A study of the Breeding Biology of a Pied Flycatcher population in Wales

BY

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ABSTRACT

The Breeding Biology of a Pied Flycatcher Population in Wales

This study concerns a population of the Pied Flycatcher (**Ficedula hypoleuca**) living in nestboxes in an area of woodland in Mid-Wales. The occupants of 180 nestboxes were monitored during 1988 and 1989. In addition to behavioural observations and records of breeding performance, individual adults and pulli were caught and measured, and a blood sample taken.

In the Pied Flycatcher, polygyny is a common mating strategy in which the two or more females mated to a single male nest in discrete territories up to 500m apart. This behaviour has been interpreted in two ways, firstly as the result of female choice for the quality of the male or his territory, and secondly, as a consequence of male deception, by which already-mated males attract secondary females who suffer reduced breeding success as a result. In this population polygyny was a rare occurrence; only 3 of 240 breeding males were recognised to be polygynous. These males defended two adjacent nestboxes. The breeding success of the three secondary females was not unusually low. These results suggest that a model of male- or territory quality might better explain the situation in this population.

The occurrence of extra-pair mating has being noted in a number of species, including the Pied Flycatcher. In this study it was found to account for 2.7% of the offspring screened by genetic fingerprinting. Another common method for detecting extra-pair paternity uses the heritability of a skeletal measurement. The results from the two methods are shown to be incompatible.

A number of weaknesses with the heritability method are described and discussed.

The increasing number of studies on the Pied Flycatcher throughout Europe reveal that the frequency of mating strategies such as polygyny and extra-pair mating differ from area to area. This suggests that environmental factors may play a major part in determining the costs and benefits of such strategies.

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PART 1

Introduction

CHAPTER 1

Introduction: Mating Systems and Paternity

Introduction

Mating plays a large part in the life of animals, and has probably influenced much of their evolution. Behavioural and physiological traits that influence mating can affect the number and quality of offspring and, if heritable, will be shaped by natural selection. The assemblage of traits is called the mating system, and study of such a system can reveal much about the processes underlying its evolution. The range of mating systems is wide and varied, even within close phylogenetic groupings. A broad classification comprises four categories: monogamy, polyandry, polygyny and promiscuity. The term "mating system" is often used to describe the typical behaviour of members of a population or a species. Within such a grouping, however, there may be widespread variation in the mating strategies adopted. A mating system results from individual patterns of mate choice, from individual resource requirements and individual adaptations to maximise lifetime reproductive success.

Within a species or population, different individuals may have quite different mating strategies, and particular animals may change strategy if conditions demand. Oring (1982) describes the many similarities between the hypotheses regarding the evolution of some mating strategies and the mathematical models described in the theory of "Evolutionary Stable Strategies". Such models have recently become widely used (Maynard Smith 1977, 1982) but were applied to similar questions by Fisher in 1930.

Mating systems are the consequences of genetic, physiological and ecological factors. Ecological factors play a large part in shaping specific mating systems. Reproductive fitness can be sub-divided into any number of components, such

as encounter rate with potential mates, female fecundity or juvenile survivorship. Vehrencamp and Bradbury (1984) list nine divisions for males and six for females. Different behavioural and physiological traits may affect one or more of these components in complex ways. Selection will favour those individuals expressing better combinations of traits. The complexity of links within mating systems makes their study particularly difficult. Ideally, all components of fitness must be identified and analysed together, but in practice, analyses are usually limited and simplistic.

This chapter reviews studies relating a variety of factors to the evolution of a wide range of mating systems. I hope to emphasise the range of approaches which are used. Some are theoretical, others evolutionary and some based upon detailed observation. This study investigates the behaviour of one particular species over three years in one Welsh valley. However, the variety of approaches covered in this chapter is intended to indicate the range of ways, not mutually exclusive, in which such a problem might be addressed. Later, in Chapter 2, I focus more closely upon the particular mating system of the Pied Flycatcher, drawing upon work on other species to compare and contrast the different theories and examine how they might be tested.

Factors Affecting the Nature of Mating Systems

Anisogamy

A fundamental influence on the nature of mating systems is anisogamy, the asymmetry between male and female gamete size. Males have small, inexpensive, mobile gametes (sperm) and females large, nutrient-rich ones (ova). Because of this inequality, the selection pressures on males and females are likely to be different. Bateman (1948) used genetically marked *Drosophila* to show the two major consequences of anisogamy. Firstly, male reproductive success is very dependent on the number of females that he fertilises, whilst

females may achieve near-maximum fertilisation rates from only one copulation. Secondly, and consequently, the variance in reproductive success is higher for males than females.

Phylogeny

The physiology of particular animal groups can dominate the evolution of breeding systems. In mammals, for example, lactation means that, before weaning, only females can feed the offspring. In groups where fertilisation is internal, females are obliged to care for the eggs at least until they are laid. No such constraints bind the male, who is free to leave after fertilisation. This is likely to have an important effect upon the evolution of parental care. The relative contributions of the sexes are governed by the physiology of the animal. Comparisons within taxa exhibiting both modes of fertilisation reveal its effect upon the types of mating system that evolve. A study of 102 families of teleost fish found a strongly significant relationship. Of the families with internal fertilisation, 86% exhibit maternal care. In the majority (70%) of the families with external fertilisation the male cares for the young (Gross and Shine 1981).

When using comparative methods to interpret life history strategies, it is important to separate phylogenetic effects from the ecological ones that are under study. Gould and Lewontin (1979) and Clutton-Brock and Harvey (1984) stress the need for caution in interpreting cross-species comparisons of life history characteristics and mating systems. The taxonomic level at which the analysis is carried out can greatly affect the relationships between variables (Clutton-Brock and Harvey 1984).

Several studies have examined the distribution of avian mating systems across groups of species and at the level of species or families. Lack (1968) found that only 9% of bird species were polygamous, but since his estimate, an

increase in the published of field data has revealed that polygamy is much more common. Møller (1986) surveyed European passerines, and found that 39% were polygynous. Passerines are less polygynous than many other orders of birds (eg. the galliformes); Møller suggests that its frequency throughout the class must be nearer to 50%.

Sex ratio

Theoretical models have shown why the sex ratio is so commonly 1:1 (Fisher 1930). Alleles which tend to cause over-production of a single sex may be favoured in the short term but over a longer time, the only stable sex ratio is that for which the production of sons and daughters are equally profitable. Mostly this is unity. However, in natural populations, the ratio can be biased in favour of one sex. It has been suggested that polygamy may sometimes be a response to transitory changes in sex ratio within a small population. Balfour and Cadbury (1979) found that in Orkney, 84% of adult male Hen Harriers (*Circus cyaneus*) were polygynous. At the time of their study, breeding females were twice as numerous as males and, it seems that females were faced with the alternatives of mating polygynously, or not breeding at all.

Reliance upon help from partners

Polygyny is much more prevalent in mammals than in birds. Part of the reason for this is physiological; young mammals are dependent on milk. Unlike male birds, male mammals are unable to contribute directly to the feeding of the offspring until they are weaned (although some help indirectly by feeding the female).

In some mammals, large litters place a burden on the mother's ability to raise offspring without additional help. Females of a primate, the Saddle-back

Tamarin (*Sanguinus oedipus*), give birth to a high proportion of unusually heavy twins, which, it appears, cannot be reared without additional help. Sometimes helpers are non-reproductive. Often, the help comes from attendant males, all of whom have been allowed to copulate with the female and, therefore, have some chance of being the father of her offspring (Goldizen 1988).

While a male Wandering Albatross (*Diomedea exulans*) is absent from the nest, the incubating female remains without food. She is dependent on the return of the male from a foraging trip. Males are known to cover between 3600 and 15000km on a single foraging trip during an incubation shift (Jouventin and Weimerskirch 1990) and can be absent for up to a month. This reliance is thought to explain the high degree of monogamy and the lifetime pair-bond in this species (Lack 1968, Tickell 1968, Wittenberger 1979).

Fidelity between seasons in the Kittiwake (*Rissa tridactyla*) is thought to be maintained because of the cost of divorce in terms of breeding success. The help of a new male, it seems, is not as efficient as that from an established partner. Pairs mating together for the first time have a lower reproductive success than established pairs of the same age (Coulson and Thomas 1984).

Ecology

Earlier in this chapter I discussed the phylogenetic correlates of polygyny. I described how physiological similarity among related species can predispose them to similar mating habits. Differences between the mating systems of closely-related species can often be attributed to dissimilarities in their ecology. Later, I discuss how, within a species, the resources available to an individual can affect their breeding success, but here I am concerned solely with cross-species comparisons in which a species is described according to general features of the ecology and breeding behaviour of its members.

To find the associations between the ecology and breeding systems of closely-related species, workers must distil the complex environment of the organism into a number of simple, but relevant, variables. They select particular environmental features which they believe might have affected the evolution of the breeding systems in the group under study. By categorising and measuring these features for each species they produce variables which can be compared and correlated with classifications based on the mating system.

For example, Jolly (1972) classified primate species into six ecological divisions ("nocturnal", "arboreal leaf-eaters", "arboreal omnivores", "semi-terrestrial leaf eaters", "semi-terrestrial omnivores" and "arid-country species"). She examined measurements of social organisation such as group size and home range, and concluded that her ecological categorisation were not useful in explaining social patterns. Perhaps the links between ecological situation and adaptation in social behaviour are too subtle to be revealed by this simple analysis.

Haartman (1969) surveyed 158 European passerines and found polygyny significantly more common in the 36 species with enclosed nests. Of these, 64% were polygynous, as compared with only 31% of birds with open nests. He suggested that the correlation may be because enclosed nests provide protection from predation and weather, resulting in a reduction in the requirement for parental care.

Orians (1969) regarded mating systems as the result of active female choice, based primarily upon the availability of resources within a defended territory. He found four ecological factors that seem associated with polygamy (Table 1.1).

Orians found polygyny more prevalent in:
Marsh nesting birds with altricial young
Inhabitants of early successional habitats
Birds in which food is widespread but nesting sites are restricted
Birds in which clutch size is influenced by factors other than the ability of parents to feed their young
Comparisons are between polygynous and monogamous species

Table 1.1: Ecological correlates of polygyny found by Orians (1969)

Marshes and early successional vegetation are both swiftly-changing habitats, in which food availability also changes rapidly in time and space. In both habitats, says Orians, female settlement will follow the patchy distribution of food, and regular spacing of male territories will result in polygyny. In other species, nesting sites are the patchily-distributed resource that can be monopolised by a single male. Orians cites the Pied Flycatcher as a prime example, although the model does not explain why a male's territories are dispersed.

The last of Orians correlations concerns the effect of male polygyny upon the defence of the nests and the rearing of the young. If the reduction in male investment caused by polygyny has little effect upon the survivorship of the young, then polygyny will be favoured. This condition will be met when predation rates are low, and food easy to come by.

Møller (1986), used quantitative methods in an examination of 112 European passerines. He found two ecological attributes that are significantly more common in the 47 polygynous species (Table 1.2).

Møller found polygyny more prevalent in:
Marsh nesting birds
Birds which winter in the tropics
Comparisons are between polygynous and monogamous species of European passerine.

Table 1.2: Ecological correlates of polygyny found by Møller (1986).

Polygyny is commoner in marsh-nesters because of the patchy distribution of food in such habitats. Good nesting sites can be clumped within one male territory. In addition, the abundant food supplies in certain parts of these areas allow broods raised by a single parent to survive, enabling males to divide their efforts between several nests without seriously affecting the survival rates of the nestlings.

Wintering in the tropics is associated with habitats that are unsuitable for the species outside the breeding season, which is often short. In circumstances such as these, the amount and accuracy of female choice may be reduced. Females may lack the time to check the mating status of males and thereby avoid polygyny, even if it is eventually detrimental to their reproductive success.

Møller finds that the polyterritorial habit is associated with certain ecological conditions (Table 1.3). The high incidence of plumage dimorphism in these species is thought to be related to the increased importance of sexual selection because male fitness is more variable, and mate choice is based less upon aspects of territory and habitat quality (Møller 1986). Size dimorphism is reduced because there is less competition between males through territorial conflict.

Møller found that polyterritorial birds:
Nest solitarily rather than colonially
Winter in the tropics
Are more plumage dimorphic
Are less size dimorphic
Comparisons are between polyterritorial and mono-territorial polygynous passerines from Europe.

Table 1.3: Ecological correlates of polyterritoriality found by Møller (1986).

Large-scale comparisons can be complicated by the variation in the knowledge of the bird fauna from region to region. The temperate European and North American avifaunas have been studied for longer and are generally better known than any other. However, many more species are found in the tropics, but these are relatively little known. The proportion of migrants is greater in the well-studied, temperate regions. Birds in the developed temperate regions are often more easily studied than, say, species living in remote forest in the tropics.

Comparative methods can be used with categorical or continuous data (Harvey and Pagel, 1991). Generally, species are categorised as "poly-" or "mono-"gamous but in reality there may be both within-individual and between-individual variation in mating behaviour. If observations of mating behaviour can described as a continuous variable then the analysis would be similar to comparing, for example, leg length. Unfortunately, it is difficult to make measurements of mating behaviour which are definitely discrete or continuous variables. Workers must forfeit the reality of their data to fit the type of analysis preferred.

Quality of resources held by mate

Much research has featured the role of resource availability on the evolution of mating systems. The reproductive success of males is often limited by access to females, while female reproduction depends upon resources, particularly energy. Males often compete for control of the limiting resource and gain the opportunity to mate with the females that use it.

One resource which can limit breeding success is the availability of suitable nesting sites. This is true for the Slimy Sculpin (*Cottus cognatus*), a common Canadian freshwater fish whose nest consists of a particular sized rock under which the male must excavate a cavity to accommodate him and the eggs (Mousseau and Collins 1987). Successful reproduction depends upon the availability of such rocks and, in certain lakes, males are faced with a shortage. In these lakes, the rate of polygyny is higher. Males which control a rock are able to mate with more than one female, while rock-less fish remain unmated.

In the Lark Bunting (*Calamospiza melanocorys*), female breeding success is affected by their ability to fledge healthy offspring on a hot, open habitat. A major cause of nestling mortality is overheating. Females whose nests are in thick cover fledge more offspring. Males, therefore, compete to monopolise suitable cover, and female settlement is dependent on it's availability. As a result, males whose territories enclose suitable bushes are able to mate with more than one female (Pleczynskya 1978).

If males hold resources, and females choose mates on the basis of these, then it follows that the number of females mating with each male depends on the size of his holding. A formal expression of this is the Polygyny Threshold Model, a widely used interpretation for avian mating systems. (Verner 1964, Verner and Willson 1966, Orians 1969). This is particularly relevant to this study and is discussed more fully in Chapter 2.

Quality of mate

In making the distinction between quality of resources held by a potential mate and quality of the individual itself, I am imposing a rather artificial division. It is likely that the genetic constitution of the animal affects the ability to acquire and maintain an important resource such as a territory or spermatophore. Thus, holding a quality resource shows the quality of the holder, and mate choice based upon the possession of the asset will result in incidental benefits. However, certain criteria for mate choice can comfortably be separated from those based on the "immediate" gains of access to a resource. Fecundity, fertility, genetic complementarity or evidence of some feature of "genetic quality" may all be used as standards for mate choice. In certain situations, the only contribution of the male is his gametes, for example in lekking species, and mate choice is based upon male dominance and/or appearance (Bradbury 1981).

Deception

The cues upon which mate choice is based may not necessarily always be honest. As stressed above, the mating pattern and investment level that is optimal for one member of a breeding coalition, may not be ideal for the other(s). The Deception Hypothesis is particularly important in the interpretation of the Pied Flycatcher mating system and is discussed in Chapter 2.

Alternative Reproductive Strategies

Mating systems have often been regarded as a fixed feature of a species. Species are classified as monogamous or polygamous, and further subdivided as, for example, polygynous or polyandrous. There is a drawback with such a

classification; deviations from the general habit of the population are not characterised, however widespread they may be. As a result, deviations are often described as "alternative" reproductive strategies, unintentionally implying that they are subsidiary, possibly even opportunist, departures from the "true" mating system. As evidence is gathered, it is becoming clear that the mating patterns within a population are complex, and that descriptions and analyses should echo this complexity.

In this section I describe some "alternative" mating strategies. I hope to emphasize that their role in the evolution and ecology of mating behaviour is not subordinate to the "mating system"; rather that both should be regarded with a holistic approach.

A feature common to many "alternative" strategies is conflict between individuals. Brood parasitism, extra-bond copulations, and infanticide (Bertram 1976, Crook and Shields 1985) are examples of such behaviours, in which the fitness of one individual is improved to the detriment of another.

Egg-dumping

Inter-specific brood parasitism has long been known in cuckoos. It has been reported very occasionally in other species, and records of Great Tit chicks being reared in the nests of Pied Flycatchers could be interpreted as such. (Järvinen 1977, Pitkänen 1982).

Brood-parasitism ("egg-dumping") is increasingly being reported within species. Apart from observing a female laying an egg in the nest of another, it can be detected in three ways. The first depends on the fact that females normally lay only one egg per day, so any additional ones should result from an egg-dump. The second clue is the occurrence of eggs different in shape and colour to those of the host female. Thirdly, and most conclusively, intra-

specific brood parasitism (IBP) can be revealed by genetic methods of parentage analysis.

Brown and Sherman (1989) report direct observations of brood parasitism, in which a female was seen entering the nest of another, whose clutch size was known. When the visiting female left, another egg had been laid. Using the second method on Swallows (*Hirundo rustica*), 3 to 31% of nests were found to have been parasitised (Møller 1987b). In a DNA fingerprinting study of a British population of the same species, Wellbourn (1991) found that only one offspring in 296 resulted from egg-dumping.

Cliff Swallows (*Hirundo pyrrhonota*) display an unusual form of brood parasitism. Females move partially incubated eggs from their own nest to those of nearby conspecifics (Brown and Brown 1988). The benefits to parasitic females are twofold, their costs of parental care are decreased and the risk of total breeding failure reduced by spreading eggs in several nests.

The subject of this study, the Pied Flycatcher, reportedly exhibits brood parasitism. Håland (1986) describes observations made by G. Høgstedt who, occasionally, found two eggs laid on the same day in a single nest. This seems to show that IBP occurred, albeit in a population whose density was raised to artificially high levels.

Extra-bond copulation and paternity

In many species with apparently stable breeding units of pairs, trios or harems, there are records of individuals than depart from these and indulge in extra-bond copulations (EBCs). In monogamous species these are called extra-pair copulations. Several studies have now quantified the effects of EBCs in terms of extra-bond offspring (EBOs). Some of these are listed in Table 1.4.

Species	EBO Rate	Method	Reference
Lesser Snow Goose *Anser caerulescens caerulescens*	2.4%	Plumage Polymorphism	Lank *et al.* (1989)
	35.2%	Genetic markers	Quinn *et al.* (1987)
Mallard *Anas platyrhynchus*	8.0%	Plumage Polymorphism	Burns *et al.* (1980)
	3.0%	Starch Gel Electrophoresis	Evarts and Williams (1987)
Swallow *Hirundo rustica*	26.0%	Tarsus length heritability	Møller (1989)
	3.4%	DNA fingerprinting	Wellbourn (in prep.)
Purple Martin *Progne progne*	15.3%	DNA fingerprinting	Morton *et al.* (1990)
Dunnock *Prunella modularis*	1.0%	DNA fingerprinting	Burke *et al.* (1990)
Willow Warbler *Phylloscopus trochilus* Wood Warbler *Phylloscopus sibilatrix*	0.0%	DNA fingerprinting	Gyllensten *et al.* (1990)

Species	EBO Rate	Method	Reference
Indigo Bunting *Passerina cyanea*	14.4%	Starch Gel Electrophoresis	Westneat (1987b)
	35.0%	DNA fingerprinting	Westneat (1990)
House Sparrow *Passer domesticus*	15.1%	DNA fingerprinting	Wetton (1990)
Zebra Finch *Taeniopygia guttata*	2.4%	DNA fingerprinting	Birkhead *et al.* (1990)
Pied Flycatcher *Ficedula hypoleuca*	24.0%	Tarsus Length Heritability	Alatalo *et al.* (1984a)
	-	Tarsus Length Heritability	Lifjeld and Slagsvold (1989b)
	18.0%	Tarsus Length Heritability	Alatalo *et al.* (1989)
	4.0%	DNA fingerprinting	Lifjeld *et al.* (1991)

Table 1.4: Estimates of the proportion of extra-bond offspring (EBO) in a number of bird species. Differences in the EBO rate within species might reflect flaws with the detection methods, or between-year or between-site variation. The study of the Pied Flycatcher by Lifjeld and Slagsvold (1989b) found that the tarsus length heritability method estimated that the EPO rate was close to zero. However, they maintained that the method was flawed, and because of this they did not conclude that the EPO frequency was zero.

The reproductive success of males is directly increased by extra-bond fertilisations, while the relationship between EBFs and female fitness is more subtle. Because of this, males stand to gain most, and are very often the initiators of EBCs. Females may respond by actively accepting or rejecting mating attempts, or they may be physically unable to resist the advances of the male, for example, in the Lesser Snow Goose (Mineau and Cooke 1979). If the female has a choice, she should base it on criteria that will improve her lifetime reproductive success. Females in extra-bond copulations rarely receive anything other than gametes, and many theories about female choice of EBC partner emphasise criteria based upon the genetic quality of the male. Alternatives to these are:

Non-genetic benefits to the female, for example, the transfer of nutrients during mating by male butterflies (Boggs and Gilbert 1979).

Safeguarding against sterility of the main partner. Wetton and Parkin (1991) found a relationship between fertility of the cuckolded male and the proportion of extra-pair offspring in his brood. This suggests that females who EPC, reduce the risk of their fitness being affected by their partner's infertility.

Promoting genetic diversity (as opposed to quality) within the brood, and therefore reducing the chances of all succumbing to disease.

Promoting an allegiance with the EBC partner that may result in paternal care by him, or in pairing with him in future.

Females have no preference, and mate at random.

What is the effect of cuckoldry upon the fitness of the cuckolded male? It is very likely that extra-pair mating increases the fitness of the male who fathers

EBOs (although he may pay a cost if his cuckoldry increases the chance of his own female mating with another). It is probable that it also increases female fitness, via a range of possible mechanisms. The reproductive success of the cuckolded male is almost certainly reduced. His reproductive output will be reduced because extra-pair offspring would otherwise probably have been fertilised by him. He will also lose because paternal investment will, in part, be directed towards raising unrelated chicks. It seems likely, therefore, that males should prevent their partner having extra-pair copulations and fertilisations. Extra-pair copulations can be deterred by mate-guarding. If EBC's have occurred, their effects can be reduced if the rate of within-pair copulation is high (Birkhead *et al.* 1987). A wide range of studies examine these phenomena in birds and other species (Birds: Birkhead *et al.* 1987, Westneat 1988; Other species: papers in Smith 1984).

Males whose mate has had EBCs also have the option of reducing their investment in the mating attempt, even to the extent of abandoning the female altogether. Møller (1988) found that male Swallows who witness an EPC by their mate, reduced their parental investment. Richardson and Coetzee (1988) document the abandonment of the breeding attempt by a male Aardwolf (*Proteles cristatus*) whose female was seen to EPC. The risks of erroneously rejecting their own legitimate offspring would make it expensive for males to reduce their investment unless very sure of their non-paternity.

The Flexibility of Mating Systems

Flexibility of mating systems is known both within and between populations. Environmental differences between areas or years might change the proportions of individuals adopting each mating strategy. As Trivers (1972) points out, the optimum for one individual may depend on those adopted by others. Such a system could result in a mixed population, in which individuals adopt different

strategies. Individuals might specialise or change strategy according to their age and condition.

In the Dunnock (*Prunella modularis*), monogamous, polygynous and polyandrous mating systems have all been recorded in the same area during the same period (Davies 1985). Extra-bond copulation is known to occur rarely (Burke *et al.* 1990). In most polygynous species, only a proportion of females mate with polygynous males; others do not share their mate. Conversely, in many typically monogamous passerines, polygyny has been recorded as a rare event (Orians 1969).

Foxes (*Vulpes vulpes*) living on an Alaskan island were typically polygynous. When El Niño caused a crash in seabird numbers, and their primary food source failed, the foxes ate rarer, smaller species, and most became monogamous (Zabel and Taggart 1989). In the Tengmalm's Owl (*Aegolius funereus*), a north European species that feeds on small mammals, the frequency of polygyny is higher in peak vole years (Korpimäki 1988). In this, and other birds of prey, when food is abundant, males can feed more than one female and polygyny is favoured (Newton 1979).

Even in the Wandering Albatross (*Diomedia exulans*), which is almost exclusively monogamous, and in which mates are faithful for many years, there is a record of a forced extra-pair copulation (Tickell 1968). Surely, if this is the case, then no mating system can be regarded as invariable?

Such examples suggest that it may be erroneous to regard mating systems as permanent features of a species or population. They may be better considered as general descriptions of the situation in a particular circumstance, capturing a generality, but masking much of the underlying complexity.

CHAPTER 2

The Mating System of the Pied Flycatcher

Introduction

Research on the Pied Flycatcher has figured prominently among studies of avian mating systems (Clutton-Brock 1988). The species is common in most of Northern Europe, nests readily in nestboxes and, in many respects, is easily studied. Most breeding male Pied Flycatchers are mated to a single female, but a small proportion are polygynous. Typically, males with two or more mates, defend separate nest sites and one female nests in each, a phenomenon that is called polyterritoriality (Haartman 1949).

To understand the evolution of mating behaviour in the Pied Flycatcher, two major topics must be addressed:

> Polygyny: Why do some females mate with males who are already mated?
>
> Polyterritoriality: Why do the females mated to the same male live on different territories?

Several explanations have been proposed for the evolution of the mating patterns seen in the Pied Flycatcher. Some consider polygyny, others polyterritoriality and some both. Theories can be differentiated by their predictions of the costs and benefits of the behaviour to the participants. Important among these are the extent to which male partners are chosen rather than merely acquired, the degree of knowledge upon which any decision is based, and the relative importance of territory quality and male quality in the choice. Similar

hypotheses and predictions have been tested in other species, and this chapter reviews both the work on the Pied Flycatcher and relevant studies of other bird species.

The breeding behaviour of the Pied Flycatcher

In April, the males arrive on the breeding grounds and establish territories. Disputes are common, and occur near to the nest site; the birds seem to defend a point rather than an area. As Von Haartman put it in 1956, "a Pied Flycatcher's home is his castle". Having occupied one or more territories, males try to attract the arriving females. Breeding commences rapidly, and the females soon lay eggs. Males begin to guard their mates less, and some spend time at a second territory, where they court later-arriving females. Those males who attract more than one mate will devote most of their time and effort to the first brood, but may help the secondary female, especially when the other brood has fledged.

Geographical variation in the frequency of polygyny

The rate of polygyny varies across the range. Figure 2.1 shows the frequency as measured in some studies in Northern Europe. Different experimental manipulations and nestbox densities may affect the proportion of polygyny differently in different studies. The proportion of young sired by these birds is the polygyny frequency multiplied by the relative numbers of offspring per male.

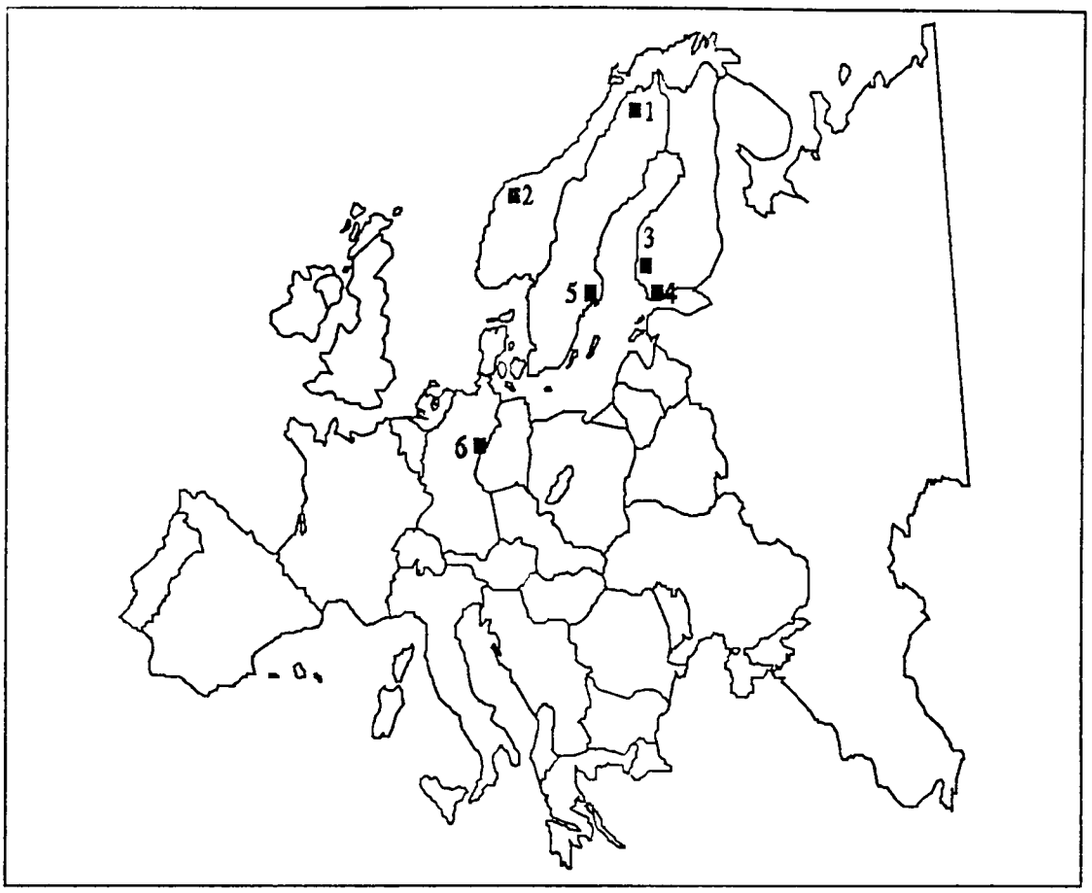


Figure 2.1: Polygyny frequency measured in some studies on the continent.

1 Northern Sweden	3%: Nyholm and Myhrberg (1983), Röskaft *et al.* (1986)
2 Central Norway	4%: Röskaft *et al.* (1986)
3 SW. Finland	7%: Haartman (1949, 1951)
4 S. Finland	7%: Röskaft *et al.* (1986)
5 Central Sweden	15%: Alatalo *et al.* (1982, 1984)
6 Lingen, Germany	10%: Winkel and Winkel (1984), Röskaft *et al.* (1986)

Territory Quality and Polygyny: the Polygyny Threshold Model

It has been suggested that female Pied Flycatchers choose to settle in whichever breeding territory will maximise their reproductive success. Female settlement pattern is determined by female choice which is based upon male territory quality. If the territory held by a male is substantially better than the next-best alternative, then it will pay a female to settle there, even if she must bear the cost of sharing with another. If the difference in quality between two territories is large enough to offset the costs of sharing, then the difference is

said to exceed the "polygyny threshold" and polygyny will be favoured. This simple theory has been expanded and developed, and has been applied in a number of ways to general and specific studies of polygynous (and polyandrous) mating systems. It is called the "Polygyny Threshold Model", and is illustrated in Figure 2.2. The model has been applied to the case of the Pied Flycatcher, but opinions differ about the degree to which it explains the mating system of the species.

