental bed? No eggs were found on 31 March but egg batches were present on 1 April. It is quite possible that laying began on 31 March but unlikely that eggs were laid earlier than this as no larvae were found on either of these two days. 31 March was the seventh day after the deposition of the wrack bed and the thirteenth day after the peak spring tide. There had been a gap of at least 15 days and probably much longer since the last wrack bed was removed from the St.Mary's Island area.

It is noteworthy that the highest wrack bed temperatures observed

were on these two days and that the first egg batches found were in the warmest part of the bed. Burnet (1960c,1961) observed in the laboratory that C. frigida females laid more eggs at 25°C than at 15°C when given a choice of laying sites. It seems that in this experimental wrack bed, although flies were present from 25 March, eggs were not laid until the temperature was in the region of 20°C. The flies may also have been "waiting" for the seaweed to reach a suitable stage of decomposition but I consider temperature to be the more important factor as females will lay eggs on fresh Laminaria stipe in the laboratory if kept at 27°C.

This lag between deposition of a wrack bed and egg laying was suggested by Dobson (1974a). It is probably a feature of all wrack beds but its length will vary. A deep bed would warm up more rapidly than a shallow one and be colonised more quickly. It may also be that in summer wrack beds warm up more rapidly and colonisation occurs earlier. This is an interesting possibility because, other things being equal, the more rapidly a bed is colonised the sooner flies will emerge. If wrack bed temperatures overall are also higher in summer development times from egg to adult will be more rapid. Both these effects would tend to decrease the advantage to fast developing genotypes ($\beta\beta$ of DD) in short lived wrack beds and might be expected to produce an increase in α sequence and Adh-B allele frequencies in the summer. This is just what has been observed (Chapter 3). It would be premature to suggest that an interaction between temperature, colonisation and development rate was the cause of the seasonal cycle in inversion frequency but it is a possible contributory factor.

By 6 April large larvae and even a few pupae were present in

the wrack bed but small, apparently recently hatched, larvae were also present. Small larvae continued to occur right up to the 17 April collections (see Table 4.2). This suggests that once egg laying had commenced it continued for some time. Towards the end some of these eggs may have been laid by females which had developed in this wrack bed but for at least the first 10 days they must have come from flies attracted to the bed from around the St.Mary's Island site. As Dobson (1974a) reported, C. frigida is strongly attracted to rotting seaweed and the experimental bed was the only actively decomposing pile of weed on that part of the coastline. It is remarkable that I was unable to collect any C. frigida on 19 March but that from 25 March onwards there was always a plentiful supply of flies around the wrack bed.

What was the range of emergence times? In the laboratory the first flies were collected on 12 April, that is 19 days after the deposition of the wrack bed and 11 days after the first egg batches were observed. A few pupae were found in the natural bed on 6 April. In normal laboratory culture at 27°C the pupal period lasts 3 to 4 days so it is reasonable to suppose that emergence began in the field at about the same time as it did in the laboratory. The next spring tide was on 15-16 April (days 22-23). From the histograms in Figs. 4.2 and 4.3 it can be seen that peak emergence in the laboratory cage had already passed by this time and about 40% of the total number of adults had emerged.

In fact the experimental bed was not removed by this spring tide but was removed by a storm, probably on 19 April (day 26). By this time about 60% of flies had emerged. Emergence continued in the laboratory cage until 13 May (day 50), that is a total span of 32 days over which flies emerged.

Why was there such a long span of development times and such a pronounced early peak? The answer probably lies in a combination of the egg laying sequence and the temperature. If the majority of egg batches were laid in the first few days after 31 March but occasional batches were laid thereafter then the distribution of emergence would be skewed. The first larvae to hatch will have experienced the lowest larval density and the highest wrack bed temperatures and will therefore have the fastest development time. Later on the wrack bed cooled down (although once in the laboratory the temperature was more or less constant), the larvae became crowded, and the nutritional quality of the seaweed may have been reduced by the presence of previous larvae. These effects would tend to strengthen the early peak of emergence and extend the long tail. In the field these effects would probably have been more marked than in the laboratory because egg laying continued after 6 April and the wrack bed cooled progressively. Flies may emerge over a very long period of time from a single generation in a natural wrack bed.

Were there any differences between genotypes? The order of mean development times is close to that expected on the basis of laboratory work, with the exception of the DD genotype as already mentioned. The simplest explanation for the apparently slow development of DD flies, both male and female, would be to assume that some second generation flies had emerged in the collection tank before the emergence of the first generation was complete. A small number of DD individuals emerging very late in the sequence would have a large effect on the mean (e.g. omitting the last 11 DD males to emerge gives a mean of 25.70 instead of 28.16 days).

I had attempted to avoid this problem by collecting flies daily. At less than 20°C it is most unlikely that females would lay eggs within 24 hrs of eclosion. However the collection of 14 April was missed due to a collecting trip to St.Mary's Island. Even a single DD x DD egg batch laid at this time would have been sufficient to give the anomalous DD results observed.

If an appreciable number of second generation egg batches had been laid and developed to eclosion this should be detectable by examining the curves of cumulative emergence in Fig.4.4. The curves show no marked discontinuities so any contribution of second generation flies can only have been small.

With such broad and skewed distributions of emergence times, comparisons of means are not very informative. A more useful approach is to ask what adult flies would be available to start the next generation if the present wrack bed were removed on any particular day. With the simplifying, and rather unrealistic, assumption that all flies remain viable and fertile throughout the period of emergence this question can be answered by looking at the cumulative allele frequencies. The cumulative Adh-B frequencies are plotted for males and females separately in Fig.4.5. On day 19, the first day of emergence, the B frequency in males was zero. It rose steeply until day 24 and then gradually through the remainder of the emergence period. In females there was a steady, slight decline in B frequency throughout.

On the assumption of random mating and if the present wrack bed was removed on any particular day, the Adh-B frequency in the next generation would be the unweighted mean of the male and female frequencies. The predicted frequencies are as follows:-

Fig.4.4 Cumulative totals of male and female flies emerging in the collection tank

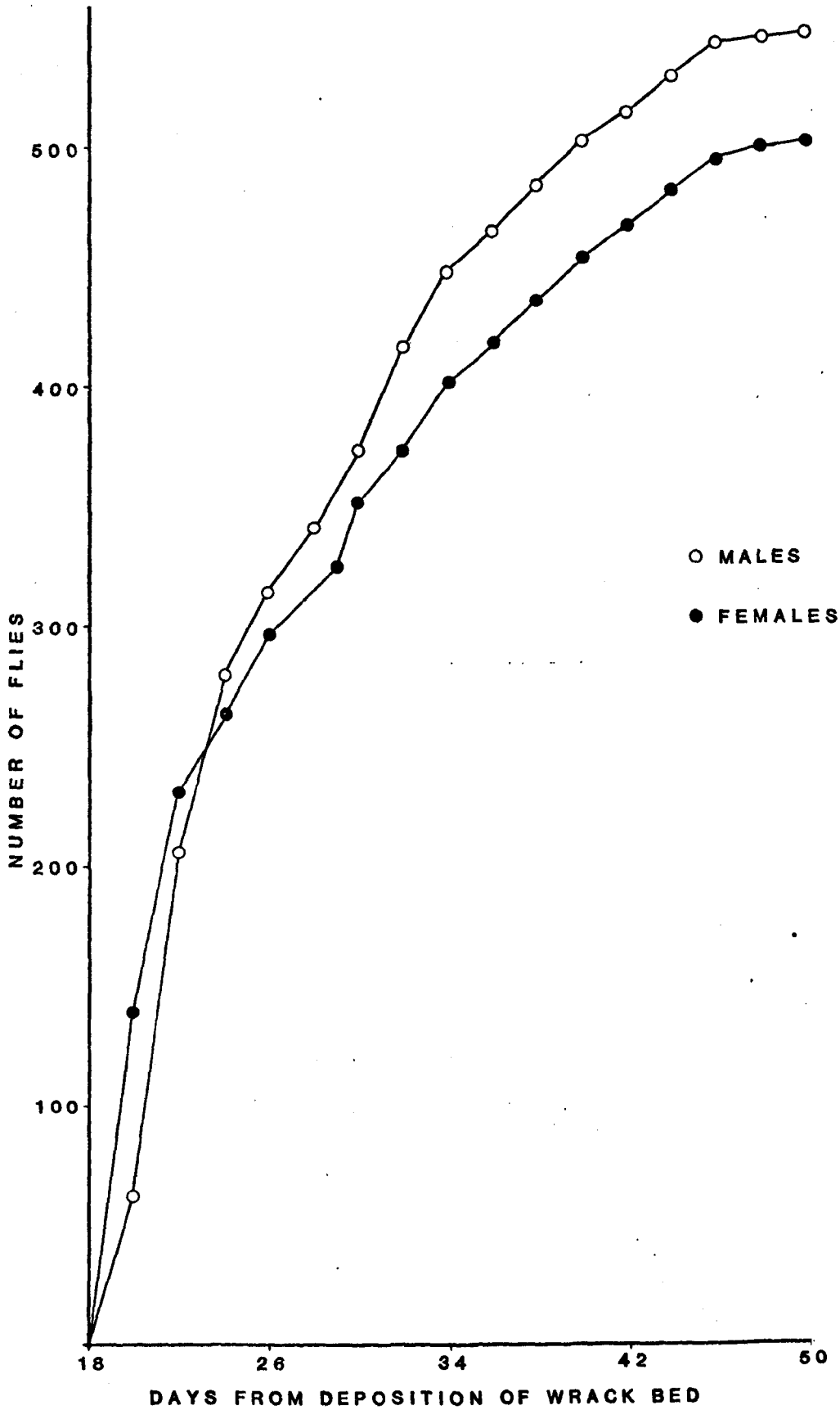
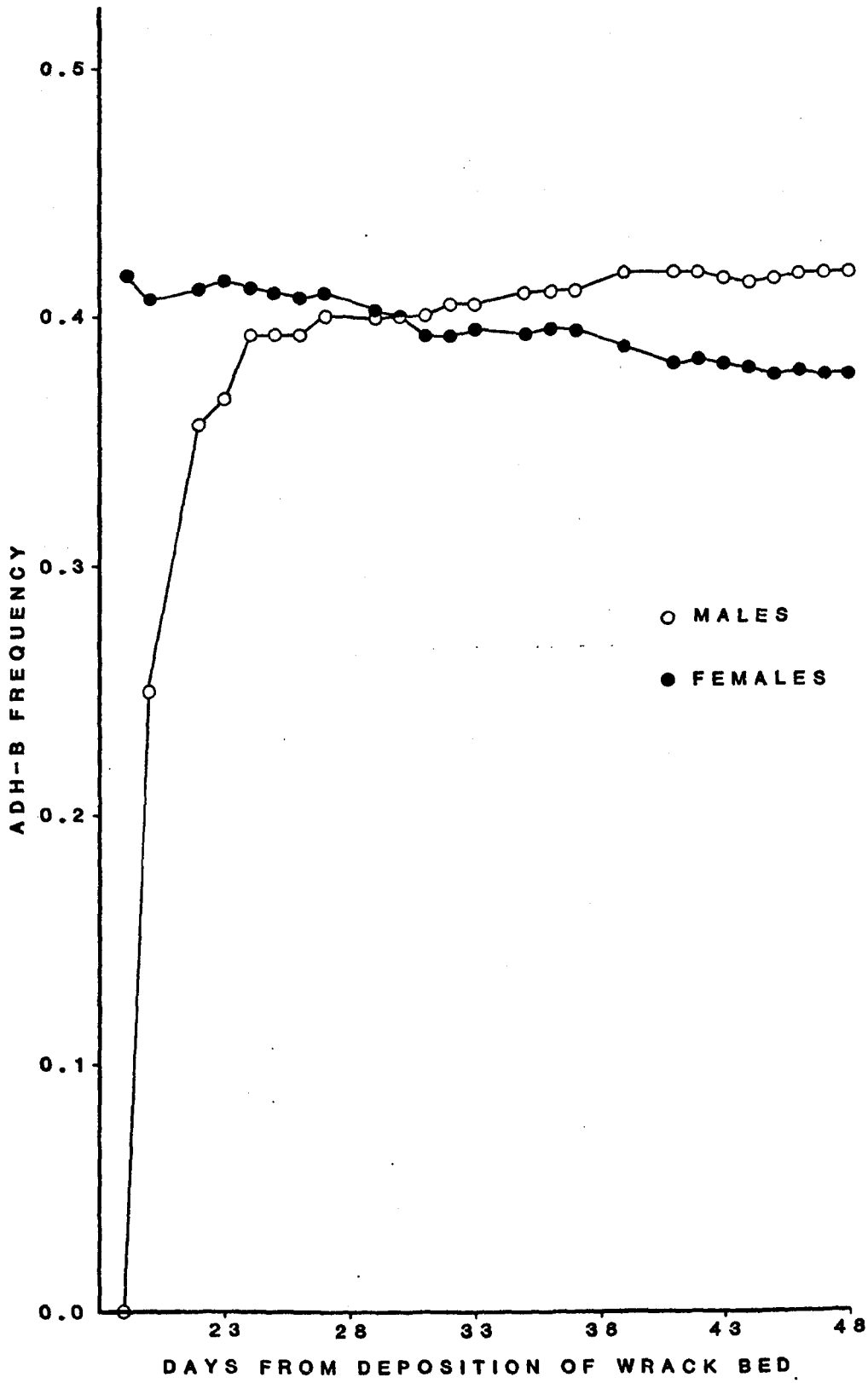


Fig.4.5 Adh-B frequencies amongst all males (or females) that had emerged up to and including the day shown



Day	Frequency of Adh-B		
	Parental females	Parental males	Predicted progeny
19	0.42	0.00	0.21
20	0.41	0.25	0.33
21	0.42	0.30	0.36
22	0.42	0.36	0.39
23	0.42	0.37	0.39
24	0.41	0.39	0.40
25	0.41	0.39	0.40
30	0.40	0.40	0.40
40	0.39	0.42	0.41

The overall Adh-B frequency in the parental generation was 0.40±0.01 (1 standard error).

These results support the arguments of Day et al (1980) and Collins (1978) that an interaction between development time and wrack bed life time can influence gene frequencies at the Adh locus. Furthermore they suggest that small differences in the timing of wrack bed removal might have large effects.

In reality the situation is more complex than suggested here. The longevity and fecundity of the adults is important. If the genotypes do not differ in longevity then the predicted B frequencies will increase further after day 24 as other, earlier emerging, males will die first. If there are differences the pattern will be more complex (see Chapter 8).

The period in which the Adh-B frequency in males was changing rapidly was 19 to 24 days after deposition of the wrack bed. Because of the delay between the previous spring tide and the seaweed deposition

this was 25 to 30 days after the spring tide and, therefore, just at the time of the next spring tide. This is the most probable time of wrack bed removal so that, if the delay and subsequent rate of development are typical of natural wrack beds, removal time will have a major influence on gene frequencies.

What was the egg to adult development time? No accurate measures of egg to adult development times in natural wrack beds are available for comparison with laboratory observations because of the uncertainty about the time of egg laying. In the study bed it seems very unlikely that any eggs were laid before 31 March and some pupae were undoubtedly present on 6 April. On 10 April a few empty pupal cases were found. This gives a minimum egg to adult development time of 10 days with pupation after 6 days. In the laboratory at 27°C and at low larval densities emergence commonly begins on the ninth day but very rarely before. The development time of at least the first few batches in the experimental bed was, therefore, very rapid and similar to that observed in the laboratory. Larvae from these batches would have experienced the highest overall temperatures and the lowest larval densities. Later on larvae would certainly have developed more slowly.

What is the relationship between the inversion and development time? Flies that are Adh-BB, BD or DD are almost certainly karyotypically αα, αβ and ββ respectively. BC flies can be either αα or αβ and CD flies either αβ or ββ (see Introduction). Collins (1978) and Day et al (1980), therefore, considered the differences in development time which they observed between Adh genotypes to result from differences between karyotypes. There is no obvious reason why the Adh locus should influence development time - alcohol

dehydrogenase is probably involved in utilisation and/or detoxification of environmental alcohols (as in Drosophila, David 1977, van Herrewege & David 1978). Development time is a complex character that is most likely to be controlled polygenically. The α/β inversion polymorphism may hold together in linkage disequilibrium alleles at loci which influence development time.

If the karyotype influences development time without there being any effect of the Adh locus then the mean development times of BC or CD flies should fall between those of BB and BD or BD and DD flies respectively. Also the variation in BC and CD flies should be greater than in the other groups because they are karyotypically heterogeneous. These predictions do not hold good either for the data presented here or for the laboratory results of Day et al (1980) with respect to means although in Day's results the variances for BC and CD are distinctly higher than for the other genotypes and it should be noted that the numbers in these classes are always rather small. In my results the frequency of the C allele is more or less constant throughout the adult emergence time as one would expect if Cα and Cβ chromosomes were equally common.

In Chapter 7 I compare the effects of inversion sequences and of Adh alleles on development time. A discussion of the relative importance of the inversion and of the Adh locus will be deferred until this additional information has been presented.

Chapter 5

Viability and Larval Competition

5.1 Introduction

Samples from natural populations of Coelopa frigida consistently have an excess of Adh-BD heterozygotes over the Hardy-Weinberg expectations. This is accompanied by a deficiency of both BB and DD homozygotes. Collins (1978) found differences in viability between these genotypes in the laboratory. The viability of the BD genotype was higher than either of the homozygotes and Collins concluded that the α/β inversion showed heterosis for viability which was likely to be the explanation for the deviations from Hardy-Weinberg expectations in natural populations.

An important aspect of Collins' experiment was that he used three different levels of larval crowding or density. At the high density the viabilities of both homozygotes relative to the heterozygote were very much lower than at low density. This suggests that the heterozygote is in some way a "better competitor" than the homozygotes and it offers the possibility of analysing the basis of the heterosis and of confirming its importance in natural populations. I have, therefore, attempted to repeat Collins' laboratory observations, to make observations on larval density in natural populations, and to analyse effects of larval density. The results of these experiments will be described in this and the following chapter but it will be helpful to discuss the concept of competition first.

5.2 Competition

The term "competition" has been used in a variety of ways in ecology and evolution which, together with its anthropomorphic connotations, have tended to reduce its usefulness. The various uses were distinguished by Birch (1957) and arranged in an order of increasing latitude:-

1) "Competition occurs when a number of animals (of the same or of different species) utilize common resources the supply of which is short; or if the resources are not in short supply, competition occurs when the animals seeking that resource nevertheless harm one another in the process." This is the most restricted meaning, requiring that the needs of the competitors overlap and are in short, or relatively short, supply. The resources may be nutrients but they may equally well be nesting sites.

2) The meaning of competition may be extended to include "the interference with one species by another even when there is no demand for a common resource." This use would not include predation and parasitism but rather something like physical disturbance of the habitat of a small sedentary organism by the activity of a larger organism. The distinction between (1) and (2) is unlikely to be important when considering intraspecific competition where there is usually a common resource.

3) Some authors include predation and parasitism within the meaning of competition which thus becomes any situation where one organism exerts a negative influence on another.

4) Finally competition has been used as equivalent to Darwin's "struggle for existence" that is the differential survival of organisms due to the action of any component of the environment,

biotic or abiotic. An important difference here is that factors are included which are not influenced by the density of the organisms involved. All the other meanings of competition contain the idea of increasing competition with increasing crowding - resource limitation will become more severe, "interference" will increase and predators or parasites will become more common.

I will use competition in the strict sense of the first definition. Within this limited concept a number of subdivisions can be recognised. Nicholson (1954) distinguished between "contest" and "scramble" competition. Where there is a contest the victors each take a share of the resource which is sufficient for their needs and the losers obtain nothing. This type of competition occurs where the resource is in discrete units e.g. nesting sites or where an area is divided into territories of a set size. Scramble competition, on the other hand, occurs where all individuals share out the resource until it is exhausted, as for example, where blowfly larvae compete for a limited amount of meat (Nicholson 1957). There is an important difference between the outcomes of these two types of competition. In a contest no matter by how much demand exceeds supply some individuals will obtain sufficient of the resource, for example each nesting site will be occupied by one pair however many pairs are present. Where there is a scramble, however, there will come a point of increasing demand where all the resource will have been used up without any of the individuals obtaining sufficient of it to survive. If a large enough number of blowfly eggs are laid on a small piece of meat none will survive to adulthood (Ullyett 1950).

Within the scramble type of competition mortality, or other

detrimental effects, may arise through shortage of the resource or through direct interference between individuals. These forms of competition have been called "exploitative" and "interference" respectively by Park (1954). When two organisms compete for a limited resource one may have a detrimental effect on the other purely by using up the resource, the exploitative type of interaction, or it may affect the other by excreting or secreting a harmful substance, by aggressive behaviour, by cannibalism or by any other type of interference. For interference competition to fall within the definition used here the competitors must share a common resource. Examples of both exploitative and interference competition in blowflies are described by Ullyett (1950). Clearly these two types of competition can occur together.

It is possible for organisms to interact in ways which have not been covered by any of the definitions given above. In particular the effect of one organism on another may be advantageous. This has been called "cooperative competition" by Allee et al (1949, p395) but this seems to be extending the term competition too far. Gustafsson (1953) calls the beneficial effect of one barley strain on another "cooperation" but the anthropomorphic overtones of this term can be avoided by using the term "facilitation" as used by Lewontin (1955) to describe increased viability of Drosophila strains with increasing larval density.

In Coelopa frigida there is a relationship between larval density and viability from egg to adult (Thompson 1951, Burnet and Thompson 1960, Rowell 1969 and see Introduction) which suggests both facilitation and competition. Facilitation occurs as viability rises with increasing density to a peak and then competition can be

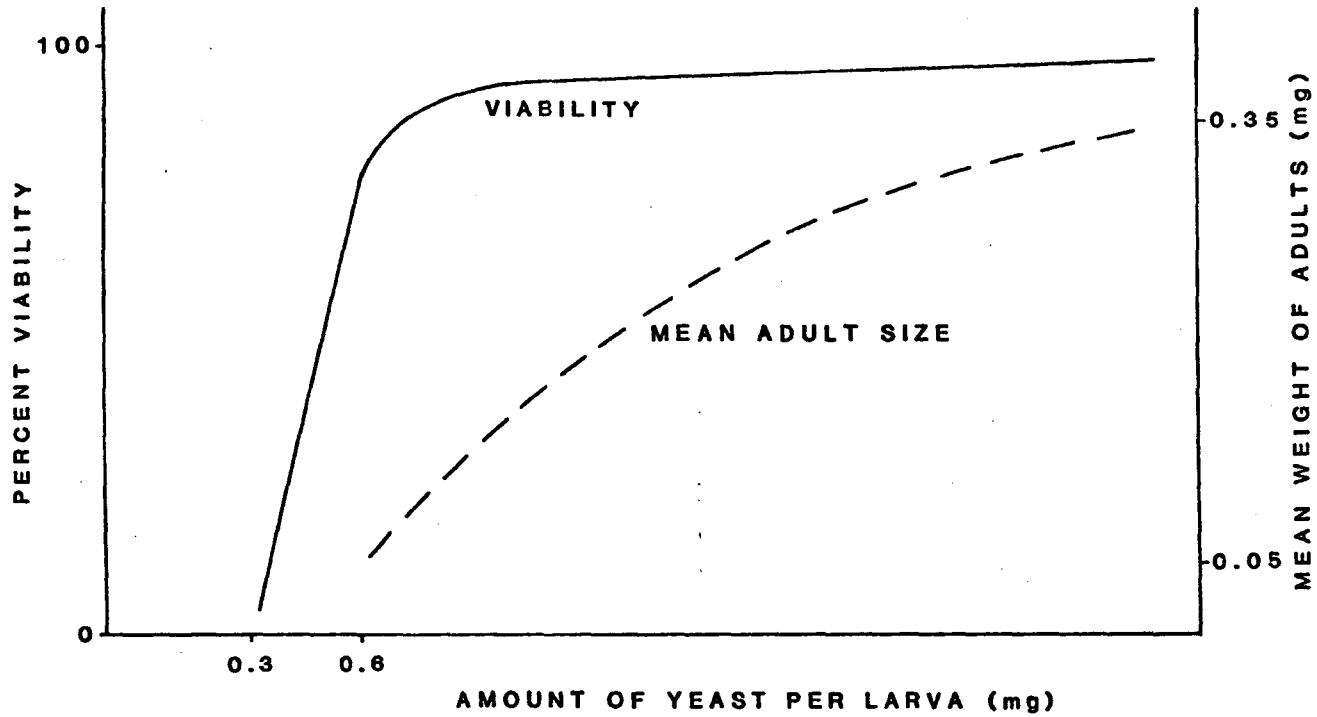
seen as viability falls beyond this peak. At least in the laboratory, the conditions in which Coelopa larvae live are very similar to those of Drosophila species and Lucilia species (blowflies) in that the larvae live within a mass of nutrient medium where they grow rapidly, spending most of their time feeding. The period of feeding is limited by the onset of pupation by which time the larvae must have reached a minimum weight for survival. There have been many investigations of the effects of larval density on viability in other Dipteran species some of which will now be reviewed to provide a background for the discussion of my observations on Coelopa.

5.3 Larval Competition in Diptera

Competition between diperan larvae is usually of the scramble type as opposed to the contest. In both Lucilia cuprina (Nicholson 1954,1957) and Drosophila melanogaster (Bakker 1961) a point of increasing larval density is reached at which no individuals survive to adulthood. Before this point the sizes of adults decline with increasing larval density until a minimum size is reached and then viability drops rapidly (Bakker 1961). This is shown diagrammatically in Fig.5.1. This type of relationship led to the concept of a minimum survival weight which larvae must attain before they can pupate successfully. Larvae "scramble" for the available food and if it is used up before they attain the minimum size required they die. However competition has important detrimental effects before causing a reduction in viability. Reduction in size may reduce the reproductive output of both males and females (Nicholson 1957, Partridge and Farquhar 1981, Rose and Charlesworth 1980, Lamb 1964).

The time taken for development may also be affected by increasing

Fig.5.1 The effect of food supply on viability and adult size in Drosophila melanogaster (after Bakker 1961).



competition and in an expanding population this can be an important element of fitness. In some cases development is delayed by higher densities - indeed delay in development can be a very sensitive indicator of adverse conditions (Sang 1956, McFarquhar and Robertson 1963). On the other hand, Bakker (1961) found a decrease in mean development time with increased density which he attributed to exhaustion of the food supply before slow developing larvae reached the minimum survival weight. This view is supported by the observation that, in competitive conditions, later emerging adults are smaller than early emergers (Sang 1950, Bakker 1961, Hughes 1980). At any one density, fast developing larvae grow beyond the minimum survival weight and emerge as large adults, slower developers produce smaller adults and the slowest never achieve the minimum weight. As density increases size decreases and development time is shortened.

This picture, presented especially by Bakker (1961), does not always apply (Caligari, 1980). As mentioned above development time is frequently lengthened by increased competition. This may be due to a difference in culture technique. Bakker used yeast spread on an agar base where all the food present is immediately available to the larvae and so rate of development is not affected by increased density. In standard maize meal molasses medium, it is possible that food becomes available progressively and that larval development is therefore affected by competition for food.

In some cases adult body size has been observed to decrease initially with increased density but then increase at higher density and this is associated with longer development time (Chiang and Hodson 1950, Hughes 1980). In certain conditions slow developing

larvae apparently survive better than fast developing ones and grow to large size. Possible changes in conditions that bring about this shift are discussed by Hughes.

The effect of larval competition on adult size can be used to detect its occurrence in natural populations. In Drosophila melanogaster and D. pseudoobscura, there is little size variation (Roff 1981, Sokoloff 1957, McFarquhar and Robertson 1963), although some seasonal fluctuation has been detected in D. melanogaster which is due largely to temperature variation, but also in part to increased densities in the summer (Tantawy 1964, Atkinson 1979). By contrast the thorax length of D. subobscura may vary by a factor of 3 (McFarquhar and Robertson 1963).

In addition to competition, facilitation has been observed in laboratory cultures of Drosophila. In D. melanogaster (Lewontin 1955) and in D. busckii (Lewontin and Matsuo 1963) viability rises with increasing density to a maximum and then falls. This is the pattern observed in Coelopa frigida (Burnet and Thompson 1960, Rowell 1969). Flies are large at densities below the optimum but as competition increases size drops. In natural populations adult size is very variable in Coelopa frigida (Mayhew 1939).

So far I have considered the effects of competition within strains. This is important for understanding population dynamics but from the point of view of population genetics competition between strains, or between genotypes, is of more interest. (However it may be necessary to study intragenotypic competition in detail in order to understand intergenotypic interactions (Caligari 1980).)

Larval crowding has been shown in the laboratory to affect the

relative fitnesses of many naturally polymorphic variants. Dobzhansky (1947c) found significant departures from Hardy-Weinberg expectations in laboratory populations of Drosophila pseudoobscura segregating for the AR and CH third chromosome arrangements, but only when larvae were crowded. Druger and Nickerson (1972) were unable to repeat this observation using a population which had been maintained in the laboratory for ten years. Laboratory populations polymorphic for the ST and CH arrangements reach an equilibrium at about 30% ST when larvae are uncrowded, but 70% ST when they are crowded (Birch 1955). This can be related to the natural seasonal cycle in ST and CH frequencies; ST is at high frequency in the summer when larval densities are high. Viabilities under conditions of larval competition appear to be affected not only by overall density but by the genotypes of the other larvae present. For example in D. pseudoobscura the presence of the AR arrangement affects the relative viabilities of the ST/ST, ST/CH and CH/CH genotypes (Levine et al 1954) and in D. persimilis the WT arrangement affected the outcome of competition between the KL and MD arrangements (Spiess 1956). In neither case could the outcome of competition in the "three way" populations be predicted from the "two way" comparisons.

Similar results have been obtained for visible polymorphisms. Da Cunha (1949) found an increase in heterosis with increased larval competition in the colour variants of D. polymorpha but he did not observe any excess of heterozygotes in natural populations. The relative viabilities of wild type (+/+) and +/e heterozygote larvae are unaffected by density but the relative viability of the ebony homozygote (e/e) is greatly reduced in crowded conditions

(Moree and King 1961). Increased heterosis is also found under crowded conditions between the colour morphs of the marine copepod Tisbe reticulata (Battaglia 1958).

More recently the effect of density on polymorphic enzyme loci has been studied. This included the Esterase 6 locus in D. melanogaster (Kojima and Huang 1972) and the Mdh-2 (Malate dehydrogenase) locus of D. pseudoobscura (Tošić and Ayala 1981). In both cases increased competition in the form of increased larval crowding, accentuated differences in viability between genotypes, and viability was reduced more by competition with like than with unlike genotypes. In other words there was evidence for frequency dependent selection, especially at high densities, which suggests that the genotypes had slightly different requirements. In the cases of the Alcohol dehydrogenase locus (review in Van Delden et al 1978) and the amylase locus (Horn and Scharloo 1981) the outcome of competition can be influenced by varying the culture medium in a way relevant to the enzyme involved i.e. by changing alcohol or starch concentration. It must be noted that in experiments with individual enzyme loci there is always the possibility that the observed effects are due to other loci in linkage disequilibrium with the studied locus. Recently it has been shown that competition is more severe between related than between unrelated Drosophila larvae (Pérez-Tomé and Toro 1982).

Slightly different requirements of different genotypes might lead to more efficient utilization of resources by mixed cultures than by single strain cultures. This would lead to the mixed cultures having a higher overall viability than the pure cultures,

especially at competitive densities. This was observed for D. pseudoobscura chromosomal variants by Beardmore (1963). The effect has been called "facilitation", but a distinction should be made between this situation where the higher viability is due to less intense competition, and facilitation as previously defined, where the presence of another organism or organisms is positively beneficial.

A general feature of the results of competition between genotypes or strains is the unpredictability of the behaviour of the mixed populations from a knowledge of the effect of density on the pure genotypes or strains. This has been clearly illustrated by extensive studies of laboratory strains of D. melanogaster (Lewontin 1955, Dawood and Strickberger 1964) and of D. busckii (Lewontin and Matsuo 1963).

Various attempts have been made to solve this problem. In general the first question to ask is whether competition is of the exploitative or interference type. Interference competition in Dipteran larvae is likely to be due either to build up of harmful substances in the medium, which may be waste products or specific growth retardant secretions, or to physical interference varying from reduction in feeding time to cannibalism.

Interference competition depends on the amount of space available per larva whereas exploitative competition depends on the amount of food per larva. If these can be separated a distinction can be made between the types of competition. Bakker (1961) used his technique of feeding D. melanogaster larvae on yeast spread on agar to achieve the separation. A wide range of larval densities (per unit volume) at a fixed amount of food per

larva had no effect on viability. In these conditions competition is exclusively of the exploitative type. On the other hand in some conditions in serial transfer population experiments with D. melanogaster adult production is limited without all the available food being utilised (Gilpin 1974, Hughes 1980) indicating an interference component of competition. This interference component may be particularly important where the population is continuous rather than synchronous. Large larvae may interfere with the feeding of small larvae or may cannibalise them as happens in some other insects (Nicholson 1957, Lucilia cuprina; Dye 1982, Aedes aegypti; McCauley and Wade 1980, Tribolium confusum).

Another approach to the distinction between exploitation and interference and to the interaction of different strains in general is the conditioned medium experiment. This experiment involves growing larvae in normal medium, removing them and then growing a further group of larvae on the same medium. If the viability of the second group (the test generation) is different from that of a control on fresh medium at the same density then the first group (the conditioning generation) must have made some stable change in the medium. The change is likely to be either depletion of food or build up of harmful substances. These can be distinguished by adding extra food for the second group. Interactions between strains can be examined by varying the strains used in the two generations.

The technique was used by Dawood and Strickberger (1964) with Drosophila melanogaster and by Weisbrot (1966) with D. melanogaster and D. pseudoobscura. In both cases comparisons were between a variety of laboratory strains. Some combinations of conditioning

and test generations gave facilitation while others showed reduced viability. There was no apparent order to the results. The rather complex interaction between larvae, yeasts and culture medium was thought to be responsible for the unclear results.

Conditioned medium experiments have also been used to investigate frequency dependent selection acting on the Esterase 6 locus in D. melanogaster (Huang et al 1971, Kojima and Huang 1972). Here the results were more conclusive. Conditioning with FF homozygotes gave the greatest reduction in viability in the test generation for FF larvae and the least for SS larvae. Conditioning with SS homozygotes had the opposite effect. These responses were only observed when a certain density of larvae in the conditioning generation was exceeded indicating a threshold density above which frequency dependence becomes important. The experiments did not distinguish between exploitative competition and interference competition, although it is clear that physical interactions between larvae cannot be important.

Dolan and Robertson (1975) could not repeat these observations on the Esterase 6 locus nor obtain any comparable results for the Adh locus. The results of Kojima's group could have been due to chance linkage disequilibrium between the Esterase 6 alleles and alleles at other loci affecting viability.

A situation very similar to the Coelopa inversion polymorphism has been studied in Drosophila pavani using the conditioned medium technique (Budnik et al 1971, Brncic and Budnik 1976). An inversion on Chromosome IV shows heterosis in crowded, but not in uncrowded, conditions and this may be important for its maintenance in natural

populations. Eggs from a segregating population were used in both the conditioning and test generation. The test generation adults showed a significant departure from Hardy-Weinberg expectations in the predicted direction while controls at the same density did not. In this experiment yeast was added throughout both generations so the effect was attributed to interference, probably "biotic residues", rather than food shortage.