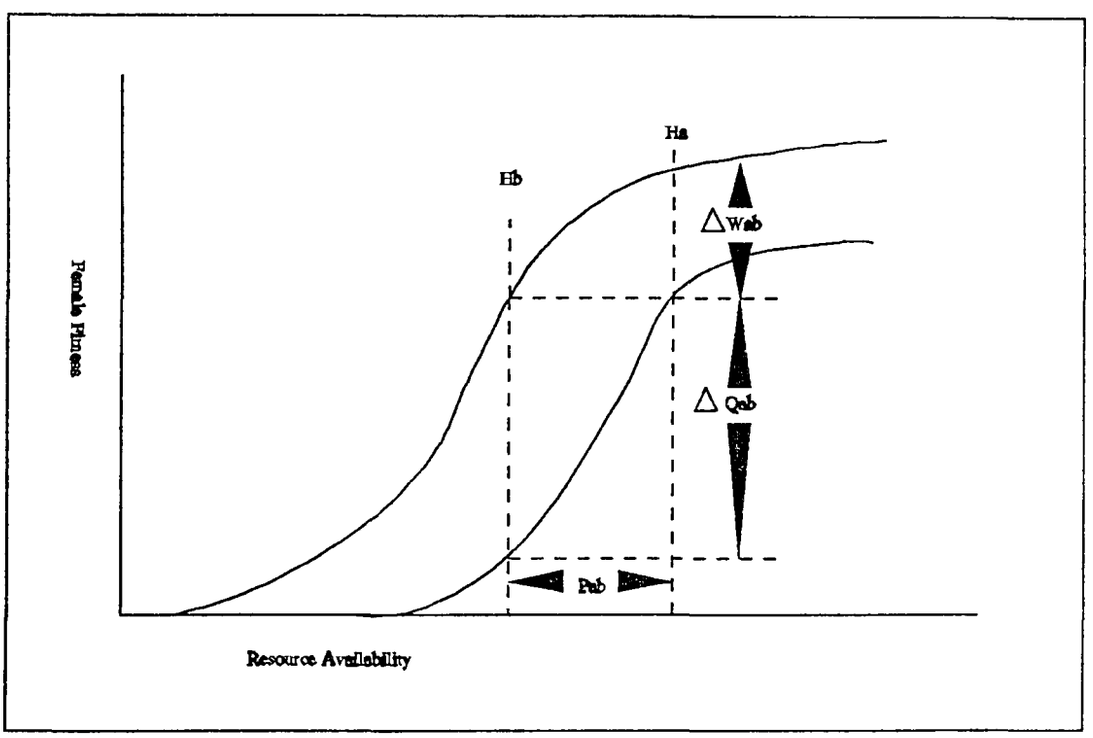


Figure 2.2: A graphical interpretation of the polygyny threshold model showing the conditions necessary for the evolution of polygyny. Polygyny will be favoured when the success of secondary females (lower curve) in habitat **a**, of quality H_a, is greater than that of monogamous females (upper curve) in less suitable habitat **b**, of quality H_b. The difference between the fitness of primary females in the two habitats is ΔW_{ab}. A female will become a secondary mate in the good habitat, **a**, if its quality is sufficient to increase her fitness by the amount Δ**Qab**. The habitats differ in quality by ΔP_{ab}.

A number of assumptions are made in the original Polygyny Threshold model proposed by Orians (1969). Davies (1989) finds that empirical evidence to support the assumptions is sparse. The first assumption is that male territories

vary in quality, and that female reproductive success is affected by this variation. The dimensions of the nesting cavity are related to clutch size and presumably fledging success in both natural and artificial nests (Gustaffson and Nilsson, 1985; but for negative evidence see Alatalo *et al.*, 1988a,1988b). Assumption number two is that females suffer a cost to sharing a territory, the evidence for which is described below. The third and fourth assumptions concern the ability of the birds to choose and settle in the optimum sites. Females must be able to accurately assess territory quality and male mating status, and must then be able to mate with the male holding the territory of their choice. Alatalo *et al.* (1988a) found that time-consuming assessment of male quality and/or mating status could prove costly, by delaying the initiation of breeding. Stenmark *et al.* (1988) and Alatalo *et al.* (1990) both find evidence for behavioural indicators of male mating status, but disagree whether females make use of these.

A review of the work done at Uppsala by Alatalo and Lundberg (1984c) cites two ways in which their data are incompatible with their interpretation of the Polygyny Threshold model. These are that secondary females have lower breeding success than monogamous and primary birds, and that their predictions of settlement pattern based on estimates of territory quality were not borne out.

Breeding success of secondary females

Alatalo *et al.* (1981) tested the prediction that polygyny should only occur if the reproductive success of secondary females is at least as high as that of simultaneously laying monogamously mated females. In the Uppsala study, secondary and primary polygynously mated females fledge fewer young than monogamously mated ones. The number of young fledged declines through the season. Brood size can be standardised in relation to the regression line for monogamously mated birds, in which case both primary and secondary females

are significantly less successful. If the number of young fledged by monogamously mated females is 100%, then primary females fledge 80.9%, and secondary females only 64.8%. In a Norwegian study, Stenmark *et al.* (1988) found that secondary females were at less of a disadvantage than those in Uppsala. Secondary birds fledged 86% as many young as monogamously mated ones.

The reduction in the number of young fledged by polygynously mated females is partly due to their laying smaller clutches. In addition, there are differences in feeding rates at nests of different status. Males at monogamous and primary nests contributed significantly more than males at secondary nests. Male provisioning at the primary nest declines as the brood approach fledging; the male transfers his attention to the secondary nest and feeding rates there increase.

Contributions of males at monogamous and primary nests are significantly higher than those at secondary nests. Male feeding at secondary nests decreases late in the nestling phase. Female feeding rate at secondary nests is greater than at the other types of nest.

It seems that if females are without help at the nest they compensate by increasing their feeding rate, but are unable to make up fully for the absence of the male. In the later stages, the rate of feeding per nestling may approach that of monogamous birds. Despite this, some young may die and the weights of fledglings from secondary nests are lower, and the tarsi of these birds are permanently shorter (Alatalo *et al.* 1982a). Again, the Norwegians find otherwise. In their study there was no significant difference between the fledging weights of offspring with different maternal mating pattern (Stenmark *et al.* 1988).

So, in terms of phenotypic quality and number of young fledged, at least in Uppsala, polygynously mated females (especially secondary females) lose out

and, according to Alatalo *et al.* (1982a), this is not compatible with the Polygyny Threshold model.

Nesting density and the frequency of polygyny

Using the Polygyny Threshold model, Alatalo and Lundberg (1984c) generated predictions about nest site distribution. To do this they made certain assumptions.

> Nest sites are (or contain) the resource which males monopolise, and on which females base their choice of mate.
>
> Habitat heterogeneity is such that boxes within a 50m radius are of similar quality.

The first assumption is borne out by the study of Alatalo, Lundberg and Glynn (1986a), who found that female settlement pattern was influenced by the quality of the nest site, rather than characteristics of the male himself.

In their 1984 study, Alatalo and Lundberg arranged nestboxes in clumps, with 2-9 boxes per group and over 100m between groups. Their prediction, was that males would choose the best secondary territory available (which would be nearby). Thus, they say, the Polygyny Threshold model is true only if males showed a tendency to choose boxes in the same clump. The tendency was, in fact, the opposite, supporting the hypothesis that successful polygyny depended upon having distant nests. This may be true, but without detailed analysis of territory quality and the topography of the study area, their predictions are very much dependent upon the validity of the assumptions.

The "Sexy Son" Hypothesis

It has been suggested that females who mate with already-mated males might fledge fewer offspring but still gain because the quality of the offspring is high. Weatherhead and Robertson (1979) proposed that females mated to polygynous males might gain if their male offspring were also polygynous; when compared with other females, they would have "Sexy Sons". Empirical evidence from studies of Pied Flycatchers does not to support this theory.

The phenotypic quality of young from polygynous nests is low. They are lighter and smaller than fledglings from monogamous nests. Although nestling size itself seems unrelated to future mating status, some other result of poor nutrition may cause a reduction in mating success.

The "Sexy Son" Hypothesis requires that heritability of male mating status is high. Heritability of phenotypic traits can often be around 70% (van Noordwijk *et al.* 1980) but heritability of mating status is likely to be lower because it is strongly influenced by environmental effects. Also, male mating status changes with age. Polygyny is commoner in older birds. The between-years correlation of mating status is 0.20 (Alatalo and Lundberg, 1986b; no standard error was given). This measurement, the repeatability, gives a maximum estimate of heritability (Falconer 1981).

Theoretical considerations show that low heritability of mating status would be expected. Heritability of characters closely related to fitness is likely to be low. There should be little genetic variation in such traits because advantageous mutants would spread to fixation (Fisher 1930, van Noordwijk *et al.* 1980).

Alatalo and Lundberg (1986b) say that if the "Sexy Son" Hypothesis is correct, to make up for the initial disadvantage to polygynously mated females, the heritability of male mating status must be above 0.80. Thus, using both the

Polygyny Threshold model and the "Sexy Son" Hypothesis, there is no way in which a secondary female can accept her status without reducing inclusive fitness.

In addition to the theoretical arguments, field data on Collared Flycatchers shows that polygynous males are more likely to have been fathered by monogamous males than polygynous ones (Gustaffson 1985).

The Polygyny Threshold Model and the Sexy Son Hypothesis: History and Tests on Other Species

This chapter is primarily concerned with the mating system of the Pied Flycatcher, but in this section I digress to discuss other related work. The three competing theories were developed by workers on other species, and have been applied, tested and criticised in a number of situations. These studies are relevant because they test and refine theoretical and practical ideas that may be applicable to the study of Pied Flycatchers.

The Polygyny Threshold model was introduced by Verner and Willson in 1966, who used it to interpret their survey of the breeding systems of North American passerines. It was presented in a graphical form by Orians (1969) and used to predict his ecological correlates of polygyny (see Chapter 1 and Table 1.1). Orians defined the Polygyny Threshold as "the minimum difference in quality of habitat held by males in the same region sufficient to make bigamous matings by females favoured by natural selection". His model assumes that females make mating decisions to optimise their inclusive fitness, that female fitness depends on the quality of the territory held by the male, and that it declines when he takes additional mates. A single female may be faced with the alternatives of mating with an unpaired male with a poor territory or an already-paired male with a better territory. If the difference in territory quality is high enough, then polygynous mating may be most profitable for her despite the low investment of her new partner. Orians' graphical model is

reproduced in Figure 2.2. If the slope of the fitness-function is monotonic, then its precise shape has no effect on the settlement order or the distribution of mates among males, and detailed knowledge of the relationship between fitness and harem size is not absolutely necessary.

The testable predictions of the original model are that:

> Polygynous females rear as many young as monogamous ones.
> Harem size increases with territory quality.

Altmann *et al.* (1977) show that the first prediction is not valid for use as a test of the model. The model predicts that females will adopt options according to their rank order, irrespective of the magnitude of the difference. Because of this, it is quite possible that, in a population where the polygyny threshold model is correct, there may still be a substantial difference in fitness between the last female to pair with a monogamous male and the first to mate with an already-paired one.

The second prediction is more readily applied to field investigations. If it is known that attributes of a territory limit breeding success, and if the distribution of these among males is measured, then the model predicts that females should settle with males in order of decreasing access to these, the "critical resources". In most cases, it has proved difficult to identify the important resources. Pleczynskya (1978) found that nesting success of female Lark Buntings (*Calamospiza melancorys*) depends on the availability of cover from the sun. Manipulation of the shade within territories can alter female settlement patterns, so this was deemed to be the "critical resource". However, settlement did not follow the predicted order; perhaps, it is suggested, reproductive fitness of females is also dependent upon male feeding assistance (Pleczynskya and Hansell 1980). Lightbody and Weatherhead (1987) suspect that in the Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*), territory quality is strongly linked to safety from predation. They used

measurements of territory features as indicators of accessibility to terrestrial predators, concealment and protection from adverse weather. They assumed that food availability was proportional to the length of waters edge, and combined all the measurements to give "territory quality". Assuming this remains constant between years they predicted that female choice for good territory would also be repeatable. However, it was not, and their measurement of territory quality was not linked to female choice or nesting success.

Not only is it difficult to measure resource availability, the X-axis of the graph, but female fitness, the Y-axis, cannot be directly estimated. In many studies, the number of young fledged by a female has been used as a convenient currency with which to represent female fitness, assuming equal survival and breeding success of fledglings from monogamous, primary and secondary nests.

When the predictions of the model are not borne out by comparisons of female fledging success, other components of fitness are invoked. Authors stress the importance of female survival (Elliot 1975), post-fledging mortality (Lenington 1980), and the genetic quality of the male (Weatherhead and Robertson 1979).

Weatherhead and Robertson (1979) found that field data did not support predictions of the polygyny threshold model based upon female fledging success, and suggested that the reasons were differential survival and breeding success. Besides the quantity of young produced, they added, fitness was dependent on the genetic quality of the birds. They suggested that females chose to mate polygynously with genetically superior males, and that they gained because their sons would inherit the same traits that made their fathers polygynous. This became known as the "Sexy Son" hypothesis and its application to the Pied Flycatcher has already been discussed.

Only if fitness and resource availability were measured directly, could the model be tested directly. The Polygyny Threshold model would be supported

if female choice between unmated and already-mated males optimised their fitness. In studies where only certain components of fitness are measured, the model cannot be disproved, one can only test the importance of those factors that were measured.

Given the inherent problems of the Polygyny Threshold Model and its derivatives, it is perhaps not surprising that the literature is confused and contradictory. The model, in its various forms remains the most commonly cited theory in the study of avian mating systems. Oring (1982) cites some elaborations and exceptions to the basic model. According to Møller (1986), these apply to approximately two-thirds of all European species. The basic model is unsuitable in three situations:

> When the quality of the breeding situation fluctuates within a breeding season.
>
> When most or all of the habitat is either very good or very bad, and marginal habitat is rare.
>
> In cases of polyterritorial polygyny (that is, in 14% of European passerines).

In the first case, when a habitat varies in quality through time, there would no longer be a correlation between fledging success and female settlement pattern. In addition, the ability of females to assess habitat quality might be compromised by the fluctuating environment. Wittenberger (1979) showed that if the habitat-quality curve is of a step-like form, and marginal habitat is rare, coloniality will be favoured, and polygyny will be largely a response to the shortage of suitable nest sites. The third criterion is based upon the unverified assumption that the polyterritorial habit results in uncertainty about the mating status of the male, and the valid criticism that the polygyny threshold model makes no predictions regarding mono- versus polyterritoriality.

Defending a few discrete territories is considered by Alatalo *et al.* (1981) to be a male adaptation to reduce the amount of information that a female can gather before she makes mating decisions. If females mated to a male are isolated from each other, it may be possible for a male to acquire two mates without either being aware of the other until she is committed to caring for the brood, with or without male help. The male will gain, the females may lose, but have little or no choice in the matter.

Evidence in support of this, the Deception Hypothesis, is difficult to gather. Generally, because it does not predict female settlement patterns, these cannot be used as test criteria and deception is accepted as the remaining option when other explanations have been ruled out. The crux of the model is that there is a high cost to being "choosy", and assessing the marital status of potential mates. Because of this, females are often unaware of the marital status of the male with whom they have mated (at least, at first). To test females' knowledge of their partners is difficult. Alatalo and Lundberg (1984b) give anecdotal evidence of some cases when secondary females must have been aware of the primary mate, but describe these as unusual. Stenmark *et al.* (1988) detail ways in which a human observer can distinguish unmated and already-mated males by their behaviour, after about an hour's observation. It seems likely, therefore that females have the potential to assess male mating status fairly rapidly; it would be remarkable if they did not. The Norwegians suggest that when females become secondary mates, they do so because it is the best option at the time.

Once a female has mated, if she is a secondary female, and if this is likely to reduce her fitness, she has two alternatives:

Abandon the nesting attempt and renest with another male.

Continue the current nesting attempt.

The factors influencing the decision are very much dependent upon time: the breeding season is short, clutch size declines with date and renesting will take several days. A female should renest only if the delay between abandoning her first clutch and relaying is short enough for her to retain her expected fledging success. Alatalo and Lundberg (1984b) corrected their 1981 estimate to 15 days, but emphasize the simplicity of the model and the need for other factors to be accounted for. The costs of searching for a new male are difficult to assess, but Stenmark *et al.* (1988) and Dale *et al.* (1990) suggest that their search pattern is restricted, presumably because it is costly.

Arguably the most elegant support of the Deception Hypothesis is the discovery that secondary females lay a reduced clutch size compared with a simultaneously laying monogamous bird. Alatalo *et al.* (1981) suggest that this is an adaptation to ameliorate the effect of low paternal investment. They propose that when it is no longer profitable for a female to abandon the nesting attempt and remate, the male "informs" her of her secondary status. She responds by reducing her clutch size to the optimum that a single parent can raise. Clutch size is likely to be entirely dependent upon the female, and under some sort of physiological control in the days before laying (Hamann *et al.* 1981).

Studies of other species have reached similar conclusions. Simmons (1988) finds little evidence to support other hypotheses and concludes that male Hen Harriers (*Circus cyaneus*) provide food to secondary females early in the breeding season, but then devote their attention to their primary mate.

Temrin and Arak (1989) examined studies on three species, including the Pied Flycatcher and concluded that the evidence for the deception hypothesis was "neither consistent among species nor among different populations of the same species".

Other Explanations for Polyterritoriality

Several hypotheses interpret polyterritoriality without attempting to explain polygyny. In the evolution of polygyny, the monoterritorial variant would normally be favoured. It allows a closer guard of the primary female and reduces cuckoldry; it makes territory defence easier and, because the two nests are closer, allows greater, more even care of the broods.

However, sometimes, the polyterritorial form of polygyny has evolved. Other than the Deception Hypothesis, four explanations have been put forward to explain this:

Separating the females reduces aggression between the primary and secondary female. Breiehagen and Slagsvold (1988) found evidence in support of this, that females were aggressive to caged females near the nest. They suggest that aggression may reflect competition for male assistance.

Females on different territories compete less for food. In the Pied Flycatcher, feeding takes place outside the territory so local competition is probably minimal.

The male chooses the best boxes, whatever their position. Disfavoured by Alatalo and Lundberg, this hypothesis is thought unlikely because good quality boxes are available in excess.

Having a second defended nest site may be advantageous even to monogamous males, as insurance against the failure to attract a mate to the first, or nest-loss due to predation. Of 190 polyterritorial males, Haartman (1956) found that only 23 were polygamous; perhaps the males who remained monogamous derived some advantage from their second nest site.

Cuckoldry in the Pied Flycatcher

Copulations in the Pied Flycatcher are seldom seen; extra-pair copulations are even less visible. Von Haartman recorded seeing two early in his research on the species (von Haartman, 1951). In two more recent studies, intensive observations of small numbers of individuals allowed the workers to witness extra-pair copulations. Alatalo *et al.* (1987) saw 24 copulations, of which 7 were extra-pair. They concluded that the likelihood of extra-pair copulation increased when the male was relatively distant from the females. Björklund and Westman (1983) artificially removed males and witnessed 7 extra-pair copulations mostly with near or next-to-near neighbours. They reported a much higher rate of copulation with extra-pair partners (0.8 per hour compared with 0.42 within-pair copulations per hour). Several studies measured the resemblance of putative fathers to offspring using there heritability of physical characteristics. This was regarded as a test of the effects of EPCs in producing extra-pair offspring, but for reasons described in chapter 6, the validity of these is much disputed. Recently, DNA fingerprinting studies have gathered limited data on extra-pair copulations. Lundberg and Alatalo (1992) report the findings of Gelter (1989) who found that broods of hybrid Pied/Collared flycatcher males contained offspring sired by a different male. Lifjeld *et al.* (1991) found that extra-pair copulations were relatively rare in their population. Only six of 135 pulli surveyed (4.4%) were extra-pair offspring.

Conclusion

Having examined comparative studies of mating systems, and studies of specific species, particularly the Pied Flycatcher, the only generalisation is that we know very little of what is really going on. The modelling approaches, in particular, fail because the predictions that they generate are difficult to test in the field. The Polygyny Threshold model demands knowledge of "territory quality" or "resource availability", despite our ignorance of what this means to

a bird. The model was designed for a particular use (generating predictions about general trends in avian mating systems), but has been applied to the study of particular species, in particular places. Furthermore, it is a model built upon monoterritoriality, but has been used in the polyterritorial Pied Flycatcher, and its use may or may not be valid. The main alternative, the Deception Hypothesis relies rather less upon theoretical assumptions about the factors influencing mate choice, but testing it is particularly difficult.

The costs of assessing a male seem relatively low, but the costs of rejecting him, and searching for another, may be high, especially when unmated males are rare. The costs of being a secondary female seem to vary depending upon the situation. If changing male is expensive, then assessing a potential mate ceases to be important; whatever his status, it is more profitable to mate with him than to look elsewhere, and the matter of whether the female is "deceived" becomes trivial.

Whatever the frequency of polygyny, whatever the selective effects upon the participants, extra-pair copulations must also be considered when comparing the breeding success of individual birds. In the few species where the proportion of extra-pair offspring has been measured precisely its frequency is higher than was previously thought (House sparrow 15%, Wetton *et al.* 1987; Swallow 3.4% , Wellbourn *pers. comm.*; Indigo bunting 14%, Westneat 1987b, 1990). Estimated EPC rate in Pied Flycatchers is in the mid-twenties (Alatalo *et al.* 1986). When this is compared with the rates of polygyny in Figure 2.1, it seems that in terms of proportion of young fathered by each method, polygyny may only be half as important as cuckoldry.

CHAPTER 3

Study Site and Fieldwork Methods

Introduction

This section describes the area in which the work was carried out, and the methods that were used. It contains details of the techniques used and justifications for their choice. It also presents statistical evidence relating to the success of the fieldwork methods.

Description of the Study Site

The Pied Flycatcher breeds in the north-western half of mainland Britain. Its breeding sites are almost exclusively in deciduous woodland on high upland hillsides and in birch and alder carr in the valleys below.

The species is most populous in Wales (Sharrock 1976). Its density is highest of all in areas in which nestboxes have been provided. One such area is the Gwenffrwd reserve of the Royal Society for the Protection of Birds (RSPB) in the upper Tywi valley, Dyfed. The valley of the Gwenffrwd and an adjoining area, the Dinas, are owned and managed by the RSPB as a nature reserve. The Pied Flycatcher here is the commonest species in the bird community, with a density of 120 pairs per km^2. All the fieldwork was done within the Gwenffrwd reserve. This was of great advantage to the study. It meant that we had access to a flycatcher population far greater than in most woods in Britain. The population was well studied, in that it has been the subject of a ringing programme for over a decade. It was also used as an experimental area during the preparation of a PhD thesis on habitat requirements of some Welsh

woodland birds (Stowe 1987). The thesis contains some data on the woodland areas covered in this study. The BTO study gathered information on the long-term history of the nestboxes and their inhabitants, but this was not available for my analyses. The position of the Gwenffrwd valley is shown in Figure 3.1. The Pied Flycatcher habitat within the valley has been arbitrarily divided into segments bounded by open ground, tracks, streams and changes in vegetation type. These divisions are used both by the RSPB in their management work and by Mr. C.J. Mead (CJM) and his colleagues from the British Trust for Ornithology (BTO) in their long-term study of the flycatchers (Mead, pers. comm.). On the advice of CJM, I chose as my study area a group of five woodland areas. These range in habitat type from low-lying boggy ground (approximately 100m above sea level) to high craggy sessile oak woodlands just below the tree level at a height of 300m above sea level. The areas are referred to by their RSPB/BTO identification, areas G, H, I, J and K. Occasionally, when this seems more apt I shall use the established names for particular parts of the valley (see Figure 3.2, the map of the study site). In Chapter 5, I discuss whether these areas have any biological relevance; whether their boundaries coincide with changes in Pied Flycatcher breeding conditions. Also in Chapter 5, I compare the study site and it's Pied Flycatcher population with other well-studied ones in Britain and on the continent.

Area G: 51 nestboxes in dense valley bottom woodland.

The G-series is the largest of the nestbox series. It comprises 51 nestboxes, mostly in dense oak woodland. Most of the series lies close to the river banks of the Afon Gwenffrwd. The mid-part of the nestbox series is situated by an artificial pool, close to the river.

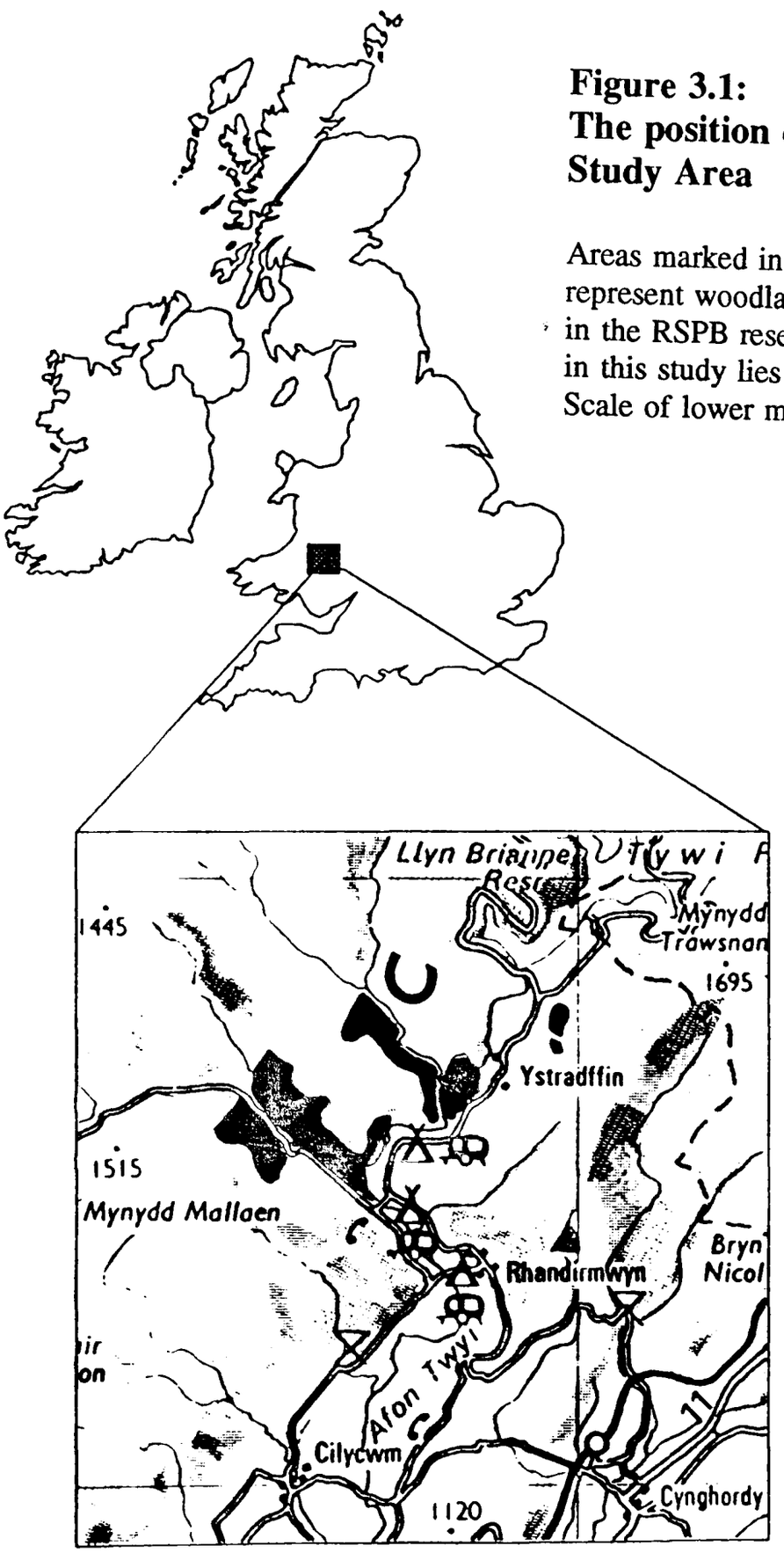
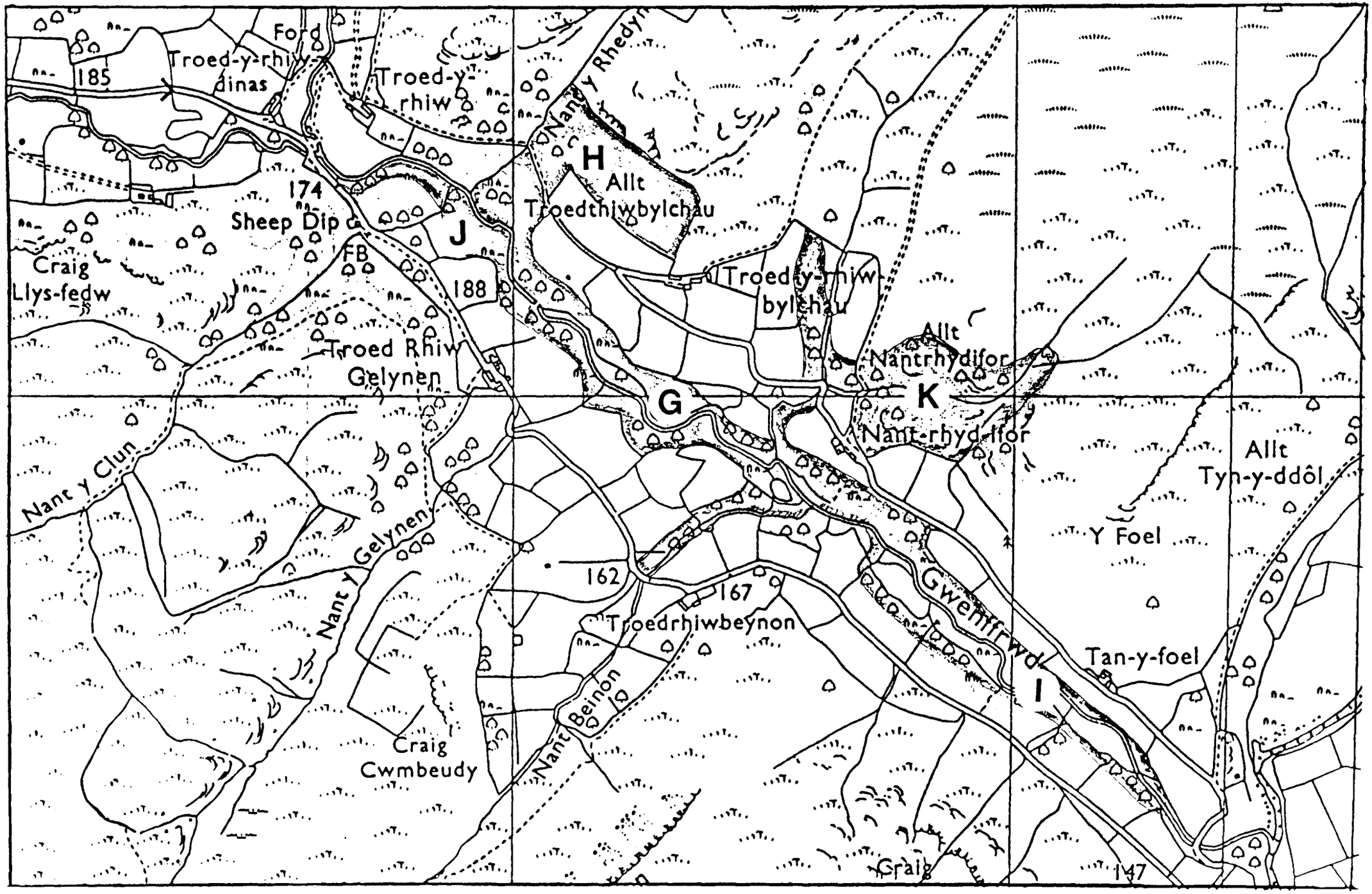


Figure 3.1:
The position of the Study Area

Areas marked in black represent woodlands in the RSPB reserve. The area used in this study lies within these. Scale of lower map 1:120,000

Figure 3.2: A map of the Study site. (90mm:1km).



Area H: 33 nestboxes in high sessile oakwood

The nestboxes of area H are spread about an open, airy wood called Allt Troed Rhiw Bylchau. They range in altitude from 200 to 300m. The western border of the area is a stream that forms the boundary of the reserve. Similar woodland continues on the other side of the stream, but is not included in either this or the long-term BTO study. This woodland probably forms a reservoir from which unknown birds can move into the study area. Above and to the north of the wood is open moorland, while to the east is an area of open scrub, recently planted with deciduous trees. A track divides the lower part of the H-series from the upper part of the G's.

Area I: 40 nest boxes in open valley-bottom woodland

The I-series is spread along the banks of the Afon Gwenffrwd, which meanders and divides. Several boxes are on occasional islands, surrounded by gravel bedded shallow water. Most of the ground is heavily grazed by sheep.

Area J: 16 nestboxes by the Afon Gwenffrwd

The J-series is the smallest of the nestbox series'. It lies alongside the river. Boxes 1 to 6 are in enclosed woodland, while 7 to 16 are in fields grazed by sheep.

Area K: 33 nestboxes in high sessile oakwood

The K-series, like the H, is high and open. It comprises 2 discrete areas; K1 to K23, and K24 to K35. The former area is the highest and steepest of the

two. The trees are stunted and the ground-cover mostly moss. The other area is more sheltered, and therefore has higher trees and more ground cover.

Description of Nestboxes, their Types and Construction

Most of the nestboxes in the areas pre-date this study. The RSPB wardens repair and replace any that are rotten or damaged. The styles of the boxes vary depending upon their age, and precise details vary considerably. They do, however, fall into four broad categories:

Type 1 boxes: Plywood construction, with a removable lid.
Type 2 boxes: Plywood construction, with a sliding front.
Type 3 boxes: Softwood construction, with a sliding front.
Type 4 boxes: Softwood construction, with a removable lid.

During the four years that I was involved in fieldwork, some responsibility for maintenance devolved to me, although a great deal was done by the RSPB. Any boxes that were irreparable or lost were replaced by another in as near to the same place as possible. It was decided that the appearance of new nestboxes during the breeding season might have unpredictable effects upon the birds. As a result, no changes were made to the nestbox density after the arrival of the first birds in 1988. In three cases, missing boxes had not been replaced by this time. They were never replaced. Boxes which subsequently went missing or were destroyed were generally reinstated when possible.

The Aims of Fieldwork

It was hoped that the fieldwork would yield the following, in approximate order of priority:

1) Blood samples from as large a population of Pied Flycatchers as possible.

2) Data on the nest histories of the population, including the significant dates, clutch and brood sizes, and the fates of the adults and pulli.

3) Biometrical data on the birds handled, to test hypotheses about the relationship between paternity and the size and condition of adults and pulli.

4) Behavioural data on the attendance of the adults, in particular the male.

A Brief Chronology of the Field Season

The field season started before the arrival of the birds. Maintenance and preparatory work was done, while surveying the area for the first arrivals. Once birds had been sighted in the valley, a regular round of nestbox visits was begun. Soon after this, traps were placed on occupied boxes to catch males to ring, measure and bleed them. The visits and trapping were continued until laying began. Once eggs were present in a nestbox, trapping attempts ceased at that box. Visits monitored the laying, incubation and hatching processes. Once nestlings were 3-4 days old trapping of adults was resumed. As nestlings grew larger they, too, were ringed, measured and bled. At a point during this period, the time demands of bird-handling meant abandoning of

regular nestbox visits, to concentrate all the effort onto processing birds. As the workload declined, visits were resumed and the fates of each, now empty, nest and its contents found.

The fieldwork can be divided into two processes; monitoring nestboxes through regular visits, and monitoring birds through trapping, ringing measurements and blood-sampling. These are described in more detail in the two sections below.

Monitoring Nestboxes

The purpose of regular visits, was to collect data on the chronology of the breeding season for each nestbox. A complete set of data would include the dates of male and female arrival, the sequence of nest-building, the commencement of laying, the times of hatching and fledging and the sizes of the clutch and brood. It would also contain information on predation and abandonment of nest, eggs or young.

Monitoring the boxes commenced when the first Pied Flycatcher had been sighted in the valley. Visits were repeated on a twice-weekly cycle and continued until the busiest part of the season when catching and bleeding adults and young took all the available time.

Each nestbox visit yielded the following information:

Date
Time (to the nearest hour)
Number of eggs
Number of young
Distance of male if visible (<1m, <2m, <3m, <4m, <5m, <10m).
Colour ring code of male if visible.

Presence or absence of female, plus ring number of female if incubating.

State of nest, observations of anything out of the ordinary.

Monitoring the Birds

The value of observing birds at the nest is greatly enhanced when more is known of the individual animals. To ring, colour mark and measure birds, and to take blood samples, it was planned to capture as many adults and young as possible.

It was particularly important that I use only methods that had little or no effect on the birds' behaviour after release. Any technique should not affect survivorship, or the ability of adults to tend their young. It should not cause desertion of the nest site, even in the pre-laying period. The processes used on young birds should not affect their growth rates or their chances of fledging.

For reasons discussed in the next section, adults were almost invariably caught using traps that confined them within their nestbox. It was decided that the following rules should govern whether traps should be set on a box, and once trapped, what procedures should be used on which birds:

Before eggs are present in the nest, the aims of trapping should be to catch, ring, measure and bleed only males. Any females who happen to be caught during this period should only be ringed.

During the laying and incubation periods and in the first three days after hatching, no traps should be put on the nestbox.

No box was to have a trap fitted for more than 40 minutes in any one day. Traps were to be checked every 20 minutes.

Pulli should be processed only after they were 10 days old, and then only if their mass was greater than 10g.

Methods of Catching Birds

The long-term BTO study of Pied Flycatchers in the valley uses small flaps, fitted to the hole of the nestbox (flap-traps). These allow birds to look into the box and to enter it, but prevent them from leaving. They are small, efficient and do not harm the birds. These were adopted as the main method for capturing adults for this study. Several could be fitted simultaneously, and when used like this, they proved an efficient way to catch several birds per hour. However, a disadvantage of these traps was that they were non-selective; any bird that entered the box was caught. In cases where one member of a pair had already been processed, there was a requirement for a trapping method that enabled specific individuals to be selected. Several methods were tried, without great success.

A radio-controlled version of the flap-trap was constructed. This utilised an ordinary radio control device as used in toy cars and boats. When activated, it caused a switch to close, sending a 12V current through a resistive wire. The wire heated up rapidly, and melted a fine monofilament line holding the standard flap-trap clear of the nestbox entrance. Thus, when the desired bird entered the box, the flap could be closed. The device worked, but was rarely used for two reasons:

The system was very time-consuming to use. Only one nestbox at a time could be trapped. The radio-control device was

capable of operating two traps, but this was only possible if the operator could watch both boxes simultaneously.

The system was prone to interference from high-powered VHF radios used by local farmers. These caused the trap to be triggered prematurely.

A second type of nestbox trap that was constructed but never used was based on a design used to great effect in Sweden (Lundberg, pers. comm.). In this system, a specially constructed nestbox was used. It could be put in place of the existing box, in which case it was presumed that the bird would enter it as it would its own. Alternatively, the box could be positioned close to the existing box, in the hope that an investigative flycatcher might enter out of curiosity. Any bird landing on the perch by the entrance would trigger a spring-operated net to rise from below the box, catching the bird gently and firmly. Unfortunately, my version of this trap proved viciously efficient when tested in the laboratory using pencils or bird-like objects, and it was decided that it would almost certainly injure a bird. It was never tested further.

The curiosity of male flycatchers could be exploited by putting a flap-trap on a standard nestbox and positioning this near to a permanent box. This system worked but was rarely used because it caught fewer birds per hour than a line of conventional flap-traps.

During the incubation period females were often found on the nest. These were easily lifted off the eggs, but to avoid stressing the bird, they were not processed at all, apart from reading their metal BTO ring, or fitting one to new birds. Pulli, of course, could be lifted straight from the nest, and processed without any form of trapping.

Logistics

The Pied Flycatcher breeding season is short and synchronous. In 1988, 30 days separated the first and last nests to hatch. 63% percent of all broods birds hatched within a 6 day period. Because of this, the jobs of trapping adults and processing pulli were restricted to a few days of concentrated effort. This created a need for planning and organisation of the work, to get maximum rewards from the limited time available. The day's work was planned with the aid of nest histories charted on paper or stored on a computer database. The method enabled birds to be processed on, or about, the ideal time in the breeding cycle, and allowed groups of boxes to be worked simultaneously. In practice, this resulted in spending around an hour in one place, working nearby nests, followed by a trip to the freezer to store the blood, and a move to another site.

The equipment used in bleeding, measuring and ringing the birds was stored in plastic toolboxes. These were either lashed to a packframe and carried on foot or, if possible, laid out in a car and taken to the nearest parking place. Using a car ultimately restricted mobility, but had three distinct advantages:

Speed of movement from place to place, especially when taking blood samples to the freezer.

An orderly, insect-free environment made processing more efficient.

Working in the car, away from the nestboxes, reduced disturbance.

Blood Sampling

Blood samples were taken for DNA fingerprinting. This technique demands only small volumes of blood, particularly when used on birds, which have nucleated erythrocytes, and therefore have more DNA per unit of blood than, for example, mammals. In 1987, when the techniques to be used in this study were being decided, a common means of taking blood was from the jugular vein. This is a particularly suitable technique for obtaining large volumes of blood (as used in enzyme analysis). Alternative techniques involved piercing a blood vessel in the foot or the brachial (wing) artery. After trials of all three techniques, brachial venipuncture was chosen. This was the least damaging technique and yielded a uniform volume of blood, after which flow could be stopped and the bird released without trauma.

The technique used is as follows:

> The feathers are parted and the wing surface cleaned with a tissue soaked in ethanol. This has the dual purpose of disinfecting the skin and causing the blood vessel to stand out. A 5ml syringe is fitted with a Sabre 25g (0.5mm x 25mm) hypodermic needle and the inside and outside surfaces of the needle are coated with anticoagulant. This is done by filling and emptying the needle from a container of heparin. Holding the bird in the correct grip the skin is pierced. Blood wells onto the surface in a bead that is removed using a capillary tube. The tube is then either sealed using Hawksley "Christaseal" putty, or emptied straight into a 1.5ml eppendorf tube containing 1ml 1x SET. The blood flow is stopped, if necessary, with a small piece of tissue paper, and the wing closed to its normal position. The blood sample is labelled and stored in a coolbox until transfer to a -20°C freezer.

Measurements and Biometrics

The biometrical measurements taken for adults were tarsus length, wing length and mass. These were chosen to provide two estimates of body size (tarsus and wing) and one of condition (mass). In pulli, the wing is constantly growing, and for any individual the measure is highly dependant upon the time it was taken. In fact, it is used in some species as a predictor of age (Wellbourn pers. comm.). In the study population, age was usually known, and so the wing length measurement was omitted when processing pulli.

Tarsi were measured using dial callipers to an accuracy of 0.1mm. Repeatability of tarsus measurements is discussed in Chapter 6. Wing length was measured by the maximum chord method using a wing-rule as supplied by the British Trust for Ornithology (BTO Ringers Manual, Spencer 1984). Birds were weighed to the nearest 0.1g using a Pesola balance. Mass is variable within and between days, particularly in pulli.

CHAPTER 4

DNA Fingerprinting Methods

Introduction

Producing a DNA fingerprint from a frozen blood sample involves many steps, which are summarised in Figure 4.1. Most, if not all, of these are modified from techniques widely used in other branches of molecular biology and molecular biologists would be familiar with all but the details. The requirements of other readers might not be for such complete coverage of the technical aspects, but for a broad description of the procedures involved. In an attempt to satisfy the needs of both technical and non-technical readers, this chapter is structured in the following way:

> The non-indented sections are intended for the general reader, and outline the methods involved and the reasons for their use.
>
> The indented sections contain the detailed protocols, including records of the equipment, chemicals and techniques that were used. Recipes and full names for all the buffers and solutions used, and their abbreviations are given in Wetton (1990) and are reproduced in the Appendix of this document.

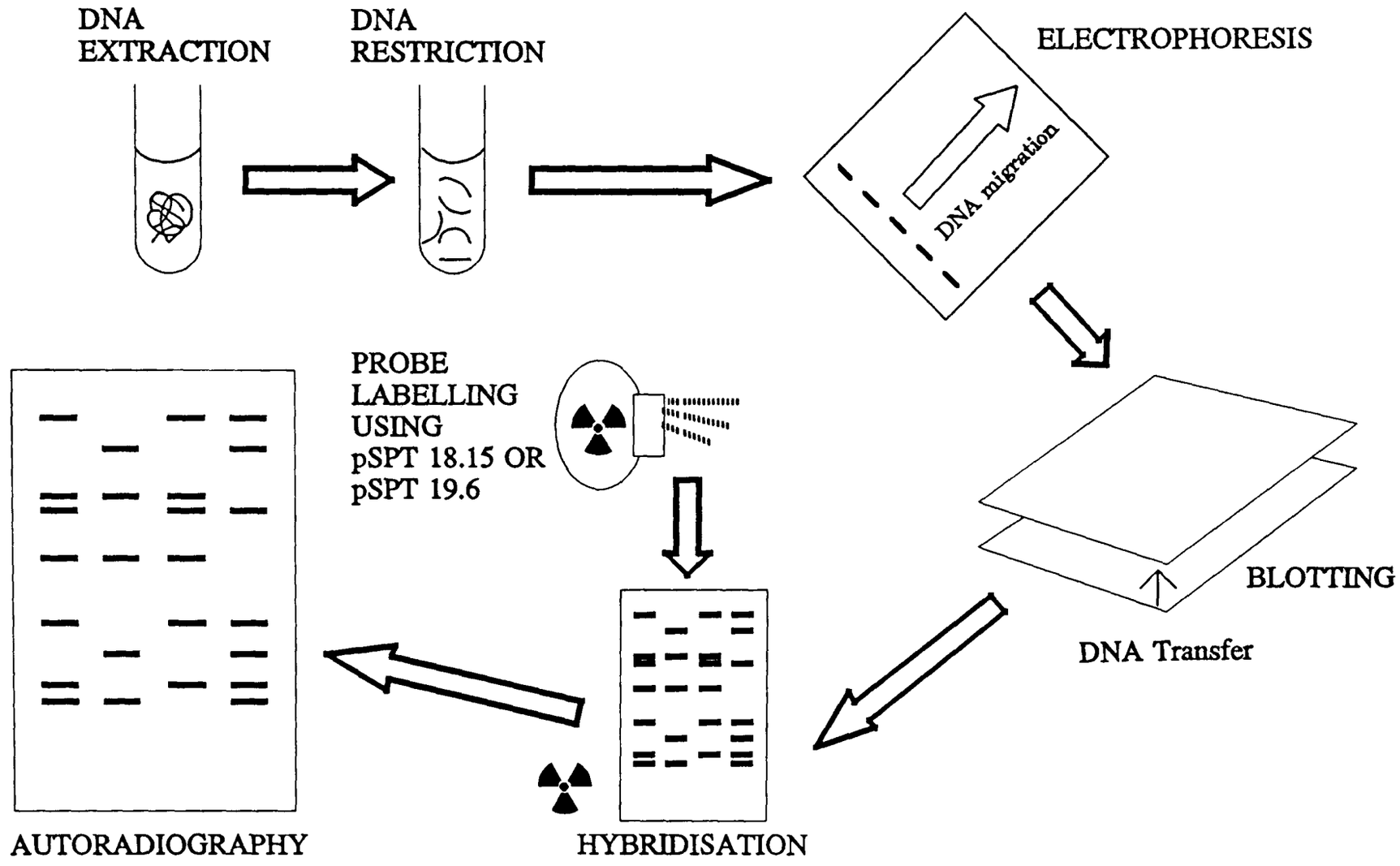


Figure 4.1: A schematic representation of the procedures involved in DNA fingerprinting.

DNA Storage

The blood sampling methods are described in detail in the fieldwork methods chapter. The blood storage methods differed in the two field seasons.

In 1988, blood was stored in the capillary tubes with which it was taken. Each tube was labelled and then protected by inserting it into the reed of a 100mm x 100mm piece of firm corrugated cardboard. The cardboard was then placed on ice for up to an hour until transfer to a -20°C freezer. During the winter of 1988/89, laboratory analysis of the blood stored in this way revealed that degradation had taken place, probably during storage in the capillary tube. Three factors might increase the rate of degradation of blood collected in capillary tubes. Firstly, the shape of the tube might increase the rate of natural deterioration. Capillary tubes have a large surface-area to volume ratio making their contents prone to rapid freezing and thawing. They also contain a relatively small volume. Empirically, it has been found that larger volumes of blood tend to be more stable than small ones (Carter, *pers. comm.*). Secondly, whilst a needle takes blood directly from the blood vessel, a capillary tube comes into contact with the surface of the skin. Substances from the skin might contaminate the blood and cause degradation. Lastly, the tubes used were coated with heparin, an anti-coagulant, which, while this is not documented, might contribute in some way to the degeneration of the DNA.

In 1989, the (un-heparinised) capillary tube containing the sample of whole blood was emptied into 1ml 1x SET (Sodium, EDTA and Tris), stored on ice and frozen within 60 minutes of sampling. It was stored in a -20°C freezer during the field season, then transferred to -80°C on return to Nottingham.

DNA Extraction

The first stage of DNA extraction is the lysis of the cells and the release of the DNA into aqueous solution. The first part of the protocol differs according to the manner in which the blood was stored, and therefore, according to the year in which the sample was collected:

1988 - Blood stored in capillary tubes. The tube containing the blood sample was allowed to thaw. The "Christaseal" plug in the capillary tube was cut out and, using a "policeman", a 10 to 15µl aliquot of blood transferred to an Eppendorf tube with 650µl of 1x SET buffer. As it contains EDTA, SET inhibits the action of nucleases. To this was added 15µl of $10mgml^{-1}$ Proteinase K stock solution, mixing gently to spread the enzyme throughout the mixture. 7.5µl of sodium dodecyl sulphate (SDS) were added. Being a detergent this lyses the cell membrane, releasing the DNA into aqueous solution. It also catalyses the action of Proteinase K and degrades nucleases and other proteins. The sample was mixed again and incubated overnight in a 55°C waterbath.

1989 - Blood stored in 1ml of 1x SET. The blood sample was allowed to thaw and was shaken using a vortex machine. An aliquot of blood was removed. The volume of this varied from sample to sample, depending on the nature of the blood/buffer mixture. The aim was to remove a 10 to 15µl piece of the gelatinous blood clot that normally occurs. This was placed in an eppendorf tube with 650µl of 1x SET buffer. As it contains EDTA, SET inhibits the action of nucleases. To this was added 15µl of $10mgml^{-1}$ Proteinase K stock solution, mixing gently to spread the enzyme throughout the mixture. 7.5µl of sodium dodecyl sulphate (SDS) were added. Being a detergent this lyses the

cell membrane, releasing the DNA into aqueous solution. It also catalyses the action of Proteinase K and degrades nucleases and other proteins. The sample was mixed again and incubated overnight in a 55°C waterbath.

Once DNA is released into solution it must be purified, and any damaging enzymes removed. This was achieved by phenol and phenol-chloroform extractions. The procedure removes proteins, lipids and carbohydrates by means of their differential solubility in aqueous and organic solutions. Some precipitate at the interface and others pass into solution in the organic layer. Each treatment removes slightly different sets of contaminants.

2. A phenol solution was prepared by dissolving phenol crystals in an equal volume of 1M Tris pH 8.0. The reaction produces two immiscible liquids, aqueous phenol being the one that forms the lower layer.

3. A 500µl volume of phenol was added to the sample, which was then mixed for 30 minutes on a rotating board. The rotation ensures a large area of contact between the immiscible aqueous and phenolic solutions. The sample was then spun in a micro-centrifuge at 11500G for 5 minutes to separate the two layers.

At this stage, the DNA is in the upper (aqueous) layer, in a loose unwound form, and can be damaged by rough treatment.

4. The upper layer was transferred to a new Eppendorf tube ensuring that none of the solids at the interface were carried over. This was done using a pipette fitted with a 1ml tip with the end cut off to increase the bore of the tip and reduce the chances of shearing the fragile DNA molecules.

5. Steps 3 and 4 were repeated until the aqueous layer was colourless. If the volume of the aqueous layer fell below 500µl it was topped up with 1x TE (Tris and EDTA) at stage 3.

When the DNA solution appears colourless, this indicates that all the phenol-soluble contaminants that can be removed have been. A treatment with phenol/chloroform removes a different set of contaminants.

6. Phenol/chloroform was prepared by mixing phenol and an equal volume of chloroform/iso-amyl alcohol (23:1, V:V); the phenol/chloroform separating out as the lower layer.

7. Steps 3 and 4 were repeated at least twice substituting the phenol/chloroform solution for phenol.

Again, when the DNA solution is colourless, this shows that all the phenol/-chloroform soluble contaminants that can be removed have been. Another set of contaminants is removed by treatment with chloroform/iso-amyl alcohol.

8. Steps 3 and 4 were repeated once, substituting chloroform/ iso-amyl alcohol (23:1 V:V) for phenol. This removed any lingering traces of phenol that would inhibit later reactions.

Having removed several contaminants, the final stage in the cleaning process involves precipitating and re-suspending the DNA in fresh, clean, sterile TE.

9. The final aqueous layer was usually about 500µl. This was transferred to a new eppendorf and twice the volume of absolute ethanol, taken straight from the -20°C freezer, was slowly added. Mixing by hand or on the rotator caused the DNA to precipitate in a white fluffy mass. At this stage, the samples could be left in the -20°C freezer.

10. The sample was spun for 7 minutes at 11500G to pellet the DNA. The ethanol was poured off and cold 75% ethanol added and left to stand for 10 minutes. This was then poured off and any remaining ethanol was removed with a disposable pipette tip.

11. The sample was dried in a 37°C incubator to remove all traces of ethanol.

12. The sample was resuspended in a volume of TE by overnight incubation at 55°C. The amount of TE added depended on the size of the pellet, the smaller the pellet the smaller the amount of TE added. Amounts added were between 10µl and 150µl.

Having been extracted the DNA was stored in a refrigerator or cold room at 4°C until required for analysis.

DNA Restriction

At this stage, the DNA is in a loose unwound form, floating in suspension. Apart from any broken by the extraction process, the DNA molecules are intact and have a high molecular weight. DNA from different individuals is indistinguishable in this form; there may be slight immeasurable differences in the length or size of the molecules, but if the intention is to identify particular individuals, the molecules must be cut up and examined in a specific way. This is achieved using restriction enzymes, proteins that break the bonds of the molecule in specific places. (Sambrook *et al.* 1989) Different restriction enzymes recognise different parts of the molecule, cutting only where a precise sequence of bases is found. The enzyme HaeIII divides the DNA only where the sequence GG\CC occurs. Throughout most of the DNA molecule this is

approximately once every 256 base pairs, but within the minisatellite regions this is not the case. Minisatellite DNA is highly repetitive. A similar sequence is repeated many times, and as a result, the recognition sites of some enzymes are very common and of others, very rare. By using one of the latter enzymes, the DNA can be cut into many small fragments, which derive from "ordinary" DNA, and a few larger fragments, some of which will be large because they include minisatellite regions. It is these fragments that have been found to differ in size between individuals, and provide the means for DNA fingerprinting. To produce the fragments, the enzyme is added to the DNA, along with other chemicals required for its activity. After a period of incubation, the enzyme should have cut the molecules and the DNA can be assayed to verify this.

The Pied Flycatcher DNA was restricted with HaeIII. Restriction involved taking a 15µl aliquot of resuspended DNA and adding 1µl of enzyme, 2µl of the appropriate 10x reaction buffer (either React2 or a proprietary HaeIII buffer). Cleavage was aided by adding 2µl of a 40mM stock solution of spermidine. The solutions were mixed gently, pulsed down in a micro-centrifuge and then placed in a 37°C water bath for digestion to occur. The enzymes need a minimum of 4 hours to work, but generally they were left overnight.

DNA Assays

The samples were assayed to determine the quality and quantity of the DNA present. Minigel assays determine whether the restriction enzyme has successfully cut the DNA and indicate the concentration of the DNA, but this is more accurately measured using fluorimetry.

The procedure by which DNA can be visualised using a minigel is adapted from Sambrook *et al.* (1989). DNA samples are loaded onto a supporting gel and subjected to an electrical field. They migrate through the gel, at a rate dependent on their size, and are thus separated. The DNA is labelled with a fluorescent chemical, which allows the distribution of fragment sizes to be visualised. The method is described below:

1. A 0.8% minigel solution was prepared by melting 0.24g LE agarose in 30ml of 1x TBE buffer in a 100ml pyrex flask in a microwave oven until it was completely dissolved. It was placed in a 55°C waterbath for 20 minutes until it had cooled.

2. The ends of a 65mm x 100mm minigel tray were sealed with masking tape in order to contain the agarose while it set. A 16- or 32- slot perspex comb was placed 10mm from one end to form wells for the samples. The agarose was poured into the tray and left for 20 minutes in order to set. The masking tape was removed and the minigel tray placed into a minigel tank containing 1x TBE and 5µl of 0.5mg/ml ethidium bromide per 100 ml of buffer.

3. Samples were prepared for assay by taking 2µl of the restriction reaction mixed with an equal volume of 2x BPB loading buffer. This buffer contains two dyes (Bromophenol blue, light blue, with a molecular weight equivalent to 500bp and Xylene cyanon, purple) which migrate through the agarose gel at different rates and allow the progress of the electrophoresis to be monitored. It also contains Ficol 400, a high molecular weight polymer which associates with the DNA and makes the sample denser than the TBE running buffer causing it to sink to the bottom of the wells. Each sample to be assayed is loaded into a well on the gel using a disposable plastic tip to reduce contamination.

4. A current was run across the gel at 80 to 100V for about 50 minutes, causing the DNA in the wells to migrate through the gel. DNA fragments of different size migrate at a different rate and are thus separated. When the purple dye had migrated to about 20mm from the end of the gel, the current was switched off and the minigel removed.

5. The ethidium bromide in the reaction buffer binds closely with the DNA, so that when the gel was placed on a transilluminator and exposed to UV light with a wavelength of 354nm, it fluoresced orange and the positions of the DNA fragments could be seen as a smear and a series of faint bands. As a permanent record of this pattern, a polaroid photograph was taken. The position of the DNA reveals whether the sample is successfully cut, partially cut or completely uncut. Partially cut or fully uncut samples have an excess of high molecular weight fragments. Samples containing only small fragments, have degraded DNA; these had to be either re-restricted or completely re-extracted.

6. In cases where the sample was partially cut, a further 2µl of enzyme was added and the "DNA restriction" protocol repeated, after which another minigel assay was done.

Once it was established by minigels that the DNA had cut successfully, the concentration of the cut DNA was assessed using a fluorimetry method. A dye was added to a small sample of the DNA. This fluoresces when in association with DNA, and the amount of fluorescence was measured using a fluorimeter. The assay enabled the concentration to be standardised by adding a volume of buffer.

The concentration of the DNA was measured using a Hoefer TKO-100 DNA fluorimeter. A 2µl aliquot of the sample to be restricted was added to a 2 ml of 1x TNE buffer containing 0.1-µgml^{-1} Hoechst-33258 dye in a disposable plastic cuvette. The cuvette was inverted several times to thoroughly mix the solutions before being placed in the machine. The dye binds preferentially to DNA and emits light at 458nm when excited by UV light at 365nm. The intensity of the light emitted is detected by a photosensitive cell and converted to a digital reading that is linearly related to the concentration of DNA. The machine was calibrated using a standard sample of calf thymus DNA (1mgml^{-1}). From this value, using an empirically derived conversion formula (Carter, pers. comm.), it was possible to calculate the volume of BPB loading buffer to add to each sample to standardise the DNA concentration.

After adding the BPB, samples were placed in the 65°C waterbath for 10 minutes, to kill any micro-organisms that may have been present in the tubes. The samples were then quenched on ice.

DNA Electrophoresis

This is a larger scale method of separating DNA fragments than the minigel described above. As with a minigel, it involves the use of an electric current to separate DNA fragments in an agarose gel. After electrophoresis, the DNA fingerprint pattern has been formed, and the remaining steps are to make it more permanent and to visualise it.

1. To make a 1% agarose maxigel, 3.75g of LE agarose was put into 375ml of 1x TAE, in a screw top 500ml Duran flask. This was weighed and heated in a microwave oven until all the

agarose had dissolved. The flask was weighed again and the mass lost due to evaporation was made up with distilled water. The flask was placed in a 55°C waterbath for 1 hour to cool.

2. A 240 x 200mm perspex gel base was sealed at each end with masking tape and with a 16 slot plastic comb fitted 20mm from one end to form the wells into which the samples were to be loaded.

3. The molten gel was poured into the tray and any air bubbles removed. It was left on a flat surface to solidify for about 2 hours. The masking tape was removed and the gel tray was placed into a LKB H4 horizontal electrophoresis tank containing 2625ml of 1x TAE. The comb was removed after the gel had been immersed, and any bubbles removed from the wells.

4. Using a sterile disposable plastic tip, 40µl of each sample was loaded in to a separate well. After leaving for 10 minutes for the samples and the running buffer to equilibrate, the voltage was turned on.

5. The gel was run for a total of 2200 volthours; usually 55 hours at 40V.

Blotting

Blotting is a means of transferring the DNA from the soft and fragile agarose gel to a robust and permanent membrane, on which it can be kept indefinitely. Using a concentration gradient, the DNA molecules are gently washed out of the gel onto the membrane. The membrane is then treated, to make the attachment more permanent.

The blotting technique used was dependent upon the type of membrane being used. The technology of membrane design and manufacture changed rapidly during the study and, several times, new and sometimes better membranes were adopted by the members of the laboratory. The protocols described below apply to the two most commonly used blotting techniques. Theoretically, DNA fingerprint pattern is unaffected by the choice of membrane but, as with different qualities of photographic film, the resolution and accuracy of the pattern is dependent on the quality of the membrane and its processing.

Southern blotting

Southern Blotting was used to transfer DNA to Schleicher and Schuell BA 85 nitrocellulose, and to Amersham Hybond-C *Extra* nylon-backed nitrocellulose.

1. When the gel had finished running, it was carefully inverted, transferred to a plastic tray and soaked in 0.2M HCl for 20 minutes. This stage, called depurination, involves the cleavage of the DNA at the sites of some purine residues, and breaks it into fragments with an average length of 400 base pairs. Since the DNA fragments at this stage may be up to 25,000 bp in length, this makes them transfer more readily to the membrane. The larger the fragments the more embedded they are within the gel matrix and consequently the more difficult the transfer. Depurination allows efficient transfer of larger fragments to the membrane.

2. Denaturation of the DNA involves the gel being left in a solution of 1.5M NaCl/0.5M NaOH for two periods of 40 minutes (solutions were replaced after the first 40 minutes). This causes the double stranded DNA to separate and reveals the complementary base pairs.

3. The gel was then left for two periods of 40 minutes in neutralising solution, which brings its pH to 7.0 and maintains the DNA in its single stranded state.

4. A 40mm strip of the gel was cut from the well end, making it 200mm square. It was placed on a strip of Whatmann 3MM paper, supported on a perspex plate above a reservoir of 20x SSC. The paper forms a wick, as its ends fold over the plate and drop into the SSC.

5. A 200 x 200mm sheet of the membrane was placed on the surface of the gel and marked with an identification code. On top of it were laid two 200 x 200mm sheets of Whatmann 3MM. Each layer was soaked in 2x SSC before placement and care was taken to eliminate all air bubbles between the layers while building the blot. A stack of dry absorbent paper towels (Kimberley Clark, UK) 100mm deep was placed on top of the blot, and a glass sheet and a 500g weight were placed on top to compress the blot.

6. After leaving overnight, the stack was dismantled and the membrane rinsed in 2x SSC. The membrane was dried for 90 minutes using a vacuum oven at 80°C, and was placed into a polythene bag for storage.

Alkaline transfer blotting

When blotting onto nylon-based membranes such as Amersham Hybond-N nylon membrane and Zetaprobe, the DNA was transferred in an alkaline buffer. The techniques used in alkaline transfer differ in the following ways:

1. The depurination stage was similar to that for Southern blotting. The gel was washed in the same solution (0.2M HCl), but for only 35 minutes, after which the gel was equilibrated in alkaline transfer solution (ATS) for 10 minutes.

2. The stack was built as before, with ATS both in the reservoir and soaking the paper layers above the gel.

3. The membrane was air dried and then dried at 80°C in a vacuum oven for 20 minutes.

Radio-labelling the DNA and making an Autoradiograph

The DNA, now fixed onto the membrane, forms an invisible smear the width of the well. The smear runs from the place on the membrane that lay above the well, to the far end of the membrane, where only the fastest-migrating fragments reached. The distance travelled by each fragment depends on its size, which in turn is a consequence of where the DNA was cut by the restriction enzyme. Because of the solutions used in blotting, the fragments of DNA making up the smear are broken into 400bp lengths, and the two chains are separated. Within each original DNA molecule were many minisatellite regions, which were fragmented somewhat by the restriction enzyme and now form part of the smear. At various places within the smear, therefore, are concentrations of these fragments that would form bands if they could be distinguished from the other DNA. As they share a similar "core" sequence, they can be visualised using a method that involves radioactively labelling only the DNA that contains the desired sequence. The radiation from this sequence-specific probe, can expose an X-ray photographic film, and reveal the pattern of the DNA on the membrane as an arrangement of lines on the film.

The method for this procedure consists of four stages; the preparation of a substrate containing the minisatellite probe sequence, the use of the substrate

to produce a radioactive RNA probe, the binding of this probe to the DNA on the membrane and the exposure of the photographic film to the labelled membrane.

Preparation of Ribo-probe Substrate

This procedure produces sufficient substrate to label approximately 1500 DNA fingerprints. Normally it was carried out for the members of the laboratory by R.E. Carter. For the sake of completeness, I reproduce the methods from Wetton (1990):

> The inserts from the Jeffreys' polycore probes were subcloned into pSPT18 and 19 transcription vectors (Carter *et al.* 1990). pSPT 19.6 and pSPT 18.5 have the inserts from the polycore probes 33.6 and 33.15 orientated so that the G-rich strand is downstream of the T7 promoter, thereby producing the hottest transcripts when labelled with α^{32}P CTP. Large quantities of the plasmids were produced using scaled-up miniprep techniques from overnight cultures of *E. coli* DH1 in 100ml LB Amp broth. The following method adapted from Ish-Horowicz and Burke (1981) was used.
>
> 1. Spin down 25ml of culture in a 30ml plastic centrifuge tube at 2000G for 6 minutes in a Sorval SS34 rotor. Discard supernatant, add a further 25ml and repeat the spin.
>
> 2. Discard supernatant and resuspend pelleted cells in 1ml of "Miniprep" buffer (50mM glucose, 25mM Tris Ph 8.0 and 10mM EDTA). Leave for 10 minutes.