The general idea of the conditioned medium experiment has also been applied to aquatic mosquito larvae where it is a simple matter to use the same water for several generations of larvae. Exploitative competition certainly occurs but it has also been suggested, for Aedes aegypti, that larvae actively secrete growth retardants at high densities (Moore and Whitacre, 1972; Kuno and Moore 1975). More recently the importance of these substances has been questioned (Dye 1982).

Clearly this technique has considerable potential although in Drosophila the complex nature of the food can make the results difficult to interpret. As yet it has not thrown much light on the factors which affect the outcome of competition between genotypes.

Bakker (1961) made a detailed analysis of the properties of two laboratory strains of Drosophila melanogaster which might affect their competitive ability. The two strains, one wild type and one marked with the Bar mutation, showed indistinguishable responses to density when in pure culture and this was shown to be exploitative rather than interference competition. When the two strains were in competition the wild type strain showed a marked advantage. Bakker argued that, as the two strains showed the same

response to food shortage, the difference in competitive ability could not be a difference in food requirement but must be a difference in the ability to acquire food. He therefore studied the following characteristics of each strain:-

(i) Efficiency of conversion of food to larval dry weight

(ii) Growth rate

(iii) Time spent in moulting (non-feeding time)

(iv) Minimum weight for successful pupation

(v) Timing of developmental stages

(vi) Resistance to starvation between exhaustion of food supply and pupation.

Of these, only (ii) and (v) differed between the strains although (iv) differed between the sexes in both strains - males were smaller than females. The wild type strain had a higher growth rate throughout the larval period than the Bar strain which, at low densities, gave larger wild type adults than Bar adults. Wild type eggs hatched about 1 hour before Bar eggs. If, as seems likely, the rate of growth at any moment is a function of size this difference in timing can explain the difference in growth rate between the strains.

Bakker showed that a difference in growth rate with the same minimum survival weight is sufficient to explain the outcome of competition between the strains. A higher proportion of the faster growing larvae will reach the minimum weight before the food is exhausted. He demonstrated the importance of growth rate by giving the Bar strain a 3 hour "start" in competition with the wild type strain. This completely eliminated the advantage of the latter strain. Later he selected strains for early and late pupation,

measured their growth rates and other characteristics and was able to predict the outcome of competition between these selected lines (Bakker 1969). Lines selected for high rate of feeding also have higher survival and produce larger adults when in competition with unselected lines (Burnet et al 1977).

Bentvelzen (1963) made a detailed statement of the importance of growth rates in competition and demonstrated that a higher variance in growth rate may give an advantage in competition even when mean growth rates do not differ. The model can also explain some cases of frequency dependence. The higher the proportion of faster growing larvae in a culture the more restricted the feeding period will be and thus the greater will be the disadvantage to the slow growers. Clearly this gives a direct relationship between frequency and relative viability; not the inverse relationship more commonly observed.

In some circumstances the feeding period of larvae may be affected by conditions other than food shortage and these conditions may also affect the feeding rate and thus the growth rate. This would amount to an interference element of competition and could allow the extension of the model to cases where development time is extended by increased density (Gilpin 1974, Hughes 1980).

In Bakker's experiments all the food was immediately available to the larvae and was utilised equally by the two strains. Clearly the competitive interactions would be more complex if food became available progressively, as probably happens in nature and in standard Drosophila or Coelopa culture conditions, or if different components of the food were utilised by different competing strains.

In Drosophila, where a defined medium is available, it is possible to investigate the effects of shortages of different components of the food (McFarquhar and Robertson 1963, Sang 1956). In Coelopa it is not clear what the larvae require or just what their relationship is with the seaweed microflora. Collins (1978) looked at the effects of varying protein content in the medium on the chromosome I inversion but he could detect no significant differences.

5.4 Larval Competition in Coelopa

The effect of larval density on viability in Coelopa is similar to that observed in Drosophila (Lewontin 1955). Viability increases slightly up to a peak at about 1 larva per gram of Laminaria. Beyond this point adult body size declines and viability falls with further increase in initial density. The fall in viability is slow at first becoming more rapid at higher densities (Burnet and Thompson 1960, Rowell 1969) probably reaching zero at about 2 larvae per gram. Clearly competition is of the scramble type.

The peak viability is lower and the fall in viability steeper where larger amounts of food are provided in the same sized container. Burnet and Thompson suggested that this is due to the greater accumulation of "toxic decomposition products", that is they suggest a greater interference element of competition with higher densities of larvae with respect to space rather than food.

Collins'(1978) experiments indicate that the competitive abilities of the chromosome I genotypes are different.

His experimental technique will be described in some detail for later comparison with my own experiments. Larvae were collected from a natural population and allowed to develop in the laboratory.

Table 5.1

Results of Collins' (1978) density experiment

a) Overall survivals and adult sizes

Density	No. of replicates	Mean no. of eggs	Mean survival %	Mean weight of adults mg
Low	5	80	94.6	8.95
Medium	5	330	70.5	4.85
High	4	726	63.0	3.25

b) Relative viabilities

Density	♂ BB	♂ BD	♂ DD	♀ BB	♀ BD	♀ DD	TOTAL BB	TOTAL BD	TOTAL DD
Low	0.83	1	0.73	0.87	1	1.09	0.85	1	0.91
Medium	0.40	1	0.35	0.52	1	0.63	0.46	1	0.48
High	0.33	1	0.32	0.56	1	0.34	0.44	1	0.33

(These values are calculated on the assumption of an initial 1:2:1 genotype ratio and 1:1 sex ratio with the viability of the heterozygote set at 1)

The adults were pair mated in mating pots and allowed to lay a batch of eggs. Adults were then electrophoresed immediately so that egg batches from Adh BDxBD crosses could be selected. These batches were used to start the density experiment. Three sets of replicates were set up using 1, 5 or 10 egg batches per canister in small canisters (9x9x13 cm). The number of eggs in each batch was recorded. The canisters contained standard culture medium and were kept at 27°C. All adults were collected and a sample electrophoresed.

Collins' results are presented in Table 5.1. In each of the low, medium and high density sets the results from the individual replicates were homogeneous and have been combined. At low density survival was high and the adult flies produced were large, also development times were short. As density increased, survival and adult size decreased and development times were longer.

At all densities there was marked heterosis. At low density heterozygotes had an advantage of about 10% despite the high overall survival. As density increased the advantage increased to well over 50%. The relative viabilities of the two homozygotes show slight differences, with that of DD higher at low density and that of BB higher at high density.

The sexes behaved somewhat differently. In general the heterozygote advantage was greater in the males than in the females. In males DDs always had a slightly lower viability than BBs whereas in females the DDs had the highest viability at low density and the lowest at high density.

Collins' technique had a number of advantages. The parents of the test generation were wild flies paired at random avoiding any

problems of inbreeding which could confuse heterosis due to the inversion with general heterosis on the crossing of inbred lines. There was a clear expectation of a 1:2:1 ratio in the offspring and the number of eggs in each replicate was accurately known. On the other hand the technique relies on obtaining a field sample and is very labour intensive. To obtain sufficient egg batches may involve starting with ten times as many pairs as the number of batches required. Parents had to be electrophoresed and replicates set up as egg batches appeared. For these reasons rather different experimental methods were used in my attempts to repeat Collins' observations. The intention was to develop techniques which would be useful in subsequent attempts to analyse factors affecting the viability differences.

5.5 Density Experiments

a) Methods Two experiments were conducted which differed in that one used egg batches to start the experimental canisters and the other used 2 day old larvae. These will be called the "egg to adult" and "larva to adult" experiments respectively.

Both experiments were conducted in round canisters containing 3 cm of standard culture medium. These canisters have a surface area of medium about 3/8 that of the small canisters used by Collins, but a similar depth of medium. The canisters were kept at a constant temperature of 27°C.

The flies used to start the experiments were from the B + D lines (see Materials and Methods, Chapter 2). The effects of inbreeding were minimised in these lines by their broad genetic base, and by ensuring that the population sizes remained large. At no time between establishing the lines and the start of these experiments

did the number of flies fall below 500. Eggs or larvae from these lines were chosen at random and the results compared with the Hardy-Weinberg expectations based on the known frequencies in the parental generation. The use of these lines was far more convenient than the wild fly method of Collins and should, therefore, allow larger and more complex experiments.

In both experiments all eclosing flies were collected daily from each canister and stored at -20°C until required for electrophoresis.

Details of the two experiments were as follows:-

(i) Parents Egg to adult - about 3000 flies were allowed to lay eggs for 24 hrs in a culture tank. SM series parents were from SM80 B + D generation 8 and the gene frequency of Adh-B was 0.42±0.04 (1 s.e.). MN series parents were from MN80 B + D generation 3 and the gene frequency of Adh-B was 0.41±0.04.

Larva to adult - about 5000 flies from the SM80 B + D line, generation 3 were allowed to lay eggs in a culture tank for 24 hrs and then removed. Parental Adh-B frequency was 0.46±0.03.

(ii) Transfers Egg to adult - at the end of the 24 hr laying period egg batches were transferred to the experimental canisters on a small amount of medium picked from the tank on a spatula. 8 replicates were set up at each of 4 densities - 2, 4, 6 and 8 egg batches per canister.

Larva to adult - at the end of the 24 hr laying period all adults were removed from the tank and it was returned to the constant temperature room. 48 hrs later the small

larvae were counted into the experimental canisters on a fine wetted paint brush. The following replicates were set up: 3 at 100 larvae per canister, 3 at 200 larvae per canister, 2 at 400 larvae per canister, and 2 at 800 larvae per canister.

(iii) Electrophoresis and Measurement Egg to adult - all flies from any one canister were pooled and a random sample was used for electrophoresis.

Larva to adult - All flies were electrophoresed after having had their wing lengths measured in the manner described in Materials and Methods, Chapter 2.

b) Results The results of the egg to adult experiment are given in Tables 5.2 to 5.5 and of the larva to adult experiment in Table 5.6. From the point of view of larval survival the two experiments will be considered together and compared with Collins' results. The information on development times and wing lengths available from the larva to adult experiment will then be presented separately.

(i) Comparison of densities The small canisters used by Collins contained about the same depth of medium as the round canisters used here but the round canisters have about 3/8 the surface area of the square canisters. The densities used by Collins were, therefore, approximately equivalent to 30, 120 and 270 eggs per round canister. The flies used to start the egg to adult experiment were small and the average size of an egg batch was probably about 50 eggs (see Chapter 8). This gave approximate densities of 100, 200, 300 and 400 eggs per canister and suggests that the 2 batch density should be comparable with Collins' Medium density and the 6 batch with his High density.

In the larva to adult experiment the number of 2 day old larvae was known exactly but there may have been some mortality between hatching and this stage so that the comparable number of eggs would have been rather higher. The 100, 200 and 400 densities should be similar to the 2, 4 and 8 batch densities respectively.

(ii) Overall survival The proportions of eggs or larvae to reach adulthood at comparable densities are given below, the values for the egg to adult experiment are necessarily only rough approximations.

Density		Percentage Survival		
		Collins	Egg to Adult	Larva to Adult
Low,	-, -	95	-	-
Medium,	2, 100	70	40	34
-	4, 200	-	30	31
High,	6, -	63	27	-
-	8, 400	-	27	23
-	-, 800	-	-	13

In all cases the viability drops with increasing density so the densities used here are in the "competitive" phase of the density/viability relationship of Burnet and Thompson (1960).

While the values from my two experiments are very similar, the survivals in Collins' experiment were much higher. There are several possible explanations for this difference. The surface area to volume ratio of the medium was similar but the amount of medium was smaller in my experiments. It seems unlikely that this would have caused such a large difference. Indeed Burnet and Thompson (1960) found a slight decrease in peak yield (larvae per gram of food) as the amount of food increased. The smaller amount of medium may have dried out more quickly. Coelopa larvae do not

survive well in dry medium. Again this seems an unlikely explanation as drying is probably related to surface area to volume ratio. It is possible that Collins' egg counts were underestimates as egg counting is difficult. However Collins was confident of his ability to obtain accurate counts. My stocks may have suffered a general inbreeding depression. This seems most unlikely in view of the way in which they were established and maintained. These stocks were maintained without difficulty for many subsequent generations. A more likely reason for the difference in overall survival is a slight change in the composition of the culture medium. Collins included powdered milk in his medium and placed a few strips of Laminaria stipe on the surface of the medium to encourage egg laying. The use of powdered milk has been discontinued because it frequently resulted in a fungal contamination of the medium which was detrimental to Coelopa survival. Laminaria stipe is not necessary for adequate egg laying provided the medium surface is thoroughly forked over. It seems possible that the omission of the powdered milk could have caused this drop in viability. Collins found that a 12 fold increase in the proportion of powdered milk in the medium caused a large reduction in overall viability at low larval density but at higher densities or with smaller proportions powdered milk could, nevertheless increase viability.

A further important distinction between the experiments was the use of flies from a different natural population. Collins' flies came from Rustington which is probably genetically isolated from both Morfa Nefyn and St.Mary's Island (Chapter 3). It is possible that these populations contain different coadapted complexes of genes which cause different responses to increased larval density. However

the results for Morfa Nefyn and St.Mary's Island are very similar despite being further separated than Rustington is from either.

(iii) Heterogeneity between replicates A considerable amount of heterogeneity between replicates was expected in the egg to adult experiment, especially in the 2 batch set, because of the different genotype ratios of individual egg batches. However there was an excess of heterozygotes amongst the parents so that the most common type of mating was BDxBD. It also seems likely (see Chapter 9) that multiple insemination is common in Coelopa which will tend to make replicates more similar. It is encouraging to note that all replicates produced flies of all three genotypes. The large number of replicates in each set was intended to smooth out the effects of heterogeneity.

The results for individual replicates are given in Tables 5.2 to 5.4 and the χ^2 values for heterogeneity are given in Table 5.5. In several cases the heterogeneity is statistically significant. Nevertheless the results have been combined over replicates for further analysis. It seems unlikely that the differences between replicates, assuming they are due to differences in initial genotype frequencies, would affect the overall conclusions about selective viability unless the relative viabilities of the genotypes are frequency dependent. Even in this case any effect in one replicate would probably be balanced by other replicates with starting frequencies on the opposite side of the mean.

In the larva to adult experiment no such heterogeneity is expected as larvae from all batches had an opportunity to mix before transfer to the experimental canisters. The results for individual replicates and the χ^2 values for heterogeneity are given

in Table 5.6. Only in the males from the 800 replicates is there significant heterogeneity. There is no difference between males and females in replicate 800/2 ($\chi_2^2 = 0.98$) but there is in replicate 800/1 ($\chi_2^2 = 12.4$, $P < 0.01$) suggesting that it is the genotype distribution of the 800/1 males which is anomalous.

(iv) Male-female differences With the exception of the 800 density in the larva to adult experiment there are no statistically significant differences between genotype distributions in males and females in either experiment (for all comparisons $\chi_2^2 < 5.5$, $P > 0.05$). Nevertheless the results are given for males and females separately in Tables 5.3, 5.4 and 5.6 for comparison with Collins' observations. As in his results, there is a slight tendency for deviations from expectation to be greater in males than in females.

Evans and Philip (1964) found a reduction in the proportion of males with increasing density in Coelopa frigida. This effect can be seen in the larva to adult experiment where the proportion of males falls from 0.45 in the 100 density to 0.40 in the 800 density and is statistically significant ($r = -0.93$ with 3 degrees of freedom, $P < 0.05$). However there is no such trend in either the MN or the SM series of the egg to adult experiment.

(v) Deviations from expected frequencies The observed numbers from the egg to adult and larva to adult experiments are compared with the expectations in Tables 5.7 and 5.8 respectively. These expectations are calculated from the parental gene frequencies using the Hardy-Weinberg equation. They, therefore, assume that there was random mating and no differences in fecundity or fertility as well as no selective mortality. These extra assumptions did not enter into Collins' experiments because he used the progeny of BDxBD matings.

Table 5.2

a) Results of Egg to adult experiment - total numbers of flies emerging

(i) MN Series

Replicate	Density 2	4	6	8
A	12	17	10	42
B	22	52	44	70
C	45	112	50	114
D	57	55	76	112
E	70	94	198	116
F	69	39	104	103
G	35	76	181	114
H	53	57	54	134
TOTAL	363	502	717	805

(ii) SM Series

Replicate	Density 2	4	6	8
A	35	36	103	127
B	34	85	47	113
C	35	47	70	98
D	30	75	32	112
E	32	44	68	133
F	46	96	104	109
G	20	34	82	123
H	39	52	70	91
TOTAL	271	469	576	906

Table 5.3

b) Results of Egg to adult experiment - MN series genotype frequencies

	MALE				FEMALE			
REPLICATE	BB	BD	DD	TOTAL	BB	BD	DD	TOTAL
2A	0	2	3	5	1	4	2	7
2B	1	4	3	8	0	10	4	14
2C	5	8	5	18	6	14	7	27
2D	8	8	5	21	5	14	8	27
2E	4	15	2	21	6	17	4	27
2F	3	24	2	29	3	13	3	19
2G	2	9	7	18	1	6	6	15
2H	9	16	2	27	6	9	6	21
TOTALS	32	86	29	147	28	87	40	155
4A	1	4	3	8	1	4	2	7
4B	6	16	2	24	4	14	6	24
4C	3	11	6	20	4	12	12	28
4D	5	3	10	18	3	17	9	29
4E	7	14	7	28	1	13	6	20
4F	5	11	6	22	4	10	3	17
4G	2	17	2	21	0	12	15	27
4H	5	10	7	22	8	15	3	26
TOTALS	34	86	43	163	25	97	56	178
6A	1	0	1	2	2	4	2	8
6B	2	9	9	20	5	6	13	24
6C	7	7	8	22	14	7	5	26
6D	3	16	4	23	1	16	8	25
6E	2	12	8	22	2	12	12	26
6F	4	14	5	23	3	16	3	22
6G	5	11	6	22	4	12	9	25
6H	12	7	5	24	8	11	5	24
TOTALS	36	76	46	158	39	84	57	180
8A	5	13	2	20	4	13	5	22
8B	5	16	8	29	5	9	5	19
8C	2	11	7	20	3	13	11	27
8D	6	11	4	21	5	16	4	25
8E	6	17	4	27	1	15	5	21
8F	2	14	6	22	2	16	8	26
8G	3	18	4	25	4	13	6	23
8H	0	10	8	18	5	17	7	29
TOTALS	29	110	43	182	29	112	51	192

(The TOTAL columns refer to the total numbers electrophoresed <u>not</u> the total number of flies emerging)

Table 5.4

c) Results of egg to adult experiment - SM series genotype frequencies

	MALE				FEMALE			
REPLICATE	BB	BD	DD	TOTAL	BB	BD	DD	TOTAL
2A	1	9	1	11	1	13	10	24
2B	4	10	4	18	4	8	4	16
2C	5	6	6	17	6	11	1	18
2D	0	12	7	19	0	8	3	11
2E	2	5	5	12	4	10	6	20
2F	1	19	4	24	1	19	7	27
2G	4	8	2	14	0	4	2	6
2H	9	17	5	31	2	2	3	7
TOTALS	26	86	34	146	18	75	36	129
4A	3	7	7	17	8	5	6	19
4B	13	10	1	24	7	16	1	24
4C	7	8	2	17	6	15	9	30
4D	7	7	4	18	5	20	5	30
4E	6	10	11	27	2	9	6	17
4F	1	8	12	21	2	17	8	27
4G	4	11	0	15	4	12	3	19
4H	1	24	5	30	3	9	6	18
TOTALS	42	85	42	169	37	103	44	184
6A	8	13	2	23	5	11	9	25
6B	3	16	6	25	3	14	5	22
6C	0	14	10	24	3	11	10	24
6D	3	13	2	18	4	9	1	14
6E	4	14	7	25	5	15	3	23
6F	4	18	2	24	3	13	8	24
6G	5	13	8	26	5	12	5	22
6H	9	10	5	24	3	18	3	24
TOTALS	36	111	42	189	31	103	44	178
8A	4	17	3	24	4	15	5	24
8B	5	14	7	26	2	13	7	22
8C	2	18	4	24	1	16	7	24
8D	1	11	6	18	7	18	5	30
8E	5	15	2	22	4	18	4	26
8F	1	9	8	18	6	16	8	30
8G	3	17	4	24	12	8	4	24
8H	10	13	3	26	6	12	4	22
TOTALS	31	114	37	182	42	116	44	202

(The TOTAL columns refer to the total numbers electrophoresed <u>not</u> the total number of flies emerging)

Table 5.5

Egg to adult experiment - heterogeneity within sets of replicates

a) MN Series

Density	Males (χ^2_{14})	Females (χ^2_{14})
2	30.0 **	11.9 N.S.
4	23.4 N.S.	24.3 *
6	25.9 *	40.6 ***
8	17.2 N.S.	10.2 N.S.

b) SM Series

Density	Males (χ^2_{14})	Females (χ^2_{14})
2	22.9 N.S.	22.2 N.S.
4	53.8 ***	21.4 N.S.
6	26.3 *	15.4 N.S.
8	24.9 *	22.5 N.S.

Probability levels:- * $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 5.6

Larva to adult experiment - genotype frequencies

	MALES				FEMALES			
Replicate	BB	BD	DD	Total	BB	BD	DD	Total
100 1	1	8	4	13	4	11	6	21
100 2	4	8	6	18	4	12	4	20
100 3	3	7	4	14	3	6	6	15
TOTAL	8	23	14	45	11	29	16	56
		χ_4^2 = 1.5 N.S.				χ_4^2 = 1.9 N.S.		
200 1	4	17	3	24	7	23	10	40
200 2	6	16	6	28	9	21	4	34
200 3	6	17	6	29	6	19	4	29
TOTAL	16	50	15	81	22	63	18	103
		χ_4^2 = 1.3 N.S.				χ_4^2 = 3.0 N.S.		
400 1	3	25	11	39	11	32	8	51
400 2	4	23	9	36	13	33	13	59
TOTAL	7	48	20	75	24	65	21	110
		χ_2^2 = 0.3 N.S.				χ_2^2 = 0.8 N.S.		
800 1	10	30	2	42	6	24	16	46
800 2	9	15	14	38	15	37	23	75
TOTAL	19	45	16	80	21	61	39	121
		χ_2^2 = 13.9 ***				χ_2^2 = 1.0 N.S.		

χ^2 values are for heterogeneity between replicates
Probability levels: N.S. $P > 0.05$ *** $P < 0.001$

(Totals are the total number of flies emerging - all flies were electrophoresed?

Table 5.7

Egg to adult experiment - comparison of observed values with expected values assuming random mating and no selective mortality.

a) MN Series

		MALES				FEMALES			
Density		BB	BD	DD	χ_2^2	BB	BD	DD	χ_2^2
2	O	32	86	29	12.76	28	87	40	4.59
	E	25.93	71.62	49.45	**	27.34	75.52	52.14	N.S.
4	O	34	86	43	4.06	25	97	56	2.77
	E	28.75	79.41	54.83	N.S.	31.40	86.72	59.88	N.S.
6	O	36	76	46	3.34	39	84	57	2.02
	E	27.87	76.98	53.15	N.S.	31.75	87.70	60.55	N.S.
8	O	29	110	43	10.85	29	112	51	7.20
	E	32.10	88.67	61.22	**	33.87	93.54	64.59	*

b) SM Series

		MALES				FEMALES			
Density		BB	BD	DD	χ_2^2	BB	BD	DD	χ_2^2
2	O	26	86	34	9.00	18	75	36	4.93
	E	24.54	70.63	50.82	*	21.68	62.41	44.90	N.S.
4	O	42	85	42	11.44	37	103	44	9.66
	E	28.41	81.76	58.83	**	30.93	89.02	64.05	**
6	O	36	111	42	13.35	31	103	44	8.55
	E	31.77	91.44	65.79	**	29.92	86.12	61.96	*
8	O	31	114	37	17.71	42	116	44	15.17
	E	30.59	88.05	61.96	***	33.96	97.73	70.32	***

N.S. $P > 0.05$

Probability levels: * $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 5.8

Larva to adult experiment - comparison of observed values with expected values assuming random mating and no selective mortality.

		MALES				FEMALES			
Density		BB	BD	DD	χ_2^2	BB	BD	DD	χ_2^2
100	O	8	23	14	0.32	11	29	16	0.12
	E	9.52	22.36	13.12	N.S.	11.85	27.82	16.33	N.S.
200	O	16	50	15	5.59	22	63	18	7.56
	E	17.14	40.24	23.62	N.S.	21.79	51.17	30.03	*
400	O	7	48	20	8.21	24	65	21	5.81
	E	15.87	37.26	21.87	*	23.28	54.65	32.08	N.S.
800 1	O	10	30	2	12.73	6	24	16	1.99
	E	8.89	20.87	12.25	**	9.73	22.85	13.41	N.S.
800 2	O	9	15	14	1.68	15	37	23	0.11
	E	8.04	18.88	11.08	N.S.	15.87	37.26	21.87	N.S.

Probability levels: N.S. $P > 0.05$

* $P < 0.05$

** $P < 0.01$

The most striking feature of these results, especially from the egg to adult experiment, is the consistent excess of BB and deficiency of DD flies both in males and in females when compared with the expectation. This was not anticipated on the basis of Collins' results where there were only slight differences in viability of the homozygotes both of which had much lower viabilities than the heterozygotes. In fact at low and medium densities DDs did somewhat better than BBs overall in his experiments. This discrepancy can be explained in one of two ways: either the viabilities in these experiments were very different from those in Collins' experiments or there was non-random mating and/or differences in fecundity or fertility between the genotypes in the parents.

Overall viabilities were much lower in my experiments and this may have been due to the change in the culture medium. This change in the food could have favoured the BB larvae rather than the DD larvae. However in the experiment mentioned above Collins did investigate the effect of varying the powdered milk content of the medium on the viability of flies of different karyotypes. Milk is an important source of protein and he was interested in the association between the inversion and the peptidase-1 locus. He found no differences in relative viabilities deppite a large change in overall viability.

Non-random mating, possibly with differences in fecundity or fertility between genotypes, seems a more likely explanation for the high frequencies of B alleles. The parental and overall offspring frequencies were:-

	Parental	Offspring
MN Series	0.42	0.46
SM Series	0.41	0.48
Larva to adult	0.46	0.48

This sort of change in gene frequency implies sexual selection in favour of BB of the order of 10-20%, or an equivalent advantage in fecundity or fertility, or some combination of these effects. Collins found no differences between genotypes in fecundity and fertility which means that some form of sexual selection is the most likely alternative. That sexual selection in favour of BB and BD males actually can occur in Coelopa frigida has now been demonstrated (Chapter 9).

The probable influence of non-random mating on the genotype frequencies in these experiments makes their interpretation difficult. However if viability differences between genotypes were as large as those observed by Collins it should be possible to detect them. In particular the effect of density should be clear because the eggs and larvae used in all replicates camd from the same matings and so any effect of sexual selection should be the same at all densities. One would expect to see an excess of heterozygotes at all densities but with a marked increase at the highest densities. The observed proportions of heterozygotes were:-

Density	MN Series	SM Series	Larva to adult	Collins
				0.53 (Low)
2, 100	0.57	0.59	0.51	0.68 (Medium)
4, 200	0.54	0.53	0.61	
6,	0.47	0.57	-	0.72 (High)
8, 400	0.59	0.60	0.61	
800	-	-	Rep1 0.61 Rep2 0.46	

While there does appear to be a consistent excess of heterozygotes (except in the MN 6 group), there is no indication of an increase with increasing larval density. It is possible that the excess of heterozygotes is a result of disassortative mating but it seems more likely to be a result of high viability relative to the homozygotes. The mortality in these experiments was uniformly higher than in Collins', and it increased with density. If anything one might have expected this to increase the viability differences between genotypes and yet the excess of heterozygotes never reaches even the level of Collins' Medium density replicates. It may be that the explanation of this difference is again to be found in the difference between the media. This would imply that the addition of a small amount of powdered milk could have a marked effect on the relative viabilities of the genotypes which was dependent on larval density. While this is possible, it does seem unlikely.

Once again this difference could be due to the isolation of the base populations. Different coadapted gene complexes within the α/β inversion system might have different competitive abilities. However the MN B + D and SM B + D lines give similar results despite the great distance between the populations on which these lines are based (Chapter 3).

It would be unwise to press the analysis of viability in these experiments too far in the absence of clear cut expectations but several points can be made: 1) the results suggest that there is sexual selection in favour of BB individuals, 2) there is a consistent excess of heterozygotes which is probably due to their superior viability, 3) the larva to adult and egg to adult techniques give similar results, 4) the MN B + D and SM B + D lines give

similar results and 5) there is no evidence for changes in relative viabilities with larval density.

The important question relating to the larval density effect is whether or not it is manifest in natural conditions. This question will be tackled later in this chapter.

(vi) Sizes and development times Collins noted that at higher larval densities flies emerged later and were smaller. The development times and sizes (wing lengths) of all adults emerging in the larva to adult experiment were, therefore recorded. Larvae were transferred into the experimental canisters for this experiment on the second day after the end of the 24 hr laying period. They then have a time from hatching of 1.5±0.5 days assuming that hatching occurs about one day after egg laying. Flies emerging on, say, the 10th day from transfer are, therefore, said to have a development time of 11.5 days. The full data for development times and sizes are given in Appendix 3 and are summarised in Table 5.9.

The relationships between density, development time and size are complex and so I will consider first the effect of density on development time and on size independently and then the interaction between development time and size.

At each density the association between genotype and development time was as predicted from previous work (Collins 1978, Day et al 1980). There was very little difference between females of different genotypes but in males BB flies emerged later than DD flies with BDs intermediate in all cases except density 200 where they were the last to emerge. In general the difference between BD and DD males was greater than between BD and BB males indicating a degree of dominance for late emergence (Table 5.9a and Figure 5.2).

Table 5.9

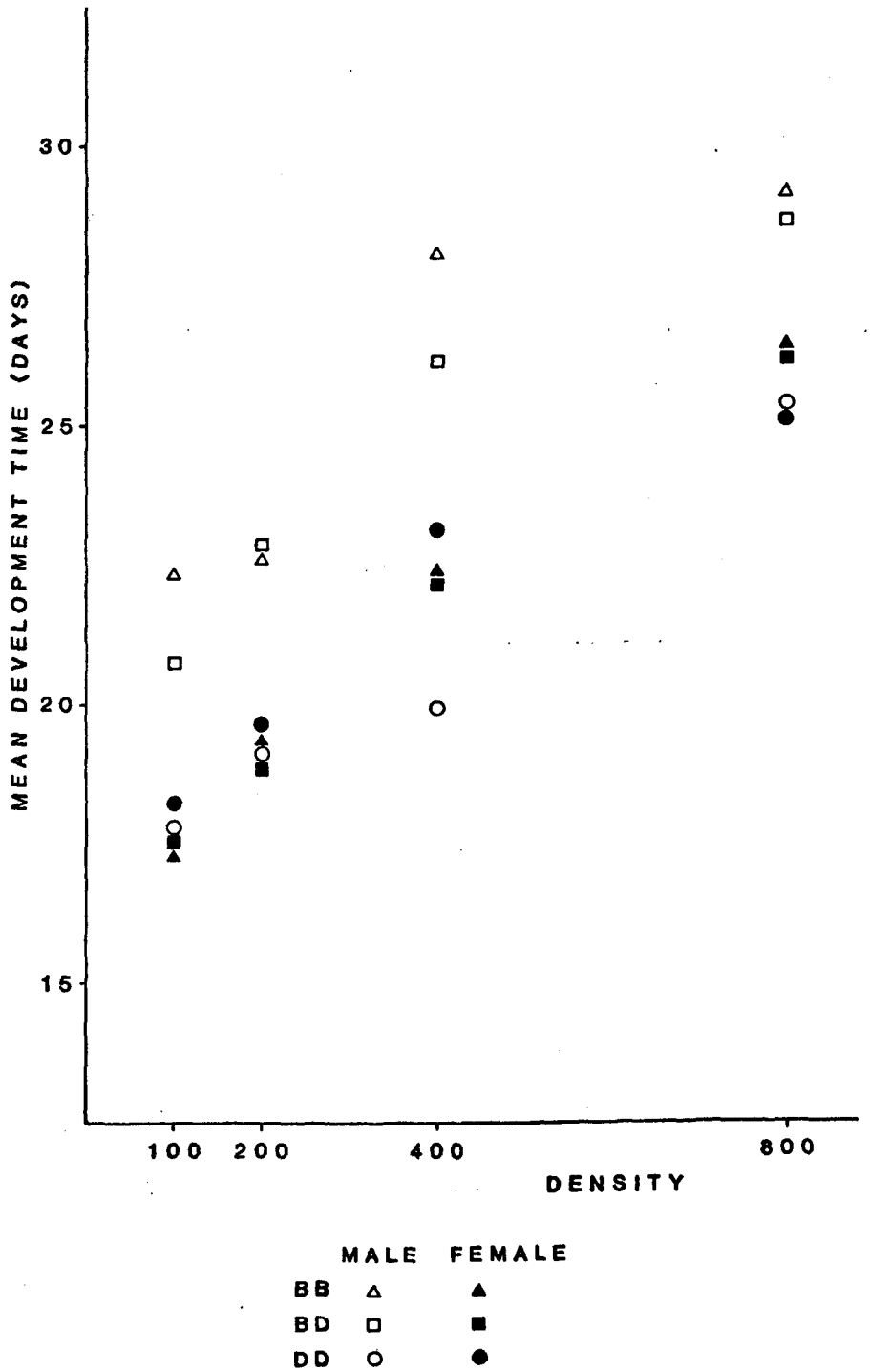
a) Larva to adult experiment - Mean development times (days)

	MALES			FEMALES		
Density	BB	BD	DD	BB	BD	DD
100	22.38	20.76	17.79	17.41	17.43	18.25
200	22.63	22.98	19.17	19.18	19.13	19.28
400	28.06	26.18	19.93	22.42	22.12	23.17
800	29.15	28.60	25.31	26.39	26.38	25.08

b) Larva to adult experiment - Mean wing lengths (graticule divisions)

	MALES			FEMALES		
Density	BB	BD	DD	BB	BD	DD
100	4.57	4.42	3.52	3.71	3.73	3.70
200	4.00	3.80	3.20	3.42	3.45	3.42
400	3.45	3.27	2.98	3.04	3.18	3.07
800	3.22	2.98	2.64	2.90	2.87	2.81

Fig.5.2 The effect of larval density on egg to adult development time.