The following steps were carried out at 4°C unless otherwise stated.

> 3. Add approximately 20mg Lysozyme (by spatula) and leave for 30 minutes.

4. Add 2ml of 0.2M NaOH, 1% SDS and mix gently. Leave at room temperature for 5 minutes.

5. Add 1.5ml of precooled 5M potassium acetate (3M potassium acetate and 2M ethanoic acid; Ph 5.6). Mix gently and keep on ice for 5 minutes.

6. Centrifuge at 6000G for 10 minutes.

7. Transfer supernatant and add to it an equal volume of phenol/chloroform, mix for 5 minutes. Spin at 6000G for 6 minutes.

8. Transfer top aqueous later and repeat the phenol/chloroform extraction.

9. Transfer aqueous layer and wash with an equal volume of chloroform. Spin at 6000G for 6 minutes.

10. Transfer top aqueous layer and add 2x volume of cold (-20°C) ethanol. Mix gently and leave overnight at -20°C.

11. Spin at 6000G for 10 minutes.

12. Remove the supernatant and wash the pellet with 70% ethanol, discard ethanol and draw off remaining liquid with a disposable pipette tip.

13. Dry pellet at 37°C for 20 minutes. Resuspend in TE overnight at 55°C.

Making and Using the Ribo-probe

The probe is a messenger RNA produced from the more guanine-rich of the two strands of substrate DNA. In it, all the CTP nucleotides are radioactive. The radioactive RNA binds tightly to homologous strands of DNA on the membrane. RNA probes are used because, as RNA binds tighter to DNA than DNA does, a ribo-probe is more efficient than its DNA counterpart.

The riboprobe labelling protocol used was devised by R.E. Carter. I reproduce here the method from Wetton (1990):

1. 50µl aliquots of the resuspended plasmid were restricted with the enzymes EcoRI (pSPT 19.6) or Hind III (pSPT 18.15). These cut in the polylinker region of the plasmid which is downstream of the insert with respect to the T7 promoter region. The DNA in these digests was assayed using a fluorimeter, and then diluted to a concentration of 0.24µgµl^{-1} with sterile TE.

2. Labelling reactions were set up by adding then mixing the following:

5.5µl restricted pSPT
1.0µl ATP (10mM)
1.0µl GTP (10mM)
1.0µl UTP (10mM)
4.4µl 5x transcription buffer
2.0µl DTT (100mM)
1.0µl RNase inhibitor (25 units)
1.0µl T7 RNA polymerase (10 units)
5.0µl ^{32}P CTP (400 Ci mmol l^{-1})

3. The reaction was terminated after a 60 minute incubation at 38°C by the addition of 20µl of Nick-stop mix (Sambrook *et al.* 1989).

4. Unincorporated nucleotides were separated from the labelled transcript by spun column chromatography. The chromatography column was formed inside the barrel of a plastic 1ml syringe by placing a pad of glass wool and then filling the barrel with TE-buffered Biogel P60 granules. The column was packed by spinning at 2000G for 5 seconds, then washed through twice with 150µl of TE by further 5 second spins. The liquid which flowed through was collected by a 15ml polypropylene tube in which the barrel was supported.

5. The probe was carefully put onto the surface of the column which was spun at 2000G for 5 seconds then washed through with an additional 50µl of TE and a further 5 second spin. In the nick-stop mix are two dyes; one co-migrates with the unincorporated nucleotides which stay in the column and the other with the labelled transcript which passes out of the syringe barrel into a 15ml tube. 1µl aliquots of the reaction before and after separation were mixed with Ecoscint scintillation fluid and assayed by scintillation counting to determine the proportion of the radioactive nucleotides that were incorporated into the transcript.

6. Up to 16 200mm x 200mm nylon membranes were pre-hybridized simultaneously in 500ml of 1x SSC, 1% SDS, 1x Blotto (Johnson *et al.* 1984) in a 220mm x 220mm plastic box. The box was gently agitated in a 65°C shaking waterbath for 5 to 8 hours. Pre-hybridization prevents the probe from binding directly to the membrane. After this, the membranes were

removed briefly from the box while a volume of the probe was added sufficient to read 6 x 10^7 counts per minute. The membranes were returned ensuring that they were thoroughly immersed and no air bubbles were trapped. Hybridization was allowed to proceed overnight at 65°C.

Washing and Autoradiography

The surplus probe was washed off the membranes which were then wrapped in cling film to protect them. A trial autoradiograph was made by placing the membrane into an X-ray cassette with a sheet of film, sandwiched between two intensifying screens (which help the image to form quickly at the expense of resolution). After an overnight exposure, the film was developed and the resulting crude autoradiograph used to determine the duration of a longer, better-quality exposure, ideally without screens.

Membranes were washed in four changes of 1x SSC, 0.1% SDS at 65°C for 25 minutes to remove any probe not bound to the DNA fragments. Whilst slightly damp they were wrapped in Saran Wrap. The membranes were then autoradiographed with two intensifying screens (either Cawo or Hi Speed X) with Fuji RX film at -80°C for 18 hours. After this the autoradiographs were developed and the quality of the image was used to determine the time required for a full exposure. If the signal was particularly intense, the intensifying screens were omitted to produce a sharper image. Screenless exposure lasted 3 to 10 days at room temperature.

Technical Aspects of the DNA Fingerprinting Results

This section describes the DNA fingerprinting results from a technical point of view. It examines the success rate of Pied Flycatcher fingerprinting in this study, and attempts to explain why it was so low. The section also includes some analysis of the genetics of the Pied Flycatcher minisatellite regions, and an assessment of the value of the range of different probes which might be used for paternity testing in this species.

Reliability of the Technique

During the study I ran 87 Pied Flycatcher gels. On only 28 of these were there tracks of acceptable quality for paternity testing. Over 1000 DNA samples were extracted, although, when the survey was discontinued, there were usable fingerprints of only 157 individuals. This success rate is unusually low in comparison with other studies at Nottingham and elsewhere (Ashworth *pers. comm.*, Austin *pers. comm.*, Carter *pers. comm.*, Hochachka *pers. comm.*, Hutchinson *pers. comm.*, May *pers. comm.*, Meng *pers. comm.*, Reily *pers comm.*, Wellbourn *pers. comm.*, Wetton *pers. comm.*, Burke *et al.* 1989, Lifjeld *et al.* 1991).

The reasons for failed extractions and fingerprint gels can be divided into two classes; general problems regarding fingerprinting within the Avian Genetics Laboratory, and specific problems associated with the Pied Flycatcher study.

On several occasions, DNA work within the laboratory was frustrated by contamination of the water supply, failures of electrical equipment, faulty membranes and enzymes. These affected everyone to some extent, and were (arguably) unavoidable.

However, besides these general problems, it became apparent that others affected only the Pied Flycatcher fingerprint survey. Many extractions failed, producing either no DNA, or small quantities that were often difficult to cut with restriction enzymes. It seems likely that this was partly related to the small volume of the blood samples. Being a comparatively small bird, weighing 10 to 12g, smaller samples were taken from Pied Flycatchers than, for example, 20g Swallows (Wellbourn *pers. comm.*) or 28g Sparrows (Wetton 1990). An empirical rule regarding the quality of DNA from blood samples seems to be that larger samples produce better DNA (Carter *pers. comm.*). This is probably because deterioration processes are related to concentration and/or the surface area to volume ratio of the original sample. In addition, sampling blood from the wing surface might have allowed greater contamination than taking blood direct from the blood vessel.

Contamination of DNA can make it difficult to digest with restriction enzymes. The method used to separate Pied Flycatcher DNA from potential contaminants is described in Chapter 4, and was similar to that used by other workers in the laboratory. The variation in extraction techniques both within- and between workers consists mainly of alterations in the number of treatments with each solvent. The aim during extraction is to perform the number of treatments which removes the most contamination whilst losing a minimum of DNA. A major contaminant is protein, which can be assayed in a DNA sample using fluorimetry. Table 4.1 shows the results of such an assay.

Worker	Sample	Absorbtion		Ratio 260/280
		260nm	280nm	
JDEH	1	0.43	0.29	1.48
	2	0.81	0.51	1.58
	3	0.36	0.27	1.33
	4	1.38	1.08	1.28
	5	0.50	0.34	1.48
	6	0.87	0.46	1.89
	7	0.40	0.27	1.48
	8	0.44	0.29	1.51
REC	1	0.69	0.49	1.40
	2	0.90	0.66	1.36
PCC	1	0.48	0.35	1.37
	2	0.70	0.50	1.40

Table 4.1: An assay of protein contamination in DNA samples. The greater the ratio of absorption at 260nm to that at 280nm, the lower the protein concentration. REC and PCC kindly donated DNA samples from Sparrowhawks and Pied Flycatchers respectively.

From Table 4.1, it seems that Pied Flycatcher DNA extracted by me was slightly less contaminated than DNA extracted by the two other workers. I tended to repeat the treatments more than the others, and this is reflected in the purity of the DNA. It therefore seems that the problems with Pied Flycatcher fingerprints are not directly related to the concentration of protein in the DNA. Other contaminants may be present, and might affect the quality of fingerprints, but these were not assayed.

DNA fingerprints of Pied Flycatchers were also attempted by Jon Wetton, Paul Cabe and Melissa Wellbourn, none of whom met with much more success than me.

When this study began, 19.6 had been found to be the better of the two probes for use on a number of bird species. This was the first to be developed into a ribo-probe (Carter pers. comm.) and as a result, it was used for most of the fingerprints in this study. More recently, each membrane has been probed with both the probes, and a comparison of the results can be made from Figures 7.6, 7.7 and 7.8.

A common feature of Pied Flycatcher fingerprints is the large number of "relict" fragments which form a dark smear near the origin on most autoradiographs (See Figure 7.6). This is present in DNA cut using HaeIII, AluI and MbOI, probed with both Jeffreys probes (pSPT 18.15 and pSPT 19.6). A similar pattern has been observed in fingerprints of Azure-winged magpies (Wetton *pers comm.*). This is least apparent when the DNA is cut with HaeIII and washed with a comparatively dilute solution of SSC (namely 0.01xSSC).

There are known to be specific problems involved in fingerprinting a number of other species. Carter (*pers comm.*) found that both pSPT 19.6 and pSPT 18.15 bind only weakly to Sparrowhawk (*Accipiter nisus*) bands which are small, numerous and relatively common in the population. All these features hinder interpretation of the fingerprints. Hutchinson (*pers. comm.*) finds difficulty in obtaining good quality DNA fingerprints from Red Squirrel (*Sciurus vulgaris*) tissue, partly because nuclease activity in these samples seems unusually high and causes rapid post-mortem degradation of the DNA.

To produce satisfactory DNA fingerprints of a particular organism seems to demand specific treatments and running conditions. In this study the ideal methods were not established.

Minisatellite Regions of the Pied Flycatcher Genome

The Pied Flycatcher fingerprints prepared in this study are similar in a number of ways. They have a dark "smear" close to the insert line and many closely-packed bands. Such patterns can quite easily be distinguished from those of several other species prepared using similar methods. This dissimilarity might be attributed to genetic or cellular differences between the species, or to technical differences in the preparation of the material.

The Pied Flycatcher fingerprints indicate the presence of minisatellite regions, which remain large and unfragmented, appearing as a dense, dark region close to the insert line. In this part of the fingerprint, bands are not discrete, coalescing to form a continuous dark streak. This represents a very diverse assortment of DNA fragments having almost the whole range of molecular weights between the limits of the "smear". This may indicate a great diversity of minisatellite regions in this size range in the Pied Flycatcher genome. Alternatively, some feature of the cellular environment and/or the extraction procedure might have caused slight degradation of minisatellite fragments, altering their size and making what should be a series of bands appear contiguous. Running fingerprint gels under different conditions, and with different probes might resolve discrete bands where currently we see the smear.

The different appearance of Pied Flycatcher fingerprints is a manifestation of inter-specific variability among minisatelites. Only a large survey would reveal to what extent the similarity between fingerprint patterns is related to phylogenetic relationships between species. When fingerprints of some closely-related species are prepared using the same techniques, there is sometimes a resemblance. For example, Carter (*pers. comm.*) noticed certain features typical of the DNA fingerprints of raptors. The interspecific diversity of minisatellite loci was examined by Gray and Jeffreys (1991). They found one minisatellite locus which was very variable in man but much less so in other great apes.

They found another which was very similar among all the great apes but varied among Old World monkeys. They concluded that the level of inter- and intra- specific variation in individual loci is largely unpredictable. As multi-locus probes reveal a number of different loci, they too will reveal both differences and similarities between closely-related species.

Multi-locus fingerprinting is still widely used and is arguably preferable for large-scale studies of the mating patterns within a population. Gray and Jeffreys (1991) findings are evidence that only a proportion of single-locus probes surveyed will prove useful in such studies. Developing such probes is a major task, and is easier if multi-locus fingerprinting is reliable in your species. However, it is possible that probes developed for use on other bird species might also prove useful for the Pied Flycatcher.

Improving the Quality of Pied Flycatcher Fingerprints

The failure to produce consistently good fingerprints from Pied Flycatchers seems to stem from problems with the quality of the DNA, and the choice of restriction enzymes and probes. As the basic techniques become more routine, and the range of alternatives broadens, an enzyme/probe combination might be found which produced useful multi- or single locus DNA fingerprints.

CHAPTER 5

The Breeding Biology of Pied Flycatchers in the Gwenffrwd

Introduction

This chapter concerns the numbers of eggs that were laid, of chicks that hatched and of nestlings that fledged. I examine variation in these measures of breeding success, relating them, in turn, to features of the study site, the nestboxes and the breeding adults.

In the chapter, some terms are repeatedly used. It seems important to define them precisely in advance. Here I examine one aspect of reproductive fitness, namely breeding success. My scope is limited because, with a few exceptions, once fledglings left the nest, their fates were unknown. In this chapter, therefore, breeding success is used as a general term encompassing several components that were recorded or estimated in the field; clutch size, brood size at hatching, and brood size at fledging. The proportion of eggs that hatched is called hatching success, and the proportion of hatchlings that fly from the nest is called fledging success.

Statistical Tests

The data described in this chapter are ecological in nature. In the field I measured a number of variables such as clutch size, laying date and wing length. All these are governed by complex biological processes, and are, in the words of Campbell (1975), "variables whose value can be regarded as the result of a large number of small and independent contributions". Whatever the distribution of the many underlying variables, the *Central Limit Theorem* shows that the distribution of the dependent variable is likely to be close to the

normal. In addition, for many distributions, the mean of a sample has a distribution very close to normality. There is therefore some reason to believe that statistics which assume a normal distribution have a use in the interpretation of data such as mine.

However, in this chapter I have, where possible, used tests which are relatively less sensitive to the underlying distribution. The null hypothesis of such a test concerns only the distribution of the variates, not the parameters which describe the underlying distribution.

Where I have used parametric tests, I have done so either because there is no distribution-free equivalent, or because they describe the relationship between the variables in a simple, easily understood way. Where both parametric and non-parametric tests were possible, the results were similar.

Factors Affecting the Number of Offspring

The quality of the nestbox and its surroundings

In this section, I describe how several features of the nestbox, and the surrounding habitats relate to measures of breeding success. The literature on habitat requirements of breeding birds is immense (Sharrock 1976). In the case of the Pied Flycatcher, several studies have shown relationships between breeding success and the size of the nestbox, the surrounding woodland vegetation type and the composition of the local bird community. An extensive study by Stowe (1987), whose study area included the Gwenffrwd, concentrated upon the habitat requirements of the species. I did not attempt to replicate his detailed measurements of habitat structure and food availability, or his faecal analysis. My intention was to find out whether any of the factors affecting breeding success also influence the occurrence of extra-pair mating and polygyny, and to make generalisations about the population that might

explain why the mating system of these birds seems to differ from the well-studied Scandinavian populations.

The quality of the nestbox

Arguably the most un-natural feature of well-studied Pied Flycatcher populations is the provision of nestboxes. How the findings of these studies relate to the natural situation is unclear, but some breeding parameters, such as fledging success, are dramatically different in the two situations (Nillson 1984). Providing nestboxes, however, makes fieldwork easier and can maintain a large study population (Campbell 1955). After adding more boxes, only to see the population increase, seemingly without limit, von Haartman (1956) stated resignedly "Few ornithologists are probably wealthy enough to supply more nestboxes in an area of $4km^2$ than the Pied Flycatchers can use".

In Britain, a major determinant of Pied Flycatcher distribution is the provision of boxes (Sharrock 1976). In many areas, including the Gwenffrwd, the management of the land during the last century resulted in a predominance of plantation-like woodland, with stands of similar age. Such areas lack the very old trees in which holes are most common, and it has been argued that providing boxes merely compensates for the lack of natural holes (Nillson 1984).

Unlike natural nest sites, nestboxes are often quite similar. Their dimensions are fairly standard, they are positioned uniformly low down on the tree, and their resistance to predation is presumably alike. Good and bad quality natural nests could result in more causes of variation in breeding success, than in nestbox populations. The difference among nestboxes was artificially increased by Gustaffson and Nillson (1985), who found the size of the nesting cavity affected clutch size and fledging success. Boxes with a bottom area of

57cm^2 had fewer eggs, and lost more nestlings through predation and desertion than boxes with a bottom area of 87cm^2.

In the Gwenffrwd study, the boxes were comparatively large. The smallest had a bottom area of 56.2cm^2, and the largest 180cm^2, with the mean being 101cm^2. In 1988, the size of a clutch was related to the bottom area of the nestbox (Spearman Rank Test: R_S=0.1923, P<0.033, n=125). However, in 1989, the relationship was non-significant (Spearman Rank Test: R_S=0.1151, P<0.207, n=115). With the data from both years pooled, the relationship was significant at the 2% level (Spearman Rank Test: R_S=0.1565, P<0.016, n=240). Bottom area is determined by two dimensions, the distance from side-to-side ("Width") and that from the front to the back of the box ("Depth"). These were examined separately. Of the two, the relationship between box width and clutch size was the stronger (Table 5.1). The mean clutch size in nestboxes wider than 115mm was 0.63 eggs greater than that in narrower ones. This is very strong evidence of an association between the two variables, but is not sufficient to claim that the width of the box determines clutch size.

Because nestboxes in the same area are often made to the same design, there is a strong relationship between area and nestbox-type and size. When clutch sizes are corrected for location, the relationship with width remains, but is no longer significant (Table 5.1). The differences in breeding success among areas are discussed in more depth later in this chapter.

Year	Length	Width	
		Uncorrected	Corrected
1988	R_S=0.066 n=125 P<0.466 NS	R_S=0.186 n=125 P<0.038 *	R_S=0.117 n=125 P<0.192 NS
1989	R_S=0.000 n=115 P<0.993 NS	R_S=0.146 n=115 P<0.119 NS	R_S=0.072 n=115 P<0.439 NS
Both Years	R_S=0.038 n=240 P<0.560 NS	R_S=0.166 n=240 P<0.010 **	R_S=0.109 n=240 P<0.090 NS

Table 5.1 Spearman Rank Correlation of clutch size and nestbox measurements in 1988 and 1989. Within "Width" the "Uncorrected" column shows the correlation of nestbox width and clutch size. The "Corrected" column shows the correlation between nestbox width and clutch size, when corrected for between-area differences. NS = Non-significant statistic, * = P<0.05, ** = P<0.01.

Additional evidence regarding the relationship between width and clutch size is ambiguous. Ten nestboxes contained clutch sizes less than five; none of these was over 115mm wide. Table 5.2 shows that this association is non-significant. However, a width effect would result in high year-to-year repeatability of clutch size in the same box which was not found (see later in this chapter). The mechanism for such an effect might be that the physical efficiency of brooding is greater in such a box, or that better quality parents choose to nest in wide boxes. A larger sample size, over a greater number of years would establish what, if any, is the effect of nestbox width.

		Clutch Size	
		<5	≥5
Nestbox Width	<115	10	200
	≥115	0	30

Table 5.2 There is no significant association between between smaller boxes and smaller clutch sizes. Fisher's Exact Test, P=0.26.

In an experiment designed to separate the effects of male and nestbox quality, Slagsvold (1986a) manipulated the attractiveness of nestboxes by tilting them. Some were left upright, some tilted to one side and some leant backwards. In the present study, our intentions were different and an effort was made to keep boxes as upright as possible, because traps work best in this way. Two boxes were at very pronounced angles. One was on a long-fallen tree, and housed tits in both years of the study. The other held the largest clutch in 1988 and fledged 9 offspring. After the tree broke during the winter it had a clutch of 7 eggs, all of which produced young that fledged. It seems that in this case, at least, a radical alteration in the attitude of the nestbox did not catastrophically affect breeding success.

The Gwenffrwd boxes fall into four distinct styles, described in Chapter 3. There was no effect of nestbox style on the breeding success of the Pied Flycatchers nesting in them. (Kruskall-Wallis test for differences in clutch size among the three common styles of nestbox $H=3.26$, $P<0.20$, not significant). Nor was there an effect of box type on the probability of use, except that new boxes, which in any one year tend to be of the same design, are less often used during their first year in place.

The surroundings of the nestbox

The variation in breeding success caused by differences among the boxes is probably less than that caused by habitat features. Several studies have attempted to measure, manipulate or control for habitat quality, often as a means to find whether females choose males or their territories. Pleczynskya (1978) found that the single most influential feature of the breeding territory of Lark Buntings (*Calamospiza melanocorys*) was the availability of shady bushes near the nest.

In the Pied Flycatcher, there is no single factor that has been identified which strongly affects breeding success within a wood; probably several are important. In some studies the existence of relationships has been shown, and in a smaller number they have been identified:

Some nestboxes were occupied more frequently over several years than would be expected from a random choice of nest site (Askenmo 1984). However, no such relationship was found during Stowe's (1987) work in the Upper Tywi valley.

Some boxes consistently produced more fledglings than others (Askenmo 1984). Again, Stowe did not find this.

Density-dependence of fledgling numbers, nestling weight and tarsus length suggests that breeding success is limited by food availability (Alatalo and Lundberg 1984a). This relationship is weak, because Pied Flycatchers do not defend a feeding territory, but forage over a wide area (von Haartman 1956).

Pied Flycatchers in deciduous woods were found to lay earlier and have higher breeding success than those in coniferous habitat (Lundberg *et al.* 1981). Males in deciduous habitat were larger.

Frequency of box use was related to the density of oaks and to the foliage cover at canopy height (Stowe 1987).

The mean laying date in a sample of woods was strongly affected by the altitude of the wood, and this affected clutch size (Stowe 1987).

In the next section, I examine three indicators of habitat effects; the differences in breeding success among the five nestbox series, the relationship between the

breeding success of neighbours and the year-to-year consistency of breeding success in each box.

Area to area differences in breeding success

It is very likely that, within the Gwenffrwd and Dinas reserves, variation in topography, vegetation, and climate, combined with differences in the distribution of competitors, parasites and predators will have measurable effects upon the breeding biology of the Pied Flycatchers. To study this in any great depth, a larger sample size would be useful. Data covering a wider area over several seasons (such as that gathered by the BTO study) would provide sufficient information to reveal some links between the local environment, the breeding behaviour of the birds and their fledging success. Such information exists, but was not available for such an analysis to be included in this study. As a result, I have analysed solely the data gathered during my study that I acknowledge is limited.

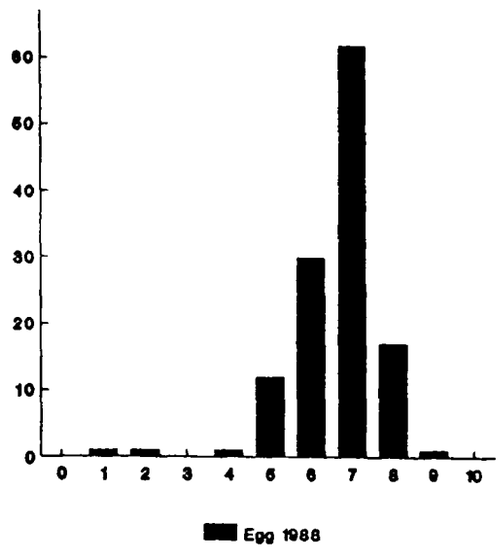
One way to reveal a relationship between habitat and breeding success is to examine the variation in breeding success on a smaller scale, comparing neighbours; this is done in a later section. Here, I examine the differences in breeding variables between the five sections within my study area. To some extent, the division may be arbitrary. Some boundaries may be biologically relevant, but some are almost certainly not. However, to my subjective eye, differences in the vegetation and aspect of the areas were sufficient to give each its own character; some variation I detected may also be important to the Pied Flycatchers. Given the circumstances, therefore, I feel that the analysis is of some value and may reveal subjects for future studies.

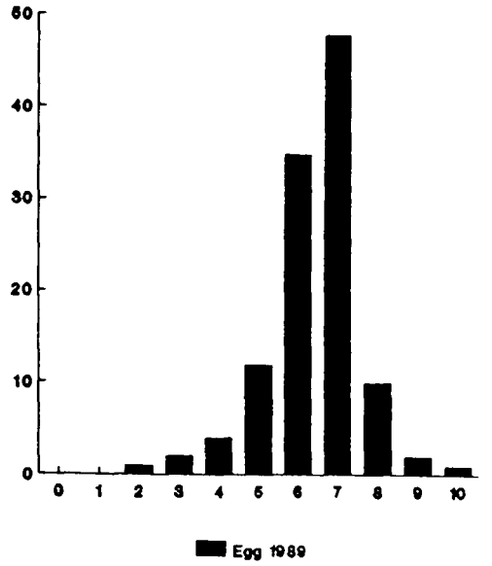
Figure 5.1 shows frequency distributions of clutch size, brood size at hatching (BSH) and brood size at fledging (BSF). It can be seen that very few nests had particularly small clutches and broods. In fact, in the two years, only three

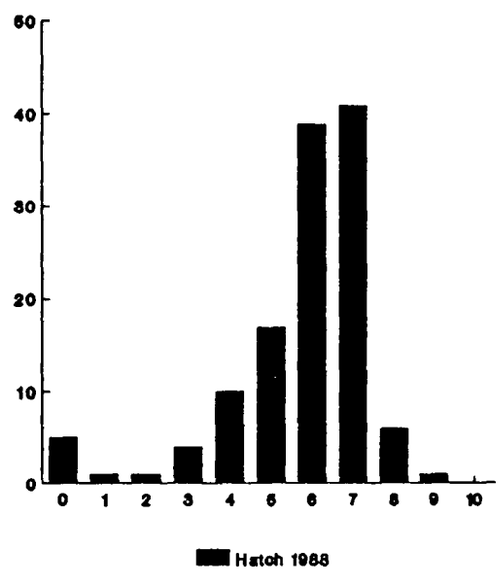
clutches were of less than three eggs. One of these was abandoned and the female concerned re-nested elsewhere. Another was involved in a possible case of polyterritoriality (G22, See Chapter 7). The third box, in which both chicks died, was on a high, exposed woodland edge.

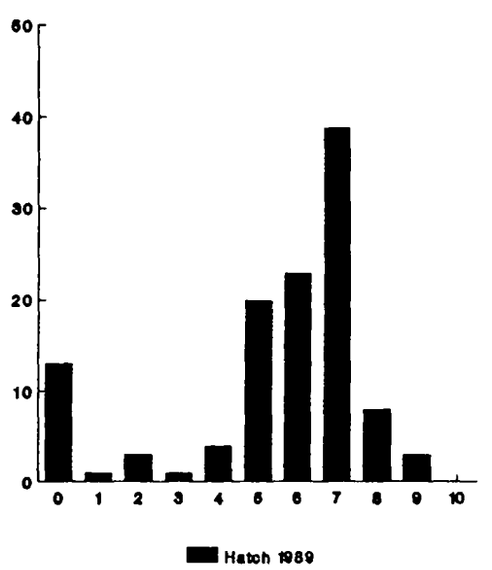
I have omitted these nests from some parametric analyses where, I believe, they would have a disproportionate effect upon the statistics, giving spurious and misleading results.

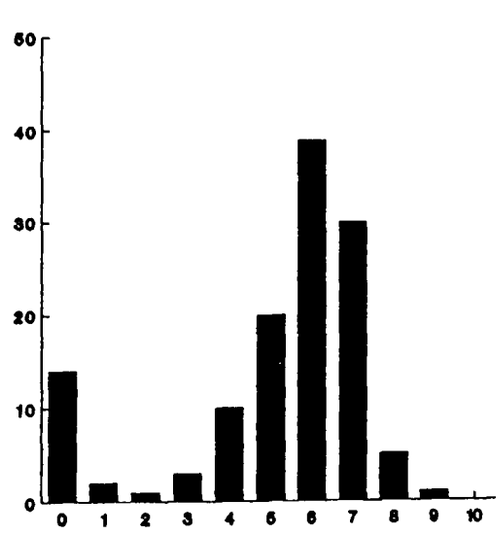
Table 5.3 shows, for each area, the mean values of clutch size, brood size at hatching and brood size at fledging. Because the mean values can be disproportionately affected by the few very small clutches and broods mentioned above, it is unwise to attribute great biological significance to the small differences between mean clutch and brood sizes in different areas.











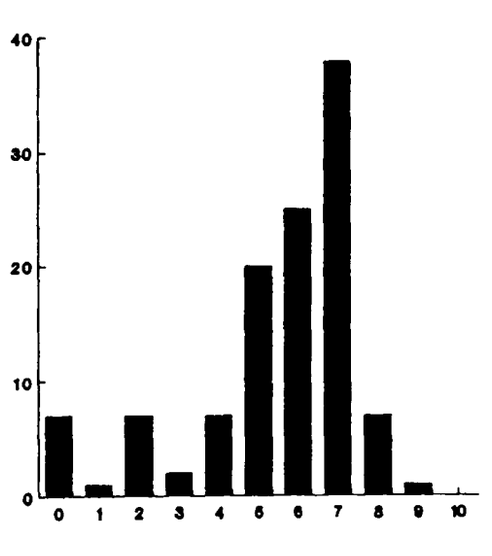


Figure 5.1: Frequency distributions of Clutch Size, Brood Size at Hatching and Brood size at fledging in 1988 and 1989. It can be seen that very few nests had small clutches and broods.

Year	Area	Mean Clutch Size ± s.e (n)	Mean Brood Size at Hatching ± s.e (n)	Mean Brood Size at Fledging ± s.e (n)
1988	G	6.63±0.21 (32)	6.23±0.22 (30)	6.12±0.26 (26)
1988	H	6.87±0.13 (23)	6.48±0.18 (23)	6.45±0.19 (22)
1988	I	6.55±0.18 (33)	5.47±0.30 (32)	5.34±0.32 (30)
1988	J	6.79±0.23 (14)	6.31±0.23 (13)	5.91±0.20 (11)
1988	K	6.30±0.31 (23)	5.68±0.23 (22)	5.45±0.23 (22)
1988	All Areas	6.61±0.10 (125)	5.98±0.12 (120)	5.82±0.13 (111)
1989	G	6.94±0.14 (33)	6.81±0.17 (32)	6.64±0.21 (33)
1989	H	6.40±0.27 (25)	5.76±0.40 (17)	4.54±0.42 (22)
1989	I	6.24±0.19 (25)	6.13±0.21 (23)	5.83±0.22 (24)
1989	J	6.75±0.25 (4)	7.00±0.00 (3)	6.50±0.44 (4)
1989	K	5.93±0.28 (28)	5.56±0.34 (27)	5.80±0.28 (25)
1989	All Areas	6.42±0.11 (115)	6.16±0.14 (102)	5.83±0.15 (108)

Table 5.3: Three aspects of breeding success in the five study areas in 1988 and 1989. All clutches and brood sizes included. CS = Clutch Size, BSH = Brood Size at Hatching amd BSF = Brood Size at Fledging. The results for all but CS 1988 are significantly different. There is a trent for CS, BSH and BSF to be greater in box-series G & H and smaller in series I & K.

The Kruskall-Wallis test is relatively unaffected by outliers and the results of this non-parametric test are shown in Table 5.4. It reveals that, for both years, there are significant differences between areas for all measurements except clutch size in 1988. Area to area differences in brood size at fledging are highly significant in both years. Comparisons of the means, and of the average ranks for each area which are computed for the Kruskall-Wallis test, indicate that clutch and brood sizes were consistently higher in areas G, H and J in 1988, and that areas G and H also had larger clutches in 1989.

Year	Clutch Size	Brood Size at Hatching	Brood Size at Fledging
1988	H=3.15 P<0.50 NS	H=11.36 P<0.02 *	H=13.68 P<0.01 **
1989	H=12.17 P<0.02 *	H=12.97 P<0.01 *	H=20.52 P<0.0001 ***

Table 5.4 Differences in breeding success among areas, in 1988 and 1989, Kruskall-Wallis test. NS = a non-significant result. * = P<0.05, ** = P<0.01, *** = P<0.001.

A pattern emerges from these results. The three upstream areas, G, H, and J, have larger clutches, and these are reflected in larger broods (Table 5.3). Exceptions to the general rule seem to be the high brood size at hatching in the K-series in 1989, and the low brood sizes in the H-series in the same year. When these are examined more closely, it is apparent that the BSH in the K-series seems high, only because that in the H-series is low. The clutch size in the H-series is slightly larger than the average, but BSH is 0.4 birds lower than average. This results from a large proportion of nests in which all, or some, of the eggs failed to hatch (Table 5.5). Partial nest failure occurred 8 times in the 25 clutches of the H-series, and 12 times in the 90 nests elsewhere; thus, the probability of partial nest failure was 2.4 times greater in the H's (G-test:G_{adj}=5.13, 1 df, P<0.025). Similarly, the probability of total nest failure (which occurred 8 times in the H's and only 5 times elsewhere) was 32%, 6

times greater (G-test:G_{adj}=11.07, 1 df, P<0.001). The probability of fledging is also lower in the H-series. See Figure 5.2 for a flow diagram of the possible outcomes of nesting attempts.

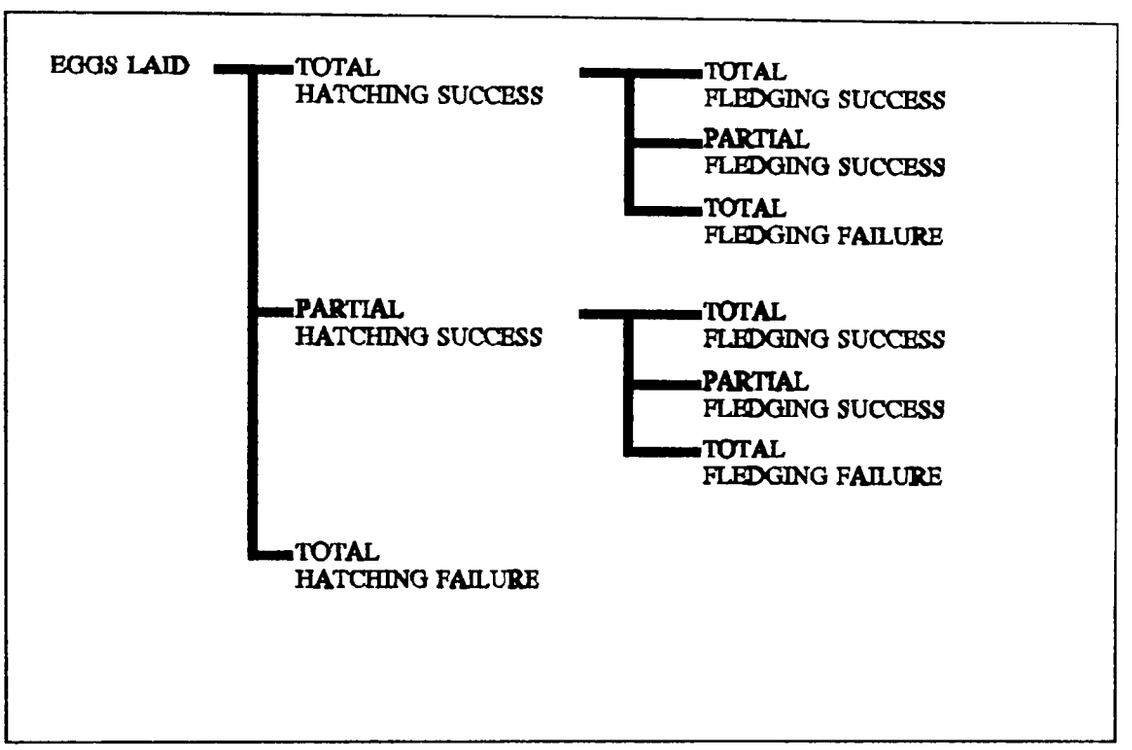


Figure 5.2: A diagrammatic representation of the possible outcomes of a nesting attempt, the probabilities of which are summarised for each nestbox series in Table 5.5.

Year	Outcome	G	H	I	J	K	All
1988	No. Nests	32	23	33	14	23	125
	THS	0.625	0.783	0.515	0.571	0.435	0.584
	PHS	0.344	0.217	0.455	0.357	0.522	0.384
	THF	0.031	0.000	0.030	0.071	0.043	0.032
	TFS_{THS}	0.600	0.611	0.529	0.625	0.800	0.616
	PFS_{THS}	0.100	0.111	0.235	0.250	0.100	0.151
	TFF_{THS}	0.300	0.278	0.176	0.125	0.100	0.219
	TFS_{PHS}	0.636	1.000	0.400	0.600	1.000	0.688
	PFS_{PHS}	0.091	0.000	0.067	0.200	0.000	0.063
	TFF_{PHS}	0.273	0.000	0.533	0.800	0.000	0.313
1989	No. Nests	33	25	25	4	28	115
	THS	0.879	0.360	0.840	0.750	0.714	0.713
	PHS	0.091	0.320	0.080	0.000	0.250	0.174
	THF	0.030	0.320	0.080	0.250	0.036	0.113
	TFS_{THS}	0.828	0.667	0.810	1.000	0.850	0.817
	PFS_{THS}	0.103	0.222	0.143	0.000	0.100	0.122
	TFF_{THS}	0.069	0.000	0.048	0.000	0.050	0.049
	TFS_{PHS}	1.000	0.375	0.500	-	0.857	0.650
	PFS_{PHS}	0.000	0.625	0.500	-	0.000	0.300
	TFF_{PHS}	0.000	0.000	0.000	-	0.143	0.050

Table 5.5 Probability of success or failure during nesting. Once eggs are laid, all may hatch successfully (Total Hatching Success= THS), or success may be partial (PHS), or hatching failure may be total (THF). For nests with THS or PHS, the fledging success may be total or partial, or there may be complete failure. Subscripts denote one outcome given a previous one; for example TFS_{THS} represents Total Fledging Success for broods whose hatching success was also total (See also Figure 5.2).

The year-to-year difference in the variation among areas is small. In two-way analyses of variance for the three measures of breeding success, the effects of year are not significant (Table 5.6). For BSH, the year effects are almost significant. This results from the lower rates of partial nest failure in 1989. The rates of total nest failure are higher in this year, but still not significantly so (Table 5.5). For brood size at fledging, the interactions between area and year are significant. This is caused, at least in part, by the change in fortunes of the H-series, from being the area with the highest proportions of total hatching and fledging success to having the least. This could be due to the weather. In a number of woods on hillsides, the Pied Flycatcher breeding success has been dramatically affected by a single spell of bad weather. Other woods with different aspects remained unaffected (Pickup *pers. comm.*). This seems to be what happened to the H-series in 1989.

Variable	Effect of		
	Year	Area	Interactions
Clutch Size	F=1.895 1,225 df P<0.17 NS	F=2.827 4,225 df P<0.03 *	F=0.776 4,225 df P<0.54 NS
Brood Size at Hatching	F=3.471 1,206 df P<0.06 NS	F=3.407 4,206 df P<0.01 *	F=1.408 4,206 df P<0.23 NS
Brood Size at Fledging	F=1.413 1,198 df P<0.23 NS	F=3.696 4,198 df P<0.01 *	F=3.155 4,198 df P<0.02 *

Table 5.6: Between-area and Between-year variation in Clutch Size, Brood Size at Hatching (BSH) and Brood Size at Fledging (BSF). NS = a non-significant result. * = P<0.05. There were significant differences between-areas for the variables analysed.

In conclusion, some feature of the G,H and J areas caused them to have higher breeding success in 1988. This is not necessarily just a habitat feature; in addition, it may be that the adults in these areas were of better quality. In the second year of the study, clutch sizes followed the same pattern, with the three upstream areas having larger-than-average clutches. However, something then caused birds in the H-series to have dramatically lower hatching and fledging success. It is possible that this woodland was particularly affected by bad weather. Analysis of the long-term data on the Gwenffrwd and Dinas populations might reveal more about the factors which affect the relative breeding success of birds nesting in different parts of the woodlands.

Similarity between neighbours

If features of the area surrounding the nest box affect the breeding biology of the Flycatchers in nestboxes, some of the features might be expected to be shared by neighbouring boxes. Clutch and brood sizes of neighbouring nests might be expected to be more similar than those of non-neighbours. To test this, each nest was paired with the nearest neighbour, and similarity between the pairs tested with the Spearman rank correlation coefficient. A potential problem with the statistics in these analyses is that an individual nest can appear as both a "nestbox" and a "neighbouring box". The two variables are not independent. Two nearby boxes can even form pairs, each being the other's neighbour. Because of this, the results should be treated with caution.

For each pair, the breeding success variables were compared with those of neighbouring birds (Table 5.7). Only in 1989 was there a significant correlation between aspects of breeding success of neighbours; the number of hatchlings and hatching success. In 1989, when the H-series had a disproportionate number of clutches that failed to hatch, it contained several neighbouring pairs who both had hatching failure. Part of the relationship is due to this. However, the H-series is not solely responsible for the

relationship, as excluding the boxes of this area still leaves a significant relationship (r_s=0.2210, n=90, P<0.037).

	1988			1989		
	n	r_s	p	n	r_s	p
Number of eggs	124	0.219	0.808	115	-0.043	0.65 NS
Hatching Success	124	-0.091	0.312	115	0.229	0.01 *
Nestling Survival	120	0.105	0.252	102	0.395	0.69 NS

Table 5.7 Spearman Rank Correlation coefficients for nestboxes and their nearest neighbour, ignoring empty or failed nests. NS = a non-significant result. * = P< 0.05. Only in 1989 is there a significant difference in the hatching success between box-series.

Neighbours of birds that did not have 100% fledging success are themselves more likely to lose hatchlings before fledging (Table 5.8). In no case did the neighbour of birds that had total fledging failure also fail completely. This suggests that it is partial fledging failure, the loss of one or two pulli, which is shared by neighbours. When the birds and neighbours that lost all the brood are excluded from the analysis, the association becomes stronger (Table 5.9).

		Hatching success of neighbour	
		100%	<100%
Hatching success of pair	100%	62 (58.4)	11 (14.6)
	<100%	10 (13.6)	7 (3.4)

Table 5.8 Hatching success in nestboxes and their nearest neighbour. G-test:G_{adj}=3.15, 1 df, P<0.07, NS.

		Hatching success of neighbour	
		100%	>0% <100%
Hatching success of pair	100%	62 (57.3)	8 (12.7)
	>0% <100%	6 (10.7)	7 (2.3)

Table 5.9 Hatching success in nestboxes and their nearest neighbour, excluding boxes with total hatching failure. G-test:G_{adj}=9.98, 1 df, P<0.005. Hatching success of neighbours is related.

Year-to-year similarity in measures of breeding success

If any characteristics of nests are related to breeding success, some might be expected to remain constant from year to year. They would cause similarity between the breeding success in the same box in successive years. However, there seems to be no relationship between the clutch sizes in the same box in successive years. Nor is there a relationship in the size of broods from year to year (Table 5.10). There is no evidence that hatching or fledging failures occur more in particular boxes (Tables 5.11 and 5.12). The only evidence of a factor that affects the suitability of a nestbox, and which is constant between years, is a relationship between usage of the nestbox in successive seasons. Some boxes are regularly unused by any species (Table 5.13). Of those that were used at least once, the settlement pattern of Pied Flycatchers or other species was not significantly different from random (Table 5.14). Table 5.15 shows 7 Blue Tit (*Parus caeruleus*) nests that were used in successive years, instead of the expected 3. With a larger sample size such patterns may be significant.

	Spearman Rank Correlation			
	n	r_s	p	
Clutch Size	78	0.1272	0.27	NS
Brood Size at Hatching	69	0.8886	0.47	NS
Brood Size at Fledging	66	-0.1158	0.35	NS

Table 5.10 Correlation of clutch size and brood sizes in the same nestbox in subsequent years. There is no correlation between clutch and brood sizes in the same nestbox in subsequent years.

Total Hatching Failures		1989	
		No Failure	Failure
1988	No Failure	69	2
	Failure	1	0

Table 5.11 Showing the association of hatching failure in 1988 with hatching failure in the same box in 1989.

Total Fledging Failures		1989	
		No Failure	Failure
1988	No Failure	62	2
	Failure	5	0

Table 5.12 Showing the association of fledging failure in 1988 with fledging failure in the same box in 1989.

		1989	
		Occupied	Empty
1988	Occupied	152	7
	Empty	13	8

Table 5.13 Condensed table of box occupancy in 1988 and 1989. G-test:G_{adj}=16.35, 1df, P<0.001. Those boxes which are occupied in one year tend to be occupied in a subsequent year.

		1989	
		PF	Other Species or Empty
1988	PF	92	38
	Other Species or Empty	28	21

Table 5.14 Condensed table of box occupancy by Pied Flycatchers in 1988 and 1989. G-test:G_{adj}=2.88, 1 df, P<0.1. Boxes that are used by Pied Flycatchers in a year are not used exclusively by that species in other years.

Observed (Expected)		1989				
		PF	GT	BT	Other	Empty
1988	PF	92 (86.7)	8 (6.5)	17 (20.2)	7 (5.8)	6 (10.8)
	GT	8 (8.0)	0 (0.6)	3 (1.9)	0 (0.5)	1 (1.0)
	BT	7 (10.0)	0 (0.8)	7 (2.3)	1 (0.6)	0 (1.3)
	Other	1 (1.3)	1 (0.1)	0 (0.3)	0 (0.1)	0 (0.2)
	Empty	12 (14.0)	0 (1.1)	1 (3.2)	0 (1.0)	8 (1.8)

Table 5.15 The species occupancy of each box in 1988 and 1989. (Expected values in parentheses). Note; 7 not 3 Blue Tit repeats.

The timing of the breeding attempt

In seasonal climates, the timing of breeding affects the availability of resources and the ecological and environmental conditions in which the young are reared. Pied Flycatchers, especially in northern regions, have only a limited period during which the conditions are suitable for nesting. Breeding must commence during the late spring or early summer, as days lengthen, the air warms and insect larvae become abundant. It is well established that Pied Flycatcher clutch size declines through the season (Lack 1966, Slagsvold 1976). It has been suggested that female mating strategies are affected by the inevitable decline in reproductive success which results from a delay in breeding (Alatalo and Lundberg, 1984). Thus, the mating patterns of Pied Flycatchers may be directly affected by the seasonality of their northern breeding areas.

A multiple regression model shows that, in 1988, laying date had a significant effect upon clutch size. The date of nest initiation was also significant, but

this was found to be due to several extreme outliers. When these 8 nests were removed from the model, only the date of nest initiation was significant (Table 5.16). In 1989, a similar relationship existed; after the removal of nine extreme boxes, clutch size was found to decline with laying date (Table 5.17). The distributions of clutch size within the samples used in tables 5.16 and 5.17 depart significantly from the normal distribution, even after the outliers were removed. There are four reasons to use linear regression for this analysis:

1) To test for a relationship between laying date and clutch size.
2) To show that, in this case, clutch size declines with date.
3) To summarise the relationship in an easily understood form.
4) To enable comparison with similar results from other studies (see Chapter 8).

Non-parametric tests provide evidence in support of the first and second assertions. The Kruskall-Wallis test is insensitive to departures from the normal, and shows significant differences in laying date among clutch sizes. The average ranks (shown in Table 5.18), reveal that extreme clutch sizes are associated with extreme laying dates, with large, early clutches and small, late ones.

Y= Clutch size	Coefficient	s.e.	t	P
Constant	9.193	0.992	9.267	0
Laying Date	-0.058	0.024	2.449	0.02 *
n=113				

Anova table for regression					
Source of Variation	SS	df	MS	F	P
Linear Regression	3.487	1	3.487	5.998	0.02 *
Deviations from regression	64.530	111	0.581		
Total	100.938	112			

Table 5.16 Upper section: Regression of Clutch Size on laying date 1988. Day 0 = April 1st. Lower table: Analysis of Variance table for regression. * = $P<0.05$.

Y= Clutch size	Coefficient	s.e.	t	P
Constant	10.166	1.005	10.113	0
Laying Date	-0.087	0.024	3.651	0.0005
n=82				

Anova table for regression					
Source of Variation	SS	df	MS	F	P
Linear Regression	9.780	1	9.780	13.327	0.0005
Deviations from regression	58.708	80	0.734		
Total	68.488	81			

Table 5.17 Upper section: Regression of Clutch Size on laying date 1989. Day 0 = April 1st. Lower table: Analysis of Variance table for regression.

Clutch Size	1988		1988	
	Sample Size	Average Rank	Sample Size	Average Rank
4	-		3	54.17
5	7	71.14	7	59.29
6	29	56.91	25	49.00
7	60	62.95	39	35.45
8	17	30.32	8	27.25
	Test Statistic=14.73		Test Statistic=12.79	
	Significance Level P<0.01		Significance LevelP<0.02	

Table 5.18 Kruskall-Wallis test for relationship between Clutch size and laying date. Differences are significant in both years and average ranks suggest a decline in clutch size with date.

There was no direct effect of date upon the other breeding variables. The only significant determinant of the number of eggs to hatch was clutch size, and the only significant factor in the number of offspring fledged was the number hatched.

In 1989, a clutch in which some eggs did not hatch seemed more likely to have chicks that did not fledge. This is supported by the association between total fledging success and total hatching success in the contingency table, although this is not quite significant (Table 5.20). There was no significant relationship in 1988 (Table 5.19).

1988		Total fledging success	
		Yes	No
Total hatching success	Yes	8 (8.4)	40 (39.6)
	No	13 (12.6)	59 (59.4)

Table 5.19 Association of total hatching success and total fledging success in 1988. There is no evidence that broods with less than total hatching success are more or less likely to have total fledging success. G-test:G_{adj}=0.038, 1 df, NS).

1989		Total fledging success	
		Yes	No
Total hatching success	Yes	7 (3.7)	13 (16.3)
	No	12 (15.3)	70 (66.7)

Table 5.20 Association of total hatching success and total fledging success in 1989. Although the relationship is not significant, it seems that broods with less than total hatching success are likely to have total fledging success. G-test:G_{adj}=3.723, 1 df, P<0.1

The Effect of the Parents on the Number of Young

The shape and size of adults

Three biometrical characters were measured: mass in grams, tarsus length in millimetres (mm), and wing length in mm. Adult tarsus length is thought be achieved around the time of fledging and to remain constant throughout the life of the bird. Maximum wing length is determined at the time of the previous moult, which in the Pied Flycatcher, is in Africa, during the British winter. Wings and tail feathers become abraded, particularly on females who are incubating. Of the three characters, mass is most changeable. In neither year was the distribution of mass, tarsus or wing length for males or females significantly different from the normal (Kolmogorov-Smirnov Test, Table 5.21).

	1988	1989
Male Tarsus Length	0.0972, P=0.9996, n=56 NS	0.1025, P=0.3065, n=89 NS
Female Tarsus Length	0.0960, P=0.4685, n=78 NS	0.0745, P=0.9998, n=87 NS
Male Mass	0.1015, P=0.9998, n=48 NS	0.0848, P=0.5292, n=91 NS
Female Mass	0.0906, P=0.9991, n=73 NS	0.0779, P=0.9995, n=93 NS
Male Wing Length	0.1673, P=0.1087, n=52 NS	0.1364, P=0.0678, n=91 NS
Female Wing Length	0.1775, P=0.0201, n=73 *	0.1609, P=0.0162, n=93 *

Table 5.21 Results of Kolmogorov-Smirnov Test for goodness-of-fit, showing that, in most cases, the distributions of the samples do not differ significantly from normality. The Variables tested are male and female tarsus length (in mm), mass (in grams) and wing length (in mm). NS = a non-significant result. * = $P<0.05$.

Tarsus length was measured using a dial calliper. Measurement accuracy is described in detail in Chapter 6. Repeatability of tarsus length measurements was 78%. In both years, the distributions of male and female tarsus length were not significantly different from the normal (Kolmogorov-Smirnov test). There is no significant difference between the tarsus length of the males and females that were associated with nestboxes in the study in 1988 (Table 5.22). However, in 1989, males had significantly longer tarsi, the mean for males being 0.08mm more than that of females. Mean male tarsus length also changed between 1988 and 1989, the means differing by 0.15mm. However,

the tarsus length of those individuals that were caught in both years does not change significantly (Table 5.23).

Tarsus Length	Female	Male	F	t
1988	17.241±0.060 (73)	17.266±0.068 (47)	1.228 73,47 df NS	0.582 118 df NS
1989	17.201±0.050 (94)	17.117±0.051 (90)	1.008 94,90 df NS	2.589 182 df P<0.01 **
F	1.123 73,94 df NS	1.085 90,47 df NS		
t	1.139 165 df NS	3.762 135 df 0.001 ***		

Table 5.22 Tarsus length of males and females associated with nests. 1988 and 1989.

Year	Tarsus Length of males measured in both years	Mean Tarsus length of other breeding males	Statistics	
			F	t
1988	17.239±0.0918 (28)	17.266±0.068 (47)	1.086 28,47 df NS	-0.162 73 df NS
1989	17.190±0.0855 (28)	17.117±0.051 (90)	1.144 28,90 df NS	0.485 116 df NS
F	1.152 28,28 df NS	1.198 47,90 df NS		
t	0.085 54 df NS	1.198 135 df NS		

Table 5.23 Tarsus length in mm for males who were handled in both 1988 and 1989, and for those who known in only one year.

Wing Length	Female	Male	F	t
1988	77.699±0.156 (73)	79.957±0.192 (47)	1.001 73,47 df NS	10.404 118 df P<0.001 ***
1989	78.000±0.152 (93)	79.674±0.152 (89)	1.043 93,89 df NS	9.318 180 df P<0.001 ***
F	1.216 93,73 df NS	1.188 89,47 df NS		
t	1.615 164 df NS	1.320 134 df NS		
Both Years	77.868±0.110 (166)	79.735±0.125 (136)	1.064 136,166 df NS	13.449 300 df P<0.001 ***

Table 5.24 Wing Length in mm for males and females associated with a nest. 1988 and 1989.

The average wing length of males is almost 2mm longer than that of females (Table 5.24). This may be due, in part, to the abrasion received to the wings of incubating females. The average wing length of females did not change from year to year. However the wings of individual females were found to be significantly longer in the second year. Of the 27 individual females who were caught in both seasons, 18 had longer wings in 1989 (Signs test: Z=3.06 P<0.001, Means: 1989=77.67mm, 1989= 78.36mm).

Because females vary in mass within years, the causes of year-to-year variation are more difficult to assess. A large proportion of the within-year variation is explained by the age of the pulli (see below). Female mass was corrected for the age of the pulli, and the standardised mass still differed significantly between years, although the means differed by less than 2% (Table 5.25).

Mass (g)	Female		Male	Uncorrected Female Mass	Corrected Female Mass
	Uncorrected	Corrected		F	F
1988	13.288±0.105 (73)	13.790±0.098 (65)	12.106±0.081 (47)	2.603 73,47df P<0.001 ***	2.000 65,47df P<0.01 **
1989	13.071±0.087 (93)	13.636±0.091 (70)	12.172±0.062 (90)	2.079 93,90df P<0.001 ***	1.688 70,90df P<0.01 **
F	1.145 73,93df NS	1.087 65,70df NS	1.094 90,47df NS		
t	4.676 164df P<0.001 **	3.182 133df P<0.01 **	1.094 135df NS		

Table 5.25 Mass of males and females associated with a nest in 1988 and 1989. Females Mass is presented in two forms, the uncorrected, and corrected for the age of the chicks at the time of ringing.

Of the breeding males weighed in 1988, 20 were weighed again in 1989. There was a significant association between the mass in the two years. (r_s=0.7657, n=20, P<0.001). Of these males, the two oldest had a large weight change between 1988 and 1989. One of these was from H24 (coincidentally, he was the mate of an unusually heavy female described in a later section on "The effect of adult biometrics on breeding biology"). Because they seemed unusual these two males were removed from the dataset. For the 18 remaining birds, the rank correlation coefficient was higher (r_s=0.8701, n=18, P<0.001). A linear regression was fitted, which had a r^2 of 79.02% (Table 5.26).

Y= Mass in 1989	Coefficient	s.e.	t	P
Constant	-53.601	22.001	2.436	0.0269
Mass in 1988	1.461	0.181	8.0648	0.0000
R^2=79.02%	n=18			

Anova table for regression					
Source of Variation	SS	df	MS	F	P
Linear Regression	683.48	1	683.48	65.04	0.0000
Deviations from regression	168.14	16	10.51		
Total	851.61	17			

Table 5.26 The mass of males in 1989 related to the mass of the same individual in the previous year.

The mass of the 18 males when they were recaptured in 1989 was significantly greater than in 1988 (Wilcoxon Signed Pairs test, n=18, T_s=15, P<0.005), and the variance was significantly greater (F=2.661, P<0.001).

The relationships among the three characters vary between the sexes, and from year to year. In 1988, all three characters were correlated for both sexes. In 1989, wing length was not correlated with either of the other variables for either sex. This may be because birds were caught earlier with respect to laying date in 1988. The median date of capture for males in 1989 was 4 days later than in 1988. Females were caught 6 days later. These differences are strongly significant (Males: $H=46.3$, $P<0.001$; Females $H=34.3$, $P<0.001$). Besides this, a small proportion of birds were caught much earlier in 1988. As a result the sample of birds for 1988 includes birds at very different stages of the breeding cycle; this may be the cause of the difference in the relationship among biometrics.

The effect of adult biometrics on breeding biology

The mass of a female Pied Flycatcher is most affected by her stage in the breeding cycle. The females were almost all caught and weighed whilst their young were in the nest. The recorded mass of a female is affected by the age of her offspring when she was weighed. The age of the offspring is easily determined from their hatching date. The accuracy to which this is known, differs from year to year. In 1988, the hatching day of most pulli was recorded on nestbox visits; in 1989, nestbox visits were stopped earlier, and hatching was rarely witnessed. However, the hatching date of a brood is directly related to other factors that were measured. It can be estimated with the equation below:

Hatching Date	=	Laying Date	+	Duration of laying period	+	Duration of incubation period

Because Pied Flycatchers lay one egg per day, the duration of the laying period is equal to the clutch size. The duration of the incubation period was measured in 1988, and was 12.2Days±0.157 (n=61). From these, it can be

seen that the hatching date can be estimated using the equation below (rounded to the nearest whole day):

Hatching Date = Laying Date + Clutch Size + 12

The estimated hatching date correlates well with the hatching dates recorded in 1988 (r^2=79.14%, n=67). This accuracy justifies its use for the 1989 data. If the hatching date is known or estimated, it is easy to calculate the age of the pulli when the female was ringed.

The mass of females is negatively related to the age of their offspring. In 1988, females lost approximately 0.2g for each day spent tending the young. In 1989, the rate of weight loss was approximately 0.1g per day, which is about 0.75% of total body mass (Tables 5.27 and 5.28).

Y= Female mass	Coefficient	s.e.	T	P
Constant	137.665	1.408	97.786	0.0000
Age of pulli	-2.009	0.417	4.814	0.0000
R^2=26.28% n=67				

Anova table for regression					
Source of Variation	SS	df	MS	F	P
Linear Regression	1534.42	1	1534.42	23.18	0.00001
Deviations from regression	4303.37	65	66.21		
Total	5837.79	66			

Table 5.27: The change in body mass of females in relation to the number of days spent feeding chicks before ringing (1988).

Y= Female mass	Coefficient	s.e.	t	P
Constant	135.541	1.551	87.352	<0.0001
Age of pulli	-0.908	0.243	3.739	<0.0001
R^2=16.85% n=71				

Anova table for regression					
Source of Variation	SS	df	MS	F	P
Linear Regression	739.98	1	739.98	13.98	0.00038
Deviations from regression	3650.37	69	52.90		
Total	4390.37	70			

Table 5.28: The change in body mass of females in relation to the number of days spent feeding chicks before ringing (1989).

Having corrected mass measurement for pullus age, there was no significant correlation between female mass and clutch size, brood size at hatching, brood size at fledging, ringing date or laying date, in either year. However, in both years there was a non-significant positive relationship between clutch size and female mass (Spearman rank: 1988, n=67, P<0.3; 1989, n=71, P<0.1). In order to examine this further, the masses and clutch sizes were standardized, so that in each year the mean was zero and the variance one. The datasets were pooled, giving a sample size of 138. The Spearman rank correlation coefficient then showed that there was no significant relationship between the variables, but a linear regression detected a positive relationship. This was found to be due to a single female, the bird in H24 in 1989, who was the heaviest female of all, and had a clutch size of 10. When this female was removed from the analysis, the slope was not significantly different from zero.

For 1988, when males caught very early in the season are excluded, there is an almost significant relationship between male mass and ringing day (Kruskall-Wallis test: H=-0.27, n=46, P=0.069). Male mass declined at a rate of 0.056g per day (n=46, P<0.047). In 1989 on the other hand, there is no significant relationship between mass and ringing date (H=0.27, n=91, P=0.40). There are no significant relationships, in either year, between male mass (both uncorrected and corrected for date) and nest initiation date, laying date, clutch size, hatching success or fledging success.

Conclusion

In this chapter I examined a number of factors which I thought might be relevant to the behaviour and reproductive success of Pied Flycatchers in the study area.

Nestbox design, an artificial aspect of the breeding environment, was not found to affect the breeding biology of the birds. There was evidence that old boxes were preferred to new ones.

The environment of the nestbox was studied indirectly, by comparing breeding data for different areas of woodland and by examining associations between the histories of adjacent boxes. Clutch size differed between areas and hatching and fledging failures were found to be more common in some localities. The productivity of a nestbox in 1989 was unrelated to the breeding success in the previous year. Measures of breeding success were lower for birds who bred later. There was no detected effect of male or female tarsus length, wing length or mass on breeding success.

CHAPTER 6

The Heritability of Tarsus Length and its use in the Estimation of EPF Rate

Introduction

In birds, the monogamous mating system is widespread (Lack 1968, Moller 1986). In a typical example of such a system, both the female and her mate can be observed tending the nest and young. In a growing number of species, field workers have reported females copulating with a male other than their mate. If these copulations result in offspring, then the "extra-pair" mating affects the reproductive success of both males, and may also have implications for the reproductive success of the female. It is now possible to use direct genetic techniques in paternity testing, but, before this technological development, estimates of cuckoldry frequency were derived using indirect methods. One of the most widespread of these uses heritability (Alatalo and Lundberg 1984c).

In simple terms, heritability measures the degree of resemblance between relatives. However, it also has a formal meaning. The heritability of a trait is defined as the ratio of additive genetic variance to phenotypic variance (Falconer 1981). In the situation described here, the heritability is estimated by doubling the slope of the regression of offspring on a single parent. Implicit in this is the assumption that the similarity represented by the regression has solely genetic causes.

Falconer partitions both genetic and phenotypic variance into smaller components that can be quantified in the field only by elaborate experiments

involving cross-fostering. In this study, and others like it, assumptions take the place of measurements.

Because pulli cannot be sexed in the hand, their sex is unknown. Thus a mixed sex sample of offspring is regressed on the single sex sample of parents. Heritability from parent to same-sex offspring may differ from the parent/different-sex value, for genetic reasons such as sex-linked or mitochondrial inheritance, or because of non-genetic effects such as egg size or differential investment in the offspring.

Even in well-designed laboratory experiments, Falconer (1981) points out that "heritability cannot easily be estimated with any great precision, and most estimates have rather large standard errors".

Alatalo and Lundberg (1984c) calculated the regression of mean offspring tarsus length on single parent tarsus length for three successive years, in a study population of Pied Flycatchers in Sweden. They used tarsus because it is known to vary little during the lifetime of a bird, and because the heritable component of tarsus length often high (Van Noordwijk 1984). In each year they found that the offspring in the sample were significantly more similar to their mothers than to the male tending the nest.

Since this publication, the technique has been used on other species, (Swallow, Møller 1987a; Great Tit, Norris and Blakey 1989), and has become rather controversial. Lifjeld and Slagsvold (1989b) found that the male/offspring and female/offspring heritability estimates in four samples of Pied Flycatcher families from Norway did not differ significantly. Dhondt (1990) presented evidence on the between-site and between-year variability in heritability values for tarsus length in the Great Tit. Despite considerable differences between male/offspring and female/offspring heritability values in particular years in particular places, he found that, overall, there was no significant difference. He concluded that the between-site and between-year variability in heritability (h^2)

values was great enough to cast doubt upon the value of comparisons as evidence of cuckoldry at a specific site in a particular year.