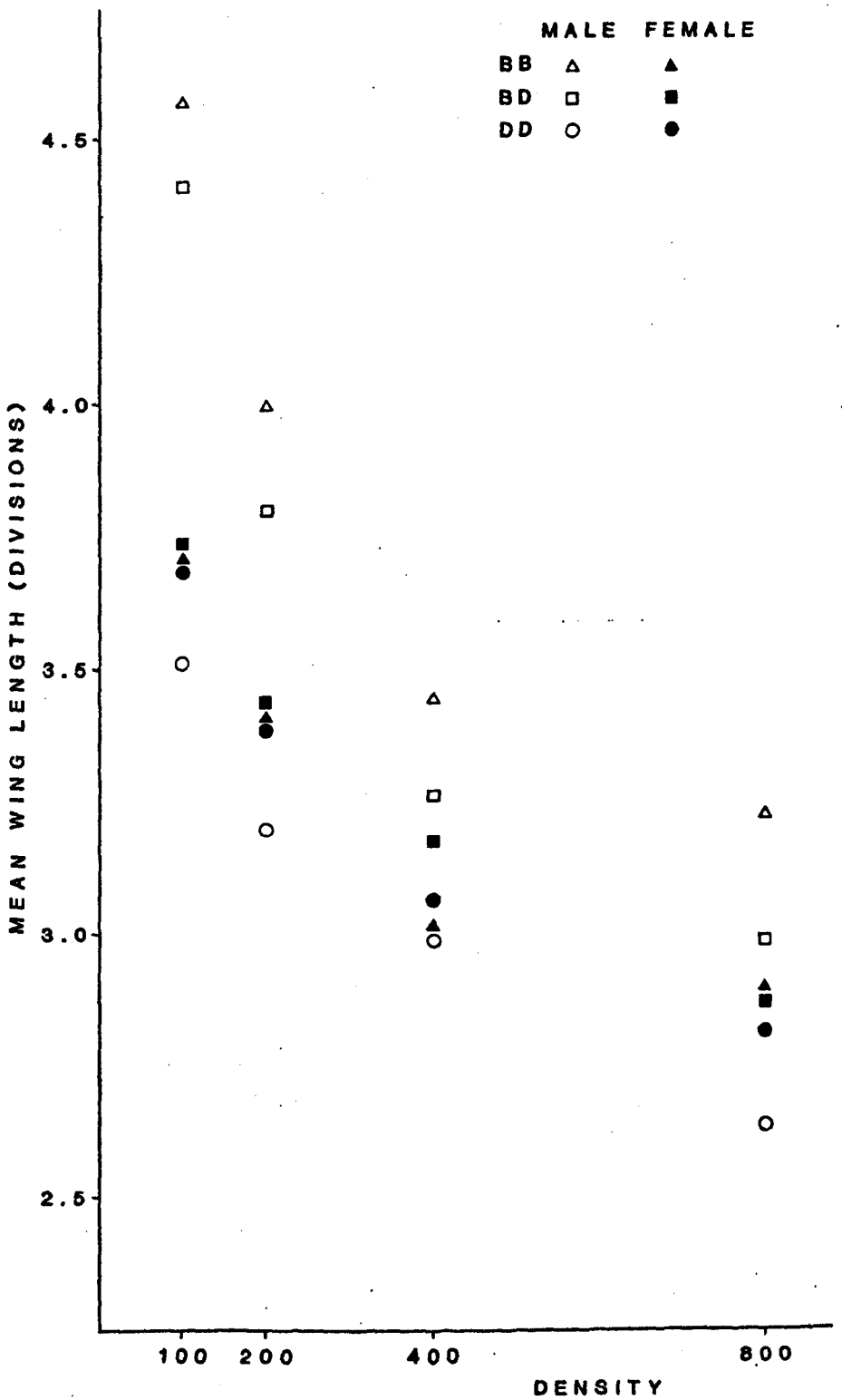
Considering all male flies, there was a highly significant increase in development time with density ($b = 10.5\pm0.80\times10^{-3}$, $P<0.001$) as there was for female flies ($b = 11.5\pm0.69\times10^{-3}$, $P<0.001$). Amongst males a significant improvement in the fit was obtained by fitting parallel lines for the three genotypes ($F_{2,282} = 52.33$, $P<0.001$). The estimated intercepts were: for BB 21.38 days, for BD 20.98 days and for DD 16.65 days. For females allowance for differences between genotypes did not improve the fit ($F_{2,389} = 0.13$) the estimated intercept for the three genotypes together was 17.06 days. There is a suggestion in Figure 5.2 that the increase in development time is tailing off by the time the density reaches 800 larvae per canister.

There were slight differences in development time between replicates ($F_{2,627} = 4.91$, $P<0.05$). This was probably due to variations in effective density due to chance mortality in the first few days after transfer.

The results for wing lengths are in many ways comparable with those for development time (Table 5.9(b) and Figure 5.3). Within any given density females of the three genotypes were very similar in size but amongst males the genotypes were clearly different in mean size. BB males were in all cases larger on average than BD males which in turn were larger than DD males. BD males were closer in size to BB than to DD males implying a degree of dominance for large size.

Adult size decreased markedly with density. For males $b = -1.47\pm0.12\times10^{-3}$ ($P<0.001$) and for females $b = -1.08\pm0.06\times10^{-3}$ ($P<0.001$). As for development time the fit is improved by allowing for genotypic differences in males ($F_{2,277} = 38.96$, $P<0.001$) but

Fig.5.3 The effect of larval density on adult size (1 division = 1.45 mm).



not in females ($F_{2,386} = 0.98$). The estimated intercepts were: for BB males 4.40 divisions, for BD males 4.14 divisions, for DD males 3.66 divisions and for all females 3.67 divisions.

The size differences between male genotypes were greatest at the lowest density and somewhat less at higher densities - especially at 400 larvae per canister. The decrease in size with increase in density appears to be reaching a minimum level in the 800 larvae per canister density but it appears that this minimum is different for the different male genotypes. Further density points would be necessary to confirm this.

There is clearly some sort of relationship between size and development time. The BB males take longest to reach adulthood and are the largest while DD males are small and develop rapidly. Within densities there is a clear relationship between mean size and mean development time (Figure 5.4). Fitting parallel regressions of size on development time for the four densities using the basic data accounts for 43% of the total variance with a slope of 0.001 (which is not significantly different from zero, $P>0.20$) and intercepts: 3.89 divisions at density 100, 3.55 divisions at density 200, 3.14 divisions at density 400 and 2.88 divisions at density 800.

The surprisingly non-significant slope in this analysis suggests a study of the size/development time relationship within genotypes and sexes for each individual replicates (replicates cannot be combined because they have significantly different mean development times - see above). The results are summarised in Table 5.10. Out of a total of 55 regressions where there were 3 or more flies available 46 were negative, that is size decreased with increasing

Fig.5.4 The relationship between adult size and egg to adult development time at each of the four larval densities.

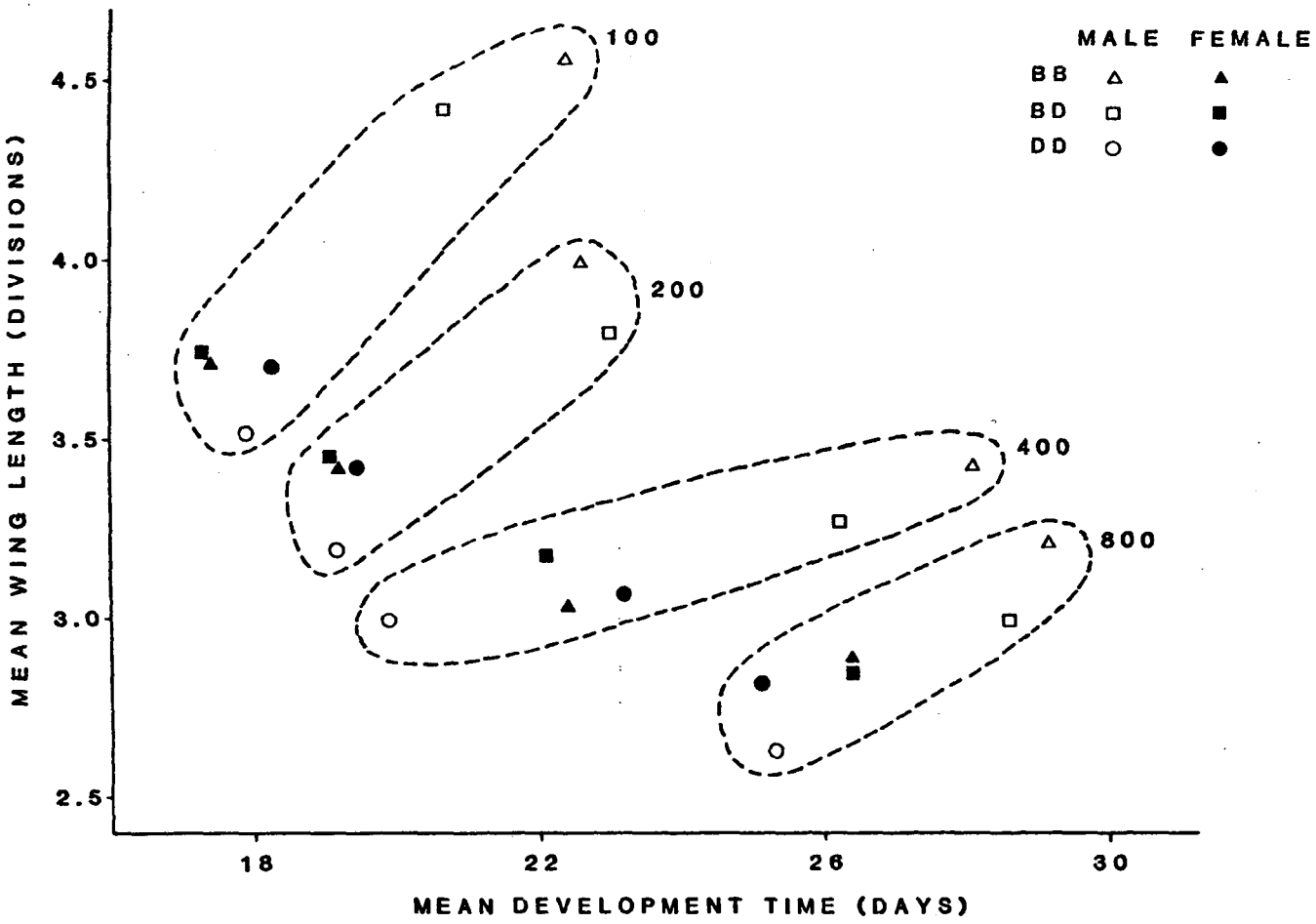


Table 5.10

The relationship between size and development time within replicates for the different Adh genotypes and sexes.

Density	Adh genotype	Males			Females		
		BB	BD	DD	BB	BD	DD
100		o+o	--+	---	--o	-*-	---
200		+--	-+-	o--	---	-** -** -***	---
400		-+	--	**-	-**	-*** -***	-+
800		--	+-	o+	--	-+	-

Notes:- Each symbol represents flies of that sex and genotype from one replicate. The symbols are:-

o less than three flies for which data are available

\+ regression of size on development time is positive

\- regression of size on development time is negative

* slope of regression is significantly different from zero with $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

development time, and only 9 were positive. The average slope was -0.044 divisions per day. 8 of the negative regressions were significant at the 5% probability level at least, while none of the positive regressions was statistically significant. The significant regressions were mainly amongst BD females - this class had the largest number of individuals in most cases.

The tendency for individual size to decrease with increasing development time is in contradiction to the trend of mean sizes which increase with development time (Figure 5.4). This means that size differences between genotypes are not simply a result of development time differences, or vice versa, but rather that the genotypes differ in both size and development time.

c) Discussion The results of these laboratory density experiments have raised a number of important questions. Firstly, although competition increases with increasing larval density as observed previously and there is a consistent excess of the BD heterogygotes at all densities, Collins'(1978) observation of an increase in heterozygote excess with density has not been repeated. This could be due to differences in experimental design but it could also be due to genetic differences between the strains of flies used. Two important questions need to be answered. Does heterozygote excess increase with larval density in natural conditions? Secondly, is the answer the same for all natural populations or is there evidence that isolated populations have different coadapted gene complexes associated with the α/β inversion? Studies on natural populations are reported later in this chapter and the question of coadaptation is addressed in Chapter 7.

Whether or not the heterozygote excess is affected by density it is a common feature not only of laboratory experiments but also of samples from natural populations (Chapter 3). What is the nature of the selection which produces this excess? Some type of superiority in competition is the most likely answer if there really is an effect of increasing density but these experiments give no indication of the nature of the competitive advantage. In the next chapter I will describe experiments which attempt to dissect the possible selective advantages of the heterozygotes.

Another possibility raised by these experiments is that non-random mating occurs in Coelopa producing an increase in Adh-B frequency amongst offspring when compared with parents. This is examined in detail in Chapters 8 and 9.

Finally the observations on development time and size must be considered. The association between Adh genotype and development time has been described before (Collins 1978, Day et al 1980) in laboratory experiments and I have shown (Chapter 4) that it is also found in natural conditions. It has important implications for the maintenance of the Adh and inversion polymorphisms (Chapters 4 and 10). The association between genotype and size has not been observed before despite the striking differences between males. If size is related to components of fitness such as longevity or reproductive success this association, if it occurs in the field, could have a major effect on gene frequencies in natural populations. As the mean sizes and mean development times of the genotypes are related, their effects on fitness might be expected to have interesting interactions. The sizes of adult flies in natural populations and the effects of size on fitness components are considered in Chapters 8 and 9.

As larval density increases adult flies emerge later and are smaller. Such a decrease in size is a common observation when competition increases in insect populations and is often accompanied by an increase in development time (Miller 1964, Sokoloff 1955, David et al 1970, Haupt & Busvine 1968). It is a feature of scramble competition - as density increases nutrients are shared amongst more individuals and therefore each individual is smaller when the nutrients are used up or when pupation occurs. The reduction in size continues until a minimum is reached and then viability falls rapidly (see Figure 5.1). My results suggest that the minimum size is being approached at a density of 800 larvae per canister but that it might differ between the genotypes and sexes. However viability was reduced at much lower densities and so the situation must have been more complex than that depicted in Fig.5.1.

Bakker (1961) found that development time decreased with increasing density in Drosophila melanogaster in conditions where competition was of the exploitative type. However an increase in development time with density is a common observation (see for example McFarquhar & Robertson 1963 for D. subobscura or Moeur & Istock 1980 for the pitcher-plant mosquito). It may represent an element of interference competition which reduces feeding and growth rates or a limiting rate of food supply rather than an absolute limit on the amount of food available.

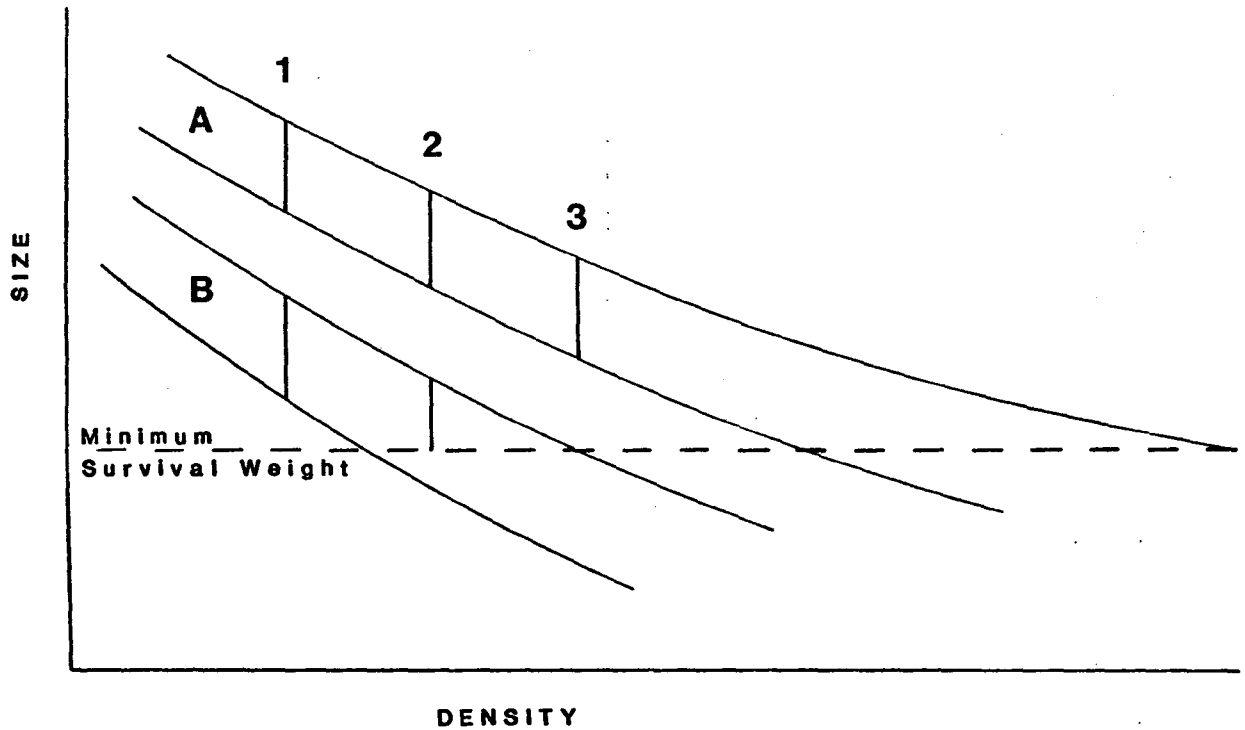
The adult size and development time of non-diapausing insects are controlled by two factors - the growth rate during the larval stages and the timing of pupation. Pupation itself is controlled by hormones - ecdysone and juvenile hormone - levels of which are ultimately controlled by the brain in response to sensory inputs,

such as distension of the abdomen after a blood meal in Rhodnius (Wigglesworth 1954). In Drosophila melanogaster larvae become committed to pupation shortly after entering the third instar (Bakker 1961) and if they fail to reach the minimum survival weight before the onset of pupation or before the exhaustion of the food supply they die. This is the basis of Bakker's and Bentvelzen's (1963) explanation for the outcome of competition in Drosophila. At low density fast growing larvae reach a larger size at pupation than slow growing larvae. As density increases some of the slower growing larvae fail to reach the minimum survival weight and so the proportion of the faster growing type increases amongst the survivors. This is shown diagrammatically in Figure 5.5. As nutrients are exhausted earlier at higher densities development time decreases with increasing density. The instantaneous rate of growth is proposed to be a function of size and so a larva that achieves an early start or gets a small advantage early on will have a large size advantage at pupation.

This theory cannot account for some of the observations in Coelopa. It does not allow for an increase in development time with density or for a negative relationship between size and development time within densities. In addition it is necessary to account for the related differences in size and in development time between genotypes and if possible to relate these differences to the relative viabilities of the genotypes.

I propose two modifications to the theory. First that the onset of pupation in Coelopa is controlled by a combination of body size and time and second that increasing larval competition decreases the rate of growth either because the rate of nutrient

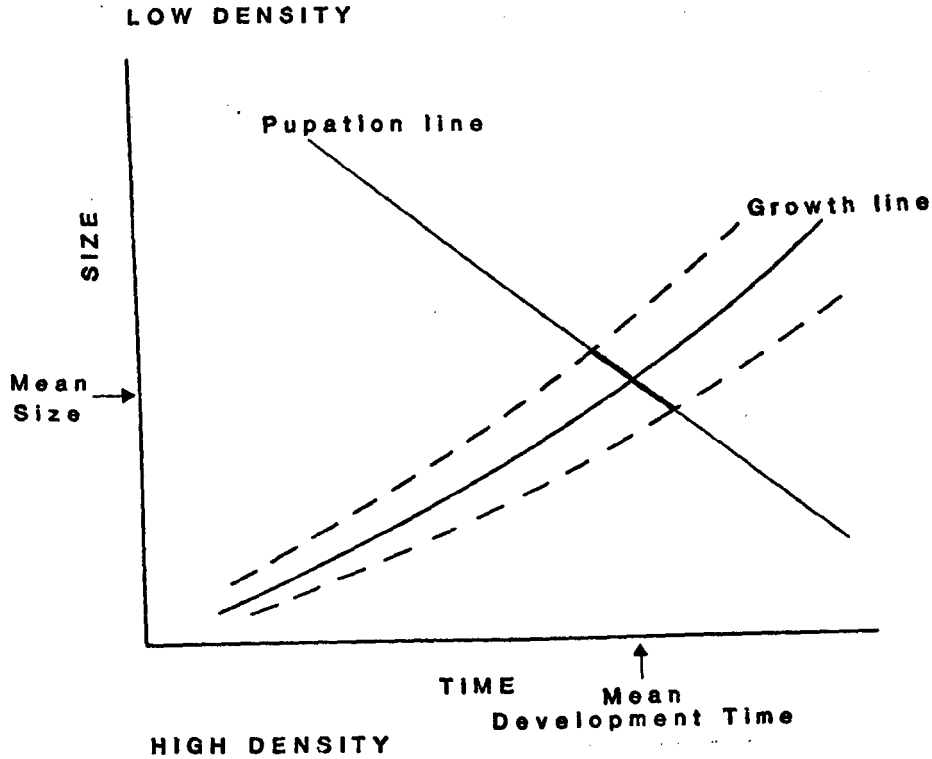
Fig.5.5 The effect of larval density on size at pupation and thus on viability (after Bakker 1961). Group A flies grow faster than group B flies and thus achieve a larger size. At density 1 all flies survive. At density 2 some of the B flies fail to reach the minimum survival weight and so the proportion of A survivors is increased. At density 3 all survivors are of group A.


1
A
2
3
B
Minimum
Survival Weight
SIZE
DENSITY

supply is limited or because there is interference between larvae. These modifications are illustrated in Figure 5.6. It can be seen that at any one density adult size decreases with increasing development time and that an increase in density both decreases size and increases development time.

Neither of the assumptions is substantiated but both appear reasonable and potentially testable by experiment. Coelopa larvae cannot feed directly on seaweed but require the action of micro-organisms to release nutrients from the seaweed. It is quite possible that the rate at which nutrients are released might limit the growth rate of larvae and that this limitation might be more severe at higher larval densities. Alternatively physical interactions between larvae in dense populations might reduce the time they have available for feeding and thus slow down their rate of growth. From the point of view of the control of pupation the proposed relationship would allow larvae which achieved a large size rapidly to pupate immediately while those that had not reached such a size could continue feeding for longer. One can envisage a possible mechanism whereby pupation occurred when some hormone dropped below a critical concentration. The concentration would fall with increased body size but would also fall with time if the hormone was unstable and was not produced after an initial pool had been established. This is, of course, purely hypothetical but it serves to demonstrate that the proposed relationship is physiologically possible.

The association of both size and development time with genotype and sex can be explained by an effect on the position of the "pupation line" of Figure 5.6. If this is higher then, with the


LOW DENSITY
Pupation line
Growth line
SIZE
Mean
Size
TIME
Mean
Development Time

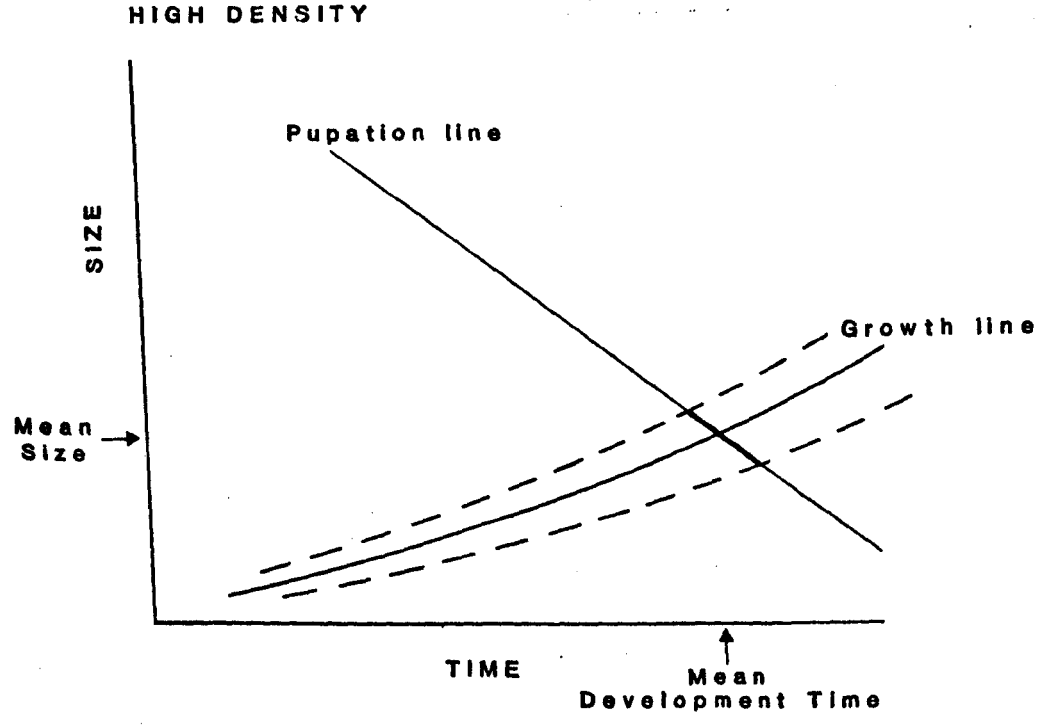

HIGH DENSITY
Pupation line
Growth line
SIZE
Mean
Size
TIME
Mean
Development Time

Fig.5.6 Proposed explanation for the effect of density on size and development time in *Coelopa frigida*. The "Growth Line" shows the growth of an average individual with time, dashed lines are the upper and lower limits of the distribution of growth rates. The "Pupation Line" is the set of points in size and time at which pupation is initiated. The thickened region of the pupation line gives the expected distribution of sizes and development times of pupae and thus of adults.

same growth rate, the development time will be longer and the size larger as is observed with BB or BD males compared with DD males (Figure 5.7). It would not be possible to account for an increase in both development time and size by assuming differences in growth rate between genotypes.

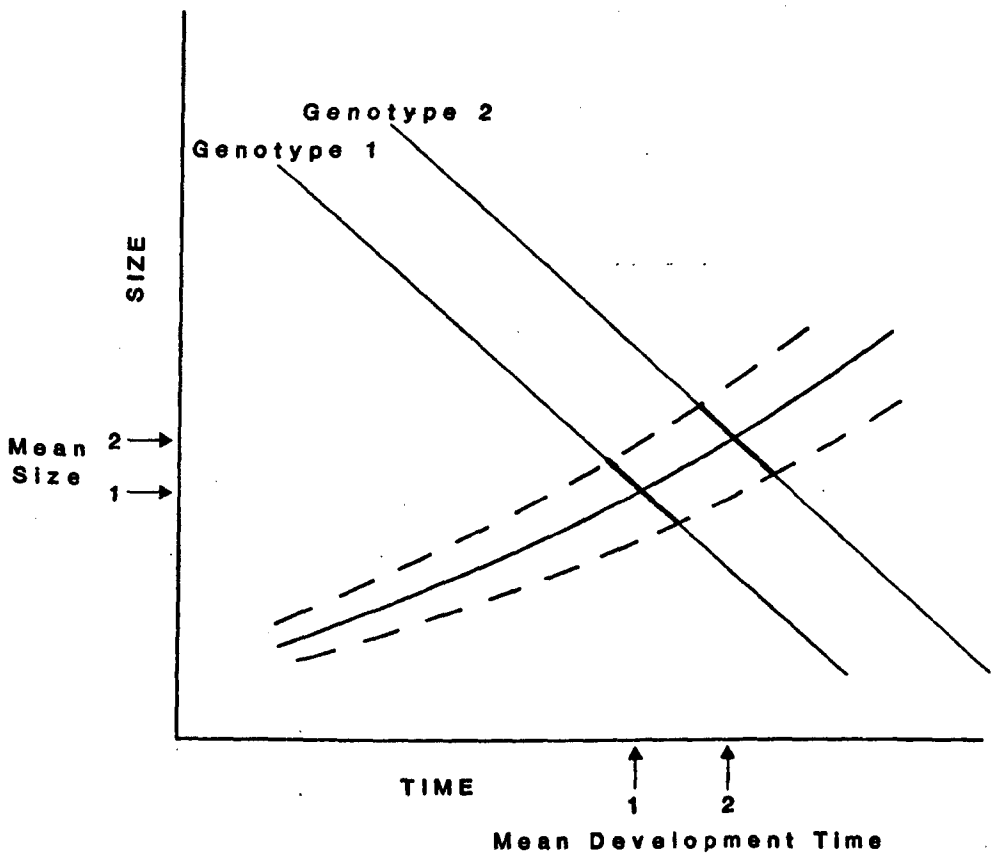
This model adequately explains the observed relationships between genotype, size, development time and density but so far I have not considered its predictions regarding the relative viabilities of the genotypes. This aspect can easily be accommodated by supposing either a minimum survival weight or a point in time at which all nutrients were exhausted, or both.

If one assumes that there is a single minimum survival weight applicable to all genotypes then it is clear that the genotype with the lowest "pupation line" would have the lowest viability at high density. Conversely if one assumes a single point in time at which all nutrients are used up then the genotype with the highest "pupation line" would suffer the highest mortality. If these two factors operated together then an intermediate genotype (the BD genotype amongst males) would have the highest viability and its advantage would increase with density.

The same result can be achieved by allowing different minimum survival weights for the different genotypes, and for this there is suggestive evidence in the data. Indeed if one allows this kind of variation almost any set of viability relationships can be accommodated.

The main difficulty with explaining viability in this way is that the females differ very little in size and development time between genotypes and yet the viability relationships are similar to those in males, if less marked. This difficulty could potentially

Fig.5.7 Extension of the explanation presented in Fig.5.6 to account for observed differences between genotypes.



be overcome by appealing to Bentvelzen's (1963) hypothesis that a strain with a higher variance in growth rates would have an advantage in severe competition over a strain with the same mean growth rate but a lower variance although it might have a disadvantage at low densities. My data do not give a clear indication of greater variability in the heterozygotes - in five out of the ten replicates the variance of development time in heterozygous females was greater than in either homozygote.

This model suggests a number of possible lines for future research. In particular direct observations on growth rates would be informative as would be a determination of the minimum survival weights of the different genotypes. The nutritional requirements of Coelopa are already under study at Nottingham and this work should provide a better understanding of the way in which limitation of resource supply affects larvae. Another important aspect of the model is the way in which the onset of pupation is controlled. An approach to this problem might be made by starving larvae of the same age but different sizes, or vice versa, to determine how rapidly they pupated. One would expect larger (or older) larvae to pupate sooner.

5.6 Viability on Laminaria

The two density experiments reported above have confirmed Collins' (1978) observation that the Adh-BD heterozygotes have a higher egg to adult viability than either of the homozygotes. However they have failed to repeat his observation that this advantage increases with increasing larval density. I have suggested that this discrepancy between the sets of results may be due to the

Table 5.11

Results of viability experiment on *Laminaria* stipe

Density	Replicate	Total adults (average % survival)	BB	BD	DD	Heterogeneity χ^2
25	1	19	4	11	4	
	2	22	1	17	4	
	3	20	6	9	5	
	T	61 (81%)	11	37	13	6.0
50	1	27	7	11	5	
	2	37	6	11	7	
	3	29	4	13	7	
	4	41	3	14	7	
	T	134 (67%)	20	49	26	3.0
100	1	71	6	14	4	
	2	88	3	17	4	
	3	80	5	14	5	
	4	69	8	12	4	
	T	308 (77%)	22	57	17	3.4
200	1	103	4	12	8	
	2	148	3	16	5	
	3	129	2	17	5	
	4	146	4	16	4	
	T	526 (66%)	13	61	22	3.4
300	1	174	28	111	19	
	2	166	30	93	39	
	T	340 (57%)	58	204	58	8.5 ($P<0.05$)

Table 5.12

Viability experiment on Laminaria stipe - comparisons with 1:2:1 expectation

Density		BB	BD	DD	χ_2^2	Percentage of BD
25	O	11	37	13		60.7
	E	15.25	30.5	15.25	2.90	
50	O	20	49	26		51.6
	E	23.75	47.5	23.75	0.85	
100	O	22	57	17		58.4
	E	24	48	24	3.90	
200	O	13	61	22		64.2
	E	24	48	24	8.73 *	
300	O	58	204	58		63.8
	E	80	160	80	24.20 ***	

* - $P < 0.05$

*** - $P < 0.001$

different culture media. This possibility makes it all the more important to ascertain whether there is density dependent heterozygote advantage in natural conditions. Some attempts to obtain evidence for such an effect in the field are described in the next section. In this section I will describe experiments conducted in the laboratory but using the seaweed Laminaria as food source rather than artificial culture media.

a) Method Stipes of the two species of brown seaweed Laminaria digitata and L. hyperborea were collected at low water of spring tides from St.Mary's Island, Tyne and Wear. The stipes were split longitudinally and cut into lengths of about 5 cm then stored at $-20^{o}C$ until required.

Two day old larvae were transferred from a stock tank on standard culture medium as in the larva to adult experiment. In this case they resulted from a mass mating of BD flies generated from the BB(lab) and DD(lab) stock lines. The following replicates were established: 4 at each of 25, 50, 100 and 200 larvae per canister and 2 at 300 larvae per canister. Small canisters were used each containing 100±5 g of Laminaria stipe.

All adult flies emerging were collected and a sample from each replicate was electrophoresed.

b) Results The results are presented in Table 5.11. Males and females were not separated. The starch gel containing all the adults from replicate 25(4) was unscorable due to a failure in the cooling system during electrophoresis.

Overall viabilities were higher than in the experiments on laboratory media but showed the expected decline with increasing density.

The observed numbers are compared with the Mendelian expectations

in Table 5.12. Only at the two highest densities were there significant deviations from expectation. At each density there was an excess of heterozygotes and this increased as density increased over the range 50 to 200 larvae per canister. The 25 larvae per canister replicates produced a surprisingly high proportion of heterozygotes despite their high overall survival, indeed replicate 25(2) produced 17 adult BD flies even though it started with only 25 larvae expected to be in the ratio 1 BB : 2 BD : 1 DD. By contrast at a density of 50 larvae per canister the results were very close to expectation while the mortality was much higher.

The numbers used in this experiment were small and the flies used to generate the test animals were from inbred laboratory stocks. The results must, therefore, be interpreted with care. However, one advantage of this experiment is that there can be no contribution of sexual selection with respect to the Adh locus. What the results do seem to indicate is that the density dependent heterozygote advantage in viability observed by Collins can still be detected when the food source for most of the larval lifespan is Laminaria stipe. Natural seaweed beds consist of Laminaria stipe and frond with varying amounts of Fucus and other seaweeds. The conditions in these laboratory canisters are still very different from natural conditions but are a significant step closer than in previous experiments. The results, therefore, suggest that an attempt to detect density effects in the field may be worthwhile.

5.7 Larval Density in Natural Populations

Genotype frequencies in almost all samples from natural populations differ from Hardy-Weinberg expectations (Chapter 3).

The BD genotype is in all cases in excess of expectation and the BB and DD genotypes are below expectation. Collins (1978) and Butlin et al (1982) have suggested that this is a result of the heterozygote advantage in egg to adult viability which was demonstrated first by Collins and which has been confirmed in my laboratory experiments. Furthermore it has been suggested that the variation in heterozygote excess observed in natural populations reflects differences in larval density. However there have been no attempts to demonstrate either differences in viability between genotypes or the proposed effect of density in the field.

Direct estimates of absolute viabilities in natural conditions would seem to be impractical because of the difficulty of estimating starting numbers. Estimates of relative viability could be obtained if the genotype frequencies amongst eggs could be ascertained. Sampling adult flies present at the time of egg laying would give an estimate of gene frequencies in the eggs but this is a difficult thing to do. The adults rarely take to the wing and so cannot be captured in reasonable numbers in a kite net. However they are active at this time because the wrack bed is warm and they are, therefore, difficult to catch in an aspirator. No satisfactory trap has been developed. Even if the parental generation genotype frequencies were known there would still be the problem of differential contribution to the eggs as in the egg to adult experiment above (section 5.5).

Another approach would be to sample larvae at different stages during the development of a single generation of larvae in a natural wrack bed. If there is differential mortality during the larval period this should be detectable by comparing the genotype frequencies in early and late samples. This is the approach which I have adopted.