There have also been theoretical and methodological criticisms of the technique. Lifjeld and Slagsvold (1989b) estimate that, given the size of the standard errors of their regression coefficients, the sample size would have to be increased from 130 to 1600 families for the observed difference between male/ offspring and female/ offspring regressions to be significant. They also present evidence for a non-genetic correlation between father and offspring tarsus length. They found that males with longer tarsi were better able to feed their young. This environmental component would act in addition to the genetic component, increasing the resemblance of males to their offspring, and would cause the heritability method to underestimate the EPO rate. Their discovery is evidence of a general flaw in the heritability method: that sex differences in the environmental component of parent/ offspring heritability can cause differences in h^2 independent of cuckoldry. Gebhardt-Henrich and Nager (1990), working on the Great Tit, describe sources of environmentally-caused similarities between parents (or foster-parents) and offspring that are particularly important in poor seasons. These include the influences of parental feeding abilities and egg weight upon the growth rate of the offspring. They do not state whether either of these is known to be responsible for a greater resemblance between one sex and the offspring.

The purpose of this chapter is to apply the technique to the study population of Pied Flycatchers, to test for differences between male/offspring and female/offspring heritabilities, to relate these to the DNA fingerprinting evidence, and to contribute to the debate on the meaning of heritability differences.

Methods

The tarsus measurement method is described in Chapter 3.

Repeatability of Tarsus Length Measurements

Tarsi reach their adult length before the nestlings leave the nest, and normally remain the same length throughout the adult life (for an exception to this, see Gebhardt-Henrich and Nager 1990).

Using the calculations described in Note 1 (at the end of this chapter), the repeatability of tarsus length measurements was estimated to be 0.781±0.051. This shows that, in a sample of 181 birds who were measured more than once, 78% of the variation in tarsus length was between individuals, rather than between measurements of the same individual.

In addition, between-measurement variation was not found to be systematic; there is no significant difference between first and second measurements of a bird (Sign Test, Z=0.936, P≈0.35). In view of this, it seems reasonable to regard the differences among multiple records of the same bird as measurement error, and to use the mean measurement as a best estimate of the true tarsus length. Because of this, for all subsequent analyses, the parental values used are the mean of one to four independent measurements, taken over the two year study period. Pulli were measured only once prior to fledging.

Year-to-Year Variation in Tarsus Length

Differences between adult tarsus lengths related to sex or year are not significant (n=269, F=0.336, 2df). Neither is there a significant difference between the tarsus length of pulli in each year (n=1083, F=0.328, 1df). There

are no significant differences between the variance of adult or pullus tarsus length within or between years (see Table 6.1).

Year	Parents		Pulli Tarsus
	Sex	Tarsus	
1988	Female	17.176±0.634 (62)	17.384±0.289 (358)
	Male	17.214±0.714 (44)	17.406±0.344 (245)
1989	Female	17.238±0.518 (83)	17.320±0.250 (482)
	Male	17.096±0.582 (80)	17.329±0.250 (466)

Table 6.1: Tarsus length (mm) of males, females and pulli in 1988 and 1989. Mean ± S.E. (n). No significant differences in mean tarsus length between years or sexes.

Assortative Mating

There was no significant relationship between the tarsus measurements of paired males and females (regression coefficient= -0.13±0.09, F=2.28, n=122, NS). These data are shown in Figure 6.1.

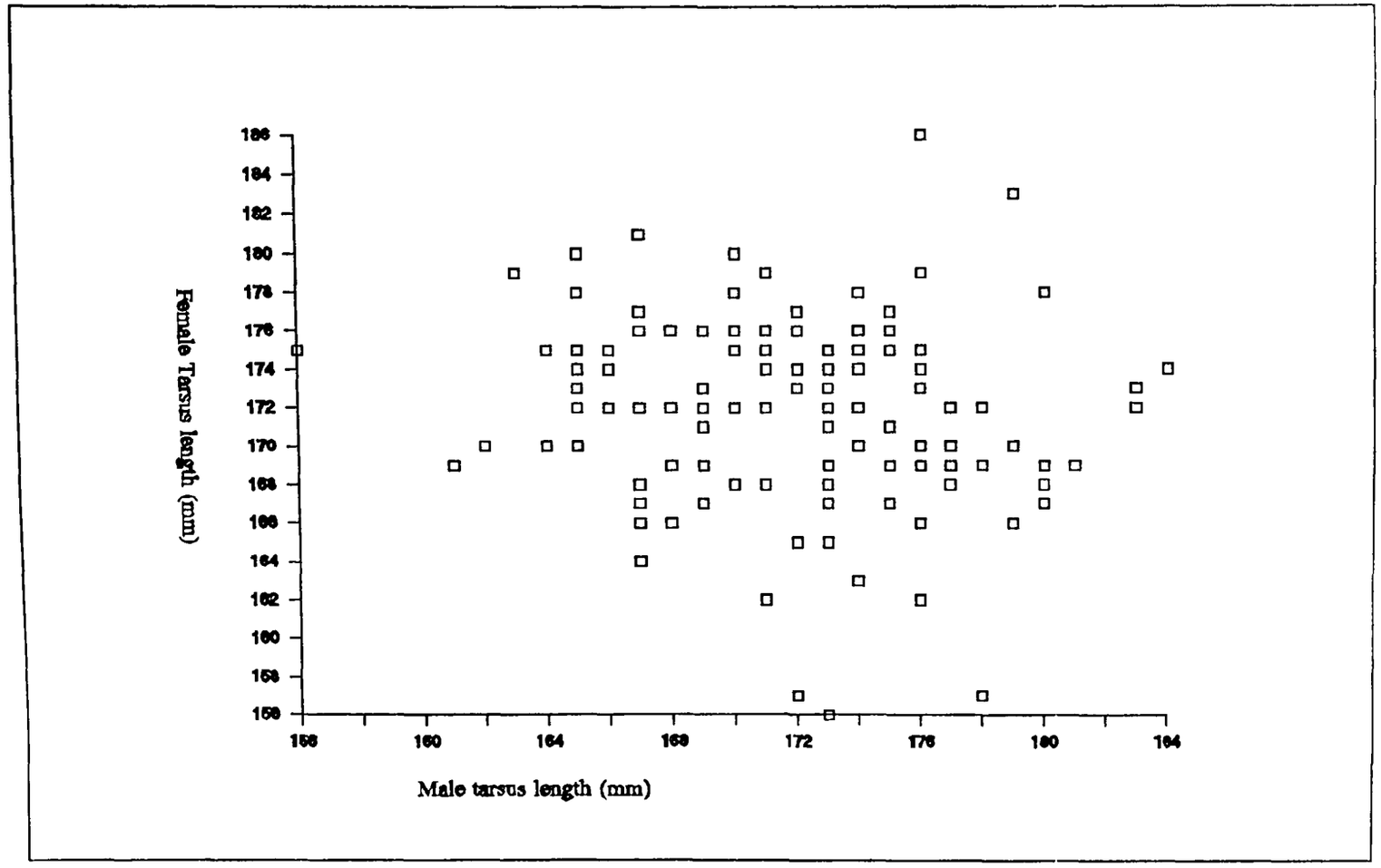


Figure 6.1: No Assortative Mating by tarsus length. Data pooled over 2 years.

Parent Offspring Regressions and Estimated EPF Rate

The female/offspring and male/offspring regression lines were calculated using a similar method to that described by Alatalo and Lundberg (1984c). Offspring within a brood are not regarded as independent points. Dependence of offspring phenotypic value on brood mean is compensated by using a regression with more than one Y-value (offspring tarsus length) to each X-value (parental tarsus length) (Sokal and Rohlf 1981, Box 14.4). In 1988, the slope of neither regression was significantly different from zero, nor was the difference between the two slopes statistically different (Table 6.2). In 1989, however, both slopes were significantly different from zero, but the difference between them was non-significant. Alatalo and Lundberg (1984c) state that the percentage difference between the heritabilities is an estimate of the EPO frequency. The EPO rates were estimated using the equation:

$$EPORate = \frac{100 * (h^2_F - h^2_M)}{h^2_F}$$

Where h^2_F is the female/offspring heritability and h^2_M that of the male.

Year	Female	Male	Difference
1988	0.37±0.20 n_1=62, n_2=358 H_0:ß=0 F=3.32 1,60 df NS (P<0.1)	0.37±0.25 n_1=44, n_2=245 H_0:ß=0 F=2.16 1,42 df NS	2.41% H_0:$ß_{female}$=$ß_{male}$ F_s=0.000 1,102 df NS
1989	0.65±0.18 n_1=83, n_2=482 H_0:ß=0 F=12.35 1,81 df P<0.001 ***	0.57±0.16 n_1=80, n_2=466 H_0:ß=0 F=12.45 1,78 df P<0.001 ***	10.9% H_0:$ß_{female}$=$ß_{male}$ F_s=0.082 1,159 df NS
Difference	H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.997 1,141 df NS	H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.493 1,120 df NS	

Table 6.2: Heritability estimates of tarsus length in 1988 and 1989. Heritability values are equal to twice the regression coefficient. n_1: number of parents, n_2: number of pulli. The null hypotheses (H_0) tested are a) that the slope of the parent/ offspring regression does not differ significantly from zero (H_0:ß=0), and b) that the two regression coefficients do not differ significantly from each other (for example, H_0:$ß_{female}$=$ß_{male}$). Only the slopes of the 1989 regressions differ significantly from 0, and in neither year are the female/offspring and male/offspring regressions significantly different. The estimated EPO rate is shown as a percentage in the "Difference" column.

Differences in Variance

According to Falconer (1981), if the variances of each sex are not equal, the regression on mid-parent cannot, strictly speaking, be used. Lifjeld and Slagsvold (1989b) point out that, in their population, the variance of male tarsus length was greater than the variance of female tarsus length, and that this would automatically lead to a lowering of the slope of the regression line. In my population, the variances of male and female tarsus length were not significantly different (see Table 6.1). Despite this, to remove any effects that might have resulted from inequalities in variance between the groups, I standardised all my measurements, so that within each group (males, females, offspring), within each year, the mean was zero and the variance one. The regression lines were re-calculated and the results are shown in Table 6.3. The slopes of the regression lines calculated from standardised data do not differ significantly from those calculated with non-standardised data and shown in Table 6.2 (Comparing the regressions of standardised and non-standardised data shows no significant differences: F_s for females in 1988=0.002 with 120 df, F_s for males in 1988=0.003 with 84 df, F_s for females in 1989=0.020 with 162 df, F_s for males in 1989=0.001 with 156 df).

Year	Female	Male	Difference
1988	0.34±0.19 H_0:ß=0 F_s=3.33 1,62 df NS	0.32±0.22 H_0:ß=0 F_s=2.16 1,42 df NS	9.37% H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.003 1,102 df NS
1989	0.55±0.16 H_0:ß=0 F_s=12.34 1,83 df P<0.001	0.55±0.16 H_0:ß=0 F_s=12.44 1,78 df P<0.001	-0.11% H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.000 1,159 df NS
Difference	H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.785 1,141 df NS	H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.745 1,120 df NS	

Table 6.3: Heritability of tarsus length in 1988 and 1989, data standardised. Sample sizes as Table 6.2. In 1988 neither slope was significantly different from zero, in 1989 both slopes were significant. In neither year did the slopes of the male/offspring and female/offspring regressions differ significantly.

Standardisation allows the pooling of the two years' data. The heritability estimates are shown in Table 6.4. The slopes do not differ significantly from each other although the male/offspring heritability is fractionally greater than the female/offspring value.

Females	Males	Difference
0.40±0.11 n_1=145, n_2=840 F_s=13.99 1,143 df P<0.001	0.44±0.12 n_1=124, n_2=711 F_s=13.59 1,122 df P<0.001	-10.25% H_0:$\beta_{females}$=β_{males} F_s=0.065 1,265 df NS

Table 6.4: Heritability of tarsus length: 1988 and 1989 data, standardised and pooled.

Sensitivity of the Results

The estimates of extra-pair paternity derived from comparisons of regression slopes are very sensitive to small changes in the slope of the regressions. These in turn, are very sensitive to the removal or addition of certain cases from the data.

The addition of individual broods to the dataset, or their removal, can greatly alter the EPO estimate. The data analysed above omit five broods, all of which contain abnormally small birds ("runts"). Families containing runts may be identified by their high within-brood variance; the runts themselves, by their place at the lower extreme of the distribution of pullus tarsus lengths. The effect of a particular brood upon the regression can be assessed using two statistics, the leverage coefficient (h_i), and the standardised residual (Sokal and Rohlf (1981) pp.539-540). These statistics are shown in Table 6.5.

Brood		Tarsus Lengths of Pulli	Brood			Pullus
			Within-Brood Variance	h_i	standardised residual	T_{pullus} (n)
1988 Females	H24	181,180,177,177,176,174,**143**	175.62	0.0096	-0.2651	5.28
	I15	181,176,176,174,**148**	172.00	0.0037	-0.3433	4.05
1988 Males	G10	178,177,174,171,**118**	657.30	0.0055	-1.0029	16.58
	H24	181,180,177,177,176,174,**143**	175.62	0.0067	0.0420	5.28
1989 Females	G31	167,166,166,164,161,159,**142**	76.57	0.0020	-1.1529	4.86
	G32	169,169,169,166,165,165,164,**151**	35.07	0.0108	0.4021	3.86
	H19	180,176,175,161,158,154,153,**152**	132.84	0.0283	0.7637	3.70
1989 Males	G31	167,166,166,164,161,159,**142**	76.57	0.0027	-1.0775	4.86
	G32	169,169,169,166,165,165,164,**151**	35.07	0.0020	-0.7672	3.86
	H19	180,176,175,161,158,154,153,**152**	132.84	0.0048	-1.0270	3.70
	I22	181,174,174,173,173,**150**	113.37	0.0021	-0.2113	4.05

Table 6.5: Outliers in the regression data sets. All broods detected as outliers in each dataset are presented, with the extreme pullus in bold type.(*Continued overleaf*)

Table 6.5 (*continued*): The I15 brood is classed as an outlier in the female 1988 data set, but not in the male 1988 dataset, because the male from I15 was not identified. Similarly, the G10 female in 1988, and the I22 female in 1989 were not identified so the broods were not included in these analyses. Columns headed "brood" contain statistics for the brood within the dataset for that sex and year. The critical value for the leverage coefficient h_i is (4/n); 1988 females: h_i=4/64=0.0625, 1988 males: h_i=4/46=0.870, 1989 females: h_i=4/86=0.0465, 1989 males: h_i=4/84=0.0476. The critical value for the standardised residual is $t_{0.05[n-2]}$; 1988 females: 1.9980, 1988 males: 2.0168, 1989 females: 1.9913, 1989 males:1.9920. The column headed "Pullus" contains "sample kurtosis" statistic (Barnett & Lewis 1984) for tarsus length of the pullus in the total pullus dataset for the year. The test is applied consecutively, with the most extreme sample being removed until the statistic no longer exceeds a critical value related to the sample size, which in this case is 3.60 (Barnett and Lewis 1984, Table XVb).

In none of the four data sets (female/offspring 1988 and 1989, male/ offspring 1988 and 1989) were there any significant leverage coefficients or standardised residuals (see Table 6.5). However, another statistic, the Sample Kurtosis, (Barnett & Lewis 1984) shows that the within-brood variances of the five broods discussed are significantly discordant, so that, while the rest of the sample come from a distribution with mean μ and variance σ^2, the five broods are more likely to come from distributions with means different to μ, or with variances different to σ^2. The same statistic also shows that the tarsus lengths of the five pulli are significantly discordant. As the table shows, the powers of the three tests differ. Only the sample kurtosis test indicates that the broods and birds are outliers. However, the effect of these birds upon the slope of the regressions, and on the resulting EPO estimates, is considerable. Table 6.6 is the equivalent of Table 6.2, showing the regression results and heritability estimates calculated using datasets that differ only in that they include these 5 additional broods.

Year	Female	Male	Difference
1988	0.32±0.19 n1=64, n_2=370 H_0:ß=0 F=2.76 1,62 df NS	0.43±0.26 n_1=46, n_2=257 H_0:ß=0 F=2.77 1,44 df NS	-30.18% (2.41%) H_0:$ß_{female}$=$ß_{male}$ F_s=0.094 1,106 df NS
1989	0.75±0.19 n_1=86, n_2=505 H_0:ß=0 F=15.39 1,84 df P<0.001 ***	0.53±0.18 n_1=84, n_2=495 H_0:ß=0 F=8.18 1,82 df P<0.01 **	30.2% (-12.2%) H_0:$ß_{female}$=$ß_{male}$ F_s=0.733 1,166 df NS
Difference	H_0:$ß_{1988}$=$ß_{1989}$ F_s=2.367 1,146 df NS	H_0:$ß_{1988}$=$ß_{1989}$ F_s=0.097 1,126 df NS	

Table 6.6: Heritability estimates of tarsus length in 1988 and 1989, using datasets that include the 5 extreme broods referred to in the text. Heritability values are equal to twice the regression coefficient. n_1: number of parents, n_2: number of pulli. The null hypotheses tested are a) that the slope of the parent/ offspring regression does not differ significantly from zero (H_0:ß=0), and b) that the two regression coefficients do not differ significantly from each other (for example, H_0:$ß_{female}$=$ß_{male}$). The slopes of the 1989 regressions differ significantly from 0. In neither year are the female/offspring and male/ offspring regressions significantly different. The EPO rate estimates derived using this dataset are wildly different to those presented in Table 6.2, which appear in brackets in the "Difference" column of this table.

Table 6.6 presents a case where a small number of broods, shown to be statistically aberrant, have a large effect upon the conclusions of the heritability analysis. A robust statistic is one that would be relatively unaffected by similar small changes in the dataset. The extent to which the estimated EPO rate is a robust statistic is difficult to measure, particularly when its underlying distribution is unknown. Some randomisation techniques, such as the bootstrap and the jackknife can be used to set confidence limits on statistics whose underlying distribution is unknown. Here, as the heritability estimate depends on two distributions, related in a complex way, an assumption of the technique is not fulfilled. An informal way to verify the robustness of the EPO rate estimate is to observe the effect of deleting a small proportion of the dataset upon the final estimate. Figures 6.2 and 6.3 show the EPO rate estimates calculated after the deletion of a single brood from a restricted sample from 1988 and 1989. In this restricted sample only broods whose parents are both known were included, and, as a result, the *i*th brood in the female dataset is the same as the *i*th brood in the male dataset.

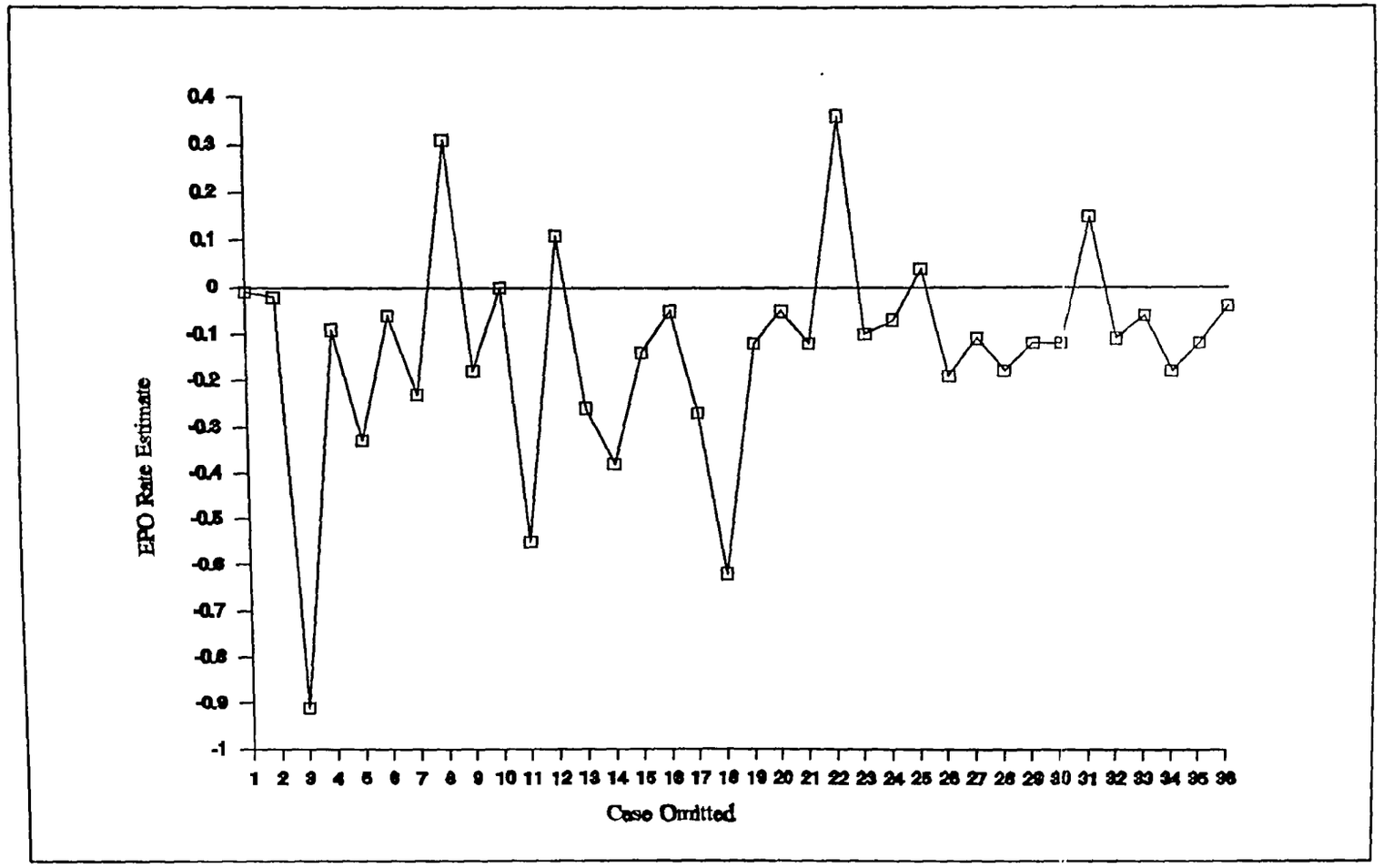
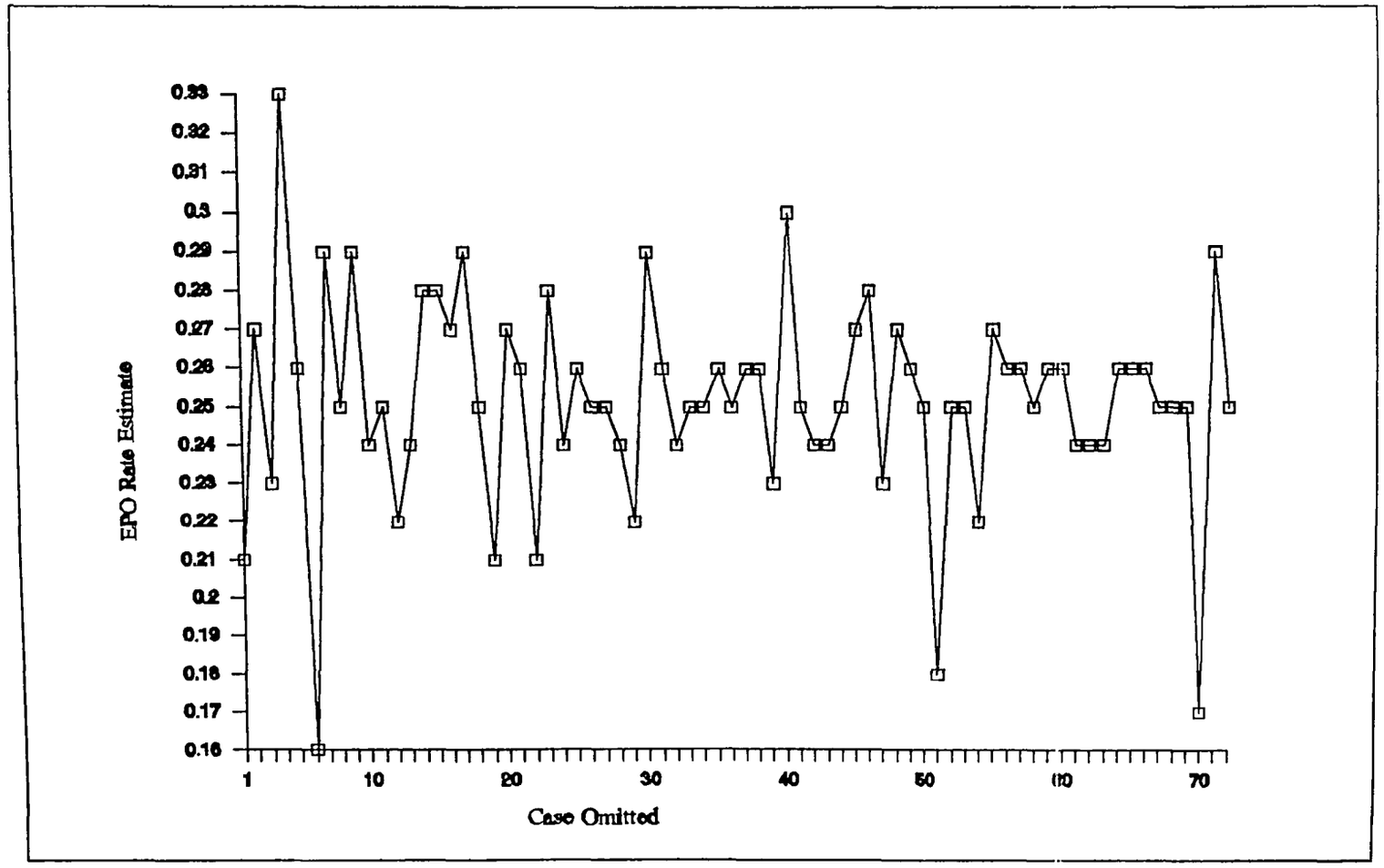


Figure 6.2: For legend see Figure 6.3.



Figures 6.2 & 6.3: The effect of deleting a single brood from the dataset upon the EPO rate estimate. The X axis identifies the brood deleted; thus when x=4, the statistic was calculated using broods 1..3 and 5..n, where n is 36 in 1988 and 72 in 1989. The Y-axis shows the EPO rate estimate. Particularly in 1988, removing a single brood can have a profound effect upon the estimated EPO rate.

In the above case, broods were removed simultaneously from both male and female datasets. In the previous analyses, the broods comprising the two datasets were not identical. In a number of broods (36 in 1988 and 72 in 1989) both parents were known. These broods therefore appear in both datasets. Other broods, whose mother or father was missed, appear in only one dataset. These could have an even greater effect upon the EPO rate estimate.

Resampling Experiments

An assumption inherent in Alatalo and Lundberg's method is that the female parents are correctly assigned to their offspring. In the Pied Flycatcher, this seems a reasonable assumption; there is little evidence to refute it. This assumption makes it feasible to use the female-offspring dataset as a source in which the level of incorrectly-assigned maternity is assumed to be zero. From these birds it is possible to take samples and reassign a given number of females to different offspring. This produces "populations" with a known rate of incorrectly-assigned maternity, for which the parent/offspring regression could be calculated. When Alatalo and Lundberg used this technique they found that the EPO rate was equal to the proportional difference between the male and female regression coefficients; the average proportion of wrongly assigned parents necessary to produce the observed 24% difference in slopes, was 24%. Their method was to replace the female parent in 10 to 50% of the nests with a randomly drawn female from the population. Using a linear regression equation on these data they estimated the proportion of females that should be wrong to reduce the slope to the same level as that for males (Alatalo and Lundberg 1984c).

Thus, for each set of broods they created, they knew the proportion of offspring with incorrectly assigned females. They could also calculate the slope of the female/offspring regression and, by comparing it with the slope of the original line, could calculate an estimated EPO rate in the same way as they

would have done with field data. I performed my resampling, creating "Artificial EPO's" (AEPO's) in a broadly similar manner, with the following exceptions:

1) By choosing to replace the parent in 10 to 50% of the nests, Alatalo and Lundberg presuppose that the real EPO rate is between these values. Without an *a priori* knowledge of the true EPO rate, it seems more reasonable to create the whole range from 0 to 100% artificial EPO's.

2) By re-assigning females to broods, Alatalo and Lundberg are creating a biologically unreal "population". In real populations the results of EPF's are likely to be a proportion of EPO's within a brood containing legitimate offspring. Re-assigning mothers alters the between-brood component of the deviation from the regression line, but does not affect the within-brood component. The result of natural EPF's is an increase in within-brood variance. Again, the slope of the regression line is unaffected by the difference in methodology, but the distribution of the variance is altered. In my resampling procedure, two offspring were randomly selected from different nests, and swapped with each other.

3) I performed many more replicates for each initial AEPO rate, to give a better idea of the spread of possible results that can be achieved with the same starting conditions. These simulations revealed that, the same EPO rate can be estimated from populations having widely different AEPO rates. The range is such that neither variable can accurately be predicted from the other. So the value of the estimated EPO rate as a predictor of the real, underlying AEPO rate is limited.

The deviations from the regression are not uniformly distributed, rather the magnitude of the deviations is related to the proportion of artificial EPO's. When the AEPO rate is very low, the regression coefficients and their significance levels are very like those for the original population but, in

general, slightly lower. When the AEPO rate is high, the range of regression coefficients is wide and a smaller proportion has significant slopes. Thus the effect of low rates of cuckoldry is, as Alatalo and Lundberg state, to decrease the slope of the regression line of the cuckolding sex on offspring. Above a threshold level, however, the slopes decrease to such an extent that the regression no longer explains a significant proportion of the variance in offspring tarsus. Figures 6.4 and 6.5 show the effect of increasing AEPO rates upon the proportion of simulations resulting in a significant regression line. In 1988 the original female/offspring regression was not significantly different from zero so, of course, neither are the regressions for populations with AEPO's. In 1989, the original regression was highly significant ($P<0.001$). At an AEPO rate of 40%, the regressions were non-significant in 5% of the simulated populations. Above 70% AEPO's, less than 50% of the regressions were significant. It can therefore be said that, using the 1989 data, if the EPO rate exceeds 70%, its effect is more likely to cause a non-significant regression than to cause a slight, but significant one. The difference in the two curves from 1988 to 1989 is due, in the main, to the greater heritability level in 1989. The heritability of a trait is a property not just of the trait, but of the particular environmental circumstances affecting the population. The greater the heritability, the higher the threshold above which regression lines tend to be non-significant.

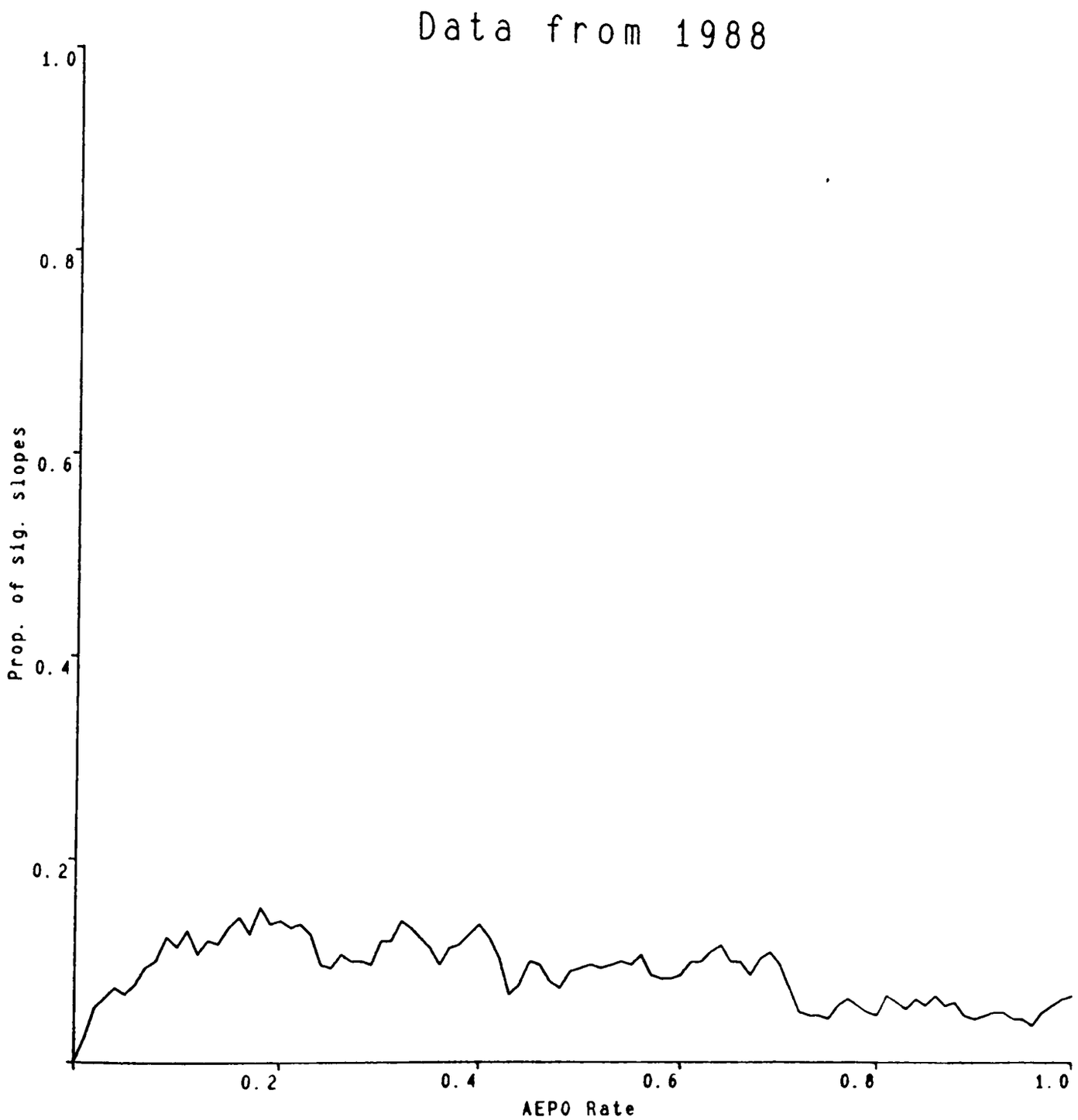


Figure 6.4: Results of a resampling experiment in which EPOs were artificially created using data from 1988 and the slope of the parent/offspring regression was calculated. The proportion of significant slopes was low and relatively unaffected by the frequency of artificial EPOs.

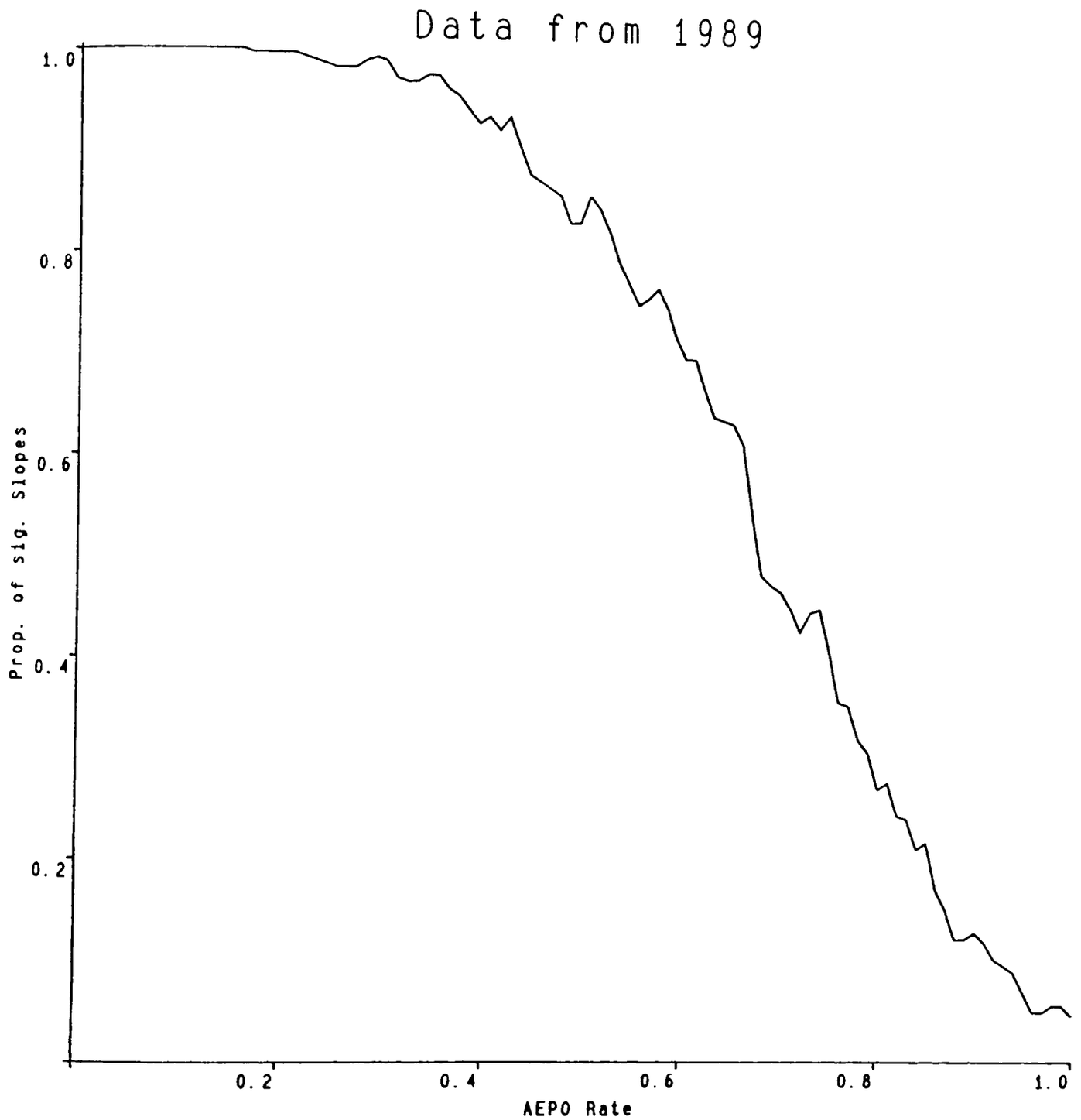


Figure 6.5: Results of a resampling experiment in which EPOs were artificially created using data from 1989 and the slope of the parent/offspring regression was calculated. The proportion of significant slopes is high and declines with increasing AEPO rate.

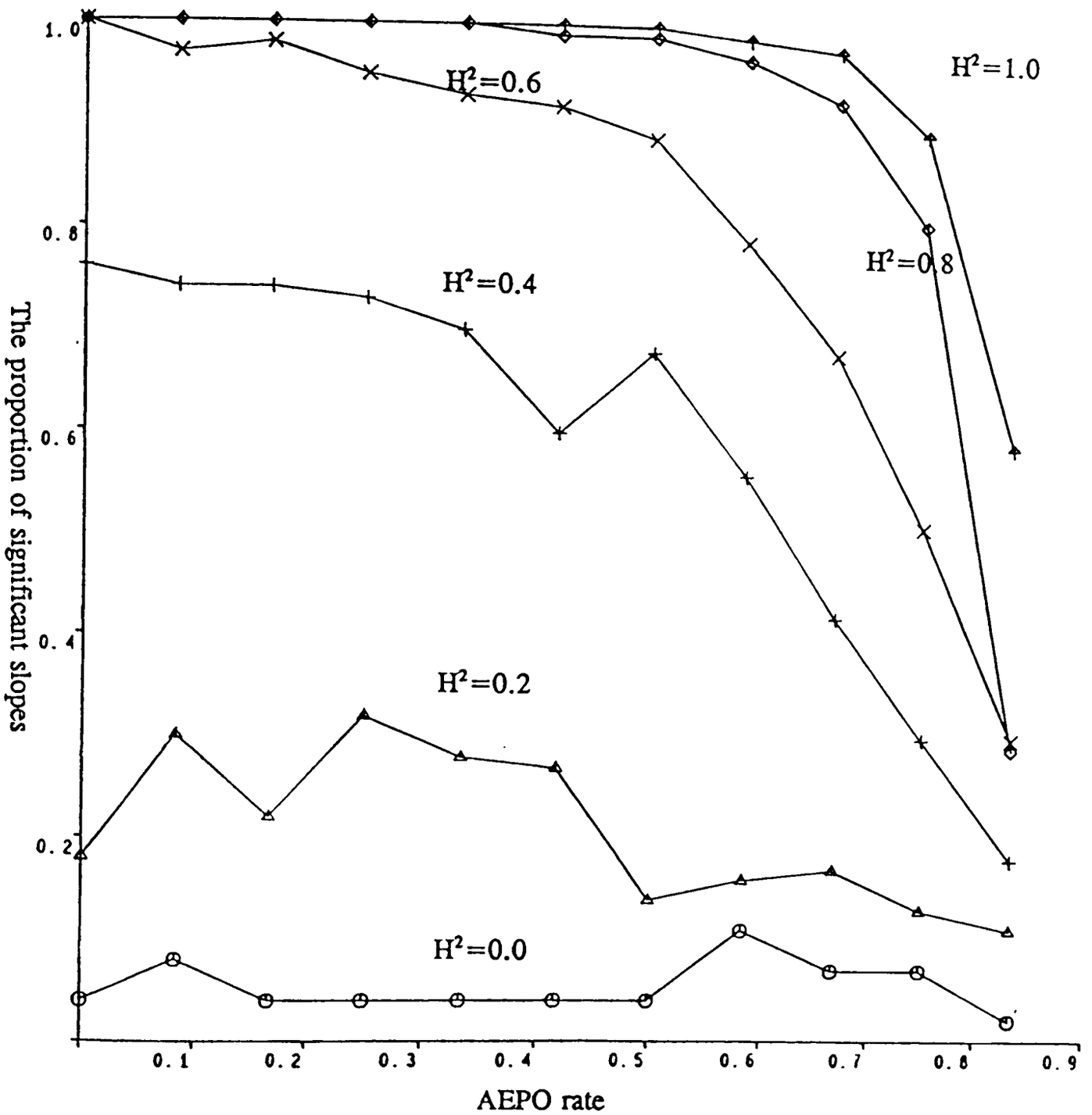


Figure 6.6: Results of a resampling experiment in which the tarsus lengths of 'parents' and 'offspring' were determined by sampling from known distributions with pre-set levels of heritability. Varying proportions of extra-pair offspring were created. The slope of the parent/offspring regression was calculated. When heritability is high, the influence of EPOs on the parent/offspring regression in large; when it is low their effect is slight.

Figure 6.6 shows the results of a simulation in which all broods were totally artificial. The females were generated randomly from a normal distribution, and the offspring, in broods of 5, were generated using the equation below, where o_{ij} is the jth offspring in brood i:

$$o_{ij} = a + bf_i + \varepsilon_i + \varepsilon_{ij}$$

Where ε_i represents the error due to variance between broods and ε_{ij} represents the error due to variance between individuals. Once created, the offspring can be reassigned in a similar manner to that described for the resampling experiments. By manipulating the heritability and the EPO rate, the relationship between these and the significance of the parent/offspring regression line can be shown. The effect of increased heritability is to increase the threshold below which the regressions are mostly significant and above which there is generally no relationship between the "parent" and offspring tarsi.

The regression lines generated in my resampling experiment are similar in slope to those shown by Alatalo and Lundberg (1984c). However, I stress that the errors on my slopes (and presumably those calculated in the same way for other populations) are so large that their value in predicting the EPO rate is limited.

Neighbours

In the manner of Alatalo and Lundberg (1984c) I also calculated the regressions of pullus tarsus on the tarsus of the nearest known male. Alatalo and Lundberg find that their neighbour/offspring regression has a non-significant positive slope, and they reason that this results from the proportion

of offspring whose genetic fathers are really the neighbour. I found that in neither year was the regression significantly different from zero. Furthermore, in 1989, the slope of the regression on neighbouring males was negative. The slopes of neighbouring female on offspring were not significantly different from zero or from the slope of the male neighbours. There was no evidence for even a non-significant trend towards more resemblance between neighbouring males and offspring than between neighbouring females and offspring (See Table 6.7).

	Neighbouring Males	Neighbouring Females
1988	0.031±0.10 n=62 H_0:ß=0 F_s=0.09 1,60 df NS	0.010±0.12 n=44 H_0:ß=0 F_s=0.10 1,42 df NS
1989	0.096±0.11 n=83 H_0:ß=0 F_s=0.80 1,81 df NS	-0.179±0.10 n=80 H_0:ß=0 F_s=3.32 1,78 df NS (P<0.1)

Table 6.7: Regression of offspring tarsus on neighbouring adult tarsus, 1988 and 1989. Neighbouring males are no more similar to the offspring than neighbouring females.

The Relation of these Data to those from Other Studies

Dhondt (1991) shows that over a number of years, across a number of study plots, the observed range in heritability values is so great that there is no significant overall difference between the slopes of male/offspring and female/offspring regressions. The heritability of tarsus length in the Pied Flycatcher has been measured by several workers, at a range of sites. Table 6.8 shows some published heritability estimates. When these are pooled using the same statistics as Dhondt (1991), the difference between male and female heritability values is not significantly different from zero (t=2.10, 9 df, P≈0.1). It seems that in this species, too, there is little or no overall difference between male/offspring and female/offspring heritabilities, despite the number of published papers having found one.

Parent/Offspring Heritability		Difference	Source
Female	Male		
0.53	0.36	0.17	Alatalo and Lundberg (1984c) 1980 study
0.57	0.42	0.15	Alatalo and Lundberg (1984c) 1981 study
0.63	0.55	0.08	Alatalo and Lundberg (1984c) 1982 study
0.56	0.32	0.24	Lifjeld and Slagsvold (1989b) Trondheim 1983
0.67	0.47	0.20	Lifjeld and Slagsvold (1989b) Trondheim 1984
0.48	0.57	-0.09	Lifjeld and Slagsvold (1989b) Oslo 1983
0.64	0.74	-0.10	Lifjeld and Slagsvold (1989b) Oslo 1984
0.37	0.37	0.00	This Study, 1988
0.65	0.57	0.08	This Study, 1989

Table 6.8: Heritability estimates for tarsus length of the Pied Flycatcher in this and published studies.

Conclusions

I find that, in the current study, there is no significant difference between the male/offspring and female/offspring heritability estimates in either year.

I find evidence from simulation studies that the heritability method is rarely able to prove a relationship between the differences in regression coefficients and the EPO rate in the population.

Within- and between-brood variation account for a large proportion of the total variance in the tarsus length of Pied Flycatcher pulli. Differences in provisioning rates account for a proportion of this variance, as do differences in nestbox site, breeding density and an unknown number of other factors. If some of this variance can be accounted for, then the real relationship between EPO's (as detected using DNA fingerprinting methods) and the phenotypic resemblance of males, females and offspring may be more easily understood.

The heritability estimate is compared with that obtained by DNA fingerprinting in Chapters 7 and 8.

Note 1: Repeatability Calculations

The repeatability of tarsus length was calculated using the methods of Falconer (1981) and Lessells and Boag (1987), in which:

$$r = \frac{S^2_A}{S^2_W + S^2_A}$$

Where S^2_W is the within-groups variance component, MS_W from the analysis of variance table (Table 6.9); S^2_W=4.788. S^2_A is a measure of the among-groups variance, corrected for differences in group size. S^2_A is calculated as:

$$S^2_A = \frac{MS_A - MS_W}{N_0}$$

Where MS_A is the among-groups variance component from the analysis of variance table and N_0 is defined as:

$$n_0 = \frac{\sum_{i=1}^{a} n_i - \frac{\sum_{i=1}^{a} n_i^2}{\sum_{i=1}^{a} n_i}}{a-1}$$

The total degrees of freedom in the analysis of variance table is 180.

Therefore:

$$\sum_{i=1}^{a} n_i = 181$$

A separate calculation determined that:

$$\sum_{i=1}^{a} n_i^2 = 405$$

The analysis of variance table shows the among-individuals degrees of freedom (a-1) to be 83, therefore N_0=2.15 and the among-groups variance component:

$$S^2_A = 17.098$$

Thus, the repeatability, r= 0.781. The standard error for this value was calculated by the jack-knife method, s_r= 0.051.

Analysis of Variance Table					
Source of Variation	SS	df	MS	F	P
Among Individuals	3453.888	83	41.613	8.69	<0.001
Within Individuals	469.250	98	4.788		
Total	3923.138	180			

Table 6.9: Analysis of Variance Table for repeatability of tarsus length measurements. r=0.781±0.051.

CHAPTER 7

Polygyny, Polyterritoriality and Paternity

Introduction

In the first part of this chapter I analyse the fieldwork data with respect to the occurrence of polygyny and polyterritoriality. To keep the behavioural results together, I then describe the results of the paternity tests.

Polygyny and Polyterritoriality

When a colour-ringed male is observed at two different nestboxes, it may be polyterritorial. Similarly, if a male is caught in two different boxes during the breeding season, then this is strong evidence that he is guarding both as his own. However sometimes this can happen when the male would not be described as polyterritorial. Males can be caught in the nests of others, which they occasionally visit out of curiosity, or during territorial disputes (pers. obs.) After a failed nesting attempt, a male may occupy another box. Thus, polyterritoriality should be defined as the simultaneous defence and maintenance of two nestboxes; neither box must be abandoned or used by another male.

Having such criteria to meet, proving polyterritoriality is not easy. With up to 120 boxes being used by breeding Pied Flycatchers, and a single worker in the field, the time that could be spent watching each box was limited. In the spring of each year, most males were not colour-ringed, so the chances of detection were further reduced. Two more problems are described in the literature: firstly, males spend less time in their second territory and so are less likely to

be seen or caught (Alatalo *et al.* 1982a), and secondly, polyterritorial males may have nests outside the study area or in natural holes (Alatalo *et al.* 1988). Bearing this in mind, it would be imprudent to claim that all polyterritorial males were found. However, my methods are vindicated by the detection of several males using more than one nest, some of which were polyterritorial. A number of these are described below.

Examples of Males Caught in Two Different Boxes

G4-G22 male 1988

This might be a case of polyterritoriality, and possible polygyny, but seems better interpreted as renesting after predation. The map in Figure 7.1 shows the area in which this bird was found.

I visited G01 on the morning of 4th May 1988, with the intention of trapping the occupants. I opened the box and found a BTO-ringed male inside. I placed him in a bag, and later colour-ringed him as "RKAS" (**R**ed,Blac**k**,**A**luminium,**S**tripe). Close by, another male was calling. I surmised that I may have interrupted a territorial dispute, and put on a trap while I ringed the bird. The caller had been caught when I returned, and I ringed him as ASRW (**A**luminium,**S**tripe,**R**ed,**W**hite). Two days later he was seen approximately 50 metres away at G04, a box in which a nest had appeared on the 3rd May. He was seen again here on the 25th May, by which time the female was incubating 6 eggs. The box was predated, probably by a rat or a weasel, on or about the 7th June. The adults were not seen here again.

Meanwhile, at G22, 160 metres across a field, a nest cup had appeared on 3rd May. No further activity of any sort was seen here until 7th June, when the G04 male, ASRW was seen by the nest. Three days later there were 2 eggs in the nest. These were the last eggs to hatch of the entire population, the young

were stunted and had a black encrustation on their bills (described in Campbell 1986 and Stowe 1984, p163). They died before fledging.

This male may have held both territories simultaneously. However, unless he built both nests at the same time, it seems more likely that he took over a complete nest sometime between 3rd May and 7th June. As the female was not caught in either nest, it is impossible to say whether he had changed partner. Lifjeld and Slagsvold (1989) found that after nest failure some birds divorced, others did not, some moved and others remained in the same box. Those who moved usually took up distant boxes. It is therefore possible that the male was polyterritorial and polygynous, but it is more likely that he was neither. Whatever the case in this year, ASRW is/was an extraordinary bird, being one of one of very few genuinely polyterritorial males the following year.

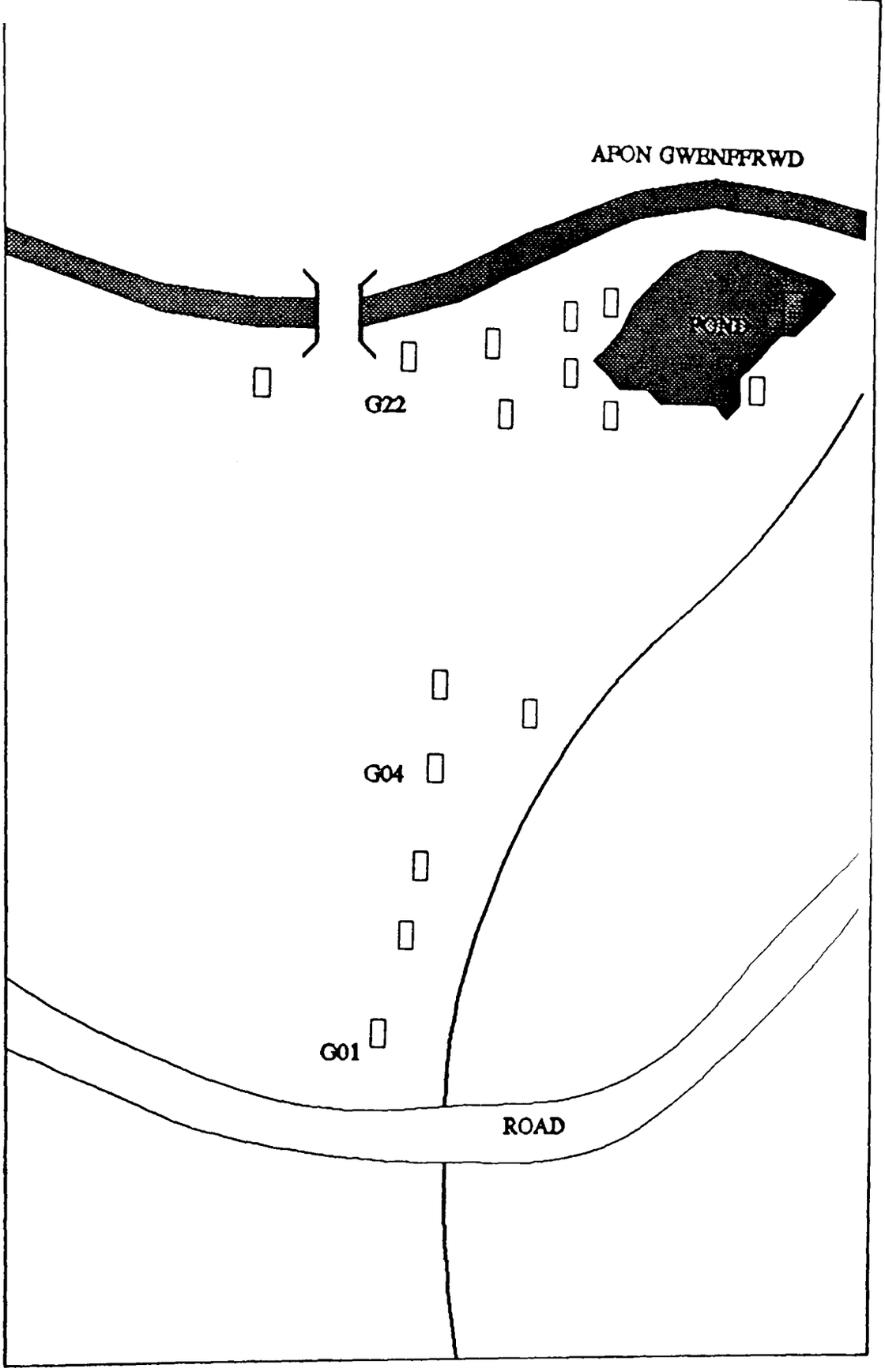


Figure 7.1: The positions of G01, G04 and G22. The distance from G01 to G04 is approximately 60m and from G04 to G22, 160m.

G10-G23 male 1988

This male, also used two nestboxes. He was certainly polyterritorial, but he was not polygynous. He was caught in G10 on 28th April, and again in G23 on the 9th May. On 16th, the female at G10 laid the first of 6 eggs, which produced 5 fledglings. No female was ever seen at G23, and no eggs were laid. It seems that this male was polyterritorial, but failed to attract a female to the secondary box.

I31-G18 male 1988

Despite being caught in two boxes, it is most probable that this male was not polyterritorial. On 6th May 1988, during a territorial dispute at I31, three birds were caught. A male and a female were trapped together, and while they were being processed, a BTO-ringed male was caught. He was colour-ringed as BOAS (Blue, Orange, Aluminium, Stripe). He was seen on 25th May at G18, 150 metres away, by the river. This nest had been initiated in late April by a bird carrying a BTO ring on his left leg, a description that would have fitted BOAS before he was colour-ringed. BOAS was seen on every subsequent visit to this site, and proved the most boldly defensive of all the males when I checked the box.

It is unlikely that BOAS ever held a territory at I31. It is more probable that he just happened to be in the area, having a dispute with the resident male at the time I was trapping.

G12-G13 Male 1989

This is a complex case; one male seems to have fathered offspring in two adjacent two nestboxes, displacing another male.

By chance, two birds with very similar colour-ring combinations nested near to each other. One was ARWS, a new bird, ringed on 28th April 1989, who I shall call the "New" male. The other was ASRW, the male that used G04 and G22 in the previous year (the "Old" male). Table 7.1 shows the histories of boxes, G12 and G13, which are less than 10m apart. Figure 7.2 shows the arrangement of the nestboxes.

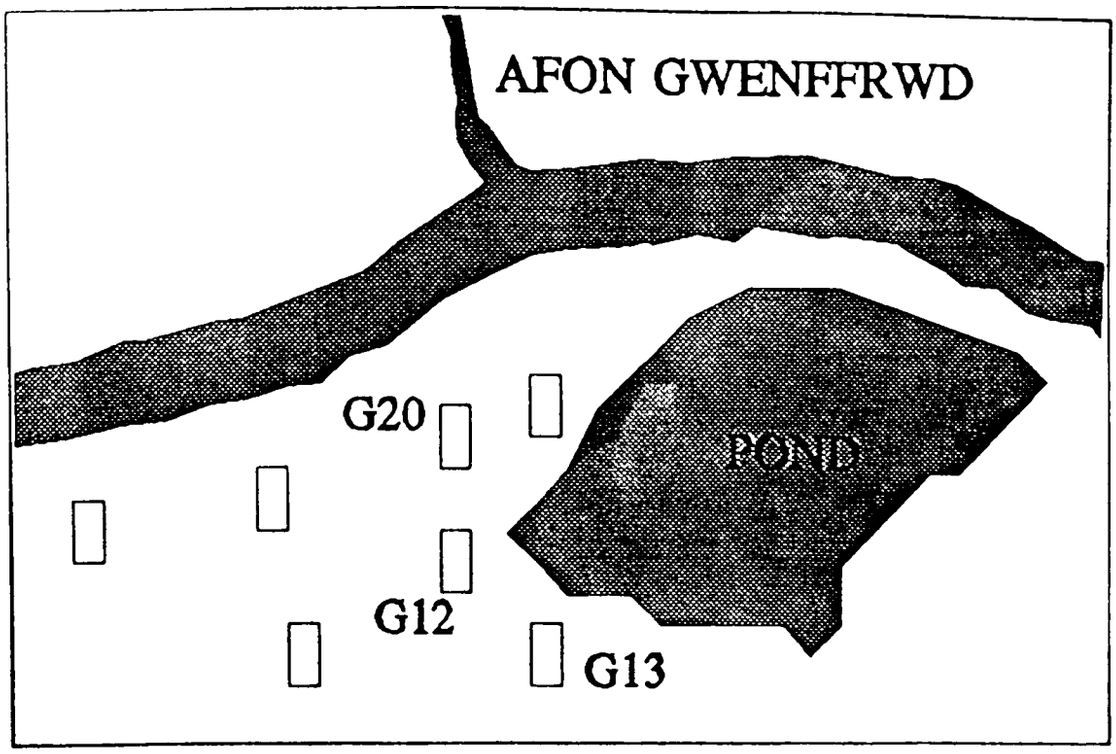


Figure 7.2: The positions of boxes G12, G13 and G22. The boxes are approximately 5m apart.

Date	G12	G13
28 April	"New" male caught	
29 April	"New" male seen	
1st May		First egg laid
2nd May		A red and White male seen, who could have been either
9th May	Nest Initiation(?)	"Old" male seen
16th May	First egg laid	"Old" male seen, Female incubating
19th May	Female incubating	"Old" male seen, Female incubating
23rd May	"Old" male seen, female incubating	
29th May		"Old" male caught, along with G13 female
7th June	"Old" male caught, along with G12 female	Pulli ringed
17th June	Pulli ringed	

Table 7.1: Histories of two nests, G12 and G13 (1989), which are thought to be the two nests of a polygynous male.

It seems very likely that the old male held both territories, and that if the new one held G13 at all, he was usurped quite early in the season, moving to G20 where he bred.

I10-I15 male 1988

This seems one of the most definite cases of polyterritorial polygyny. A bird ringed in 1988 at I18, was handled in 1989 at two boxes, I10 and I15. These are 40 metres apart, on either side of the Afon Gwenffrwd. He was caught

twice in one box and once in the other, and seen at both (see Table 7.2 and Figure 7.3).

Date	I15	I10
30 April	Male Caught	
1st May	Male seen	
8th May		Nest initiation
10th May		First egg laid
18th May	Nest initiation (?) Male seen, but not positively identified.	Female incubating
21st May	First egg laid	
22nd May		Male seen
25th May	Female incubating	Female incubating
3rd June		Male and female caught
11th June	Female caught	Pulli Ringed
12th June	Male caught	
17th June	Pulli ringed	

Table 7.2: Histories of two nests, I15 and I10 in 1989, which are thought to be the two nests of a polygynous male.

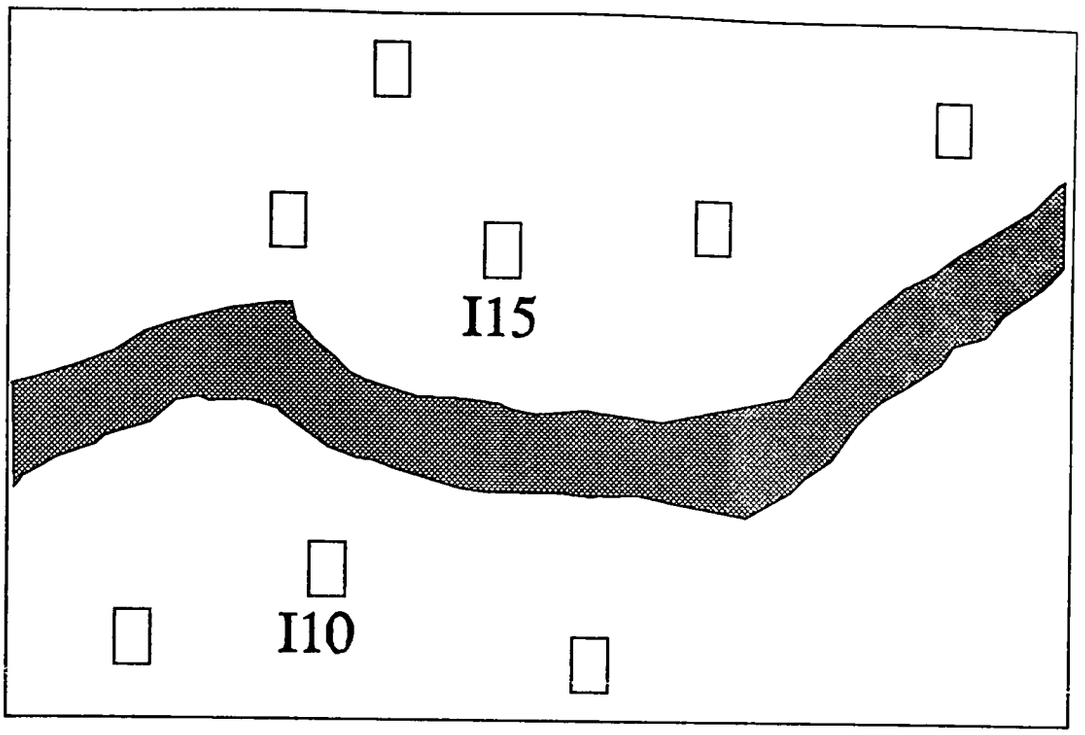


Figure 7.3: Map showing the positions of I10 and I15. The boxes are approximately 40m apart.

K21-K23 male 1988

This, too, seems a probable case of polyterritoriality, and polygyny. A BTO ringed male was caught for the first time in this study at K21 on 13th June. The next day he was caught 20 metres away, across a gulley, at K23 (Table 7.3 and Figure 7.4).

Date	K21	K23
5th May	Nest initiation BTO-ringed male seen	Nest initiation
16th May	Female incubating BTO-ringed male seen	
24th May	Female incubating	BTO-ringed male seen
10th May		First egg laid
13th June	Male and female caught	Pulli ringed
14th June		Male and Female caught
18th June	Pulli ringed	

Table 7.3: Histories of two nests, K21 and K23 (1989), which are thought to be the two nests of a polygynous male.