To ascertain whether viability differences in natural populations are density dependent it was necessary to find a method of measuring larval density in the field which would be comparable between samples.

a) Methods To estimate larval density I have used the technique described by Burnet (1960c, 1961) using a soil corer. The corer used was a steel tube with an internal diameter of 4.75 cm and sharpened at one end. This was pushed right through the wrack bed by means of a cross bar and into the sand or single below in order to give a clean end to the core of seaweed. The core was then pushed out with a plunger into a round canister for return to the laboratory. The length of core was not standardised because it was not possible to withdraw a core of seaweed unless the corer had cut right through the bed. However most wrack beds in this country are fairly shallow - in the range 25-50 cm deep - and in deep beds the larvae occupy a limited horizon of approximately the same depth. As the corer was pushed through the weed the core itself was quite tightly compacted and this may have killed some larvae and pupae.

The coring technique also provides information on the dispersal of larvae within wrack beds. This needs to be taken into account in assessing overall density.

(i) The experimental wrack bed at St.Mary's Island Four sets of core samples, 10 cores each, were taken from the experimental wrack bed at St.Mary's Island whose history was described in Chapter 4. The sampels were taken on 6, 10, 14 and 17 April, that is 6, 10, 14 and 17 days after the appearance of the first egg batches in the wrack bed. As mentioned previously a collection of parental flies was made but these failed to give scorable electrophoretograms, probably because of their age.

The cores were taken as described above. On return to the laboratory the numbers of larvae and pupae in each core were counted as soon as possible. The larvae were divided into "large" and "small". There were two reasons for this; first, because the "small" larvae were not large enough for electrophoresis and secondly because they represented a later stage in the sequence of egg laying and might, therefore, experience different conditions during growth. The small larvae of one collection might be compared with the large larvae of later collections which were likely to be of the same cohort. The criterion for distinguishing the groups was that "large" meant ready for immediate electrophoresis - in practice larvae over about 6 mm long.

Large larvae and pupae were stored at -20°C until required. Small larvae were transferred to round canisters containing culture medium, one canister for each core, and kept at 27°C. All flies emerging were collected daily and stored at -20°C.

Clearly some of the larvae collected were not Coelopa frigida. These were detected either as adults emerging in the round canisters or on electrophoresis of the large larvae. The ADH isozyme patterns of the other common dipteran species are clearly distinguishable from Coelopa frigida.

(ii) Natural wrack beds During 1981 six sets of 10 cores each were taken from natural wrack beds from which mass samples were also taken. The sites and dates of collection were:-

18 March	Whitburn and St.Mary's Island
14 May	Whitburn and St.Mary's Island (South Bay)
27 August	St.Mary's Island
4 November	St.Mary's Island

The results of the mass samples were presented in Chapter 3. The cores were taken in the same way as from the experimental bed but were treated differently on return to the laboratory. Each core was placed in a small canister with 3 cm depth of culture medium and kept at 27°C. This was intended to reduce the larval density and thus the mortality. All flies emerging were removed daily and counted before being discarded. The cores were retained until no further adults emerged. This was a much less tedious technique than sorting the larvae from the cores and was intended to give an estimate of larval density in the wrack bed as a whole which could be correlated with the genotype frequencies in the corresponding mass sample. No attempt was made to correlate the density within an individual core with the genotypes of larvae from that core as in the samples from the experimental bed.

I had hoped to obtain more than six sets of cores during 1981 but the soil corer turned out to be unsuitable for use on some wrack beds. Where the seaweed was deposited on or between large pebbles or boulders it was often possible to obtain a good mass sample but quite impossible to use the corer effectively. Some wrack beds contain large unbroken Laminaria stripes; these also make use of the corer difficult. In other cases larvae were collected from thin wrack flake from which cores could not be taken. The result is a rather small set of samples from a restricted range of types of wrack bed.

b) Results The full results of the cores from the experimental wrack bed are given in Appendix 4 and are summarised in Table 5.13.

The results of the 1981 core samples are given in Table 5.14.

(i) Aggregation of larvae Seaweed fly larvae are not distributed

at random within wrack beds but are aggregated as a result of egg laying in batches and the tendency of females to lay egg batches in "hot spots" (Burnet 1961). This aggregation can be seen by examining the index of dispersion (the ratio of the variance to the mean) of the number of individuals per core. If the distribution is random the numbers are expected to follow a Poisson series and the ratio ($s^2/\bar{x}$) will equal 1. The difference between the observed index and the expectation is significant if it exceeds $2\sqrt{(2n/(n-1)^2)}$ where n is the number of cores in the series (Burnet 1961).

Table 5.15 shows the mean, standard error and index of dispersion for each set of cores taken from the experimental wrack bed and for the 1981 sets. These statistics are quoted in each case for Coelopa frigida larvae alone and for all dipteran larvae. In the 1981 cores the only other common species was C. pilipes but in cores from the experimental bed the most common additional species was Thoracochaeta zosterae amongst the small larvae. A few Coelopa pilipes and Heterochila buccata were also found.

The large and small larvae are combined in the results from the experimental bed - the proportions in these separate groups will be discussed later. The "all larvae" figures were obtained simply by adding the numbers of small larvae, large larvae and pupae removed from each core. The C. frigida figures were obtained by the following equation:

$$\text{Number of large larvae and pupae - number of non-C.frigida larvae \& pupae} + \left| \frac{\text{Number of small larvae x Number of C.frigida adults emerging}}{\text{Total number of flies emerging}} \right|$$

The figures are, therefore, comparable in that they both relate to the number of individuals present at the time when the cores were taken. The estimate of the number of C. frigida individuals at that

time assumes that the other dipteran species survive equally well on laboratory culture medium. This is probably not true as the medium was designed specifically for C. frigida and the estimates of the numbers of C. frigida are probably overestimates. The extent of this error is difficult to assess but I think it is probably small. All the species involved can be reared on the standard laboratory medium as sole food source. Heterochila buccata does not do at all well but Coelopa pilipes and Thoracochaeta zosterae are reasonably successful.

The different technique used to deal with the 1981 cores means that in this case the figures quoted are the numbers of adult flies which emerged in the laboratory. There must certainly have been some mortality between collection and emergence. This was minimised by placing the cores in canisters with extra medium but this procedure again raises the problem of differential viability of the species. As C. pilipes was the only other common species in these cores this is probably only a small error.

The mean number of larvae per core was smaller in all 1981 samples than in the experimental bed reflecting the difference in technique. My subjective assessment in the field was that the density in the experimental bed was similar to the density in the May and August 1981 wrack beds at St.Mary's Island.

The index of dispersion is in all cases much greater than 1 indicating a highly clumped distribution of larvae. The results are very similar to those of Burnet (1961) whose values for the index ranged from 20 to 100 compared with mine which range from 6 to 175.

It is interesting to note that where there were non-C.frigida larvae the indices of dispersion tended to be lower. The main exceptions to this trend were the two sets of samples from Whitburn

in which the numbers of C. frigida were unusually low. A lower index indicates a more evenly spread distribution and suggests that the other species were not so clumped as Coelopa frigida (or that they were clumped in different places). Burnet (1961) did not find significant clumping of C. pilipes in his November samples or in his June samples where the core temperature was over 22°C but he did find clumping in samples taken in June with a temperature below 22°C. C. frigida larvae showed significant clumping in all of these sets of samples. The two Whitburn samples in which there is evidence for clumping of C. pilipes larvae were taken in March and May and had mean temperatures of 22-23°C.

On the whole it seems likely that other species do not show the highly clumped distribution of Coelopa frigida which is at least in part due to the fact that it lays its eggs in batches. The other common species lay their eggs singly, including C. pilipes. However it seems likely that all species show some tendency to lay eggs in especially favourable parts of the wrack beds where conditions of temperature, humidity, etc. are most suitable. A completely random distribution of larvae will therefore be unlikely.

The non-random distribution of larvae between cores suggests that special methods should be employed in further analysis of the data. The results have therefore been transformed by the method employed by Burnet (1961) which is appropriate to the negative binomial distribution.

The transformation is:-

$$y = \frac{1}{\beta} \sinh^{-1} \beta \sqrt{(x + 3/8)}$$

where β is the slope of the standard deviation on the mean and x is the number of larvae per core. β was estimated from the data in Table 5.15 to be 0.55.

Table 5.13

Genotype frequencies for the five common genotypes in core samples (expressed as percentages)

Date	Stage*	BB	BC	BD	CD	DD	Sample size
6/4	P	12.5	0	50.0	25.0	12.5	8
	L	14.6	6.2	38.0	12.6	24.4	698
	M	13.1	8.0	35.9	12.2	27.8	206
	F	13.6	6.8	39.4	8.9	28.4	211
	TOTAL	14.1	6.6	37.9	12.0	25.6	1123
10/4	P	9.4	7.3	22.9	16.7	41.7	96
	L	10.8	6.6	42.6	10.6	26.0	1111
	M	10.1	8.8	41.0	11.3	25.6	407
	F	11.1	6.2	39.4	12.6	24.9	325
	TOTAL	10.6	7.0	40.7	11.4	26.5	1939
14/4	P	7.2	7.6	32.6	11.2	36.2	304
	L	16.1	9.3	37.5	13.2	19.8	485
	M	8.6	6.8	46.2	9.5	26.5	325
	F	6.2	5.8	48.1	10.4	26.5	260
	TOTAL	10.5	7.6	40.5	11.4	26.3	1374
17/4	P	11.8	7.1	32.9	12.5	33.2	280
	L	17.9	8.8	41.5	11.2	16.5	491
	M	9.5	4.0	37.3	11.1	33.3	252
	F	10.0	6.8	39.8	11.2	27.3	249
	TOTAL	13.4	7.1	38.4	11.5	25.6	1272
TOTAL	P	9.4	7.2	31.5	12.7	35.2	688
	L	13.9	7.3	40.3	11.7	22.9	2785
	M	10.2	7.1	40.6	11.0	27.8	1190
	F	10.2	6.4	41.6	10.9	26.6	1045
	TOTAL	11.9	7.1	39.6	11.5	26.1	5708
Hardy-Weinberg Expectation†		12.7	7.8	37.1	11.4	27.2	

* The stages are P - pupae, L - large larvae, M - males from small larvae, F - females from small larvae

† Expectations based on the overall gene frequencies in all core samples

Table 5.14

Results of 1981 core samples - total adults emerging per core

CORE	SITE	WHITBURN	ST.MARY'S	WHITBURN	ST.MARY'S	ST.MARY'S	ST.MARY'S
	DATE	18.3.81	18.3.81	14.5.81	14.5.81	27.8.81	4.11.81
1	F	1	19	0	124	144	17
	P	2	0	6	2	14	0
2	F	12	73	4	33	69	114
	P	0	0	37	0	69	2
3	F	19	15	1	12	132	35
	P	2	0	1	0	3	0
4	F	8	17	15	1	43	28
	P	1	0	11	0	0	0
5	F	2	45	1	15	150	52
	P	0	0	28	0	1	0
6	F	2	57	7	243	166	77
	P	0	1	33	0	14	1
7	F	9	9	0	7	62	49
	P	15	0	5	0	0	0
8	F	0	10	1	109	19	109
	P	0	0	0	1	2	0
9	F	2	47	3	100	0	7
	P	1	1	92	1	23	0
10	F	6	9	1	432	0	21
	P	7	0	13	0	0	0
TOTAL	F	61	301	33	1076	785	509
	P	28	2	226	5	126	3
BD excess		1.12	1.12	1.15	1.23	1.27	1.18

F = Coelopa frigida P = Coelopa pilipes

BD excess = $\frac{\text{Observed number of BD flies}}{\text{Hardy-Weinberg expectation}}$

Table 5.15

Index of dispersion of larvae in core samples

Series		$\bar{x}$	s	$s^2/\bar{x}$
Experimental bed:				
6/4	F	159.3	95.1	56.79
	T	190.5	91.3	43.77
10/4	F	295.9	172.4	100.43
	T	325.9	158.7	77.27
14/4	F	204.4	160.1	125.44
	T	231.8	152.0	99.65
17/4	F	190.2	130.6	89.68
	T	224.2	128.8	73.99
1981:				
St.Mary's 18.3	F	30.1	23.3	18.05
	T	30.3	23.5	18.25
Whitburn 18.3	F	6.1	6.0	5.95
	T	8.9	8.5	8.05
Whitburn 14.5	F	3.3	4.6	6.55
	T	25.9	28.6	31.65
St.Mary's 14.5	F	107.6	136.9	174.16
	T	108.0	136.9	173.58
St.Mary's 27.8	F	78.5	64.5	52.95
	T	91.1	67.6	50.13
St.Mary's 4.11	F	50.9	37.7	27.92
	T	51.2	38.2	28.43

F = *Coelopa frigida* larvae only T = All dipteran larvae

In all cases the number of cores was 10 and $2\sqrt{(2n/(n-1)^2)} = 0.99$

(ii) The experimental wrack bed The results from the four sets of ten cores taken from the experimental wrack bed at St.Mary's Island in April 1980 will be considered in three sections: (a) the numbers of individuals present and the proportions of small larvae, large larvae and pupae; (b) variations in gene and genotype frequencies between developmental stages and between dates; and (c) variations in genotype frequencies with larval density.

The average number of Coelopa frigida individuals per core on 6 April was 160. This rose to 295 on 10 April and then fell again to 202 and 195 on 14 and 17 April respectively. Out of these totals the proportion of large larvae and pupae was 46.6% in the first set of samples falling to 42.4% and 40.1% in the second and third sets of samples and then rising to 44.4% on 17 April. Pupae were very rare at first but gradually increased to account for 17.4% of all individuals in the last set of cores (Figure 5.8).

These results fit in with the observations made in Chapter 4 on the experimental bed. Egg laying must have continued from its start on 1 April throughout most of the sampling period. It probbaly reached a peak between 6 and 10 April giving the peak of overall density on 10 April but recruitment to small larvae exceeded growth through to large larvae until the 14 April sample where a minimum proportion of large larvae was found. Initially growth must have been rapid to account for the numbers of large larvae present in the first sample but it probably slowed down as the wrack bed aged, cooled down and became more densely populated. The numbers of pupae are rather lower than might have been expected. This is probably because larvae tend to migrate to the drier outer areas of the wrack bed to pupate and therefore might escape sampling. As the

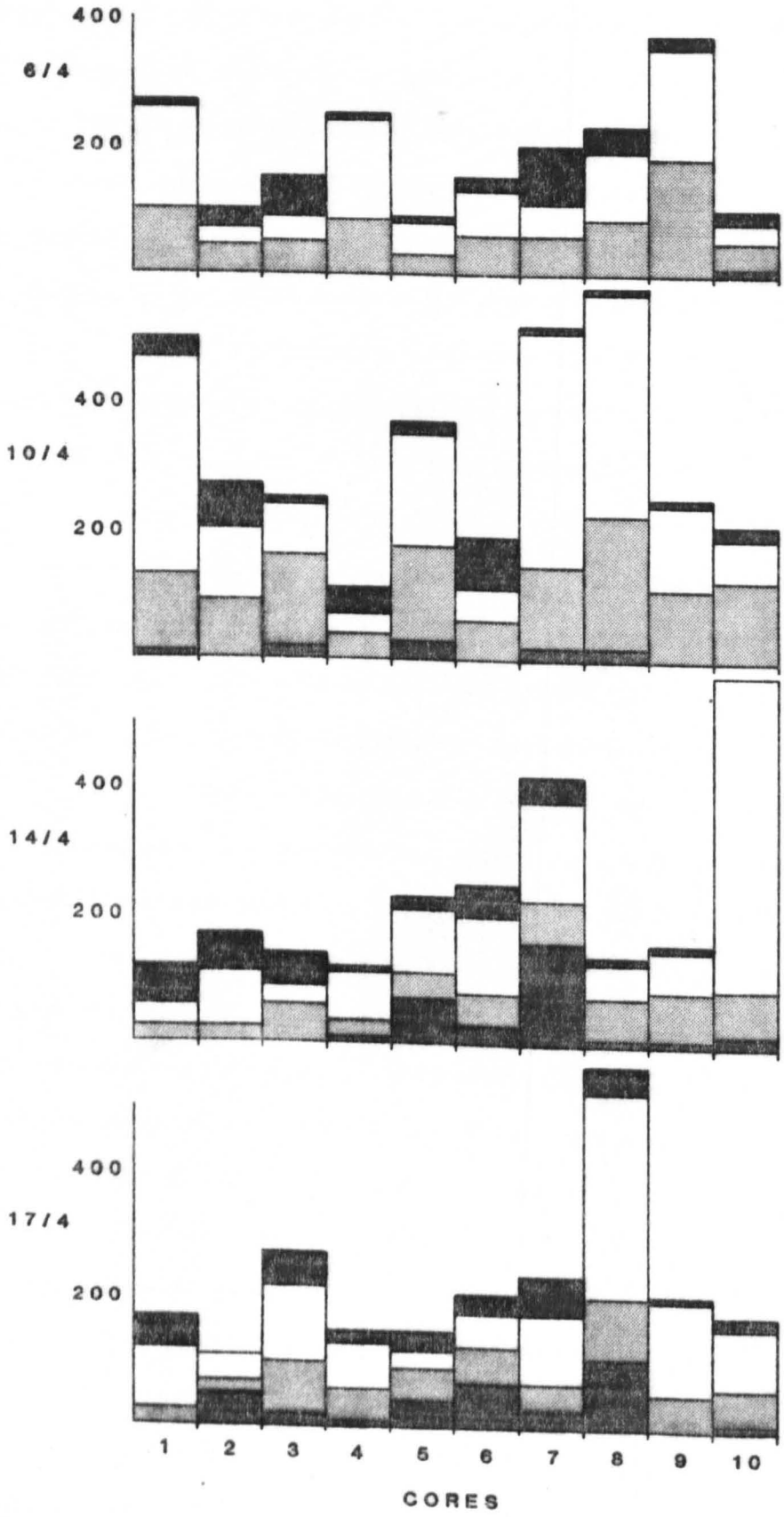

400
6/4
200
400
10/4
200
400
14/4
200
400
17/4
200
1
2
3
4
5
6
7
8
9
10
CORES

Fig.5.8 Numbers of individuals per core of the three developmental stages and of species other than Coelopa frigida.

Other species - black

C. frigida — Small larvae - unshaded

Large larvae - light shading

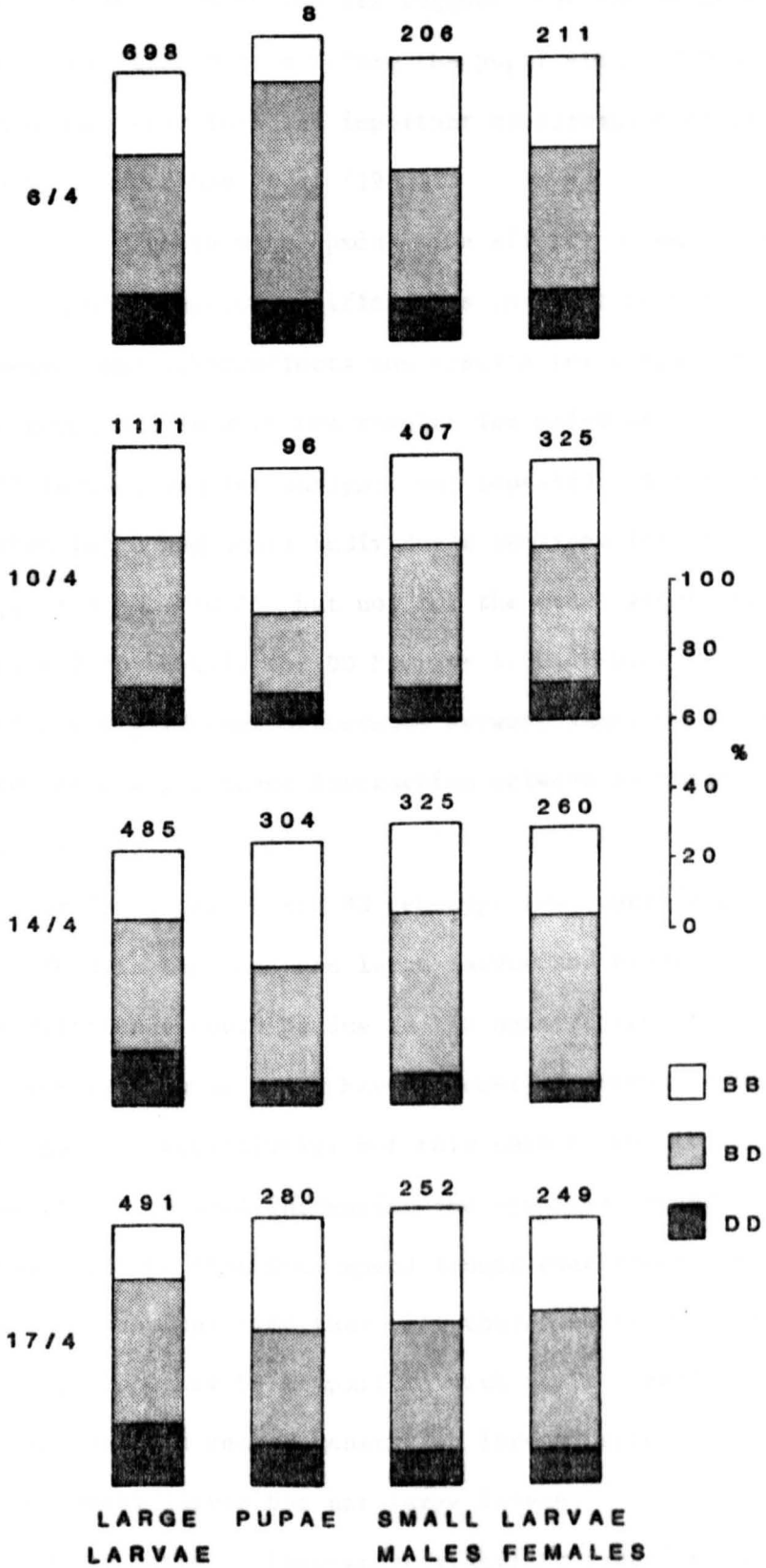
Pupae - heavy shading

first pupae were already present on 6 April emergence of new adults probably began around 10 April and from the third set of samples on 14 April onwards it is possible that some of the larvae were second generation individuals.

The proportions of the common genotypes, that is those containing only the three Adh alleles B, C and D, have been compared between sampling dates and between developmental stages using an analysis of deviance with binomial error distribution and logit transformation as described in Chapter 2. The three genotypes involving the C allele, BC, CC and CD, do not differ significantly in frequency either between samples or between stages. The proportions of the other genotypes are shown in Fig.5.9. There are significant differences between stages for these three genotypes (for BB, $F_{3,126} = 5.54$, $P<0.01$; for BD $F_{3,126} = 7.43$, $P<0.001$; for DD, $F_{3,126} = 14.41$, $P<0.001$) but differences between sampling dates are only significant for BB ($F_{3,126} = 2.98$, $P<0.05$). The interaction between stages and dates is significant for the DD genotype frequency ($F_{9,126} = 2.63$, $P<0.01$) and close to significance for the BD genotype ($F_{9,126} = 1.93$, $P<0.10$).

The genotype frequencies in males and females from the small larvae are in all cases very similar. The most striking differences are in the pupae. With the exception of the 6 April samples where only 8 pupae were found, the frequency of DD is much higher, and that of BD much lower than in either the large or small larvae (Fig.5.9). This difference diminishes in the later samples. These observations can be explained in terms of the development time differences between the genotypes (Chapter 4). DD flies are known to emerge before BD flies with BB flies emerging last and this is especially

Fig.5.9 Proportions of the three common Adh genotypes, BB, BD and DD classified by developmental stage and date of sampling.



true of males. These results suggest that the difference in development time occurs before the pupal stage rather than during it and are, therefore, an important confirmation of the laboratory observations of Day et al (1980).

Clearly these development time effects potentially account for most of the significant differences in genotype frequencies. In order to detect any other effects the results for large larvae and pupae were combined, as were the results for males and females from the small larvae, and the analysis was repeated. A significant difference between large and small individuals remained for the BB genotype ($F_{1,63} = 6.98$, $P<0.05$) but not for the other genotypes (for BD $F_{1,63} = 2.85$, $P>0.1$; for DD $F_{1,63} = 1.50$, $P>0.2$). In no case was there any significant difference between sampling dates but for BD there was a significant interaction between sample date and stage ($F_{3,63} = 3.69$, $P<0.05$).

The frequency of the BB genotype was lower in the small larvae (10%) than in the combined large larvae and pupae (13%). Part of this difference could be due to the underrepresentation of pupae in the core samples as pupae have a lower BB frequency than large larvae (10% and 14% respectively) but this cannot explain the full difference. An additional effect increasing the observed proportion of large BB larvae might be that they spend longer than other larvae at this stage. At any one time they will therefore be overrepresented in the large category by comparison with their overall frequency. There may also be some second generation larvae, with a high Adh-D frequency, amongst small larvae but not large larvae.

On the basis of laboratory viability experiments one would expect the proportion of BD larvae to be consistently higher in large larvae

than in small larvae with the opposite trend in the homozygotes. The observed proportions of BD individuals are given in Table 5.16. The frequency amongst large larvae does exceed that of small larvae on three of the four sampling dates but on 14 April there was a marked reversal with an unusually high BD frequency in small larvae and an unusually low frequency in large larvae. When pupae are added in with large larvae the difference on 6, 10 and 17 April largely disappears and the discrepancy on 14 April is increased. These effects are consistent between individual cores within sampling dates. Despite the large sample size (a total of 5708 individuals were gelled), there is no evidence for differences in relative viability between the genotypes from the "small larvae" to the "large larva or pupa" stage.

The overall frequency of BD heterozygotes exceeds the Hardy-Weinberg expectation by 9.5% which is only a modest excess when compared with other samples from natural populations (Chapter 3) where the average excess is 18.4%. Taking the 40 cores individually there are significant deviations from Hardy-Weinberg equilibrium in only 4 cases (three at the 5% level and 1 at the 1% level) for the large larvae and pupae combined and 5 cases (2 at 5%, 2 at 1% and 1 at 0.1% levels) for the small larvae. BD heterozygotes were in excess of expectation in 30 cores for large larvae and pupae and 32 cores for small larvae. Thus it can be seen that, although the expected heterozygote excess was found, it was small and occurred in both small and large larvae.

There are three possible explanations for the lack of difference between small and large larvae. Firstly it could indicate that the heterozygote excess is not a result of viability differences but is caused by disassortative mating. Whilst this possibility cannot be discounted at present there is no other evidence of disassortative

mating. Secondly the differential viability might occur before larvae have grown large enough for inclusion in even the "small" group. Effectively this means larvae of less than about 1 day from hatching, a high proportion of which will have been missed when searching these cores (but will have been included in the cores from the 1981 series). Hatch rates in C. frigida are high (about 98%, Collins 1978, Thompson 1951) so the selective mortality would have to be in the first day of larval life. This possibility has been examined in laboratory experiments reported in Chapter 6. Finally there is the possibility that there was significant selective mortality in the round canisters after transfer of larvae from the cores. Certainly there was considerable overall mortality - from the 6118 larvae transferred 2264 adult C. frigida emerged and 650 adults of other species. If C. frigida survived only as well as the other species this represents approximately 50% mortality. However C. frigida almost certainly survives better than the other flies and also there must have been some random mortality caused by handling of the small larvae. Nevertheless it is clearly possible that sufficient selective mortality occurred to explain the observed heterozygote excess despite the fact that the density in these canisters was considerably lower than in the cores themselves. If this experiment were to be repeated it would be better to restrict the number of larvae per small canister to a maximum of say 50 or 100.

The frequency of the Adh-C allele does not vary significantly between dates or between developmental stages either when all four stages are considered or when they are grouped into "large" and "small". Both the B and D alleles show variations between the four stages (for B $F_{3,126} = 11.39$, $P<0.001$; for D $F_{3,126} = 10.32$, $P<0.001$)

Table 5.16

Proportions of flies of genotype Adh-BD in the core samples from the experimental wrack bed (Hardy-Weinberg expectation overall = 0.364).

Developmental Stage	Sample date 6/4	10/4	14/4	17/4	Total
Small larvae - male	0.37	0.41	0.46	0.37	0.41
- female	0.41	0.39	0.48	0.40	0.42
- combined	0.39	0.40	0.47	0.39	0.41
Large larvae	0.40	0.43	0.38	0.42	0.41
Pupae	0.50	0.23	0.33	0.33	0.32
Large larvae and pupae	0.40	0.41	0.36	0.38	0.39
Total	0.39	0.41	0.40	0.38	0.40

Table 5.17

Adh-D gene frequencies in the core samples from the experimental wrack bed

	Sample date				
Developmental Stage	6/4	10/4	14/4	17/4	Total
Small larvae - male	.52	.52	.54	.58	.54
Small larvae - female	.51	.51	.56	.53	.53
Large larvae	.50	.53	.45	.43	.49
Pupae	.50	.61	.58	.56	.58
Total	.51	.53	.52	.51	.52

and interactions between stages and sampling dates (for B $F_{9,126} = 2.11$, $P<0.05$; for D $F_{9,126} = 2.97$, $P<0.01$). The gene frequencies for Adh-D are given in Table 5.17. Clearly most of the differences result from the effects of development time discussed above. When large larvae and pupae and males and females are combined the only effect which remains significant is the difference in D frequency between stages ($F_{1,63} = 4.46$, $P<0.05$), the large group having a frequency of 0.51 and the small group 0.54. This is probably due to the under-representation of pupae in the core samples as was the excess of the BB genotype in the large group. From the observed numbers of pupae a fairly steady reduction in D frequency might have been expected over the four sets of samples. In fact there is a marked drop in frequency between 10 and 14 April suggesting that pupation began in this interval for the large cohort of larvae laid before 6 April. The Adh-D frequency amongst small larvae, both male and female, increases on the later dates and this probably represents the appearance of some second generation larvae with a high D frequency.

Although these results do not provide evidence for differential survival between the "small" and "large" stages it may be possible to detect density dependent viability differences by making comparisons between cores for each stage. As there are differences in gene frequencies between dates within stages it is necessary to consider the dates separately. When dealing with the "small" group it should be noted that any density effects may have occurred in the round canisters after collection. Certainly the mortality increased in these canisters with increasing density. The regression of numbers emerging on starting numbers has a slope of 0.68±0.06 and as this is markedly less than 1 it indicates a lower proportion of survivors at higher density.

Table 5.18

The relationship between the proportion of BD heterozygotes and the larval density of individual cores

a) Density[1] of C. frigida alone

Date	Large larvae and pupae	Small larvae	Combined
6 April	-0.05	-0.10	-0.04
10 April	-0.09	0.19	0.08
14 April	-0.38*	0.08	-0.07
17 April	-0.09	0.31	0.04

b) Density[1] of all dipteran larvae

Date	Large larvae and pupae	Small larvae	Combined
6 April	-0.05	-0.19	-0.08
10 April	-0.09	0.17	0.09
14 April	-0.38*	0.07	-0.11
17 April	-0.09	0.39	0.03

[1]The densities were on the transformed scale described on page 204 The figures quoted are the regresssion coefficients after logit transformation of the proportions and assuming a binomial error distribution.

* indicates that the regression is significantly different from zero at the 0.05 level.

The best indicator of selective mortality is likely to be the proportion of BD heterozygotes. If there was an effect of density similar to that observed by Collins (1978) it should be possible to detect an increase in this proportion with increasing density. No correction has been made for variation in gene frequency between cores because no significant differences were detected within stages and dates in the analysis described above. The proportion of BD heterozygotes was compared, on a logit scale and with binomial errors, with the densities after the transformation appropriate to the negative binomial distribution described above. The regression coefficients (Table 5.18) were not significantly different from zero except in the "large" group on 14 April and in this case the slope was negative. Inclusion of other dipteran larvae in the density estimates had little or no effect on the regressions. In most cases the regressions do not even approach significance and about equal numbers of slopes are positive and negative. The single significant value may well have arisen by chance. There is, therefore, no evidence from this data that larval density in natural populations increases the relative viability of BD heterozygotes.

The lack of evidence for density dependence in these field results corresponds with a similar result from the laboratory experiments involving flies derived from the St.Mary's Island population (section 5.5 of this chapter). This tends to support the possibility that the extreme effect of density observed by Collins was a property of the Rustington population which he used, or perhaps of South Coast populations in general. However two shortcomings of the present data should be considered. Firstly the density estimates used for large larvae are estimates made after the mortality had occurred. This may also be true of the small larvae

if early mortality is important as suggested above. Any particular post-mortality density could result from a range of possible pre-mortality densities. This makes the present method rather weak - it is really necessary to compare relative viabilities with initial densities, ideally with viable eggs laid per unit of food. Secondly my method assumed that the larvae collected from a particular core had experienced the density within that core for all, or at least a significant part of their lives. This is effectively assuming that larvae have very limited mobility. However observation suggests that they are very active and can move considerable distances. In the laboratory it is common to see third instar larvae moving around the surface of the food medium or the walls of the tanks and they can move the length of a tank (30 cm) in only a few minutes. If larvae are mobile within wrack beds, especially if they move away from unfavourable, overcrowded areas then it may be preferable to consider the larval density of the wrack bed as a whole influencing the viability of all larvae in the bed. This was the idea behind the different coring technique employed for the 1981 series of samples.

(iii) 1981 core samples One possible reason why the core samples from the experimental wrack bed failed to show an effect of density on the proportion of BD heterozygotes was that larvae were sufficiently mobile within the small wrack bed for them all to experience the same level of effective competition. This possibility is avoided in the 1981 core series which estimated the densities in whole wrack beds for comparison with the proportions of heterozygotes in mass samples which were also representative of the whole bed. The proportions of BD individuals in the mass samples (Chapter 3 and Appendix 1) differ considerably between samples but some of this variation is due to

differences in Adh-B and D gene frequencies. To allow for this I have used the ratio of the observed number of BD individuals to the Hardy-Weinberg expectation as an estimate of the proportion of BDs present. In Chapter 3 this ratio was found to be approximately normally distributed in large samples. The measure of density used is the mean number of flies per core on the transformed scale introduced in section 5.7(i) above.

The data are plotted in Figure 5.10. Coelopa pilipes were very numerous in some wrack beds and in these cases separate density points have been plotted with and without this species. Excluding C. pilipes the regression of heterozygotes on density is not significant ($b = 0.039 \pm 0.025$, $P > 0.10$) but including C. pilipes it is significant ($b = 0.074 \pm 0.027$, $P < 0.05$). This result indicates that the relative viability of BD larvae does increase with density in natural conditions and that C. pilipes competes with C. frigida for a resource, or resources, which is relevant to the relative success of C. frigida genotypes.

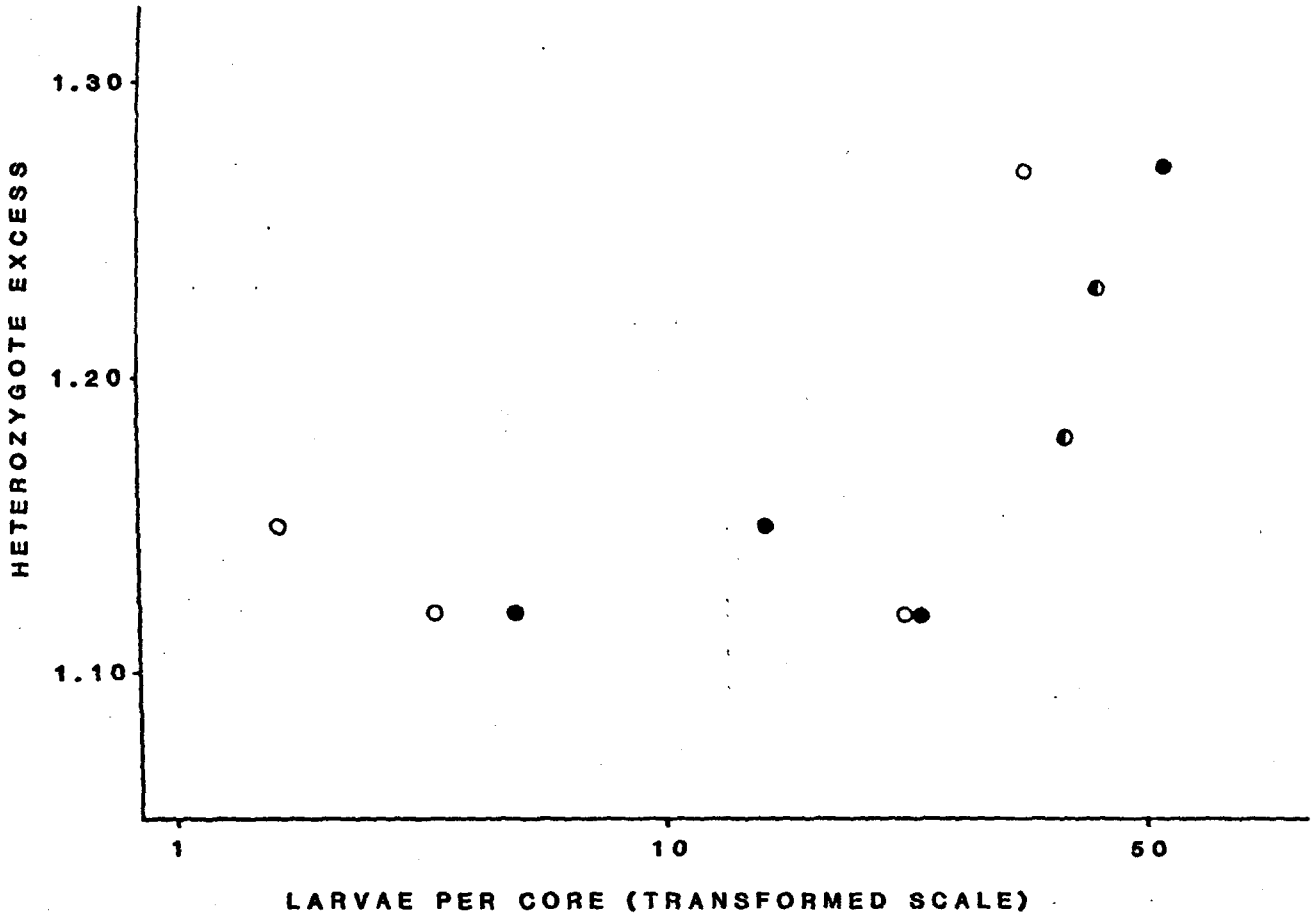
This conclusion, which is based only on six points, must be set against my inability to demonstrate density dependence both in the laboratory and in the samples from the experimental wrack bed. The explanation for the difference between these cores and those from the experimental bed could lie in the effect of larval mobility mentioned above. There were two main possible explanations for the lack of density dependence in my laboratory experiments when it had been so marked in Collins' (1978) experiment. One was that density dependent heterozygote advantage in viability was a feature of the flies used by Collins from Rustington and did not occur in the populations on which my stocks were based. One of these stocks was from St.Mary's Island and so the results of these core series go

Fig.5.10 Relationship between larval density and heterozygote excess in the 1981 core samples.

Open circles - *C. frigida* only

Closed circles - *C. frigida* and *C. pilipes*

Half closed circles - *C. frigida* (samples with less than one *C. pilipes* per core)


1.30
1.20
1.10
HETEROZYGOTE EXCESS
1
10
50
LARVAE PER CORE (TRANSFORMED SCALE)

against this hypothesis. The other possibility was a difference in culture conditions and if these core results are to be relied on they imply that Collins' conditions were a more satisfactory approximation to a natural wrack bed than were mine. Further results relevant to these two possibilities are presented in the next two chapters.

A further observation made on these core series which may be of interest was that C. pilipes reached a peak of emergence about four days after C. frigida in all the cores in which appreciable numbers of both species were found. This confirms the observation made by Dobson (1974b) that C. pilipes develops more slowly in natural conditions than does C. frigida and may help to explain the correlation between the proportion of C. pilipes in field samples and the Adh-B allele frequency which was noted in Chapter 3. The possibility will be further examined in Chapter 10.

c) Discussion The main objectives of these field sampling programmes were: (a) to establish whether or not the heterozygote excess commonly observed in mass samples was due to viability differences between genotypes, and (b) to obtain evidence for the effect of larval density on relative viabilities in the field. The results do not give unequivocal answers to either of these problems. The samples from the experimental wrack bed did not provide evidence either for viability differences or for any effect of density. On the other hand the 1981 core series did show a relationship between larval density and heterozygote excess which is difficult to interpret except in terms of viability differences between genotypes in competition.

The shortcomings of the techniques involved in obtaining the two

sets of data have been discussed. Together with Collins' results and my laboratory experiments these field results raise some important questions which will be considered in subsequent chapters:-

(i) Do flies from different geographical populations differ in the relative viabilities of the genotypes? It is possible that the chromosome I inversions of different populations contain different coadapted gene complexes which affect the competitive abilities of the larvae. This could explain some of the differences between laboratory experiments which used stocks from different sites. However if this was the reason for the lack of density dependence in laboratory experiments involving St.Mary's Island strains it could explain the negative results from the experimental wrack bed but not the positive result from the 1981 core series which were from St.Mary's Island and the nearby Whitburn site. It should be possible to detect coadaptation by making suitable crosses between strains derived from different populations and this type of experiment will be reported in Chapter 7.

(ii) Does the composition of the laboratory medium affect relative viabilities? In the laboratory density dependence was detected by Collins and by me when using Laminaria stipe as food. The 1981 core series suggest that it also occurs in field conditions. However it has not been detected in laboratory experiments using medium which does not contain the dried milk protein source used by Collins. Clearly this does not explain the lack of density dependence in the experimental wrack bed. Also it was not the only difference between my experiments and Collins'.

(iii) Is there significant selective mortality in the first few days of larval life? This possibility has been mentioned several times. It would go a long way towards explaining the lack of difference

between "small" and "large" larvae from the experimental bed and the differences between my "larva-to-adult" experiments and Collins' experiments. However it does not explain the difference between my "egg-to-adult" results and Collins' results nor the fact that viability differences were observed in the experiment using Laminaria stipe which was started with 2 day old larvae. Nevertheless this interesting possibility will be examined further in the following chapter.

(iv) Is there non-random mating? Non-random mating might explain a number of surprising observations especially the high Adh-B frequency in some of the laboratory experiments. There is now good evidence for sexual selection in favour of large (BB) males (Butlin et al 1982b, Chapters 8 and 9) but there is no evidence for disassortative mating and so an explanation for heterozygote excess involving viability differences is still necessary.

(v) Is there competition between C. frigida and other wrack bed dipterans? The 1981 core series suggest the interesting possibility that C. pilipes affects the outcome of competition between different genotypes of C. frigida. This could be a fruitful area for future research.

In addition to results on viability the core sampling programmes have given important confirmations of development time differences. Adh-DD larvae apparently pupate earlier than Adh-BB larvae with Adh-BD intermediate. Adh-C carrying genotypes do not show any particular pattern. This is in very good agreement with the results on adult emergence times reported in Chapter 4 which were based on a mass sample from the experimental wrack bed. The fact that development time differences occur before pupation lends support to

the hypothesis advanced earlier in this chapter to explain the relationship between development time and adult size.

The core samples from the experimental wrack bed also confirmed the impression gained from the mass sample that egg laying continued practically throughout the life of the bed. In the past the assumption has generally been made that egg laying is relatively synchronised in a short period after wrack bed deposition but my results show that there is a lag of several days before laying begins and that it then continues for at least 10 days and probably merges into second generation egg laying.

Finally there is the interesting confirmation of Dobson's (1974b) observation that C. pilipes adults emerge about 4 days later than C. frigida adults.

Chapter 6

Analysis of Viability Differences

6.1 Introduction

Competition of the scramble type occurs between Coelopa frigida larvae as it does between other Dipteran larvae living in comparable conditions. Collins' (1978) results indicate that this is important for the maintenance of the α/β inversion polymorphism because the competitive abilities of the karyotypes differ. As competition increases so the advantage to the heterokaryotype increases. However, my laboratory experiments do not show this effect when using laboratory culture medium. Using Laminaria stipe as food source there is a weak tendency in the direction of increasing viability of heterokaryotypes relative to homokaryotypes with increasing competition. There is some evidence from field studies for a superior competitive ability for the heterokaryotype but, as yet, this evidence is rather limited.

What is the basis of differences in competitive ability between the genotypes? A first step towards answering this question is to assess the relative importance of exploitative and interference elements of competition. This has been attempted using two experimental approaches: a "food-space" experiment comparable to that of Bakker (1961) where the amount of food available per larva is varied independent of the amount of space, and a conditioned medium experiment. The latter can also be used to assess the effects of specific genotypes in the conditioning generation. In addition to these two types of experiment I have also conducted "time-course" experiments aimed at detecting mortality at different stages of

the life cycle. In the Drosophila work described in the previous chapter it has been assumed that most, if not all, mortality in laboratory conditions occurred when food was exhausted and some larvae were too small to pupate. I have investigated the possibility that in Coelopa some larvae die long before this point.

The results of these experiments may also help to explain the discrepancies between my laboratory experiments and those of Collins.

6.2 Conditioned Medium Experiments

The basic design of these experiments was simple. Individuals of a known genotype, or a known combination of genotypes, were reared in canisters containing standard culture medium for a period of time and then removed. Another set of individuals from a test cross was then introduced to the same canisters and reared on the "conditioned" medium. The viabilities of the different genotypes in the "test" generation were then compared with the viabilities on fresh medium at the same density.

A reduction in viability in the test generation could indicate exploitative competition or interference competition but would exclude any type of physical interaction between larvae in the latter category. Comparison of genotype frequencies in the test generation between experiments with different genotypes in the conditioning generation should reveal any genotype specific, frequency dependent, effects.

The experiments could be extended by adding extracts of conditioned medium to canisters of fresh medium or by supplementing medium with various components of the food. Investigations of this sort could distinguish the alternative hypotheses of exploitative

and interference competition.

In practice there were many technical difficulties with these experiments. The central problem was to remove all individuals of the first, conditioning, generation without undue disturbance of the medium and then either to have the test generation ready to start at just the right time or to store the conditioned medium in a way which did not alter its character.

Four different experimental designs were used and they will now be described.

a) EXPERIMENT 1

(i) Method For the conditioning generation 10 pairs of flies were allowed to lay eggs for 24 hours in each of 20 small canisters containing 300±5 g of standard culture medium and then discarded. In 10 canisters BB(lab) flies were used, in the other 10 canisters DD(lab) flies were used but this stock was found to be contaminated. This latter set of 10 canisters was, therefore, conditioned by a mixture of BB, BD and DD larvae and was not used for a test generation. Instead ten days after completion of egg laying, when pupation had begun, the medium from 4 of these canisters was mixed with 800 ml of distilled water and stirred for 30 minutes and then filtered through muslin to produce an "extract" of conditioned medium. This was stored at -20°C until required.

All pupae appearing in the ten BB canisters were removed each day until the ninth day from completion of egg laying. On the ninth day the canisters were flooded with 50 ml of distilled water to bring the remaining larvae to the surface where they were picked out with forceps. The canisters were then returned to the constant temperature room overnight and on the next day any more larvae or

pupae which could be found were removed. The canisters were stored at -20°C until required.

A check was made on the number of larvae remaining in the medium after this procedure by passing the medium through a fine gauge sieve. Two canisters were checked. They had had 393 and 431 larvae and pupae removed and were found to have 10 and 51 larvae remaining respectively.

The test generation was started in the following way. BD flies were produced by crossing virgin flies from the BB(lab) and DD(lab) lines after the latter had been reestablished following the contamination. BD flies were mass mated and allowed to lay eggs for 24 hours in a culture tank. Two days after completion of egg laying the small larvae were counted into the conditioned and control canisters, 100 to each canister, using a paint brush.

The following canisters were established:-

Controls - five small canisters containing 300 g fresh medium.

BB Controls - five small canisters containing 300 g of fresh medium to which 50 BB third instar larvae had been added and that had then been frozen at -20°C.

Flooded Controls - four small canisters containing 300 g fresh medium that had been flooded with 50 ml distilled water and then left overnight at 27°C.

Extract - four small canisters containing 300 g medium made with 100 ml of water per 300 g medium replaced with extract of conditioned medium.

BB Conditioned - eight small canisters produced as above.

The BB Conditioned and BB Control canisters were thawed for 24 hours before larvae were added.

Table 6.1

Results of Conditioned Medium Experiment 1

	REPLICATE	NUMBER IN CONDITIONING GENERATION	TEST GENERATION BB	BD	DD	TOTAL	χ^2 HETEROGENEITY	
CONTROL	1		11	58	20	89		
	2		23	45	22	90		
	3		14	56	21	91		
	4		22	38	22	82		
	5		15	46	19	80		
	T		85	243	104	432	11.2	P>0.1
BB CONTROL	1		12	52	25	89		
	2		11	41	16	68		
	3		12	48	20	80		
	4		12	44	24	80		
	5		17	52	21	90		
	T		64	237	106	407	2.2	P>0.1
FLOODED CONTROL	1		18	45	17	80		
	2		18	42	25	85		
	3		22	35	23	80		
	4		18	48	25	91		
	T		76	176	90	342	3.9	P>0.1
ALL CONTROLS	T		225	656	300	1181	6.3	P>0.1
BB CONDITIONED	1	463	15	37	19	73		
	2	519	14	42	21	79		
	3	208	11	20	9	42		
	4	601	18	36	20	74		
	5	395	17	39	13	70		
	6	430	13	44	16	77		
	7	492	13	38	15	66		
	T		101	256	113	470	5.4	P>0.1
EXTRACT	1		16	46	26	88		
	2		21	38	28	87		
	3		16	61	20	97		
	4		16	48	20	84		
	T		69	193	94	356	7.7	P>0.1

Flies were collected daily from all canisters and stored at -20°C until required for electrophoresis.

(ii) Results The results are given in Table 6.1. In no case was there significant heterogeneity between replicates and there was no significant difference between the three types of control replicate. In all the controls the viability was high - between 80 and 90% (mean 83.9%) - as it was in the Extract replicates (mean 89%). In the BB Conditioned replicates the viability was reduced (mean 68.7%).

These viabilities can be compared with those recorded in the density experiments described in Chapter 5. The conditions here were very similar to those of Collins' experiments. His low density replicates started with 60-100 eggs and gave viabilities of 90-97% and were therefore very similar to the controls in this experiment. The viability in the BB Conditioned replicates was around 70% which is similar to Collins' Medium density replicates. These were started with 270-430 eggs. So the viability in these conditioned canisters is closer to the level one would predict from the number of individuals in the conditioning generation (200-600 at the time of removal) than to that expected from the test generation density of 100 larvae. Competition between larvae is, therefore, likely to be mediated through their effect on the medium rather than by physical interaction between individuals. Addition of the extract apparently had no effect on overall viability. This may mean that competition is of the exploitative rather than the interference type as no harmful chemical seems to have been transmitted with the extract but if such a chemical had existed it may have been destroyed in the extraction process. It is also possible that interference occurs by a physical or biological change in the medium not a chemical change.

There is no significant difference between the genotype distribution of the controls and that of the BB Conditioned replicates ($\chi_2^2 = 1.19$) or the Extract replicates ($\chi_2^2 = 0.2$). This is surprising in view of the reduction in viability in the BB Conditioned set - a similar reduction was accompanied by a major change in relative viabilities in Collins' experiment. Clearly the conditioning larvae made some difference to the medium which reduced the survival of the test generation but this was not equivalent to the selective effect of increased larval density. The extract apparently had no effect on absolute or relative viabilities and neither did the various operations involved in the experiment, especially flooding and freezing and the presence of dead larvae in the medium. It is possible that the extra mortality in the conditioned canisters was not related to competitive interactions between larvae as such but to some other effect of conditioning such as a change in consistency of the medium or decomposition by microorganisms. Possibly the larval density in the conditioning generation was too low to produce a significant effect in the test generation.

The genotype frequencies in all groups of replicates differ significantly from the expected Mendelian ratio of 1:2:1 (Controls $\chi_2^2 = 23.8$, $P<0.001$; BB Conditioned $\chi_2^2 = 6.6$, $P<0.05$; Extract $\chi_2^2 = 6.0$, $P<0.05$). In each case there is an excess of heterozygotes of the order of 10%, DD homozygotes differ little from expectation and there is a deficiency of BB homozygotes. If anything there is a tendency for BB individuals to have survived better and DDs worse in the replicates conditioned with BB larvae.

The results of this experiment can be summarised in terms of relative viabilities for comparison with the other experiments to

be described below:-

Type of replicate	Overall Viability	Relative Viability		
		BB	BD	DD
Control	0.84	0.69	1	0.91
BB Conditioned	0.69	0.79	1	0.88
Extract	0.89	0.72	1	0.97

b) EXPERIMENT 2

(i) Method This experiment was broadly similar to the first but was conducted in culture tanks rather than in small canisters. For the conditioning generation three tanks were started by allowing 100 pairs of flies to lay eggs in each tank for 24 hours. One tank had DD(lab) flies, one BB(lab) flies and the third DD(lab) males and BB(lab) virgin females. The tanks were kept in standard conditions until the first adults emerged. These were removed together with the large numbers of pupae on the sides of the tanks and the tanks were frozen to $-20^{o}C$ to kill the remaining larvae.

The test generation was the progeny of a BDxBD mass cross obtained exactly as in experiment 1. 1000 two day old larvae were moved to each of the three conditioned tanks, which had been thawed overnight, and to a control tank with fresh medium. Freezing of the medium and the presence of dead larvae were assumed to have no effect on the basis of the controls in experiment 1.

(ii) Results The test generation in the BB conditioned cage produced only 9 adult flies because it was seriously infected with fungi. The results for the other cages are given below:-

Type of Replicate	Total flies emerged	Results of electrophoresis			
		BB	BD	DD	Sample Total
Control	689	19	54	22	95
BD Conditioned	793	24	49	23	96
DD Conditioned	633	95	137	47	279

There was not the reduction in viability resulting from conditioning as observed in the first experiment, indeed the viability of the test generation was higher in the BD Conditioned tank than in the control tank. On the other hand there are differences between the genotype frequencies. After conditioning with BD larvae the excess of BD adults seen in the control disappeared and the proportion of BBs increased. The difference between this tank and the control tank was not statistically significant ($\chi_2^2 = 1.8$) but for the DD conditioned tank the differences were highly significant ($\chi_2^2 = 35.3$, $P<0.001$). In this case there was a marked deficiency of DD adults and excess of BB adults. The relative viabilities were as follows:-

Type of replicate	Overall viability	Relative viability		
		BB	BD	DD
Control	0.69	0.70	1	0.81
BD Conditioned	0.79	0.98	1	0.94
DD Conditioned	0.63	1.39	1	0.69

These results support the hypothesis of a stable genotype specific change in the medium produced by the conditioning larvae which might be removal of a particular resource (exploitation) or excretion of a particular toxic waste product (interference). In either case the increased viability of the BD conditioned tank is not explained.

Table 6.2

Results of Conditioned Medium Experiment 3

			TEST GENERATION					
	REPLICATE	NUMBER IN CONDITIONING GENERATION	BB	BD	DD	TOTAL	χ^2 HETEROGENEITY	
CONTROL	1		8	32	13	53		
	2		8	31	7	46		
	3		9	26	8	43		
	4		12	29	16	57		
	T		37	118	44	199	4.2	P>0.1
FROZEN CONTROL	BB1		17	40	23	80		
	BB2		12	37	25	74		
	BD1		17	67	14	98		
	BD2		21	46	27	94		
	DD1		11	54	24	89		
	DD2		19	55	20	94		
	T		97	299	133	529	16.6	P>0.05
ALL CONTROLS	T		134	417	177	728	3.2	P>0.1
BB CONDITIONED	1	313	2	3	2	7		
	4	242	2	8	1	11		
	T		4	11	3	18	0.35	P>0.1
BD CONDITIONED	1	149	10	24	6	40		
	2	221	3	12	2	17		
	3	200	15	14	11	40		
	4	117	6	9	2	17		
	T		34	59	21	114	8.8	P>0.1
DD CONDITIONED	2	170	1	20	1	22		
	3	162	8	28	5	41		
	4	179	5	11	0	16		
	T		14	59	6	79	7.6	P>0.1

In the first experiment conditioning with BB larvae favoured BB relative to DD individuals in the test generation. The opposite would have been predicted from the results of this experiment. Also the lack of any detectable effect of the extract suggests that the effect of conditioning on the medium is not the result of deposition of a stable, water soluble waste product.

The test generation densities in the two experiments were probably comparable because the area of medium in a tank is about 12 times that in a small canister and the relative viabilities in the controls were similar. The conditioning densities may have been different. No objective assessment of the density was made in this latter experiment but it appeared that densities were higher in the tanks than in the canisters.

c) EXPERIMENT 3

(i) Method This experiment was identical to experiment 1 except that the densities were increased both in the conditioning and test generations by using round canisters in place of small canisters. As in experiment 1, 10 pairs were used to start the conditioning generation and 100 second day larvae for the test generation. Conditioning was with BB, BD or DD larvae from the BB(lab) and DD(lab) lines and a cross between them. Controls were fresh medium or frozen medium with 50 dead BB, BD or DD third instar larvae per canister.

(ii) Results The full results are given in Table 6.2. Two of the BB Conditioned and one of the DD Conditioned replicates were contaminated with fungi and failed to produce any adults in the test generation.

The increased density in this experiment was intended to increase

the mortality in the test generation in the hope that this would produce greater differences between the genotypes. The viability in the fresh controls was indeed lower than in experiment 1, and the conditioned canisters showed a very large reduction in viability. The frozen controls had a markedly higher overall viability. This may have been due to the extra nutrients provided by the dead larvae in these canisters or to the physical consistency of the medium which is less gelatinous after freezing and thawing. In any case the genotype frequencies are apparently unaffected by freezing as they do not differ significantly from the fresh controls ($\chi_2^2 = 3.2$).

The two successful BB Conditioned replicates produced very few adults which may be related to the relatively high number of larvae in the conditioning generation. The genotype frequencies do not differ significantly from the combined controls ($\chi_2^2 = 0.62$). Both the BD and DD Conditioned replicates do differ significantly from the expectation based on the controls (for BD Conditioned $\chi_2^2 = 10.3$, $P<0.01$; for DD Conditioned $\chi_2^2 = 13.2$, $P<0.01$) but in the case of the BD group this effect is caused mainly by one replicate, BD3, and must therefore be interpreted with caution. In the BD Conditioned replicates overall there are less BD and DD individuals than expected and more BBs. In the DD Conditioned set there are more BB and BD and less DD individuals than expected from the controls. The relative viabilities were:-

Type of Replicate	Overall Viability	Relative Viabilities		
		BB	BD	DD
Controls	0.50	0.63	1	0.75
BB Conditioned	0.09	0.73	1	0.55
BD Conditioned	0.29	1.15	1	0.71
DD Conditioned	0.26	0.47	1	0.20

These results support the suggestion of experiment 2 that DD larvae affect the medium in a way which reduces the viability of DD individuals in the test generation. This is also true for BD Conditioning in both experiments. In this experiment BD individuals had a much higher viability in DD Conditioned medium than in experiment 2 and BB individuals had a much higher viability in BD Conditioned medium. These differences may be a result of higher density in both generations in this experiment increasing the advantage to BD relative to the homozygotes and to BB relative to DD as in Collins' density experiment.

The effect of conditioning the medium with BB larvae remains anomalous. In neither experiment 1 nor experiment 3 has the relative viability of BB been reduced by conditioning with BB larvae as might be expected by analogy with the other genotypes.

d) EXPERIMENT 4

(i) Method The first three experiments have all had two technical shortcomings in common: they have used inbred laboratory lines in both conditioning and test generations and they have involved some sort of physical change in the medium, flooding and/or freezing, between generations. This last experiment was intended as a pilot experiment for a technique which did not suffer these problems. The PORT.EE Isogenic line was used for the conditioning generation and the Portland B+D line for the test generation (derivations of both lines are given in Chapter 2). The two generations could, therefore, be distinguished by electrophoresis and it was not necessary to remove all the members of the conditioning generation before adding the test generation.

The experiment was conducted in round canisters to each of

which 10 egg batches were added from an EE stock tank. Eight days later, when the majority of conditioning larvae had pupated, 100 test generation larvae were added to each canister. These were second day larvae from a mass mating of the ninth laboratory generation of the Portland B+D line. 6 control replicates with fresh medium were also established. All adults emerging up to day 14, from the start of the conditioning generation, were discarded. Flies emerging after that were collected and electrophoresed.

(ii) Results The results are presented in Table 6.3. The viability in the control replicates was surprisingly low, 28% on average

Table 6.3

Results of Conditioned Medium Experiment 4

Type	Replicate	BB	BD	DD	TOTAL	χ^2 heterogeneity
Control	1	3	12	3	18	
	2	3	18	8	29	
	3	1	16	6	23	
	4	1	15	12	28	
	5	2	18	15	35	
	6	2	18	15	35	
	T	12	97	59	168	9.5 P>0.1
EE Conditioned	1	13	29	15	57	
	2	8	19	12	39	
	3	11	30	16	57	
	4	7	36	19	62	
	T	39	114	62	215	3.2 P>0.1

compared with 50% in the fresh controls in experiment 3 which had the same initial density. The genotype frequencies differed significantly from the Hardy-Weinberg expectations based on the parental gene frequencies (0.41 B, 0.59 D) - χ_2^2 = 12.3, P<0.001.

There was an excess of heterozygotes and a deficiency of BB homozygotes giving relative viabilities similar to the controls in the other experiments.

The overall viability in the EE Conditioned replicates was higher than in the controls (54% on average) and the genotype frequencies differed significantly from the controls ($\chi_2^2 = 39.6$, $P<0.001$). Heterozygotes were still in excess of the Hardy-Weinberg expectation but in these conditioned replicates DD homozygotes were below and BB homozygotes above expectations. The PORT.EE line is fixed for the β inversion sequence - that is the same sequence as is associated with the Adh-D allele. These results are, therefore, comparable with the DD Conditioned replicates of experiments 2 and 3 and show the same effect - a reduction in viability of the like karyotype. Some caution is necessary in this case because of the higher viability of the conditioned replicates. Indeed the genotype frequencies in these replicates do not differ significantly from Hardy-Weinberg expectations ($\chi_2^2 = 3.51$). With an overall viability of less than 60% this is itself unusual, especially with the relatively high number of BB individuals. I feel that the results do demonstrate a genuine effect of conditioning and that this is in the same direction as in the other experiments.

The relative viabilities were:-

Type of replicate	Overall Viability	Relative Viabilities		
		BB	BD	DD
Control	0.28	0.36	1	0.85
EE Conditioned(ββ)	0.54	0.99	1	0.75

e) DISCUSSION The results of the four experiments are summarised below:-

Conditioning Genotype	Change in Overall Viability	Change in Relative Viability		
		BB	BD	DD
BB	↓↓	= ↑	= =	= ↓
BD	↓↑	↑↑	↓ ↓	↓ =
DD(and EE)	↑↓↓	↑↑=	↓↓↑	↓↓↓

↑(↓) represents an increase (decrease) in viability compared with the controls in the combined results of one experiment.
= represents a difference of less than 5% of the expectation.

In general the effect of conditioning was to reduce the overall viability of the test generation by comparison with the controls. This was not an effect of the freezing or flooding procedures which did not give such a reduction. Indeed freezing increased viability in experiment 3. It is possible that a similar effect of freezing caused the high overall viability of the BD conditioned tank in experiment 2. However in experiment 4, where there was no freezing, there was again an increase in overall viability compared with the controls. This could have been due to "facilitation" of some sort but it would be necessary to suppose that the facilitation was either a specific property of the PORT.EE line or that the effect was destroyed by freezing in the other experiments. An alternative, perhaps more plausible, explanation is that there was something wrong with the batch of fresh medium used for the controls. Although every effort was made to keep batches of medium uniform some variation was inevitable if only because the Marinure is itself variable. In the conditioned medium type of experiment the control

and test canisters were necessarily made up with different batches of medium.

The fact that conditioning generally reduces viability demonstrates a stable change in the medium caused by the larvae. This is unlikely to be a physical change because it was not affected by flooding or freezing. It is most likely that the change was chemical or biological. A chemical change could have been either the depletion of a nutrient or nutrients, or build up of a harmful secretion or excretion. It could, therefore, represent either exploitative or interference competition. A further possibility is that the larvae brought about a change in the microflora of the medium. It is known that Coelopa larvae cannot survive in the absence of microorganisms and so a change in the quantity or quality of the microflora could affect the viability of the test generation. Flooding and freezing, especially where dead larvae are present, might also be expected to influence the microflora. However, Rowell (1969) found that a typical larval gut microflora was already developed in two day old larvae. The larvae used in all these experiments were in their second day when transferred to the test canisters and this technique might be expected to minimise the effect of any differences in the microflora of the medium.

The relative viabilities of the three Adh genotypes were also affected by conditioning. Considering the different techniques used in the four experiments there is an encouraging degree of correspondence between the effects observed. On the other hand these differences in method could have influenced the results and so any conclusions should be taken as tentative rather than conclusive.

By comparison with the controls, the test generation viability

of the DD genotype was generally lower, that of the BB genotype higher. The viability of the BD genotype was in most cases lower than expected but in one experiment it was higher. This pattern applies to conditioning by any of the three genotypes but the differences were largest where conditioning was with DD larvae, smallest where BB larvae were used and intermediate with BD larvae. These differences between conditioning genotypes could represent differences in density in the conditioning generation. In experiments 1 and 3 the numbers of conditioning larvae removed were recorded and do not support this suggestion but initial rather than final numbers of larvae would probably have been a better measure of conditioning density. Only in experiment 3 were all three genotypes used successfully at one time and the differences between genotypes could, therefore, reflect differences between experiments. Although in individual experiments there did appear to be evidence for a genotype specific effect, it seems that when the evidence of the four experiments is combined this conclusion is unjustified.

The effect of conditioning can be taken as being the same for all three conditioning genotypes. In this case, on the basis of Collins' observations on changes of viability with increased density, one would expect the BD genotype to have the highest viability in the test generation. While my density experiments (Chapter 5) did not show the same marked increase in heterozygote excess with density the BD genotype nevertheless, had the highest viability at all densities. On either count it is surprising to find that the viability of the BB genotype is increased by conditioning and the viability of the BD and DD genotypes decreased. My experiments did in some cases show an excess of BB individuals

over the Hardy-Weinberg expectation but this was not influenced by density and was most probably due to non-random mating.

In Collins' density experiment the increase in relative viability of the BD heterozygote was accompanied by a switch in the order of relative viabilities of the two homozygotes. Thus at low density the order was BD > DD > BB but at high density it was BD > BB > DD. It is possible that the change in the medium which caused the effect of conditioning was also the cause of this switch in order in Collins' results. The flies used in all four conditioned medium experiments were derived from South Coast populations although those used in the first 3 experiments had been maintained in the laboratory for many generations. This could explain a better fit of these results with Collins' results than with my density results as Collins used flies from Rustington whereas my flies were from St.Mary's Island and Morfa Nefyn. The following interpretation can be put forward:- 1) increasing larval competition causes a stable change in the medium which is probably either depletion of nutrients or build up of harmful products but may be a change in the microflora; 2) this change is independent of the genotype of the larvae but is tolerated best by BB larvae, less well by BD larvae and least by DD larvae; 3) there is an additional effect of larval competition which favours BD larvae and which is not mimicked by these conditioned medium experiments.

This interpretation raises two important questions: what is the nature of the competitive advantage of the BD larvae? and is the effect mimicked by conditioning exploitative or interference competition? In addition the question of genetic differences between inversion sequences from geographically separated populations, which was raised in the previous chapter, may be important here. This

question will be discussed further in the next chapter. The "food-space" experiments described in the next section were an attempt to distinguish between exploitative and interference competition. Three possible explanations for the failure to observe an increase in the BD genotype following conditioning can be considered:-

i) The advantage to the BD larvae occurs in the first two days of larval life and is, therefore, not included in the assessments of viability in these experiments. This possibility is considered in section 6.4.

ii) The advantage to the BD larvae is due to some kind of physical interaction between larvae which may be anything from time wasting following collisions to cannibalism. This type of effect should be detectable in the "food-space" experiments.

iii) The relevant change in the medium caused by competing larvae is unstable. The BD larvae may be more tolerant of a harmful but unstable secretion or excretion of the competing larvae or they may be able to compete better for a nutrient or nutrients which is supplied continuously but at a limiting rate. For example the microorganisms in the medium may produce a vitamin required by the larvae at a limiting rate and the BD larvae may be better at acquiring this vitamin or need less of it. This possibility has not been tested directly.

It is clear from this discussion that the conditioned medium experiments had a number of shortcomings. In particular they did not consider the whole egg to adult period; in all except the last experiment the character of the medium was changed by flooding and/or freezing; in no case were all Adh genotypes used for conditioning in one experiment; the flies used were mainly from

inbred laboratory lines; and flies from different natural populations were not compared. The interesting results obtained despite these problems suggest that a more comprehensive experiment would reward the considerable effort which would be required. With the variety of isogenic lines now available for the conditioning generation and broadly based B+D lines from several populations available for the test generation it should be possible to conduct such an experiment along the general lines of experiment 4. The main modification necessary would be to start each generation with egg batches rather than larvae and preferably to count these eggs. Ideally BDxBD egg batches should be selected for the test generation to avoid possible confusion of non-random mating with viability differences.

6.3 "Food-Space" Experiments

These experiments were intended to distinguish between what appeared to be the three most probable types of larval density effect namely depletion of nutrients, build up of toxic products, or increased contact (decrease in "space") between individuals. To achieve this the volume of medium was varied independently of its nutrient content. If a decrease in the available nutrients gave an effect comparable to increasing density without changing the amount of space available then nutrient availability would be the more important factor. On the other hand if a decrease in space without a decrease in nutrients mimicked increasing density then either larval contacts or concentration of toxins would be important. The experiments are similar to those of Bakker (1961) which demonstrated the importance of exploitative rather than interference competition in Drosophila but in this case the food used was less defined and probably much more complex.

Two experiments have been conducted which will be described together.

a) Methods To achieve the variation in space independent of variation in nutrient content a series of diluted media were devised the constituents of which are given in Table 6.4. Medium 1 was very similar to the standard culture medium. In the other media the amount of Marinure has been reduced to a set fraction of medium 1 for the same final volume and the amount of maize meal has been decreased in proportion. The volume was made up with water and the amount of agar increased to give approximately the same final concentration and thus a similar consistency. It is possible that

Table 6.4

Constituents of "Food-Space" media

Concentration	1	3/4	1/2	3/8	1/4	3/16
Water	560	606	652.5	676	699	711.5
Agar	9	13	16.5	18	20	20
Maize Meal	40	30	20	15	10	7.5
Marinure	160	120	80	60	40	30

all quantities in ml

makes sufficient medium for 4 round canisters

the larvae obtained some nutritive value from the agar, which is a seaweed derivative, and this may have been significant at the lowest marinure concentrations. The 3/8 and 3/16 concentrations were arranged so that a small canister filled to an equal depth with one of these would contain the same amount of marinure and maizemeal as a round canister filled with medium of 1 or 1/2 concentration. The surface area of medium in a round canister is about 3/8 that

in a square canister. Depths were kept the same because if the medium is too deep larvae do not utilize the bottom layer.

The media were made up exactly as for the standard medium (see Materials and Methods) but were allowed to set in large beakers with regular stirring to obtain an even distribution of the solid particles of marinure. Once set the media were "mashed" and then spooned into the canisters using 120±5 g in a round canister or 320±5 g in a small canister.

The replicates in the two experiments were as follows:-

	No. of replicates	Medium	Canister
Experiment 1	4	1	round
	4	3/8	small
	4	3/8	round
Experiment 2	4	1	round
	4	3/4	round
	4	1/2	round
	4	1/4	round
	4	3/8	small
	4	3/16	small

Experiment 1 was started by counting 400 two day old larvae into each replicate canister. These were from eggs laid in a 24 hour period by the flies of the sixth laboratory generation of the PORT.B+D line. Parental allele frequencies were 0.38 B : 0.62 D in a sample of 96 flies.

Experiment 2 was started with five egg batches per canister from the ninth laboratory generation of the SM.B+D line. The parental frequencies were 0.34 B : 0.66 D in a sample of 95 flies.

In both experiments the canisters were kept in a randomised lay out on one shelf in the constant temperature room at 27°C and

adults were collected daily for electrophoresis. In experiment 1 all flies were electrophoresed,in experiment 2 only a sample was electrophoresed where the total from one replicate exceeded 150 flies.

b) Results - Experiment 1 The results of the first experiment are set out in Table 6.5. The overall viabilities were highest in the medium 1, round canister replicates (mean 24%), reduced in the medium 3/8 small canisters (mean 16%), and even lower in the medium 3/8, round canisters (mean 12%). The generally low values were expected from the high starting density. There were no significant differences in genotype frequencies between replicates or between sexes for any of the three sets. In all cases the totals differed significantly from the Hardy-Weinberg expectations based on the parental frequencies (for medium 1, round canisters $\chi_2^2 = 20.58$, $P<0.001$; for medium 3/8, round canister $\chi_2^2 = 48.32$, $P<0.001$; for medium 3/8, small canisters $\chi_2^2 = 9.36$, $P<0.01$) and both sets of replicates with the low concentration medium differed from the replicates on standard medium (for round canisters $\chi_2^2 = 19.43$, $P<0.001$; for small canisters $\chi_2^2 = 7.59$, $P<0.05$). The pattern of differences can best be seen from the relative viabilities (on the assumption that departures from Hardy-Weinberg expectations are due to viability differences alone):-

	Relative Viabilities		
	BB	BD	DD
Medium 1, round canisters	0.97	1	0.60
Medium 3/8, round canisters	0.48	1	0.30
Medium 3/8, small canisters	0.64	1	0.70

Table 6.5

Results of food-space experiment 1

	REPLICATE	MALES			FEMALES			HETEROGENEITY χ^2
		BB	BD	DD	BB	BD	DD	
Medium 1 Round Canisters	1	14	48	16	10	36	17	Male χ_6^2= 6.00
	2	8	11	4	2	15	4	Female χ_6^2= 6.94
	3	13	47	20	13	33	25	
	4	2	12	6	3	16	14	Male vs.Female χ_2^2=4.15
	T	37	118	46	28	100	60	
Medium 3/8 Round Canisters	1	3	18	3	3	18	8	Male χ_6^2= 6.88
	2	4	14	7	1	24	2	Female χ_6^2= 5.64
	3	1	15	2	2	15	5	
	4	5	20	2	1	12	4	Male vs.Female χ_2^2=2.58
	T	13	67	14	7	69	19	
Medium 3/8 Small Canisters	1	3	23	13	1	22	11	Male χ_6^2= 6.43
	2	3	16	8	5	21	12	Female χ_6^2= 4.76
	3	9	21	13	7	24	15	
	4	0	11	5	1	10	8	Male vs.Female χ_2^2=0.30
	T	15	71	39	14	77	46	

There is an excess of heterozygotes in all cases but this is much greater in the medium 3/8, round canister replicates. These replicates had reduced food but equal space compared with the medium 1, round canisters. The other two sets of replicates had similar proportions of heterozygotes (56.0 and 56.5%) but where there was more space (small canisters) the proportion of BB homozygotes was reduced and that of DD homozygotes increased.

c) Results - Experiment 2 The replicates in this experiment fall into two groups: those in round canisters on media 1, 3/4, 1/2 and 1/4 which represent a series of decreasing amounts of food within the same space, and those in small canisters on media 3/8 and 3/16 which have the same amounts of food as media 1 and 1/2 respectively in round canisters but have more space. For simplicity I will refer to each set of replicates by the medium number alone.

The results are presented in Table 6.6. As in experiment 1 overall viability decreases as food decreases: for 1 mean progeny/replicate = 221, for 3/4 mean pregeny/replicate = 178.5, for 1/2 mean progeny/replicate = 33, for 1/4 mean progeny/replicate = 9.75 (as the starting number is not accurately known viabilities cannot be calculated - the number of eggs per canister was probably about 350-400). The viabilities in 3/8 and 3/16 are rather lower than those in 1 and 1/2 respectively which had the same amount of food: for 3/8 mean progeny/replicate = 102, for 3/16 mean progeny/replicate = 29.

There was significant heterogeneity between replicates for genotype frequencies in both 1 and 3/4 sets and in 3/4 there was also a significant difference between males and females (Table 6.6). Some heterogeneity between replicates was expected because of the effect of selecting five egg batches for each replicate as in the

density experiments described in Chapter 5. The results have, therefore, been combined despite these differences but bearing in mind that this procedure reduces the weight of the evidence from these results.

In all cases the genotype frequencies differed from the Hardy-Weinberg expectations, having a slight deficiency of BB, an excess of BD and a large deficiency of DD individuals. The differences were significant ($\chi_2^2 \geq 15.2$, $P<0.001$) in all cases except 1/4 ($\chi_2^2 = 3.65$, $P>0.1$) where the numbers were small.

In the series of replicates with decreasing amounts of food in the same volume (1, 3/4, 1/2, 1/4) only the 3/4 set differed significantly from the standard conditions (set 1) (for 3/4 $\chi_2^2 = 9.38$, $P<0.01$; for 1/2 $\chi_2^2 = 0.81$, $P>0.2$; for 1/4 $\chi_2^2 = 1.54$, $P>0.2$). This difference was due to an excess of BB individuals contributed mainly by the females and particularly by replicate 3. Because of the heterogeneity within this set of replicates little can be concluded from this result. It probably indicates that, by chance, several BBxBB egg batches were included in this replicate at the start of the experiment.

The 1/2 and 1/4 replicates show an increase in heterozygote excess as expected from experiment 1 although the differences are not significant. In the 1/2 series both homozygotes show reduced viabilities but in the 1/4 series BB is reduced and DD increased. This increase in DD is due almost entirely to the females of the 1/4.2 replicate.

The 3/8 and 3/16 replicates do not differ significantly from the 1 and 1/2 replicates respectively which had the same amounts of food but different volumes (for 1 and 3/8 $\chi_2^2 = 0.75$, $P>0.2$; for 1/2 and 3/16 $\chi_2^2 = 0.16$, $P>0.2$).

Table 6.6

Results of food-space experiment 2

MEDIUM	REPLICATE	MALES BB	MALES BD	MALES DD	FEMALES BB	FEMALES BD	FEMALES DD	HETEROGENEITY χ^2
1	1	10	23	7	7	15	10	Males χ_6^2=20.1 P<0.01
	2	5	29	9	0	18	11	Females χ_6^2=11.1
	3	1	18	17	2	23	11	
	4	1	24	9	3	25	10	Males vs.Females χ_2^2=0.7
	T	17	94	42	12	81	42	
3/4	1	3	12	14	8	16	19	Males χ_6^2=13.4 P<0.05
	2	3	26	3	6	33	5	Females χ_6^2= 24.1 P<0.001
	3	2	29	12	12	11	6	
	4	4	22	10	7	21	8	Males vs.Females χ_2^2=9.7
	T	12	89	39	33	81	38	P<0.01
1/2	1	0	10	3	3	14	3	Males χ_6^2=11.6
	2	0	10	5	0	11	7	Females χ_6^2=6.3
	3	4	6	3	2	15	4	
	4	1	12	5	2	6	4	Males vs.Females χ_2^2=0.1
	T	5	38	16	7	46	18	
1/4	1	0	2	2	1	7	1	Males χ_6^2=
	2	0	4	0	1	5	9	Females χ_6^2=8.4
	3	0	1	0	0	1	2	
	4	0	1	0	0	2	0	Males vs.Females χ_2^2=2.6
	T	0	8	2	2	15	12	
3/8	1	6	29	16	10	25	15	Males χ_6^2=6.0
	2	4	26	15	1	22	11	Females χ_6^2=8.8
	3	10	40	12	9	44	19	
	4	1	19	9	2	26	14	Males vs.Females χ_2^2=0.2
	T	21	114	52	22	117	59	
3/16	1	1	6	3	3	6	5	Males χ_6^2=5.5
	2	1	12	5	1	12	4	Females χ_6^2=6.4
	3	3	7	3	2	12	3	
	4	0	5	5	0	11	3	Males vs.Females χ_2^2=0.6
	T	5	30	16	6	39	15	

For comparison with experiment 1 the data can be summarised as relative viabilities, assuming that all deviations from Hardy-Weinberg expectations are due to differences in viability:-

Medium	Relative Viability		
	BB	BD	DD
1	0.64	1	0.49
3/4	1.03	1	0.47
1/2	0.56	1	0.42
1/4	0.34	1	0.63
3/8	0.72	1	0.50
3/16	0.60	1	0.45

d) Discussion Considering first the overall viabilities in the two experiments there are close similarities. In both cases restriction of the amount of food available per larva without changing the volume of medium caused a marked reduction in viability. It should be noted that in addition to reducing the total amount of food available supplying a diluted medium also makes food more difficult to obtain because a larger volume has to be processed for the same nutritive value. Nevertheless this reduction in viability is indicative of food limitation rather than interference.

Supplying larvae with an equal amount of food but in a larger space also gave a reduction in viability in both experiments, but not such a large reduction as reduced food. Again this is evidence for the importance of food in competition because had interference, either chemical or physical, been important an increased viability would have been expected in these replicates. The observed reduction in viability might be the effect of reduced food availability in the dilute medium.

So competition between larvae appears to be principally exploitative

as in Drosophila but how does this affect the relative viabilities of the different genotypes? Here the similarity between the results of the two experiments ends except that in both cases under standard conditions there is a higher frequency of B than expected. This is most likely to be due to non-random mating as in the density experiments of the previous chapter but does not invalidate comparisons between treatments as the effect should be the same in all cases.

In experiment 1 the decrease in viability with reduction in food was accompanied by a significant increase in heterozygote success. In the standard conditions the proportion of heterozygotes was comparable with Collins' Low density replicates and in the reduced food with his Medium density replicates. This experiment used flies from a South Coast population (Portland) and was started with two day old larvae. The advantage to the BD genotype does not, therefore, appear to be restricted to the first two days of larval life or to be due to physical interactions between larvae. Of the suggestions made in the previous section this leaves only the alternative of an unstable change in the medium. This experiment indicates that food limitation is important rather than harmful excretions and so the combined evidence points to a limiting rate of production of some unidentified resource.

The "increased space" replicates of experiment 1 support this conclusion as the proportion of heterozygotes was unaffected. There was, however, a significant increase in viability of DD relative to BB homozygotes. This is the reverse of the effect of conditioning the medium and suggests that the effect of conditioning is the build up of some harmful substance which in these, increased volume, replicates is diluted. This conclusion is based on just four

canisters whose combined results differed from the controls at the 5% level of probability. It must, therefore, be regarded as speculative until it can be confirmed directly.

The second experiment did not show these effects. Indeed there were no significant differences attributable either to reduction in food or increase in available space. The experiments differed in two important respects. The first used two day old larvae, the second egg batches; and the first used the Portland B+D line while the second used the St.Mary's Island B+D line. It is difficult to see how the use of egg batches could abolish the effects on relative viability unless there was massive mortality in the first two days so that from then on there was very little competition. Although observations of the experimental canisters suggested quite heavy mortality early in larval life it seems unlikely to have been sufficient to remove all the competition at later stages. There was certainly some mortality later on in larval life and probably also in the pupal stage.

A more plausible explanation of the lack of change in genotype frequencies in the second experiment is the use of a different population of flies. The first experiment used flies from a South Coast population (Portland) as in Collins' density experiment (Rustington) whereas the second experiment used flies from St.Mary's Island on the North East Coast - the same laboratory population as failed to show an increase in heterosis with density in my experiments described in the previous chapter. It seems increasingly likely that the α and β inversion sequences contain different coadapted complexes of genes in the South Coast and North East Coast populations and that it is only the combination of genes in the South Coast population that confers a competitive advantage on the heterokaryotype.

Nevertheless there is a consistent excess of heterozygotes in all the experiments involving the SM B+D line which seems likely to be due to a viability difference. The next section investigates the possibility of viability differences in the crucial early days of larval life.

6.4 "Time Course of Mortality" experiments

This set of experiments had two initial objectives: first to establish the stage of larval life at which differential mortality occurred, and second to ascertain whether mortality in the first two days of larval life could explain any of the differences between experiments started with egg batches and those started with larvae. If the increased selective mortality observed at high larval density is due to competition for food or some other resource, or if it is due to build up of harmful products in the medium, then, at first sight, one would expect the mortality to be in the late stages of larval growth. In Bakker's (1961) competition experiments with *Drosophila* mortality was almost entirely due to starvation of third instar larvae after the food had been used up or to unsuccessful pupation below the minimum survival weight. It seems unlikely that this is the case in *Coelopa* in view of the size and development time relationships discussed in the previous chapter.

Two types of experiment have been conducted. In the first a high density culture was established and then sampled at intervals during larval growth. The samples were grown on at greatly reduced density until large enough for electrophoresis or until adult and the genotype distributions at the various time points were compared. The second type of experiment involved taking second day larvae and growing them on at different densities.

a) Methods The first experiment (designated Time Course 1) was started with adult flies from the eighth laboratory generation of the PORT.B+D line with parental frequencies of 0.41 B and 0.59 D in a sample of 96 individuals. Over 2000 flies were allowed to lay eggs for 24 hours in a standard tank culture (BC1) and then transferred to another tank for a further 24 hours (BC2). These two tanks were kept together in the constant temperature room at 27°C. On the second, fourth, sixth and eighth days after egg laying a scoop of medium and larvae in a mating pot was moved from the tank into each of four round canisters containing 3 cm depth of fresh culture medium.

The first pupae appeared in BC1 and BC2 on the eighth day. Thereafter no further larval samples were taken but all flies were removed daily and stored at -20°C. When no further flies emerged they were pooled and a sample of 192 used for electrophoresis.

The larval samples were maintained at 27°C until the larvae were large enough for electrophoresis and then a sample of 48 was removed from each round canister and stored until required.

The "Time Course 2" experiment was essentially similar in design except that the adults used were the progeny of a mass cross between the SM DD Mixed line and the SM/MN BB Mixed line. The genotypes of the progeny were, therefore, expected to be 1 BB : 2 BD : 1 DD. Only one tank was used and the larvae were counted out into each sample canister on days 2, 4 and 6 at 50 larvae per canister. On day 8 the usual sample of 200 larvae was taken in four lots of 50 but these were already large enough for electrophoresis and were frozen immediately.

For the third experiment (Time Course 3) over 2000 flies of the seventh laboratory generation of the SM B+D line were allowed to

lay eggs in a tank for 24 hours. Parental gene frequencies were 0.40 B and 0.60 D in a sample of 96 flies. On the second day 100 larvae were counted out, one larva to a mating pot half filled with fresh medium (Singles). Further larvae were then counted into round canisters of fresh medium - three replicates at 100 larvae per canister and three at 400 larvae per canister. The single larvae were kept at 27°C until large enough for electrophoresis. The canisters were also kept at 27°C and all flies emerging were collected daily and electrophoresed.

Finally (Time Course 4) a low density tank culture was established by allowing 100 pairs of adults, from the BD flies used to start the Time Course 2 experiments, to lay eggs for 24 hours. On the second day 50 larvae were counted into each of four round canisters. These canisters were kept at 27°C until the larvae were large enough for electrophoresis. The adults emerging from the tank were collected daily and a sample of all those that emerged was electrophoresed.

b) Results The results of the Time Course 1 experiment are presented in Table 6.7. In no case was there significant heterogeneity between replicates but in all sets of replicates and in the adult samples the observed values differed significantly from Hardy-Weinberg expectations based on the parental frequencies (except BC2, day 8, where $\chi_2^2 = 5.47$, $P<0.10$). The most striking feature of the results is that the proportion of heterozygotes is already in excess of expectation in the day 2 samples and changes hardly at all through the other larval samples or to the adult sample. This is true in both cultures. In BC1 there was significant heterogeneity between the sets of larval samples ($\chi_6^2 = 17.1$, $P<0.01$) but not in BC2 ($\chi_6^2 = 9.2$, $P>0.10$). In neither case did the adult

sample differ significantly from the day 8 set of samples (for BC1 $\chi_2^2 = 4.2$, $P>0.10$; for BC2 $\chi_2^2 = 1.1$, $P>0.20$). What difference there was between larval samples was mainly in the proportions of the two homozygotes. BB was less, and DD more common in the day 4 and day 6 samples than in the day 2 and day 8 samples from both BC1 and BC2. The high value of BB for day 8 may have been due to the fact that pupation had already begun and DD larvae are known to start pupation first. The fact that the adult BB frequency was lower than the day 8 frequency supports this interpretation. The drop in BB frequency from day 2 to day 4, especially in BC1, could have been due to selective mortality at this stage in the base cultures, or to differences between the day 2 sample canisters and the other sets of samples.

The heterozygote excess in the adults, when compared to the Hardy-Weinberg expectation, was low - 2% in BC1 and 9% in BC2 - the main difference being a reduction in B frequency compared with the parents. This suggests that the larval density was also low. Nevertheless it is clear that much of the difference between parents and adult offspring was already present in the day 2 samples. There are several possible explanations of this result:- 1) density was not affected by transfer to the sample canisters, 2) there was non-random mating, 3) there was significant selective mortality in the first two days of larval life, or 4) differences between genotypes developed in the first two days which determined their subsequent competitive ability. In the Time Course 2 experiment where the parents were all Adh BD, non-random mating could not have been involved. In both experiments there was the additional possibility of gametic selection distorting the Mendelian ratios within egg

Table 6.7

Results of Time Course 1 experiment

DAY	REP.	BC1 BB	BD	DD	χ^2	BC2 BB	BD	DD	χ^2
2	A	10	24	14		5	27	16	
	B	6	32	10		1	25	22	
	C	5	28	15		0	28	20	
	D	7	28	13		7	22	19	
	T	28	112	52	(i) 4.2 (ii) 7.74*	13	102	77	(i) 11.9 (ii) 13.64**
4	A	2	25	21		0	26	22	
	B	4	22	22		3	27	18	
	C	3	28	17		3	26	19	
	D	3	26	19		2	30	16	
	T	12	101	79	(i) 2.1 (ii) 15.34***	8	109	75	(i) 4.4 (ii) 21.71***
6	A	4	24	20		6	27	15	
	B	4	28	16		3	24	21	
	C	2	23	23		2	27	19	
	D	4	26	18		7	25	16	
	T	14	101	77	(i) 2.8 (ii) 12.31**	18	103	71	(i) 5.3 (ii) 7.44**
8	A	5	31	12		6	27	15	
	B	5	25	18		8	29	11	
	C	9	25	14		4	29	15	
	D	4	28	16		4	22	22	
	T	23	109	60	(i) 4.8 (ii) 6.02*	22	107	63	(i) 7.2 (ii) 5.47
ADULT		18	95	79	(ii) 8.32*	18	100	71	(ii) 6.53*

(i) χ^2 values for heterogeneity between replicates with 6 degrees of freedom. In all cases P*0.05.

(ii) χ^2 values for comparison with Hardy-Weinberg expectations based on parental frequencies and with 2 degrees of freedom.
* P<0.05, ** P<0.01, *** P<0.001

batches. A precedent for such an effect exists in the "Sex Ratio" inversions on the X chromosome of Drosophila pseudoobscura (Wallace 1948).

The results of the Time Course 2 experiment are presented in Table 6.8. Again there was no significant heterogeneity between replicate samples but all larval samples and the adult sample differed significantly from the 1:2:1 expectation. There were differences between days ($\chi_6^2 = 42.8$, $P<0.001$) and the adults were significantly different from the day 8 larvae ($\chi_2^2 = 6.7$, $P<0.05$). The similarities between this experiment and Time Course 1 are striking. There was a 16% excess of heterozygotes on day 2 which was very little changed through to the adults except for an anomalous result on day 4 when there was a 4% deficiency (on days 6 and 8 and in the adults the excess was 13%). The excess was greater throughout than in the previous experiment suggesting a higher larval density but as before the full difference was already apparent in the day 2 samples.

There were more BB and less DD individuals than expected on day 2 but from then on through to the adults BBs declined and DDs increased in proportion. This effect was very marked indeed - the parental Adh-B frequency was, of course, 0.50, on day 2 it was 0.56 and in the adult progeny it was 0.30. The changes in proportions of the homozygotes were much smaller in the previous experiment but were in the same general direction of decreasing BB.

The much larger changes in this second experiment compared to the first may have been due to a higher larval density. This was not measured although similar numbers of flies were used to start the experimental cultures. It is also possible that the differences were due to the different stock lines used in the two experiments

Table 6.8

Results of Time Course 2 experiment

DAY	REP.	BB	BD	DD	T	χ^2
2	A	11	30	8	49	
	B	13	26	7	46	
	C	13	27	6	46	
	D	13	25	8	46	
	T	50	108	29	187	(i) 0.97 (ii) 9.24**
4	A	6	13	15	34	
	B	4	23	11	38	
	C	7	15	16	38	
	D	5	18	13	36	
	T	22	69	55	146	(i) 4.9 (ii) 15.29***
6	A	3	21	13	37	
	B	4	23	12	39	
	C	3	19	13	35	
	D	7	20	9	36	
	T	17	83	47	147	(i) 3.7 (ii) 14.71***
8	A	4	24	16	44	
	B	3	22	20	45	
	C	5	33	9	47	
	D	4	29	22	55	
	T	16	108	67	191	(i) 7.7 (ii) 30.50***
ADULT		3	81	60	144	(ii) 47.38***

(i) χ^2 values for heterogeneity between replicates with 6 degrees of freedom. In all cases $P>0.05$

(ii) χ^2 values for comparison with expected 1:2:1 ratio and with 2 degrees of freedom.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$

(PORT B+D in Time Course 1, SM DD and SM/MN BB Mixed in Time Course 2) or to the fact that the starting gene frequencies were different and it may be that viabilities are related to allele frequencies. The data do not distinguish between these possibilities.

In this second experiment the densities in the sample canisters were known and were the same at each time point. Mortality between sampling and electrophoresis was 6.5% in the day 2 samples and 26% in the day 4 and day 6 samples. This mortality in the day 2 samples cannot explain the observed departure from the 1:2:1 expectation and it, therefore, seems unlikely that either the first or fourth of the possible explanations put forward for the results of the first experiment can apply. As the explanation based on non-random mating cannot apply here either, the conclusion must be that there was significant selective mortality in the first two days of larval life.

This conclusion applies to the excess of heterozygotes and indeed the early selective mortality apparently explains all of the observed excess in the adult progeny. That is after the first two days the BDs no longer have an advantage.

There was, in addition to the heterozygote excess, a tendency for the DD homozygotes to increase relative to the BB homozygotes in this second experiment. This change occurred progressively through larval life and, therefore, appears to have a different basis from the heterozygote excess.

The extent to which these conclusions can be extended to the results of experiment 1 is unclear because of the different lines used and the possible differences in density both of the base cultures and the sample canisters. In view of the results of previous sections it seems not unlikely that lines based on South Coast populations

Table 6.9

Results of Time Course 3 and 4 experiments

EXPERIMENT	REP.	BB	BD	DD	TOTAL	χ^2(i)	χ^2(ii)	χ^2(iii)
TC3								
SINGLES		18	51	12	81		15.8***	
100	1	9	37	6	52		14.44***	1.52
	2	8	31	16	55		1.60	9.53**
	3	17	36	6	59		19.14***	2.03
	T	34	104	28	166	11.5*	26.38***	0.70
400	1	22	67	15	104		21.03***	0.10
	2	13	57	14	84		15.62***	2.26
	3	17	67	20	104		13.96***	3.02
	T	52	191	49	292	1.9	48.95***	3.59
TC4	1	4	25	13	42			
	2	7	22	13	42			
	3	15	17	7	39			
	4	12	21	7	40			
	T	38	85	40	163	12.7*		
ADULTS		26	74	32	132			

(i) χ^2 values for heterogeneity between replicates and have 4 degrees of freedom in TC3, 6 degrees of freedom in TC4.

(ii) χ^2 values for comparison with Hardy-Weinberg expectations based on parental gene frequencies with 2 degrees of freedom.

(iii) χ^2 values for comparison with SINGLES with 2 degrees of freedom

* P<0.05, ** P<0.01, *** P<0.001

will behave differently from those based on North East coast populations. However the results of the two experiments do indicate a broadly similar pattern, that is an excess of heterozygotes present in the day 2 samples and unchanged thereafter, plus a progressive increase in DD relative to BB homozygotes.

Is the early advantage to the BD genotype dependent on larval density, and if so is this the only density dependent effect? The third and fourth experiments were intended to answer these questions. The results of both these experiments are given in Table 6.9.

Out of the 100 day 2 larvae set up in individual pots in the Time Course 3 experiment, 81 grew to a large enough size for electrophoresis. In these 81 the genotype frequencies differed significantly from the Hardy-Weinberg expectations based on the parental frequencies ($\chi_2^2 = 15.8$, $P<0.001$). As in the previous experiments there was a large excess of BD, a small excess of BB, and a deficiency of DD individuals but in this case it is quite clear that the selective mortality occurred in the two days before sampling not subsequently. Each larva had an excess of food and space and no possible interaction with other larvae from the second day onwards.

There was significant heterogeneity between replicates in the canisters with 100 larvae ($\chi_4^2 = 11.5$, $P<0.05$) but not in those with 400 larvae. All individual replicates differed significantly from Hardy-Weinberg expectations but did not differ from the genotype frequencies in the Single larvae with the sole exception of replicate 100/2. The totals for the two sets of replicates also differed from Hardy-Weinberg expectations but not from the Single larvae.

The excess of BD individuals in the single larvae, compared with the Hardy-Weinberg expectation, (31%) was maintained with little

change in the 100 and 400 sets of canisters (30% and 36% respectively). It was much higher throughout than in the previous experiments possibly because the larval density in the base tank was very high indeed. As in the previous experiments there seems to have been a drop in proportion of BB and rise in DD individuals after the first two days of larval life. In this case it was only slight - 22% BB in the singles, 20% in the 100 replicates and 18% in the 400 replicates - but it was greater in the 400 than the 100 replicates suggesting that it is affected by larval density.

These results confirm the conclusions of the previous experiments and also demonstrate that much larger excesses of heterozygotes can be generated in the first two days of larval life and that the excess of heterozygotes is unaffected by larval competition after this critical period.

To confirm that density affects the mortality in early larval life the Time Course 4 experiment used a very low density base culture but was otherwise identical to the Time Course 2 experiment. Only the day 2 samples were taken. The results are presented in Table 6.9. There was significant heterogeneity between the four replicates although the χ^2 value only just exceeds the 5% level. This is probably due to overrepresentation of individual egg batches in the replicates. The larvae were very clearly clumped in the very low density tank culture and an effort was made to use only a few larvae from each clump in each canister, however it is quite possible that any one canister contained more larvae from one clump than another. The results were, therefore, combined. The totals do not differ significantly from 1:2:1 ($\chi_2^2 = 0.35$, $P>0.2$) nor do the frequencies in the adult sample ($\chi_2^2 = 2.48$, $P>0.2$) and the adult

and day 2 samples do not differ ($\chi_2^2 = 0.65$, $P>0.2$) although the proportion of BB is lower in the adults. This result suggests that selective mortality in the first two days of larval life is density dependent and is, therefore, likely to be the result of competition between larvae. Density dependence is not predicted by the gametic selection explanation mentioned above. However it should be noted that the genotype frequencies in day 2 larvae in this experiment and in the time-course 2 experiment, which was directly comparable except in having a higher initial density, do not differ significantly ($\chi_2^2 = 4.5$, $P>0.1$). Further experiments with variable initial numbers of larvae would be desirable to confirm this effect of density.

The time-course 3 and 4 experiments both used flies from lines derived from North East Coast populations and their results should, therefore, be compared with those of the time-course 2 experiment and previous experiments using North East Coast lines rather than with the time-course 1 experiment.

c) Discussion The four Time Course experiments give a consistent indication of the importance of the first two days or so of larval life. By the time larvae reach this age there has already been considerable selective mortality which is density dependent and which favours the BD heterozygote. It seems certain that this is a viability difference not a hatching difference as previous workers have consistently reported very high hatch rates for Coelopa in the region of 98-99% (Collins 1978, Thompson 1951). The fact that the mortality appears to be density dependent suggests some form of competition between larvae. Clearly the absolute amounts of nutrients are not limiting at this stage but amounts of nutrients available to

small larvae may be, or there may be some other, less obvious, limiting resource. Very small larvae always feed on the surfaces of the medium and seem unable to burrow into it. This is probably the reason for the improved performance of cultures in which the medium surface is forked up before introducing flies. Perhaps suitable surface feeding sites are the limiting resource. A clue to the importance of the first days of larval life was given by Rowell (1969). Newly hatched larvae do not have the characteristic gut microflora of older larvae but this develops by the end of the second day of larval life. If sterile eggs are placed on sterile medium the larvae hatch but fail to develop a gut microflora and die within two to three days of hatching. Rowell concluded that Coelopa larvae were unable to utilise some essential component of their food in the absence of microorganisms in the gut or in the medium. Both yeasts and bacteria are present but which are required is not known, and the food component(s) involved has not been identified.

In my experiments the limiting resource for early larvae could have been microorganisms themselves or their products. Transfer of larvae on day 2 to fresh medium could then represent a serious set-back to the larvae unless they have developed an adequate gut flora. It may be that the conditions in natural wrack beds are very different in this respect because egg batches are not usually laid in the field until decomposition of seaweed is already advanced. This could mean that a pool of available nutrients has built up before the larvae hatch. It will not be possible to examine these possibilities until the relationship between Coelopa larvae and the wrack bed microflora has been studied in much greater detail and the nutritional requirements of the larvae are known.

Later in larval life when, presumably, different limiting factors become important BB larvae suffer the highest mortality and DD the lowest. This effect varies between the experiments - it is much more marked in Time Course 2 than in Time Course 1. This may reflect a dependence on larval density but it could also be due to the higher initial BB frequency in Time Course 2 - that is selection against BB at this stage may be frequency dependent, or it could be due to differences between the natural populations from which the stocks used in these experiments were derived. In these experiments the proportion of BD individuals remained more or less constant during the later days of larval life indicating that BD mortality was close to the average. This seems to have been true irrespective of larval density in this period and of the level of heterozygote excess on day 2.

6.5 General Discussion

The different types of experiment reported in this chapter have consistently indicated the importance of two effects:

(i) Mortality in early larval life

(ii) Limiting rate of supply of nutrients

There have also been indications of two other effects:-

(iii) Differential mortality in later larval life between the homozygotes and probably due to build up of waste products

(iv) Differences in the results obtained from stocks derived from geographically distinct populations.

Several experiments had indicated the possibility that the first one or two days of larval life were important. This was a possible explanation for the negative results of the larva-to-adult viability experiments, the core samples from the experimental wrack bed and

the first three conditioned medium experiments. The effect was confirmed by the time course experiments. Most of the heterozygote excess observed in late larvae and adults is already present on the second day of larval life although in some cases, probably at high densities, the proportion of heterozygotes increases further after this. The advantage to heterozygotes at this early stage is density dependent.

Two results appear to contradict this conclusion. Firstly if this is the reason for the failure of the larva-to-adult experiment to show density dependent heterozygote advantage then why did the egg-to-adult experiments not show this effect? Secondly in the Food Space experiments the effects were actually more marked when the trials were started with two day old larvae than when they were started with eggs. The explanations for these discrepancies might be the same because in both cases where eggs were used they were collected from segregating populations and their numbers were not accurately known. This means that in these experiments there was considerable variation between replicates in initial densities and in genotype frequencies making it more difficult to detect viability differences. In addition where eggs are moved to start an experiment the larvae hatch onto much fresher medium than usual and medium which has not been infected with microorganisms by contact with adult flies. These effects could lead to considerable random mortality around the time of hatching which would further reduce the chances of detecting differences between treatments.

The conclusion that larvae compete for nutrients which are supplied at a limiting rate is based primarily on the first Food Space experiment combined with the conditioned medium results. In the Food Space experiment a reduction in total food was accompanied

by a decrease in overall viability and an increase in the relative viability of the heterozygotes. This suggests that competition was for a limited nutrient or nutrients. However, if that was the case then the conditioned medium results should have shown an excess of BD adults from the test generations, which they did not. The best explanation would seem to be that a nutrient or nutrients is made available to the larvae, presumably by microbial activity, at a limiting rate. This suggests a mechanism that would lead to the early selective mortality being density dependent. It is difficult to envisage competition amongst very young larvae for a resource which is in limited supply, but it is clearly possible that they might compete for a resource which is only being produced or released slowly. Possibly at this stage the microflora of the seaweed or marinure is poorly developed and so the supply of nutrients is even more limited than at a later stage. Alternatively the lack of an established gut microflora might make early larvae more dependent on external microorganisms and thus make competition between larvae more intense.

Competition for a resource which is being supplied at a limiting rate does not fall into either of the usual categories of exploitative and interference competition. The competition is exploitative in the sense that competition is for a limited resource but its effects are more like those of interference competition in that an individual's rate of feeding and rate of growth are slowed down by the presence of other larvae. Unlike the classic case of exploitative competition (Bakker 1961) individuals do not necessarily die at or around the time of pupation when the food supply is exhausted, but may also die at other times if they are unable to obtain sufficient nutrients to

maintain their metabolic activities or minimum rate of growth.

Adaptations to cope with this type of competition might be expected to fall into two general classes: 1) adaptations to minimise the basal requirement for nutrients or to survive starvation, and 2) adaptations to maximise the rate of acquisition of the limiting resource. In practice (2) would probably be achieved by increasing the rate at which the food medium is processed either by feeding more rapidly or by being larger than other larvae. Being larger than competing larvae is likely to be most easily achieved by starting larval life earlier but the advantage of an early start will be limited by the fact that earlier and earlier larvae will find a smaller and smaller microflora available in the food. The BD genotype (α/β karyotype) might have an advantage in either or both of these respects, perhaps due to an intermediate phenotype, and have a higher survival than the homozygotes especially in the early days of larval life when competition is most severe.

In the context of competition (or facilitation) between larvae in the first day or so after hatching, it may be interesting to note that C. pilipes eggs are laid singly, in contrast to the egg batches of C. frigida. C. pilipes larvae may be more tolerant of low density and this could be a result of different nutritional requirements, or perhaps later egg laying when seaweed decomposition is more advanced. In view of the correlation between Adh-B frequency and C. pilipes frequency (Chapter 3) and the possibility of competitive interactions between the two species (Chapter 5), it would be interesting to know more about differences between their ecologies.

The idea of a limited supply of nutrients due to the state of the microflora helps to explain some other observations. Burnet and Thompson (1960) and Rowell (1969) found that at low densities there

is some "facilitation", that is the viability increases with density. This could be because the activities of Coelopa larvae increase the microbial population by making more surfaces available and spreading microbes through the seaweed. Drosophila larvae have this sort of effect on yeast populations (Weisbrot 1966). Clearly the possibilities for interactions between larvae and microorganisms make the effects of competition potentially much more complex and the design of experiments like the conditioned medium and food space experiments more difficult.

The hypothesis put forward in the previous chapter to explain the effect of density on development time and adult size required that the growth rate was dependent on the density. This idea will be further extended in Chapter 9 where the effect on growth rate is assumed to be a result of interference competition. The suggestion that competition is for a resource supplied at a limiting rate is consistent with these hypotheses.

The various experiments in this chapter are all consistent with the idea of a limiting rate of supply which is most stringent in the first few days of larval life. In populations where large numbers of larvae survive the early period the supply may be limiting at later stages, as in the Time Course 3 experiment perhaps. In the Food Space experiments the complex interaction between the larvae and microorganisms was further disturbed by altering the volume of medium containing a given amount of nutrient. The reduced viability in replicates with equal nutrient in a larger volume may have been a reflection of this effective reduction in nutrient availability.

I believe there is considerable potential for further research

in this area. At present the microflora of decomposing seaweed is very poorly known. It is not known whether Coelopa requires a specific organism or group of organisms or what it is that this organism provides which the larvae are otherwise unable to obtain. Rowell (1969) tackled these questions but made only very limited progress. At the same time it is still not at all clear what properties of the BD genotype (α/β karyotype) give it its competitive advantage. A study of hatching times and growth rates may be helpful here and would also clarify the relationship between size and development time.

Competition for a limiting nutrient supply may be the basis of the advantage of the BD heterozygote over the two homozygotes but some of the results in this chapter suggest that larvae might interact in other ways as well. In particular conditioning reduced the viability of DD larvae much more than BB larvae and in the low concentration replicates of the first Food Space experiment the viability of BB homozygotes was lower relative to DD than in the controls. These results suggest that waste product accumulation in the medium can affect viability and that BB homozygotes are more tolerant of this than DD homozygotes with heterozygotes intermediate. However this effect was not observed in the second Food Space experiment and in the Time Course experiments there was an indication of the opposite effect, that is a better survival of DD than BB homozygotes in later larval life. Further experiments will be needed to clarify this discrepancy.

In this chapter and the previous one a possible explanation for some of the apparently discordant results has been the existence of different coadapted gene complexes in the various geographical populations used. This type of explanation may still be appropriate

for some sets of results, especially the egg-to-adult viability experiments and the second Food Space experiment. These experiments used flies from the St.Mary's Island population, and the Morfa Nefyn population in the viability experiments, whereas the first Food Space experiment used flies from Portland and Collins' viability experiment used flies from Rustington. The general impression from these results is that viability differences between genotypes are greater, and more strongly influenced by density, in South Coast populations (Rustington, Portland and Lulworth - from which the BB(lab) and DD(lab) lines were derived) than in the St.Mary's Island or Morfa Nefyn populations. However as no direct comparisons have been made the evidence for this difference is weak. If it is a genuine difference the most plausible explanation is that Coelopa colonies, or at least the "regions" of the South, North East and West Coasts, are sufficiently isolated genetically for the evolution of distinct coadapted gene complexes and that these genetic differences affect the relative viabilities of the Adh/inversion genotypes in competitive conditions. If such differences between populations exist they should be detectable in interpopulation crosses - the results of such crosses are the subject of the next chapter.

The results of the previous two chapters have all been reported in terms of genotypes at the Alcohol dehydrogenase locus because this was the form in which they were obtained. However the B and D alleles at this locus are completely associated with the α and β arrangements of chromosome I. This means that in all except the field experiments there was no way of distinguishing between effects of the Adh locus itself and effects of the inversion or indeed of loci in linkage disequilibrium with either Adh or the inversion. In

practice the distinction between these possibilities is of little relevance to the maintenance of the polymorphism in natural populations because of the complete association between allozymes and sequences. The distinction is relevant where the Adh-C allele is involved because the presence of an alternative allele on each chromosome type allows variation in Adh-B and D frequencies independently of the inversion frequencies. It was intended that the crosses to be discussed in the next chapter would help to separate any effects of the Adh locus from those of the inversion.

Chapter 7

Coadaptation

7.1 Introduction

Two questions have been raised in preceding chapters - 1) do the same gene arrangements from different populations contain similar or different coadapted sets of genes? and 2) does the alcohol dehydrogenase locus make any direct contribution to the selection acting on the inversion? The experiments reported in this chapter are a preliminary attempt at answering these questions.

In effect the first question is similar to that asked by Dobzhansky (1950) with Drosophila pseudoobscura when attempting to explain the indeterminate outcome of population cage experiments using chromosomes derived from different populations (see Introduction for a description of Dobzhansky's experiments). I have made comparable crosses within and between populations and gene arrangements including control crosses in which heterokaryotypic flies are generated carrying α and β sequences from the same population but in a mixed background. These crosses were made using inbred lines fixed for an arrangement on chromosome I and for an Adh allele which made it possible to look for effects of different Adh alleles in alternate karyotypic combinations.

The relevance of the Adh locus and the existence of coadaptative differences between populations are both linked to Wasserman's (1968) distinction of three levels at which selection can act to maintain an inversion polymorphism. These are the levels of genic dominance or overdominance, chromosomes or "supergenes" as described in the Introduction (Chapter 1). My crosses are similar to those used by

Wasserman to test the distinction between the genic and chromosomal levels in Drosophila subobscura (Wasserman 1972) and D. pseudoobscura (Wasserman & Koepfer 1975). I have, therefore, been able to conduct a similar analysis on my results, although my crosses cannot distinguish supergenic selection.

This extensive experiment was carried out towards the end of the period available to me for practical work. It therefore suffers from some limitations which were essential if it was to be attempted at all. Firstly the choice of populations was dictated by the flies available at the time. The Morfa Nefyn population has been compared with the St.Mary's Island population whereas, from choice, a South Coast population, ideally Rustington or Portland, would have been included. Secondly the number of lines derived and crosses made was limited. In some cases this had led to underrepresentation of important groups. Nevertheless the results are potentially important if only because there is no comparable analysis outside the genus Drosophila.

7.2 Methods

The strains used in these experiments were the "isogenic lines" whose derivation was described in Chapter 2. These strains were fixed for Adh alleles which were identical by descent. However they were not necessarily fixed for all loci within the inversion because of the possibility of recombination in homokaryotypes in the crosses required to achieve fixation for Adh. 16 strains were derived from a collection of flies taken at St.Mary's Island in September 1980, 4 from a collection at Morfa Nefyn in December 1980 and one from Portland in August 1979. The experimental crosses were carried out between April and August 1981. As each generation takes approximately

three weeks in the laboratory and the majority of strains were fixed after 3 or 4 generations this means that they had been maintained for one to ten generations before testing. The Portland Adh-EE line was an exception. It had been maintained for about 30 generations.

Some of the isogenic lines proved very difficult to maintain, especially S39BB, S39CC and MN122DD. These lines had to be expanded from very low numbers on several occasions and were then unavailable for experimental crosses.

This experiment was prompted in the first place by the suggestion of differences in relative viabilities of the chromosome I karyotypes from different populations. Therefore the main character to be assessed from the crosses was viability. Previous results on viability of Coelopa frigida suggested that it was important to measure the whole period from egg to adult and to allow for effects of larval density. To some extent these requirements are incompatible because Coelopa females lay their eggs in batches and this makes it difficult to count out a fixed number of individual eggs. Instead of attempting to control the starting numbers I therefore decided to allow the number of eggs in each replicate to vary and to make a statistical adjustment for any effect in the results.

By contrast to the relative viabilities of the karyotypes, their development times so far appear to be remarkably similar in all populations studied (Day et al 1980). This characteristic has been included in the present experiment.

The procedure for each cross was as follows:-

(i) One to five pairs of flies from the requisite strain or strains were placed in a round canister filled to a depth of 3 cm with fresh medium. The medium had been thoroughly forked over and then lightly

packed down to give a uniform surface on which any eggs could easily be seen.

(ii) The canister was kept at 27°C for 18 hours (overnight) and the flies were then removed and either discarded or used for another replicate.

(iii) The eggs laid in the canister were counted as soon as possible after removal of the flies and in all cases within 6 hours. In this way most egg batches were counted before they hatched which I found more reliable. Counting was carried out under a low power dissecting microscope. Egg batches were sometimes moved gently with a mounted needle to achieve a better orientation. It is unlikely that the number of eggs was overestimated by this counting method but it is possible, especially with large egg batches, that some eggs were missed. I estimate that in general the error was less than 5% of the number of eggs present but that in some cases it could have been up to 10% (for example where several batches had hatched before counting). In a few cases flies may have laid eggs in deep cracks in the medium surface and whole egg batches may have been missed. This will generally lead to impossible viability results (i.e. more than 100%) which can be discarded.

(iv) The canister was stored in the dark at 27°C until adult flies began to emerge. All adults were collected every day, sexed and recorded. Flies which were required for further experimental generations were stored at 4°C with the sexes separate and supplied with 0.5% mannitol solution. Flies for electrophoresis were stored at -20°C and eny remaining flies were discarded. Collection was continued until a canister failed to produce any flies on three consecutive days. A few daily collections were missed. Where this

is likely to have affected the estimate of development time the results for the relevant canister have been excluded from the analysis. Any eggs that were laid in the canisters on such occasions were removed. In no canister were second generation larvae observed.

Four types of cross were conducted:-

1) Parental (P) - within line crosses using 20 of the 21 strains. One to eight replicates per line were completed with a total of 131 replicates. It was not possible to conduct a satisfactory test with the MN122DD line.

2) F1 - 118 individual replicates of 62 different crosses between lines were carried out, including four pairs of reciprocal crosses.

3) F2 - F1 progeny were crossed from 43 of the different combinations with 121 replicates in total. The progeny of these crosses were all gelled where the total surviving was less than 72 or a sample of 60 where the total was greater. All the F1 flies used in F2 crosses were heterozygous for Adh but not necessarily for the inversion.

4) FX - 10 crosses, with two or three replicates each, were carried out between different F1 progenies. These were of the type α^S/β^M x α^M/β^S (where S and M indicate chromosomes originating from the St.Mary's Island and Morfa Nefyn populations respectively). The progeny of these crosses were gelled as for the F2. In the progeny each heterokaryotypic individual carried an α and a β sequence from the same population but in a mixed genetic background. The heterokaryotypes were expected to be half α^M/β^M and half α^S/β^S in each cross. In some crosses these types were distinguishable because of the Adh alleles carried by the original lines. For example in an $\alpha^M(B)/\beta^S(D)$ x $\alpha^S(B)/\beta^M(C)$ cross α^M/β^M progeny were Adh-BC while α^S/β^S progeny were Adh-BD.

Table 7.1

Summary of crosses showing the number of line combinations of each type

a) Parental generation

karyotype	Adh genotype / Population	AA	BB	CC	DD	EE
αα	S	1	3	1	-	-
	M	-	2	-	-	-
	P	-	-	-	-	-
ββ	S	-	-	3	7	1
	M	-	-	1	-	-
	P	-	-	-	-	1

S - St.Mary's Island
M - Morfa Nefyn
P - Portland
I - Interpopulation

b) F1 generation

karyotype	Adh genotype / Population	AC	AD	BB	BC	BD	BE	CC	CD	CE	DD	DE
αα	S	-	-	-	-	-	-	-	-	-	-	-
	I	-	-	1	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-	-	-	-
αβ	S	-	3	-	4	5	1	-	1	-	-	-
	I	1	-	-	4	10	2	1	1	-	-	-
ββ	M	-	-	-	1	-	-	-	-	-	-	-
	S	-	-	-	-	-	-	1	8	1	5	2
	I	-	-	-	-	-	-	2	4	1	-	2
	M	-	-	-	-	-	-	-	-	-	-	-

c) F2 generation

karyotype	Adh genotype / Population	AC	AD	BC	BD	BE	CD	CE
αβ	S	-	3	3	5	-	2	-
	I	1	-	4	9	2	1	-
	M	-	-	1	-	-	-	-
ββ	S	-	-	-	-	-	7	1
	I	-	-	-	-	-	4	-
	M	-	-	-	-	-	-	-

A breakdown of the crosses conducted in each of these classes is given in Table 7.1.

7.3 Results

The full data are presented in Appendix 5. The results will be analysed first in terms of viability and then for development times. The viability results will be considered first for differences in overall viabilities within and between generations and then for the relative viabilities of the segregating genotypes in the F2 and FX generations.

1) Overall Viabilities There was a clear expectation from earlier work with Coelopa (Burnet & Thompson 1960, Rowell 1969, Collins 1978) that overall viability would be related to larval density - in this case to the number of eggs per canister. I therefore attempted to keep the mean numbers of eggs and ranges of numbers comparable between generations. This was largely successful (Table 7.2), although the relatively small number of FX canisters had a lower mean and smaller range. Table 7.2 also gives the overall mean viability in each generation and it is immediately clear that the viability of the parental lines is much lower than the other generations. To test the significance of these differences it was necessary to examine the relationship between viability and density.

Rowell (1969) found that observed viabilities fitted best to a cubic function of density, the parameters of which varied with the absolute amount of food supplied. In these experiments all replicates had the same quantity of food medium. I have fitted a linear model to the data with a logit transformation and binomial error distribution using the Genstat computer package as described in Chapter 2. A model including the number of eggs and its square

Table 7.2

Summary of egg counts and overall viabilities

Generation	Mean no.of eggs per canister	Minimum no.of eggs per canister	Maximum no.of eggs per canister	Mean viability
P	127.4	24	370	31%
F1	133.3	23	374	53%
F2	126.3	27	463	53%
FX	102.5	40	210	53%

Table 7.3

Regressions of viability on number of eggs per canister

	Intercept ± standard error	Regression coefficients ± standard errors			Deviance Ratio
		x	x^2	x^3	
P	0.284 ±0.121	-1.71×10^{-2} $\pm0.24\times10^{-2}$	6.31×10^{-5} $\pm1.40\times10^{-5}$	-7.82×10^{-8} $\pm2.38\times10^{-8}$	3.17*
F1	1.310 ±0.185	-2.58×10^{-2} $\pm0.33\times10^{-2}$	15.5×10^{-5} $\pm1.74\times10^{-5}$	-26.6×10^{-8} $\pm2.77\times10^{-8}$	1.10
F2	0.155 ±0.109	-0.23×10^{-2} $\pm0.18\times10^{-2}$	2.26×10^{-5} $\pm0.87\times10^{-5}$	-5.45×10^{-8} $\pm1.20\times10^{-8}$	3.77*
FX	4.92 ±0.89	-12.9×10^{-2} $\pm2.36\times10^{-2}$	104.9×10^{-5} $\pm19.0\times10^{-5}$	-267.5×10^{-8} $\pm47.5\times10^{-8}$	0.93

The "deviance ratio" is the ratio of the mean deviance accounted for by the regression to the residual mean deviance. It is approximately distributed as the variance ratio F. * indicates a probability of less than 0.05 in the F test.

and cube as independent variates gives the best fit to the observed proportional viability in each of the four generations (Table 7.3) in accordance with Rowell's findings. The regression lines are plotted in Figure 7.1. The regression coefficients are all significantly different from zero, except the x coefficient for the F2 generation, but the deviance accounted for by the regression is only a small proportion of the total deviance in each case (4 to 12%). There are significant differences between the regressions ($F_{12,341} = 9.41$, $P < 0.001$). It is clear from the overall viabilities and the regression lines (Fig.7.1) that most of the difference is due to the poor survival of the parental lines. This low viability could be due to general inbreeding depression or to homozygosity for the αβ inversion (or both).

In the parental generation there were significant differences in viability between αα and ββ lines ($F_{4,100} = 3.18$, $P < 0.05$). ββ lines survived better at all except very low densities (Fig.7.2). This may represent a difference in competitive ability between the karyotypes, for example the smaller size at which ββ larvae pupate might allow more individuals to survive on a given amount of food. However it is possible that the αα lines were more homozygous because more generations of sib mating had elapsed in their derivation (Table 2.3) and this could explain their poor survival. There were no significant differences between lines of different Adh genotype ($F_{13,100} = 1.33$, $P > 0.05$) or between lines from different populations ($F_{8,100} = 1.66$, $P > 0.05$).

The F1 crosses were of three types with respect to the inversion, αα, αβ or ββ, although there were very few αα crosses. These karyotypes did not differ significantly in overall viability ($F_{5,50} = 1.14$, $P > 0.05$), nor were there significant differences between the various

Fig.7.1 Regressions of the proportion surviving to adulthood on the number of eggs per canister for the four generations

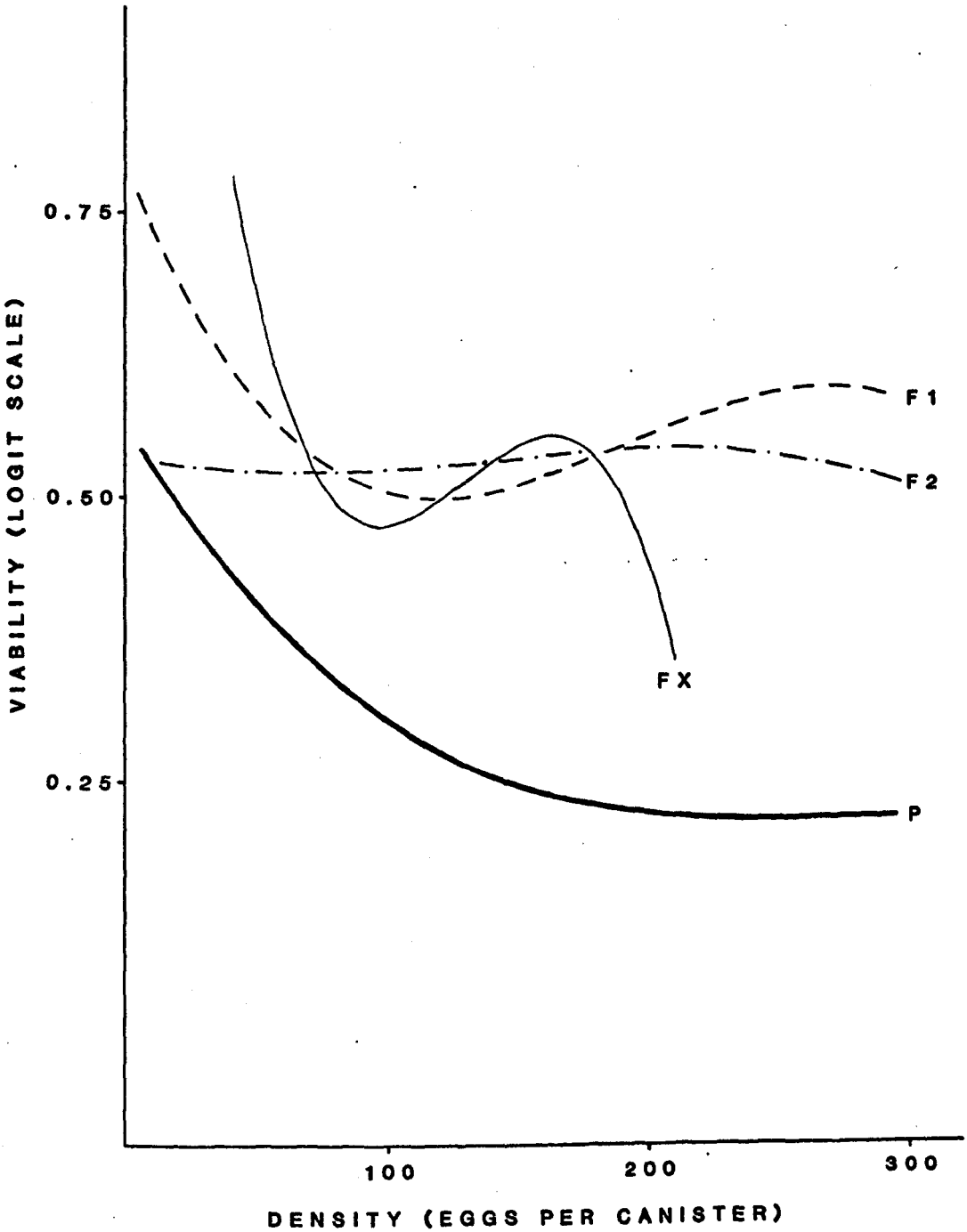
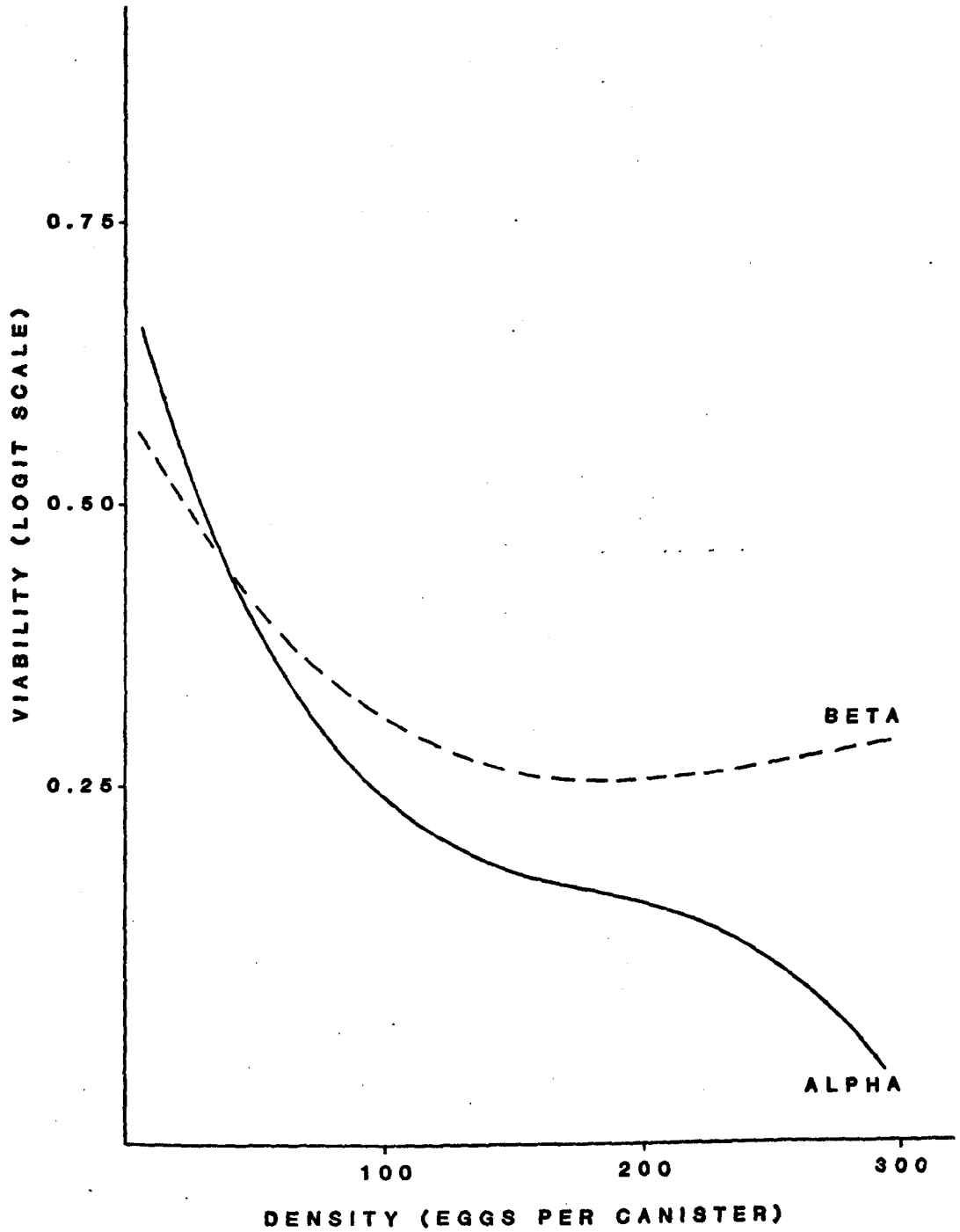


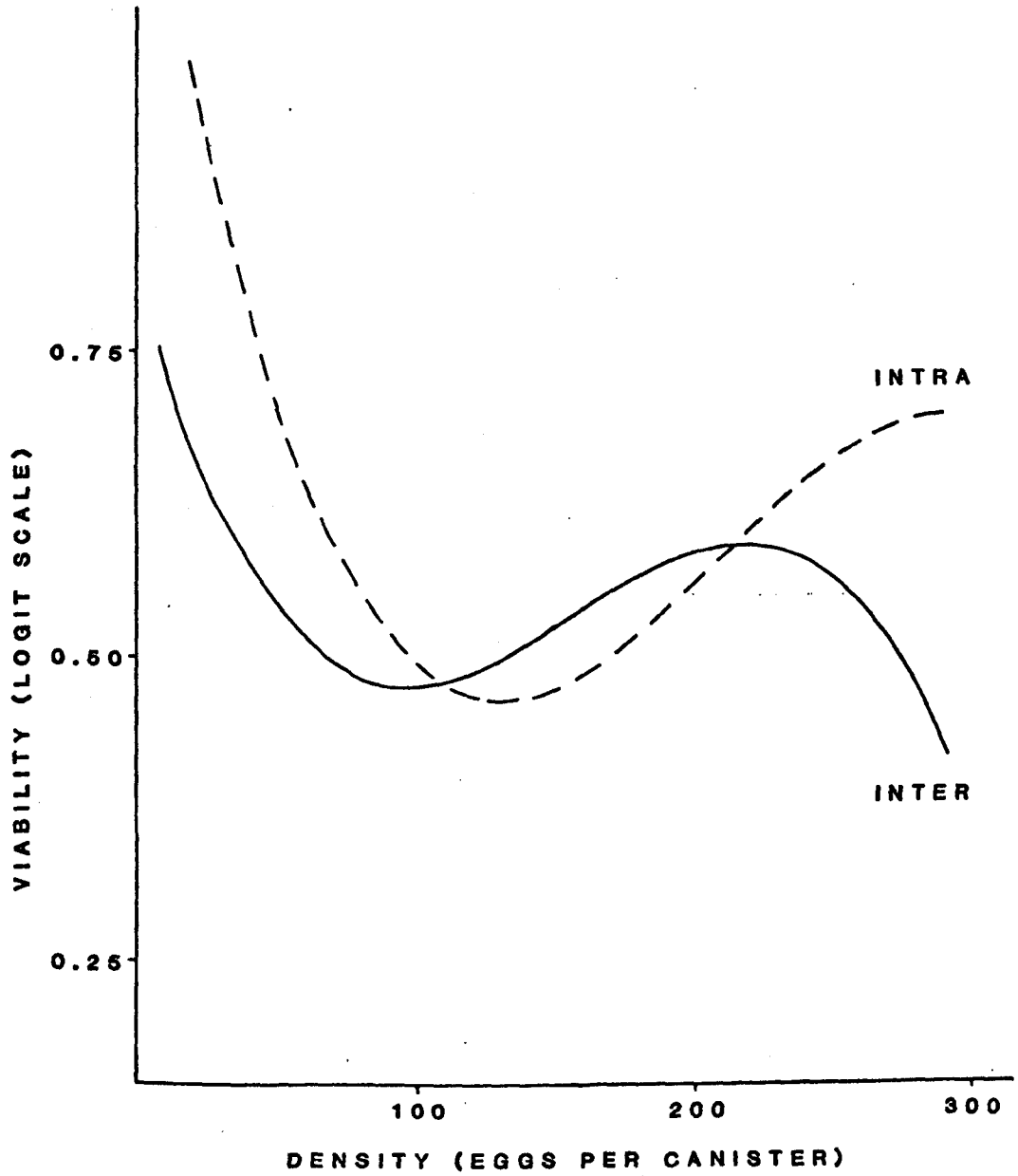
Fig.7.2 Regressions of the proportion surviving to adulthood on the number of eggs per canister for the two karyotypes in the Parental generation



Adh genotypes represented ($F_{25,50} = 1.16$, $P > 0.05$). However the population of origin did affect viability ($F_{5,50} = 3.66$, $P < 0.01$). There was only one cross involving two Morfa Nefyn lines and so this is effectively a comparison of St.Mary's Island intrapopulation crosses with interpopulation crosses - either St.Mary's Island x Morfa Nefyn or St.Mary's Island x Portland. Interpopulation F1 crosses had higher viability than intrapopulation crosses at intermediate densities (100-200 larvae per canister) but lower viability at extreme densities (Fig.7.3). The overall mean viability was higher for intra- (57%) than for interpopulation (51%) crosses, the difference being mainly in the αβ crosses.

The lack of difference between homo- and heterokaryotypes in the F1 suggests that homozygosity for the inversion as such was not the reason for the low viability of the parental lines. The parental lines were the most homozygous for loci within the inversion, although not necessarily homozygous for all such loci. The F1 homokaryotypes were more heterozygous but the F1 heterokaryotypes should have been the most heterozygous. If the large difference between the P and F1 viabilities was due to genic heterosis within the inversion then a corresponding difference should have been found between the F1 homo- and heterokaryotypes. It seems most likely that the low viability in the P generation was due to homozygosity for loci outside the inversion. However the lack of difference between karyotypes in the F1 remains surprising. Either genic or karyotypic selection on the inversion would be expected to produce higher viabilities in the heterokaryotypes and this was certainly expected from observations on segregating populations in the field and in the laboratory. The explanation may be that heterokaryotypes are better "intergenotypic competitors" but are not better "intragenotypic competitors". In this case competing different F1 crosses against a standard strain

Fig.7.3 Regressions of the proportion surviving to adulthood on the number of eggs per canister for inter- and intra-population crosses in the F1 generation



would be a more satisfactory technique than measuring viability in pure cultures.

The population effect is comparable with the F1 "luxuriance" found in other studies (see Introduction). It may reflect a greater degree of heterozygosity in interpopulation crosses than in intrapopulation crosses. It is interesting that the higher viability is restricted to the intermediate, and perhaps less stressful, density conditions and that the coadapted intrapopulation crosses do better at the extremes.

In the F2 generation no significant differences in overall viability were detected between αβxαβ and ββxββ crosses ($F_{4,80} = 1.48$, $P > 0.05$), between crosses of different Adh genotype ($F_{20,80} = 1.42$, $P > 0.05$), or between inter- and intrapopulation crosses ($F_{6,80} = 0.33$). An inversion effect might have been expected here because of reduced competition in the replicates segregating for the inversion. However all F2 replicates were segregating for Adh and were derived from two parental lines which may have differed in numerous loci both within and outside the inversion irrespective of karyotype. It is possible therefore that the genetic variability between larvae in the αβxαβ F2 replicates was not significantly greater than that in other replicates.

There was no evidence for reduced viability in interpopulation F2 replicates as might have been expected from the breakdown of coadapted gene complexes by recombination. In fact interpopulation crosses had slightly higher viabilities over most of the density range.

2) Sex Ratio There was no evidence in any of the four generations that sex ratio (analysed as the proportion of males amongst emerging

Table 7.4

Average sex ratios (percentage of males)

a) P

	Population			
	S	M	P	Total
αα	51.4	29.6	-	41.8
ββ	47.6	47.7	53.1	48.1
Total	48.4	35.3	53.1	46.4

b) F1

	Population			
	S	I	M	Total
αα	-	50.9	-	50.9
αβ	54.1	56.6	49.4	55.7
ββ	49.1	47.4	-	48.4
Total	52.0	54.9	49.4	53.6

c) F2

	Population			
	S	I	M	Total
αβ	50.0	51.3	32.8	50.4
ββ	46.1	42.9	-	45.1
Total	48.6	49.9	32.8	49.0

S - St.Mary's Island

I - Interpopulation (S/M or S/P)

M - Morfa Nefyn

P - Portland

adults) was related to larval density. This is in contrast with earlier results (Evans & Philip 1964) in which the proportion of males declined at high densities. However there were significant differences between generations ($F_{3,363} = 9.64$, $P < 0.001$; Table 7.4). The average proportion of males was low in the parental lines (46.4%) indicating that males suffer more from inbreeding depression than do females. Variation between individual lines was considerable with a significant interaction between karyotype and population of origin ($F_{2,120} = 9.67$, $P < 0.01$). This was mainly due to the very low proportion of males (29.6%) from Morfa Nefyn αα lines, especially M179BB, compared with St.Mary's Island αα lines (51.4%). The Portland EE line (ββ) also had an unusually high proportion of males (53.1%).

The average sex ratio in the F1 (53.6% males) was higher than either the P or F2 generations and this was due to a high proportion of males in the αβ crosses (55.7%) compared to the αα and ββ crosses (50.9 and 48.4% respectively; $F_{2,74} = 22.58$, $P < 0.001$). This is consistent with the idea that male survival is more affected by homozygosity than female survival. The P generation, the most homozygous, has the lowest proportion of males and the F1 heterokaryotypes, the most heterozygous, have the highest. Although the difference is not statistically significant interpopulation F1 crosses have a higher proportion of males (54.9%) than intrapopulation crosses (52.0%), again consistent with the trend.

In the F2 the overall mean sex ratio was 49.0% males. It was higher in αβxαβ crosses than in ββxββ crosses (50.4% and 45.1% respectively; $F_{1,105} = 3.95$, $P \sim 0.05$) but there was little difference between inter- and intrapopulation crosses. In the FX generation the

mean proportion of males was 53.2%. These results are also consistent with an effect of overall heterozygosity. There is no indication that a break up of coadapted gene complexes in interpopulation F2 crosses affects sex ratio.

Deleterious effects of homozygosity might be expected to be more pronounced in the heterogametic sex because it is always effectively homozygous for the whole of the X chromosome. It will therefore be more homozygous overall for a given amount of inbreeding than the homogametic sex. This effect might be compared with the disturbance of sex ratios in hybrid animals (Haldane 1922). In Coelopa no morphologically distinct sex chromosomes have been described but it is most likely, in view of the system in Drosophila and other Diptera, to be XY male, XX female. The trend observed can be viewed as the effect of increased homozygosity reducing male viability more than female viability. It is surprising, however, that in the most heterozygous crosses the sex ratio does not approach 1:1 but instead there is an excess of males. Consistently high sex ratios have not been observed previously in the laboratory or in the field. It may be important to note that they occur only in the pure cultures; they are not seen in the segregating F2 cultures where the proportion of males (49%) is remarkably similar to that observed by Evans and Philip (1964).

3) Genotype frequencies Male and female genotype frequencies in the F2 differed significantly ($\chi_2^2 > 5.99$, $P < 0.05$) in only 2 out of 118 replicates. This is fewer than would have been expected by chance, and so male and female data have been combined in subsequent analyses.

Adh genotype frequencies differed significantly from the 1:2:1 expectation in 23 cases out of 118 (13 at $P < 0.05$, 6 at $P < 0.01$, 4 at $P < 0.001$). In 21 of these replicates there was an excess of heterozygotes. These results are partitioned between αβ and ββ crosses and between inter- and intrapopulation crosses in Table 7.5. The tendency towards heterozygote excess is clear - more than 3/4 of all replicates showing an excess. However there is no marked difference between inter- and intrapopulation crosses and ββ crosses tend to produce heterozygote excess as well as αβ crosses although it is less marked.

To assess the significance of differences between classes of replicates, genotype frequencies have been analysed using the analysis of deviance technique as for viabilities. There is little evidence that genotype frequencies are affected by density. Fitting a linear regression of the frequency of heterozygotes on density (eggs per canister) accounts for less than 2% of the total variance and the slope is not significantly different from zero ($b = 4.8 \times 10^{-4} \pm 3.3 \times 10^{-4}$). However when the analysis is restricted to αβ crosses only the slope of the regression becomes just significant ($b = 8.1 \times 10^{-4} \pm 3.9 \times 10^{-4}$, $P < 0.05$) although it still only accounts for 3.4% of the total variance. As this correlation is so weak it has not been included in subsequent analyses. It is interesting that this experiment has revealed an effect of density on heterozygote excess although it is nowhere near as strong as expected from Collins' (1978) results. If this is anywhere near the true extent of the effect then it is not surprising that the laboratory and field studies described in Chapter 5 did not reveal it.

Mean genotype frequencies are given in Table 7.6. There is

Table 7.5

Comparison of F2 Adh genotype frequencies with Mendelian expectations

	No.of replicates in which proportion of heterozygotes exceeded 50%	No.of replicates in which deviation from 1:2:1 was significant at $P < 0.05$*	Total no.of replicates
Intrapopulation crosses			
αβ	28 (78%)	8	38
ββ	14 (70%)	2(+1)	20
Interpopulation crosses			
αβ	42 (81%)	10(+1)	52
ββ	6 (60%)	1	10

*Numbers in brackets refer to replicates where the proportion of heterozygotes was less than 50%.

Table 7.6

Mean genotype frequencies in the F2 generation

a) αβ x αβ crosses

		αα	αβ	ββ
St.Mary's Island	M	0.21	0.57	0.22
	F	0.21	0.57	0.22
	T	0.21	0.57	0.22
Interpopulation	M	0.20	0.59	0.21
	F	0.22	0.57	0.21
	T	0.21	0.58	0.21

b) ββ x ββ crosses

		CC	CD or CE	DD or EE
St.Mary's Island CD	M	0.20	0.56	0.24
	F	0.28	0.48	0.24
	T	0.24	0.52	0.24
St.Mary's Island CE	M	0.32	0.48	0.20
	F	0.30	0.55	0.16
	T	0.31	0.52	0.17
Interpopulation CD	M	0.25	0.53	0.22
	F	0.26	0.51	0.23
	T	0.26	0.52	0.23

clearly a higher frequency of heterozygotes in αβ crosses as expected and this is highly significant ($F_{1,75} = 15.69$, $P < 0.001$). The heterozygote excess tends to be greater in males than in females. In the ββ crosses heterozygotes are in excess overall in males but not in females.

Within the αβ crosses there are no significant differences in the frequencies of any of the three genotypes between crosses involving different Adh genotypes, different populations or different individual lines. There is, therefore, no support in these data for the suggestion that the Adh locus contributes to viability selection on the inversion. Neither is there any indication of a breakdown of coadaptation in the interpopulation F2 crosses. Indeed these crosses show a very similar level of heterozygote excess to that of the intrapopulation crosses.

There are no significant effects of Adh genotype or population in the ββ crosses either, although the EE homozygotes do have noticeably lower viability than the CC or DD homozygotes (Table 7.6b). In each type of cross there is a slight overall heterozygote excess.

In the FX generation inversion heterozygotes have both chromosomes derived from the same population but in a mixed genetic background. This type of cross was used by Dobzhansky (1950) to show that the lack of heterozygote excess in interpopulation F2 crosses was due to lack of coadaptation of the inverted segments rather than mixing of the genetic background. In this case the interpopulation F2 crosses did not show any reduction in heterosis and so there was no reason to expect any reduction in the FX generation. However of the 26 replicates only 2 differed significantly ($P < 0.05$) from the Mendelian expectation and the mean genotype

frequencies were very close to expectation (Table 7.7). The frequency of heterozygotes differed from both the intrapopulation ($F_{1,57} = 5.52$, $P < 0.05$) and the interpopulation ($F_{1,75} = 7.85$, $P < 0.01$) αβ crosses in the F2.

Table 7.7

Mean genotype frequencies in the FX generation

	αα	αβ	ββ
Male	0.25	0.52	0.23
Female	0.26	0.50	0.24
Total	0.25	0.51	0.23

From the point of view of Adh genotypes, there were three classes of FX cross: ACxBC, BDxBC and BDxBD. The first two of these produced electrophoretically distinguishable α^M/β^M and α^S/β^S heterokaryotypes but in no case did these classes differ significantly from the expected 1:1 ratio and there was no overall tendency for either type of heterokaryotype to be in excess. Interestingly the overall proportion of heterozygotes was higher in the BDxBD crosses (56%) than in the others (49% for ACxBC; 52% for BDxBC). However this effect was not statistically significant ($F_{2,22} = 0.59$).

The most plausible explanation for the low frequency of heterokaryotypes in the FX relative to the F2 lies in the population of origin of the chromosomes. The heterokaryotypes in the FX have both chromosomes from the same population whereas the homokaryotypes have chromosomes from different populations. This is the reverse of the situation in the interpopulation F2 crosses, and in the intrapopulation F2, of course, all chromosomes originate from one population. If the advantage to the heterokaryotypes in the F2 is a result of a higher

level of heterozygosity, then this advantage will be reduced in the FX because the chromosomes in the FX homokaryotypes differ at more loci than the F2 homokaryotypes as a result of their separate origin. This amounts to selection at the genic level rather than the karyotypic level. If this explanation is correct then equivalent results should have been detected in other generations. In particular the interpopulation F2 crosses should have had a higher proportion of heterozygotes than the intrapopulation F2 crosses and similarly interpopulation F1 replicates should have had a higher viability than intrapopulation crosses. In neither case was there a clear cut effect: in the F2 there was no significant difference although the interpopulation crosses had a marginally higher proportion of heterokaryotypes; in the F1 there was a significant difference but interpopulation crosses were only more viable over part of the density range and the mean viability was lower than in the intrapopulation crosses.

The difference between the three Adh types in the FX suggests that an alternative explanation is possible. The BRxBD crosses have a similar proportion of heterokaryotypes to the F2 crosses and are also similar in having just three Adh genotypes in the offspring, two homozygotes and the heterozygote. The other FX crosses have four genotypes amongst the offspring including one homokaryotype which is heterozygous for Adh. It is possible that this reduces the advantage to the heterokaryotypes by changing the competitive conditions.

4) Development times Development times are known to differ between males and females and to be affected by larval density (Day et al 1980, Chapter 4). In this analysis males and females have been treated

separately. The FX generation has not been included in the analysis and the F2 generation, because of the complication of segregating genotypes, will be discussed below. In the P and F1 generations, in both males and females, there are significant regressions of development time on larval density (Table 7.8) accounting for 30-45% of the total variance. Inclusion of the square and cube of larval density in the regression model does not significantly improve the fit. The effect of density is clearly very similar in all four cases.

As expected there was a significant difference between inversion types in P generation males ($F_{2,108} = 25.86$, $P < 0.001$) but not in females ($F_{2,107} = 0.17$). αα males have a longer development time than ββ males and are slightly more influenced by density (regression equation for αα, $y = 13.56 + 0.024x$; for ββ, $y = 11.22 + 0.019x$). Adh genotypes do not differ significantly either in males or females after removal of inversion effects. This tends to confirm that development time differences are a property of the inversion not the Adh locus. However there is a significant difference between lines from different populations both in males ($F_{4,108} = 2.80$, $P < 0.05$) and females ($F_{4,107} = 3.33$, $P < 0.05$). This is because Morfa Nefyn αα flies develop more slowly at low densities than St.Mary's Island αα flies (Table 7.9) both male and female but are less affected by increased density. The much smaller difference in ββ flies is in the opposite direction. This result suggests a genetic difference between the populations which is probably associated with the inversion because the effect differs between karyotypes. An alternative possibility is that early mortality differs between St.Mary's Island and Morfa Nefyn αα replicates such that Morfa Nefyn larvae experience higher effective densities at low egg numbers. However

Table 7.8

Effect of density on development time (in days)

		Intercept (± standard error)	Regression Coefficient (± standard error)	t (difference of slope from zero)
P	Males	11.62±0.41	0.021±0.003	8.00***
P	Females	11.16±0.32	0.021±0.002	10.19***
F1	Males	13.81±0.63	0.024±0.004	5.87***
F1	Females	12.55±0.55	0.023±0.004	6.56***

***P < 0.001

Table 7.9

Development time differences (in days) between populations in the parental generation

		Intercept (± standard error)	Regression coefficient (± standard error)
αα males	S	12.46 ± 1.60	0.029 ± 0.011
	M	15.40 ± 1.76	0.017 ± 0.009
	P	-	-
ββ males	S	11.27 ± 0.31	0.017 ± 0.002
	M	10.53 ± 1.72	0.030 ± 0.013
	P	9.88 ± 0.51	0.044 ± 0.004
αα females	S	10.30 ± 1.02	0.024 ± 0.007
	M	12.67 ± 1.40	0.014 ± 0.007
	P	-	-
ββ females	S	11.12 ± 0.32	0.020 ± 0.002
	M	10.51 ± 1.79	0.031 ± 0.014
	P	10.42 ± 0.60	0.042 ± 0.004

S - St.Mary's Island, M - Morfa Nefyn, P - Portland

no population differences in viability have been detected in the parental generation.

The inversion effect was again significant in F1 males ($F_{3,62} = 7.29$, $P < 0.001$) but not in F1 females ($F_{3,62} = 0.18$). There was only one αα replicate, in which males had a mean development time of 22.7 days. αβ males had a longer development time than ββ males and were more affected by density (regression equation for αβ, $y = 14.21 + 0.025x$; for ββ, $y = 12.92 + 0.017x$). As in the parental generation no differences were detected between crosses involving different Adh genotypes, nor were there any differences between populations in either males or females.

ββ flies in the F1 developed more slowly than ββ flies in the parental generation for both males ($F_{2,114} = 7.56$, $P < 0.001$) and females ($F_{2,110} = 8.47$, $P < 0.001$). The difference was about 1.5 days in both sexes and the effects of density were similar. This is probably an artefact of measuring density in terms of number of eggs per canister whereas it is during the third instar that density has most effect on development rate. The F1 had a lower mortality than the P generation and therefore a higher effective density and longer development time.

The F1 results agree with those of the parental generation in that there is no difference in development time between Adh genotypes within the same karyotype. This was to be expected because development time is probably a polygenic trait on which a single locus would have little effect, but an inversion could have a large effect by holding together a combination of alleles. The fact that the karyotype differences were detected in pure culture in both generations demonstrates that they are direct effects on development time rather

than side effects of competition with other genotypes. However this does not mean that the differences in development rate do not affect the outcome of inter-karyotypic competition.

The significant population differences observed in the parental lines were not detected in the F1 generation. In this generation the population comparison is essentially between St.Mary's Island intrapopulation crosses and St.Mary's Island x Morfa Nefyn interpopulation crosses. In fact the interpopulation crosses did have slightly higher intercepts than St.Mary's Island lines in both males (14.23 days compared with 13.00 days) and females (13.02 days compared with 12.02 days). This tends to support the existence of a genetic difference between the populations.

In the F2 generation the analysis of development times was complicated by the segregation of different genotypes. As expected from previous work there were significant differences between genotypes in the male offspring of αβxαβ crosses ($F_{4,146} = 30.35$, $P < 0.001$) but not in the females ($F_{4,143} = 0.65$). Male αα offspring developed more slowly than ββ offspring, with heterokaryotypes intermediate. In ββxββ crosses there was no segregation of chromosomal arrangements and no difference between genotypes amongst the offspring (for males $F_{4,28} = 0.22$, for females $F_{4,30} = 0.22$).

Within the αβxαβ crosses there were differences in the development times of both males and females of all three karyotypes between groups of crosses of different Adh genotypes: for αα males $F_{9,42} = 2.65$, $P < 0.05$; for αβ males $F_{9,48} = 4.15$, $P < 0.001$; for ββ males $F_{9,42} = 3.13$, $P < 0.01$; for αα females $F_{9,40} = 2.99$, $P < 0.01$; for αβ females $F_{9,46} = 3.68$, $P < 0.01$; for ββ females $F_{9,42} = 2.85$, $P < 0.05$. The regression lines for male and female heterokaryotypes are plotted in

Figs.7.4-7.6 Regressions of mean development times on the number of eggs per canister for αβ x αβ crosses in the F2 generation

Fig.7.4 Female heterokaryotypes. Adh genotypes are given for each regression. The dashed line is the regression for the Adh-CD genotype in ββ x ββ crosses.

Fig.7.5 Male heterokaryotypes. Details as in Fig.7.4.

Fig.7.6 Male homokaryotypes. Solid lines are for Adh-BB homozygotes and dashed lines for Adh-DD homozygotes.

Fig. 7.4

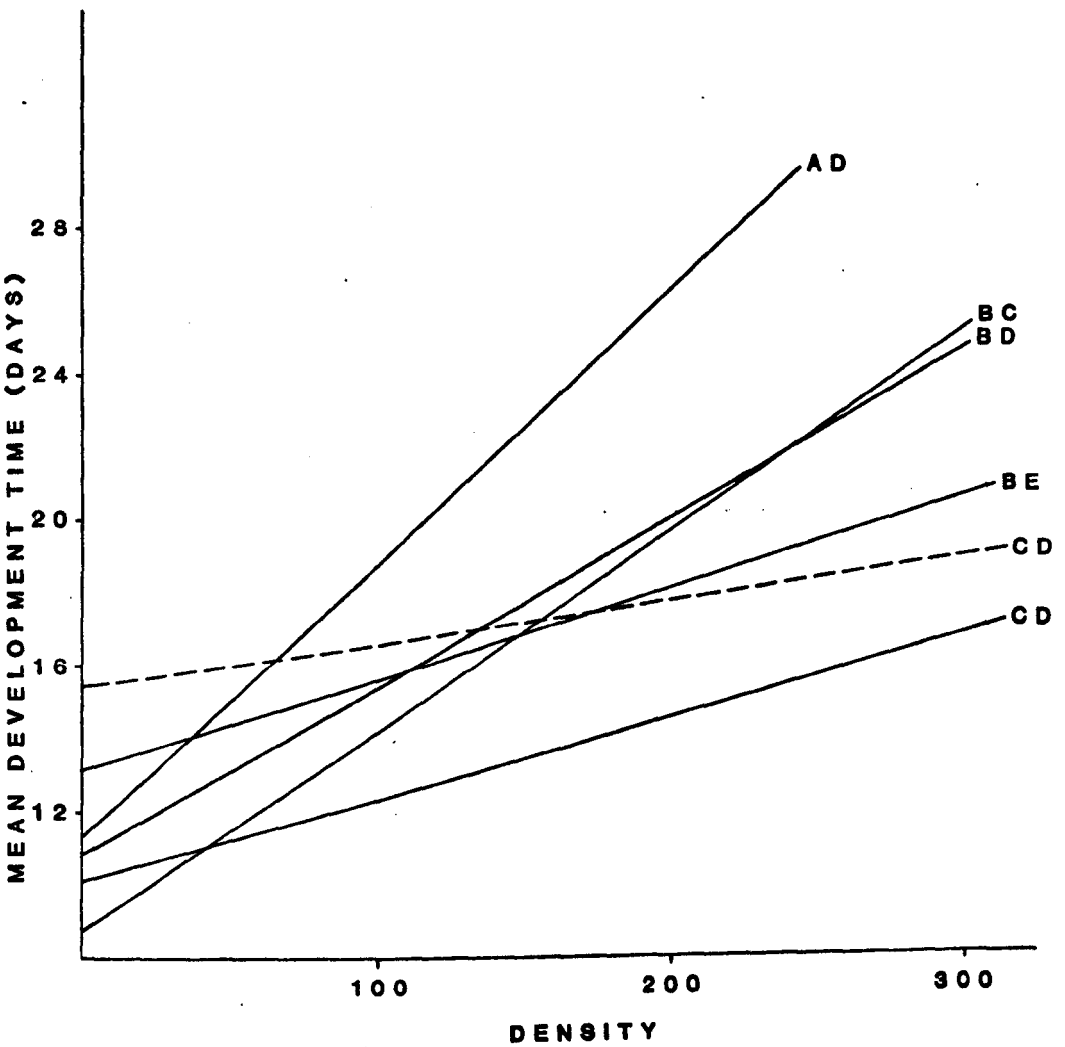

MEAN DEVELOPMENT TIME (DAYS)
28
24
20
16
12
100
200
300
DENSITY
AD
BC
BD
BE
CD
CD

Fig. 7.5

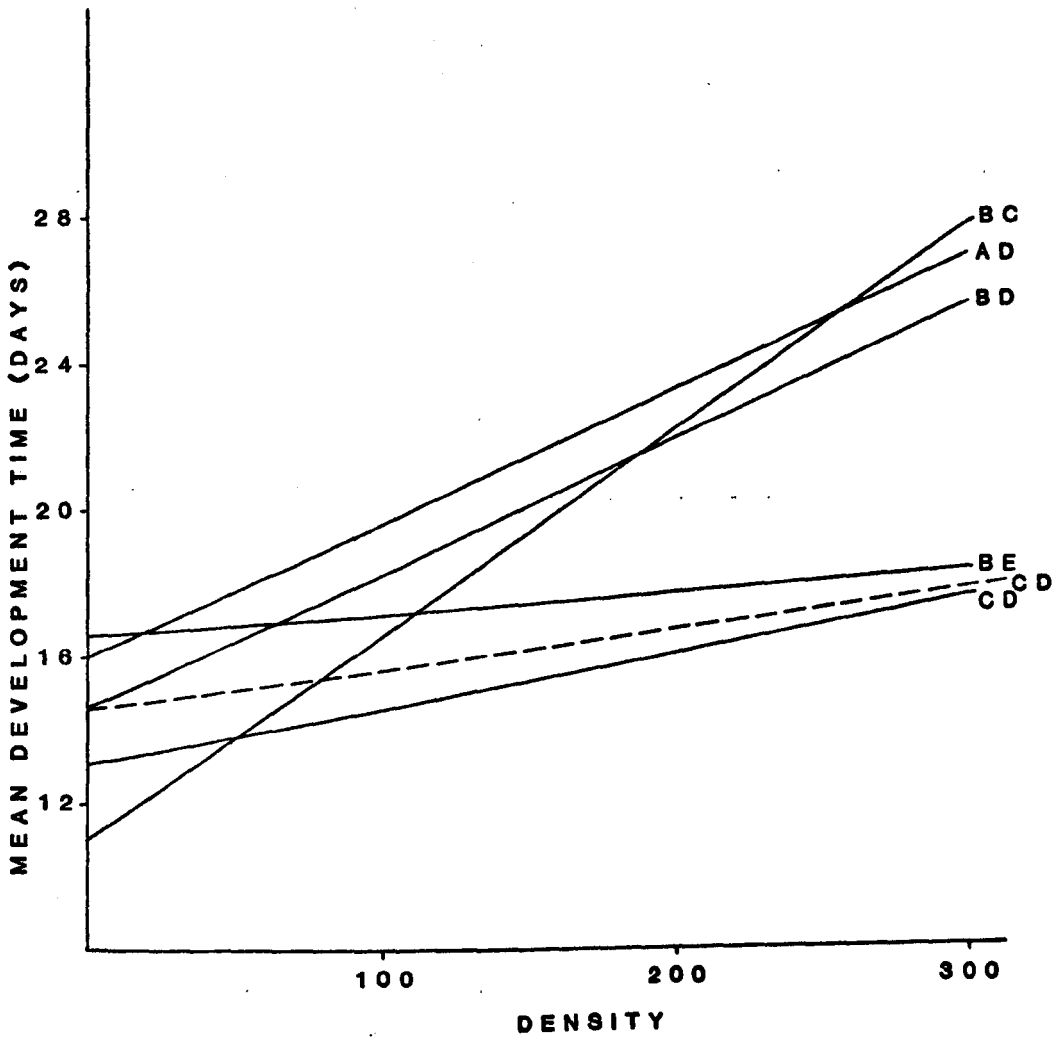
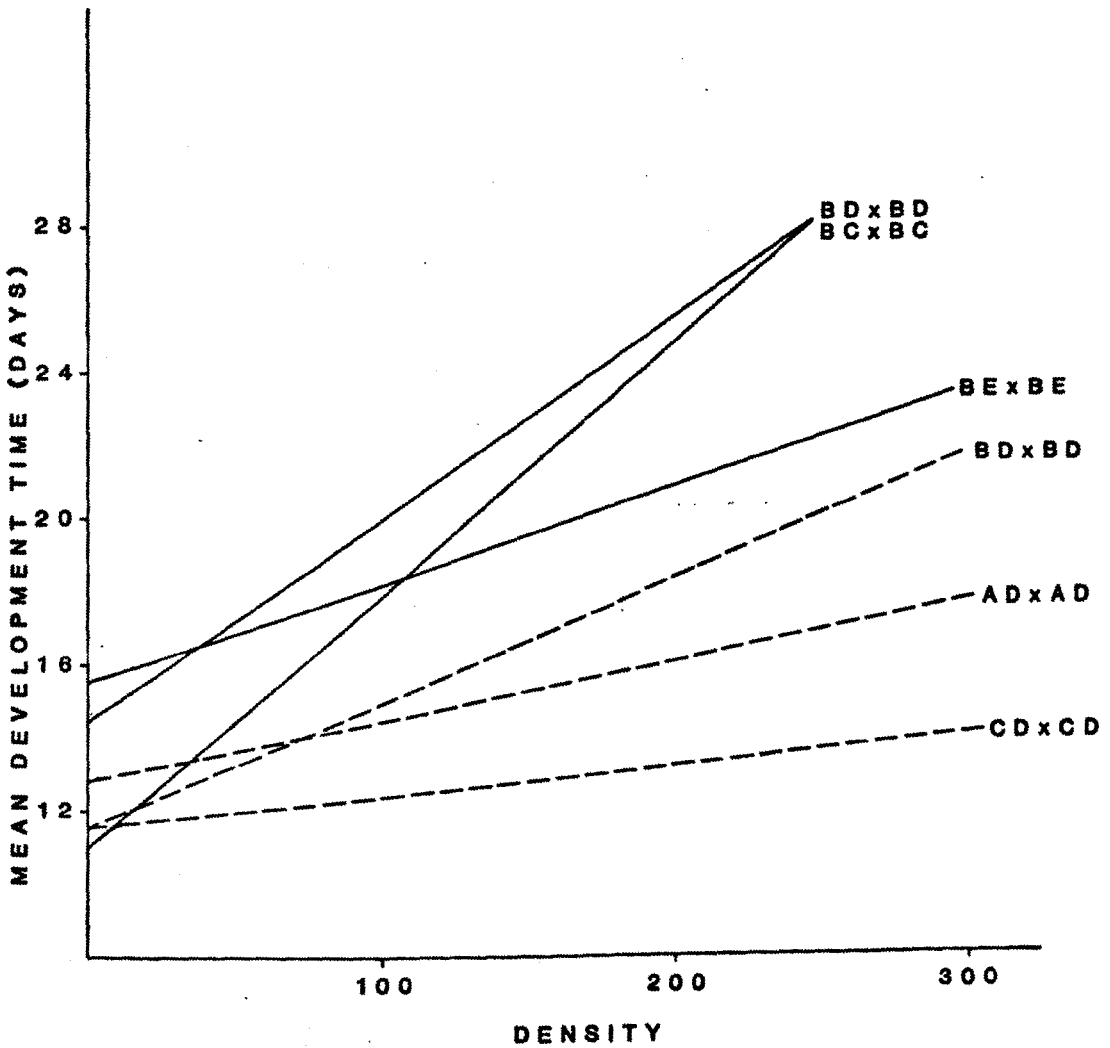

MEAN DEVELOPMENT TIME (DAYS)
28
24
20
16
12
100
200
300
DENSITY
BC
AD
BD
BE
CD
CD

Fig. 7.6


MEAN DEVELOPMENT TIME (DAYS)
28
24
20
16
12
100
200
300
DENSITY
BD x BD
BC x BC
BE x BE
BD x BD
AD x AD
CD x CD

Figures 7.4 and 7.5. There is clearly a similarity in the relative positions of the lines and this extends to the homokaryotypes as well (Fig.7.6). In other words all the offspring in some replicates developed more slowly or more rapidly than the offspring in other replicates. There is only a weak tendency for individuals of the same Adh genotype, from replicates with different combinations of genotypes, to develop at the same rate (Fig.7.6). It is interesting that the CDxCD ($\alpha\beta x\alpha\beta$) replicates show the most divergent, fast, development times, and that these are comparable with CDxCD ($\beta\beta x\beta\beta$) replicates (Figs.7.4 and 7.5). It seems possible that the Adh-C carrying α chromosome slows development less than Adh-B or Adh-A carrying chromosomes. Unfortunately only one AdhCC/$\alpha\alpha$ line was isolated and so this suggestion may apply only to the particular line and not to Adh-C/α chromosomes in general. There is the possibility, as with other development time differences, that the effects are by-products of viability differences but no such differences have been detected.

After removal of the variation due to karyotype and Adh genotype there was a significant difference between crosses from different populations, effectively between St.Mary's Island intrapopulation crosses and interpopulation crosses, in both males ($F_{4,146} = 3.54$, $P < 0.01$) and females ($F_{4,143} = 3.97$, $P < 0.01$). St.Mary's Island flies developed more slowly than flies from interpopulation crosses at all except very low densities and their development time increased more rapidly with increasing density (Table 7.10). Had there been a breakdown of coadaptation in the interpopulation crosses an increase in development time might have been expected reflecting a general reduction in vigour. The observed effect is in the opposite direction

Table 7.10

Regressions of development time (days) on density for inter- and intrapopulation crosses in the F2

	αα	αβ	ββ
Males			
S	14.0 + 0.065x	13.9 + 0.044x	11.2 + 0.034x
I	15.1 + 0.031x	15.1 + 0.017x	12.5 + 0.016x
Females			
S	12.5 + 0.049x	11.0 + 0.048x	11.1 + 0.045x
I	11.8 + 0.026x	11.7 + 0.026x	12.5 + 0.019x

S - St.Mary's Island intrapopulation crosses

I - Interpopulation crosses

and is reminiscent of the difference between the St.Mary's Island and Morfa Nefyn αα lines in the parental generation. The results, therefore, suggest that there is no breakdown of coadaptation with respect to development rate but that genes for rapid development present in the Morfa Nefyn αα strains are segregating in the F2.

5) Variance in Development Time The variances of development time have been analysed for the P and F1 generations only. Variances were transformed to a logarithmic scale as recommended by Bliss (1967) to make their distributions more nearly normal. The wide range of degrees of freedom in the samples makes this procedure less reliable but as the results are clear cut this should not affect the interpretation.

In all cases there is a strong relationship between the variances and the means of development time that accounts for 40-50% of the variation (for parental generation males $b = 0.09 \pm 0.01$, $P < 0.001$; for females $b = 0.14 \pm 0.01$, $P < 0.001$; for F1 males $b = 0.10 \pm 0.01$, $P < 0.001$; and for F1 females $b = 0.10 \pm 0.01$, $P < 0.001$). This is not surprising as a similar variation in, for example, growth rate will produce a greater difference over a long development time than a short development time. The coefficient of variation is relatively constant in both generations at about 5-15%.

After allowing for the effect of mean development time there are no significant differences between inversions in the parental generation or in F1 males.

In F1 females, ββ crosses had lower variances than αβ crosses ($F_{1,64} = 16.41$, $P < 0.001$). This may be partly an artefact of sampling only once a day which introduces greater errors into the estimation of variances as they become smaller at shorter mean

development times. However it may be that selection for rapid development has been associated with selection for low variance because there is a limit to how early successful pupation can be achieved. It is interesting that the difference in variance is in the opposite direction in males (although not statistically significant in that case) despite the fact that the differences in mean development time are much more marked.

There were no significant differences in the variance of development times between populations in either generation. In particular there was no increase in variance in F1 interpopulation crosses compared with intrapopulation crosses. Such an increase has been found in some studies to accompany F1 luxuriance in crosses between populations with different coadapted gene complexes (Endler 1977).

7.4 Discussion

Most of the lines used in this experiment came from either St.Mary's Island or Morfa Nefyn. These two populations are on opposite sides of the country and are separated by several hundred kilometres of coastline. Indeed it would be difficult to find two sites much further apart within Great Britain. It seems likely that Coelopa frigida individuals rarely move more than a few kilometres (see Introduction) although occasionally strong winds may transport flies several tens of kilometres. The chances of any gene flow between St.Mary's and Morfa Nefyn must, therefore, be remote. They are not even connected by strings of adjacent populations because of long stretches of sandy coast. If populations of Coelopa do develop different coadapted complexes of genes then such differences should be detectable in comparisons between these two populations.

Coadaptation of the genome in general is expected to lead to F1 heterosis, or "luxuriance", and F2 breakdown although a variety of results are possible in theory (Hedrick et al 1978) and have been observed experimentally (Endler 1977). Although the F1 viability in these experiments was much higher than the parental lines this was probably due to inbreeding of the latter. It is the inter- versus intra-population comparison within the F1 that is important. Had these experiments been conducted at a fixed, intermediate larval density then a classic case of F1 luxuriance would have been deduced. However at low and high densities (and indeed taking the overall average) the intrapopulation crosses had the higher viability. Certainly at high densities competition is severe and at very low densities conditions may also be stressful. Perhaps the advantage of the interpopulation F1 from increased heterozygosity is outweighed under stress by the advantage of a coherent coadapted genotype.

The difference in F1 viabilities is the only evidence for general coadaptation in these results. No F2 breakdown in viability was observed. There is a suggestion in the F and F2 generations that the two populations carry some different genes for development time but it is not clear whether or not they are associated with the inversion. There were no differences in the variance of development time between F1 intra- and inter-population crosses.

From the point of view of the inversion the most sensitive test of coadaptation was the comparison of heterokaryotype excess between inter- and intra-population F2 crosses. There was no detectable difference. This lack of evidence for coadaptation can be explained in two rather different, but not mutually exclusive, ways. Firstly, if selection on the inversion is at the karyotypic level in the sense

of Wasserman (1968), then the two sequences contain sets of alleles which are adapted to different components of the environment and which are coadapted in that they produce a heterotic heterokaryotype. The same sequence in two isolated populations may be adapted to different components of the environment or to the same components but in different ways. Combining chromosomes from such isolated populations does not produce heterotic heterokaryotypes because the chromosomes have been selected for their combining ability with the alternate sequences from their own population and not from the distant one. This is the situation in Drosophila pseudoobscura. On this model a lack of evidence for coadaptation may mean (1) that the populations are not sufficiently isolated for differences to have evolved, (2) that the populations have not been isolated for long enough for differences to have evolved, (3) that the polymorphism has only recently spread to one or both of the populations, or (4) that the selective conditions in the two populations are, and have been, so similar that divergence has been prevented. Some such explanation may apply to Drosophila subobscura in which there is evidence for selection at the karyotypic level (Wasserman 1972) but a lack of evidence for coadaptation (Krimbas & Loukas 1979). In Coelopa it seems unlikely as stated above that there is significant gene flow between the populations and the wide distribution of the inversion polymorphism argues for its antiquity. It is possible that the selective regimes at the two sites are very similar but, of course, this would be difficult to prove or disprove.

The second possibility is that selection on the inversion polymorphism is at the genic level (Wasserman 1968). In this case the advantage to the heterokaryotype lies in the fact that it masks

deleterious recessive genes present on the alternative sequences and/or is heterozygous for individual heterotic loci. This hypothesis does not require that the alleles within the inversion sequences are coadapted in the sense of the karyotypic selection model. The same sequences from different populations may contain different sets of alleles but there is no reason to expect interpopulation heterokaryotypes in the F2 to be less heterotic than intrapopulation heterokaryotypes. This possibility is supported by the lack of heterosis in the FX generation which can be explained by suggesting that the α and β sequences from isolated populations contained different sets of deleterious alleles. The homokaryotypes, being $\alpha^{M}\alpha^{S}$ or $\beta^{M}\beta^{S}$, did not suffer from homozygosity for these deleterious alleles.

Selection at the genic level may have been detected in other parts of this experiment. It would predict higher viability in interpopulation rather than intrapopulation F1 crosses. This effect is confused with any effects of coadaptation of the genetic background and, as discussed above, the results are complicated by the effects of larval density. In the F2 ββxββ crosses showed a slight heterozygote excess. This may be more easily explained on the genic than the karyotypic model if the two β sequences involved carried slightly different sets of deleterious alleles although these may have been broken up by recombination in the F1.

Development times in the segregating genotypes of the F2 showed very similar relationships in inter- and intra-population crosses although shifted to slightly later emergence in inter-population crosses especially at high densities. It seems certain that in both populations the α sequence holds together a set of alleles for

late emergence and the β sequence for early emergence. This represents karyotypic selection rather than genic selection. The alleles involved must be similar in the two populations in that there is no breakdown of the differences between karyotypes but they appear to have diverged to some extent as seen by the population effects in both the parental and F2 generations.

It is not surprising to find a mixture of karyotypic and genic selection acting on an inversion polymorphism. Epistatic interactions between loci, effectively coadaptation, are probably essential for the establishment of a new inversion (Charlesworth & Charlesworth 1973) but once the polymorphism is established the alternative sequences will tend to accumulate deleterious recessive alleles because of the restriction of recombination and the relative infrequency of homokaryotypes. Nevertheless there are no other examples of a marked genic effect in the maintenance of an inversion polymorphism as described here.

One of the reasons for looking at population differences was the discrepancy between my results on relative viabilities (Chapter 5) and those of Collins (1978). My stocks were taken from the two populations used here, St.Mary's Island and Morfa Nefyn, whereas Collins' came from Rustington. The observations of this chapter do not support an explanation of this discrepancy in terms of population differences but on the other hand, they do not rule out the possibility that the Rustington population contains alleles not present at either of these sites. The effect of larval density on heterozygote excess in these experiments was only very slight and is, therefore, more consistent with my other observations than with Collins' results.

The other objective of this experiment was to look for potentially selective effects associated with the Adh locus independent of the inversion, especially involving the Adh-C allele. In no case were significant Adh effects on viability detected but there were two suggestive observations: Adh heterozygotes were in excess over homozygotes in the ββxββ F2 crosses, and there were apparent differences between Adh genotypes amongst the FX crosses. Both of these situations involved the Adh-C allele and they suggest that further work on viability of Adh-C bearing genotypes will be necessary to understand the distribution of gene and genotype frequencies in the field. A significant effect of Adh genotype on development time was observed in the F2. However this was not a difference between individual Adh genotypes but between crosses of different types. Again the Adh-C allele was involved in the most discrepant crosses. It seems necessary to extend laboratory experiments on development time, as well as viability, to include Adh-C alleles associated with known inversion sequences.