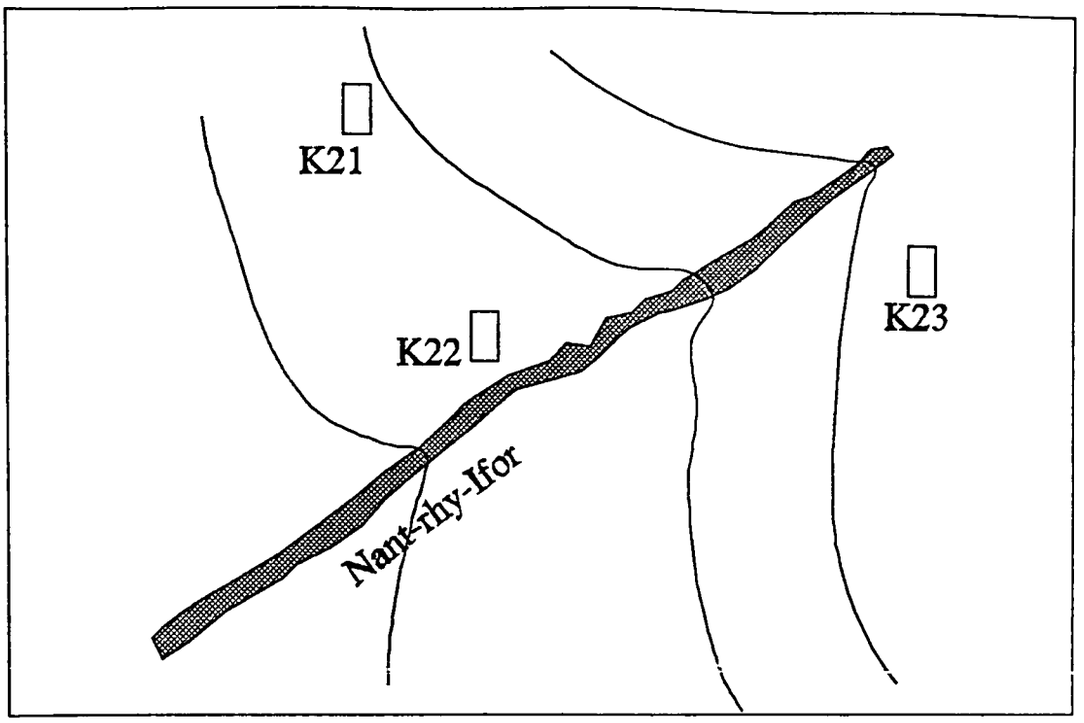


Figure 7.4: Map showing the positions of boxes K21 and K23. Fine lines represent contours, with the ground shelving steeply down from top right. The boxes are approximately 10m apart.

Of the 10 birds that were caught in more than one box, it seems that 3 were polygynous, 2 were polyterritorial but had no secondary mate and 5 just seem to have moved. All three cases of polygyny occurred in 1989 when 115 nests were recorded. The frequency of polygyny in this year was therefore 2.7%. When the results of both years are pooled, only 1.3% of males were known to be polygynous (n=240).

Figure 7.5 shows the distances between the boxes of the 10 males described above. Males who have moved seem to travel the furthest between their first and second boxes. If they are responding to nest predation or to handling, then there may be advantages to moving a large distance, well away from the threat. Predation was rare in this study, but twice when it occurred, several neighbouring boxes were destroyed in succession.

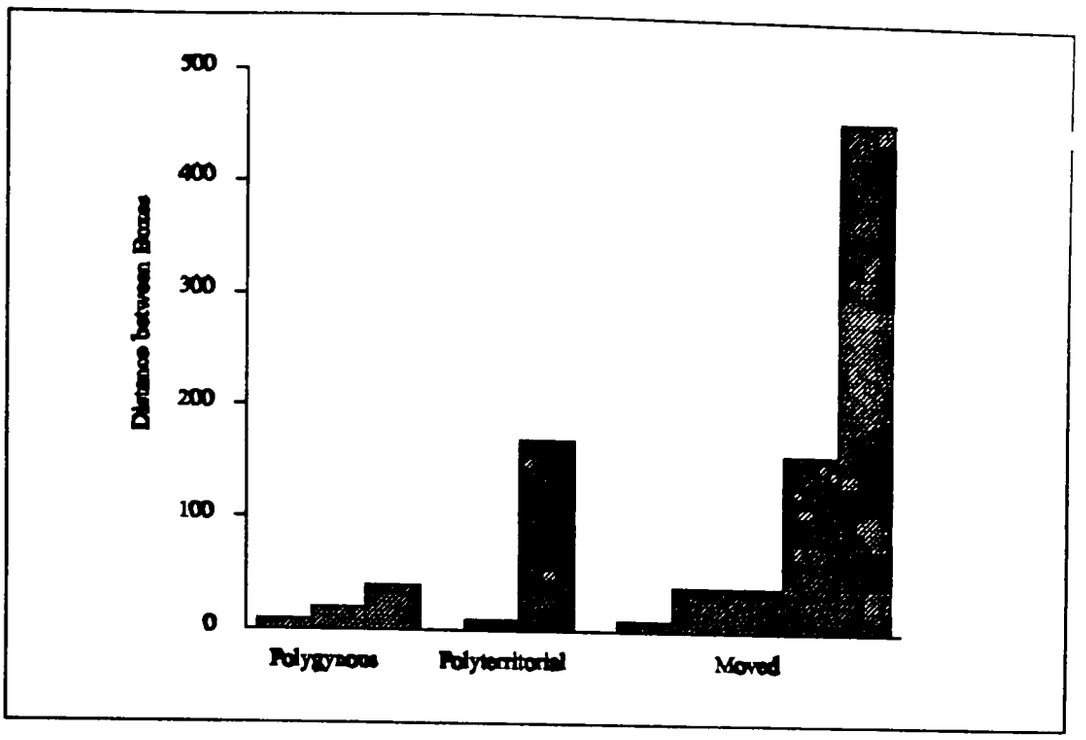


Figure 7.5: The distance (in metres) between boxes in which the same males was trapped.

In the 5 cases where males were polyterritorial, the distance between the boxes was much less than that found in other studies. It also seems that polygynous males might have closer nestboxes than those who were merely polyterritorial. Of all 5 cases, the smallest distance was less that 10m, and the largest 160. The mean distance was 56m. For the 3 males who were polygynous, the mean distance between nests was about 25m. Never was there another defended territory directly between. Von Haartman (1956) reports the distance between the territories of 158 males, which have a mean of about 400-500m. Of these, 23 were held by polygynous birds, with territories, on average 200-300m apart. Slagsvold and Lifjeld (1986) found that distances between primary and secondary nests ranged from 39 to 250m (n=13). In 1988, Stenmark *et al.* report distances ranging from 61 to 573 metres, with a mean of 193m (n=13). They describe an area of "good habitat" in which the situation seems very like this population; few males held more than one nestbox and those that did so mostly held an adjacent one. In woods like these, polygynous males might be

more accurately described as monoterritorial, because their two boxes are close together and probably can be defended as a unit.

The costs and benefits of polygyny have been measured in several different ways in Pied Flycatcher populations (see Chapter 2). In two Scandinavian studies, the fledging success of secondary females in proportion to that of monogamous and primary females was 65% (Alatalo *et al.* 1981, Alatalo and Lundberg 1984b) and 84% (Stenmark *et al.* 1988). At secondary nests, males had a lower investment in both incubation feeding and providing for the nestlings. In this study, the rarity of polygyny make it difficult to test its effects on male and female reproductive success with formal statistics. However, in the small sample, it can be said that the effect on fledging success is not disastrous, and that upon the fitness of the male is great. The polygynous males were the three most productive fathers in 1989.

In 5 of the 10 cases where males were caught in more than one box, the simplest explanation for the observations is that the male had moved boxes (see Table 7.4). As the table shows, these were all birds that were caught very early in the season. At this stage in the nesting cycle, birds have invested little in a nestbox, and may be more prone to moving. Indeed, trapping and handling may contribute to this.

Ring Number	Moved	Date	Notes
E001200	I30 to I31	28th April 1988 1st male handled in 1988	Displaced E004039, who moved to G18
E950005	H25 to H24	3rd May 1988 6th male handled in 1988	
E004039	I31 to G18	6th May 1988 10th male handled in 1988	Moved to G18 (see text)
F014001	G12 to G20	28th April 1989 1st Male handled in 1989	This is the "New" male described in the text
F014002	G18 to I24	30th April 1989 2nd Male handled in 1989	

Table 7.4 Males who moved from a nestbox after being ringed and were found breeding elsewhere.

What if Polygynous Males Were Not Detected?

The factors described at the beginning of this chapter make it quite likely that some polyterritorial and/or polygynous males were not detected. In several cases, no known male could be associated with a nest. In these cases, either the male trapped at the box had moved or none was caught, and no males ringed elsewhere were seen to be resident. It is feasible that the boxes where this happened may be the secondary nest of a polyterritorial male. On the other hand, there are some alternative reasons (including my inefficiency at trapping). As the effects of polygyny in other populations are well documented, these can be used to test whether the boxes where the male was not caught resemble secondary nests (Alatalo *et al.* 1982a). Males invest less in their secondary nest causing a substantial drop in the reproductive success

of the female. I assume that the number of times I saw a male when I visited his box reflects the amount of time he spends there, and is an indicator of his investment. If the probability of sighting a male is lower at boxes where the male was never identified, this suggests that these males spend less time at the nest and is circumstantial evidence that they are polyterritorial. When the frequency of sightings at "known male" and "unknown male" boxes are compared, the differences are significant in both years, but in different directions (Table 7.5). In 1988, the number of "known male" boxes was much lower, and sightings of these birds rarer. I think the reason for this lies with my inefficiency at trapping, rather than the elusiveness of the birds (Table 7.5). However, in 1989, I think that when I failed to identify the resident male it was in spite of considerable effort. At these boxes, males were also seen less often near the nest. Thus, there is some evidence to support the hypothesis that boxes where the male was unknown included secondary boxes of polygynous males.

Year	Class of box	Number of Boxes	Number of visits when		Probability of sighting
			A male was seen	No male was seen	
1988	Male Unknown	62	223	820	27.2%
	Male Known	68	200	955	20.9%
1989	Male Unknown	26	36	142	25.4%
	Male Known	94	172	464	37.1%

Table 7.5: The number of visits at which a male was sighted, or not sighted, for two classes of nest in 1988 and 1989. The "Male Unknown" nests are those at which the resident male was never positively identified. At the "Male known" nests, I was reasonably certain of the identity of the resident male. In both years, the number of sightings per visit differs significantly between the two classes. 1988: G_{adj}=5.81 with 1 df; 1988: G_{adj}=3.51 with 1 df.

Having shown that, at least in 1989, the males that were never identified spent less time at the box, the next question to ask is whether the breeding success of the females at these boxes reduced as a consequence. If these were secondary nests, then according to the findings of Alatalo *et al.* (1981), the female would be expected to lay fewer eggs than primary or monogamous individuals. The mean clutch size in nests with known males, was 6.52±0.11 (n=92), in the 23 boxes with no known male it was 6.00±0.32. There is no significant difference between the groups (Kruskall-Wallis H=0.94, P<0.35). When clutch size is corrected for laying date, the difference is further from significance (H=0.09, P<0.75). Neither is there a significant difference in hatching success (H=1.37, P<0.24). Beyond the hatching stage, comparisons of breeding statistics between the "Known Male" and "Unknown Male" groups would be misleading. If hatchlings die young, then there is very little opportunity for trapping the male, and as a result, in nests where no young fledge the male will be more likely to be unknown.

Polygynous males, at their secondary nest, provide less food for the incubating female and her growing offspring. As a result, the female and the pulli are lighter (Lifjeld and Slagsvold 1986). The difference between the masses of females in the two groups is not significant, especially when corrected for the age of the pulli ($H=2.93$, $P<0.09$, Corrected: $H=0.06$, $P<0.80$). Similarly, offspring mass does not differ significantly ($F=1.61$, 1,620 df, $P<0.20$).

The probability of catching the female at a nest is affected by the status of the male (Table 7.6). If the male is unknown, then the female is more likely to be unknown too. This does not support the hypothesis that males who were not identified were polyterritorial, because, if anything, the female in this case would spend more time at the nest and be more likely to get caught.

		Status of Male	
		Known	Unknown
Status of Female	Known	84	15
	Unknown	8	13

Table 7.6 Distribution of nests where both, one or neither parent was known. Pairs where both were known and pairs where neither are known are overrepresented. If the male is unknown, then it is more likely that the female will be unknown, too. G_{adj}=17.59, 1 df.

The simplest explanation for the results described above, is that some males are less likely to be seen. They and their partner are also less likely to be caught. They may be more timid or I may have unintentionally paid less attention to these nests both during visits and when trapping. There is no evidence to support the hypothesis that males that were not seen or caught might have been polyterritorial.

Might Polyterritorial Males have Nests Not Included in the Study?

As distances between territories of over 0.5km have been quoted by the Scandinavians, it is quite possible that males nesting in the study area (the G,H,I,J and K series) might have secondary nests in another nearby wood. Within 0.5km of the boundary of the study area are approximately 150 nestboxes and abutting the H-series is a wood with no boxes that may provide natural nest sites. Never was a male that was breeding in my area caught elsewhere. However, the trapping effort in the other areas of the reserve was much lower than that in mine, so this possibility cannot be ruled out. It seems peculiar, however, that if there were several males with two distant territories, not one of them chose to site both nests within my 1.5km long study area; all the polyterritorial males I found defended sites less than 200m apart.

The availability of natural nest holes is difficult to assess. During the study two natural nest holes were known. It is possible that they are more common, and that some polyterritorial birds used them. High nesting sites are attractive (Alatalo *et al.* 1982a, Alatalo *et al.* 1986a), for they are thought to be less prone to predation. It is possible to imagine reasons why the rate of polyterritoriality might differ between artificial boxes and natural nests in the same wood.

Paternity Testing and Extra-Pair Offspring

Figure 7.6 is an example of a typical autoradiograph showing DNA fingerprints of a family of Pied Flycatchers. All the bands carried by offspring can be traced to one or both of the parents.

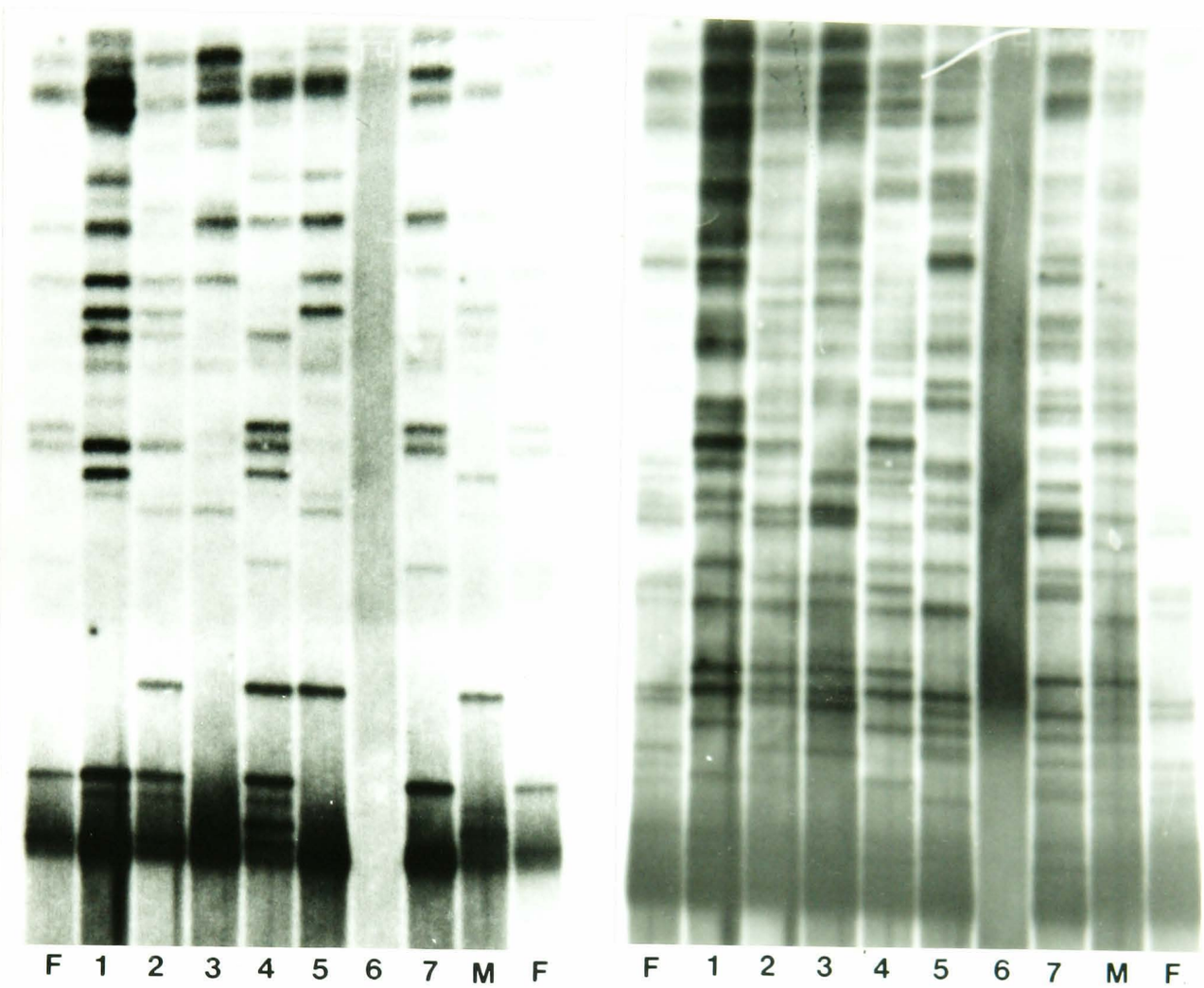


Figure 7.6: DNA fingerprints of a Pied Flycatcher family. The same membrane was hybridised with probes pSPT 18.15 and pSPT 19.6. The former typically produces a few discrete bands, while the latter produces many closely-packed bands. Some bands are revealed by both probes, while others are probe-specific. Both probes produce the "smear" close to the insert line that is typical of the survey gels.

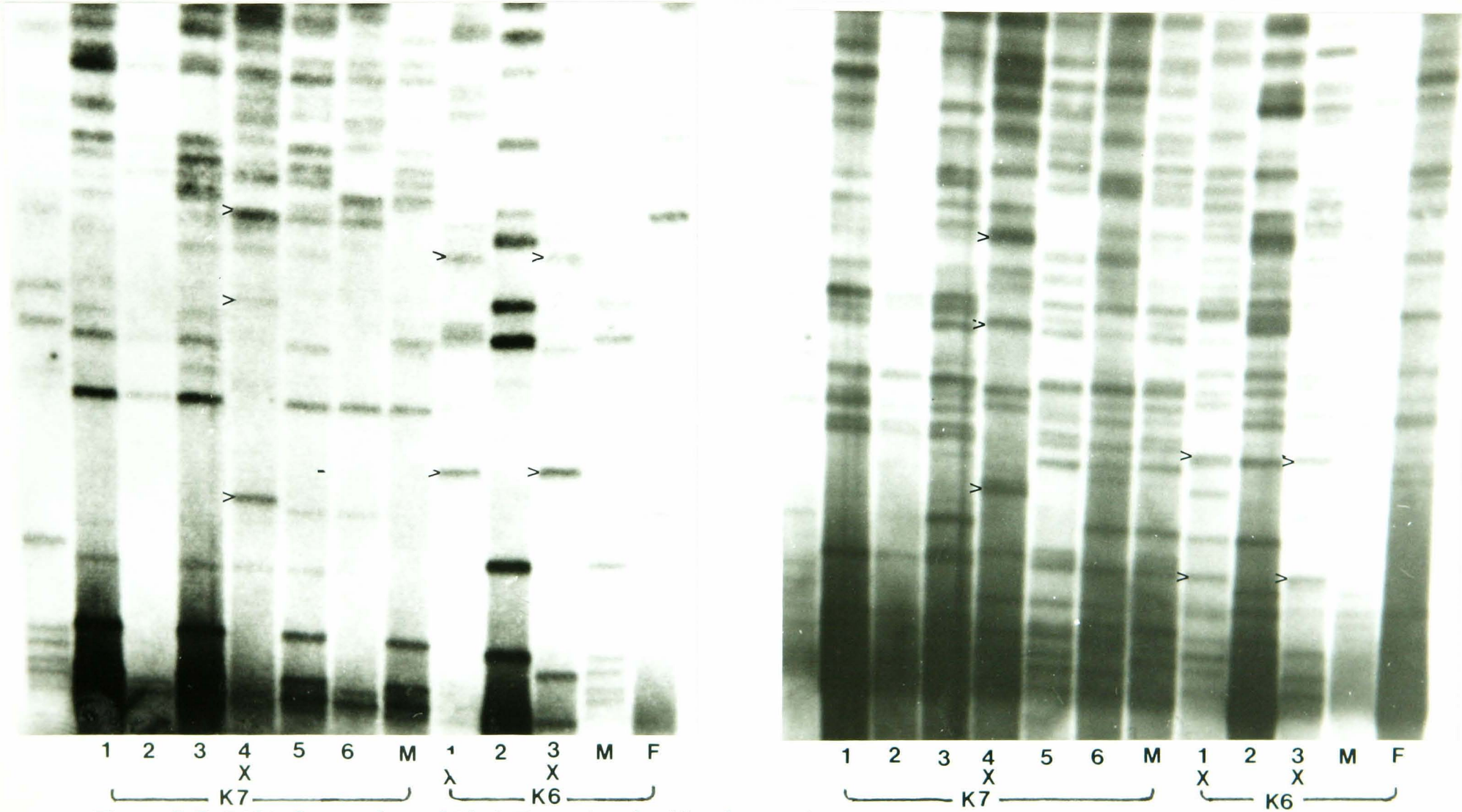


Figure 7.7: DNA fingerprints of Pied Flycatcher families from K6 and K7, probed with pSPT 18.15 and pSPT 19.6. M= Male, F= Female, Offspring numbered. Mismatching offfspring are marked with an X. Bands marked with arrows are unique to mismatching birds.

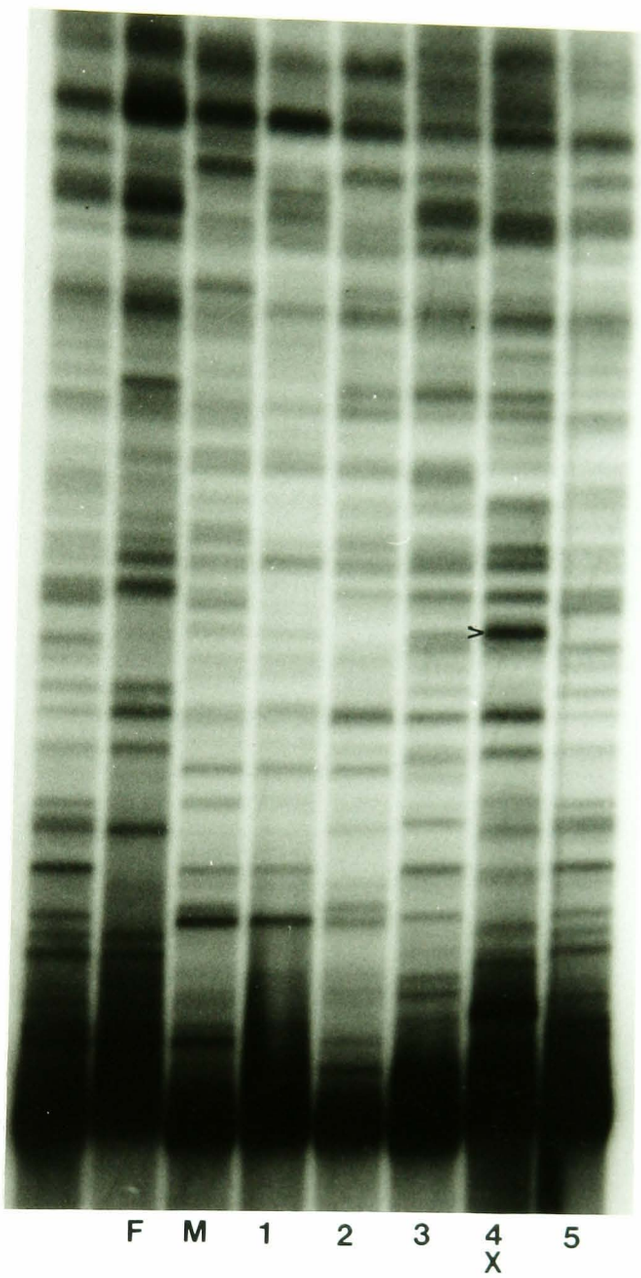


Figure 7.8: DNA fingerprints of a Pied Flycatcher family from K14, probed with pSPT 19.6. M= Male, F= Female, Offspring numbered. Mismatching offfspring are marked with an X. Bands marked with arrows are unique to mismatching birds.

When a DNA fingerprint band is possessed by a pullus but not by either of the putative parents, it must result from mutation or extra-pair fertilisation. EPFs are a likelier explanation if the mutation rate is very low, and if more than one "new" band is present. Of the 75 offspring that were compared with both parents, 4 were found to have such mis-matching bands. These are in broods K6, K7 and K14, shown in Figures 7.7 and 7.8.

The two mismatching pulli in K6 shared 2 bands within the scored region of the pSPT 19.6 fingerprint, which were unique to them. Several other bands can be discerned in regions of the fingerprint too indistinct to score formally. Probe pSPT 18.15 reveals 2 nonparental bands shared by the mismatching K6 pulli and 3 which are unique to the one in K7. The mismatch in K14 had a single unaccountable band within the region that was scored, and a further 6 unique bands outside this region.

In cases where the female is unknown, possible extra-pair offspring (EPOs) also can be detected if they are sufficiently dissimilar to their putative father. In theory, the coefficient of relatedness of first-degree relatives is 0.5, so parents and offspring might be expected to share 50% of their bands. Unrelated birds would be expected to have no bands in common. Wetton (1990) found that, in the House Sparrow, males and offspring shared 60.8% of their bands, while females and offspring shared 54.9%. Because unrelated birds share 15% of bands, the similarity of parents and offspring is greater than 50%. The father/offspring value is further increased because bands carried on the Z-chromosome are always paternally inherited. As a result, if the coefficient of similarity is closer to that of unrelated birds than to that of father and offspring, then this supports the hypothesis that the pullus results from an EPF.

Of 106 offspring that were compared with their putative father, only one comparison had no bands in common. This bird was the mismatching pullus from K7, and the DNA fingerprints of this brood are shown in Figure 7.7. Figure 7.9 shows the frequency distribution of father/offspring similarity

coefficients for "legitimate" within-pair offspring (WPOs) and the three supposed EPOs. The data presented were obtained using probe pSPT 19.6. The K7 pullus who shared no bands with his putative father shared bands with all of his sibs. He also possessed at least 4 unique bands. There is evidence of a linkage group shared by the father and the "legitimate" sibs, hampering statistical interpretation of the fingerprints. Nevertheless, it seems almost certain that pullus 3 mis-matches; formal analysis would merely confirm this.

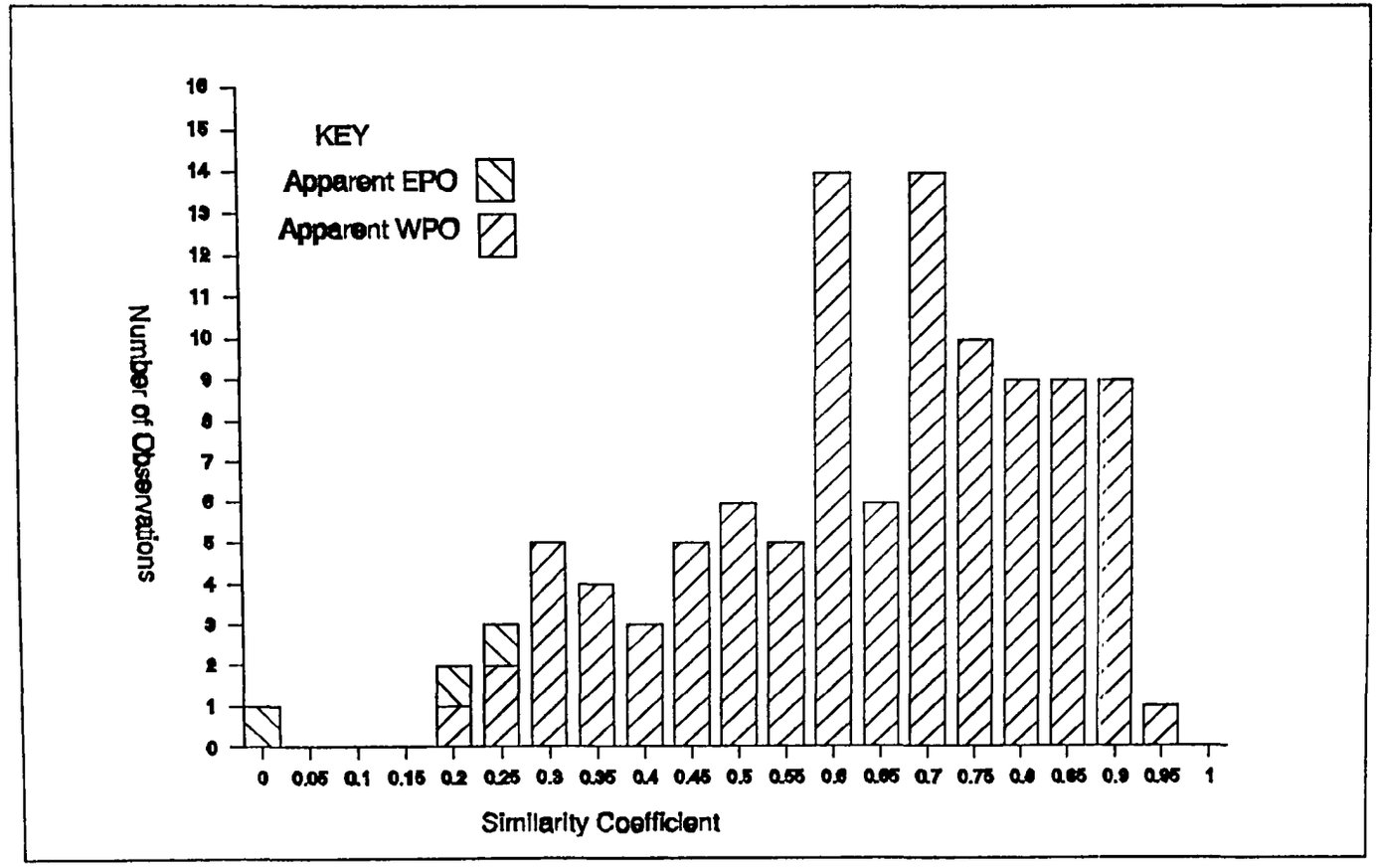


Figure 7.9: Distribution of similarity coefficients between offspring and putative father.

To test formally whether a particular offspring is a WPO or an EPO, it is necessary to estimate a threshold level of similarity, above which the bird is most likely to be a WPO, and below which, an EPO. Factors which determine this critical value include the degree of similarity between unrelated birds, the amount of linkage and sex-linkage, the mutation rate and the number of scorable bands. The current data are insufficient for all these to be accurately estimated.

Estimates of similarity between unrelated individuals are summarised in Table 7.7. These were obtained in two ways, firstly by comparison of the adults in the survey, and secondly, from a sample of 7 randomly chosen individuals. The difference between the two estimates may be due to several factors. An unlikely explanation is that similarity between pairs is inflated by assortative mating between related birds, as in captive Japanese Quail (*Coturnix coturnix japonica*) (Bateson 1980). It is more likely here that the difference results from scoring bias and/or chance. Knowing that birds are supposedly "not related" might make one less likely to say that a band is shared by two individuals on a gel. Neither sample size is large, and the number of scorable bands on some gels was small. The "random" gel is unusual in having several widely-spaced, scorable bands.

Source of Estimate	Number of comparisons	Similarity Coefficient ± s.e.
Adults in survey	14	0.315 ± 0.062
Random Adults	28	0.157 ± 0.156
Pooled Data	42	0.210 ± 0.043

Table 7.7: Summary of coefficients of similarity between unrelated birds. Results calculated using probe pSPT 19.6.

Wetton (1990) found that EPOs, who had bands that were present in neither parent, formed a discrete distribution, having distinctly lower father/offspring coefficients of similarity than WPOs. As shown in Figure 7.9, there is no such bimodal distribution in the Pied Flycatcher data, probably because the sample size is small and the number of scorable bands is low.

Figure 7.10 shows a similar distribution of similarity coefficients for mothers and offspring calculated using data from probe pSPT 19.6. All offspring shared at least some bands with their mother. It seems unlikely that any of the offspring in the survey resulted from brood parasitism. Table 7.8 shows the mean similarity coefficients for three classes of relationship, Mother/Offspring, Father/Offspring and Offspring/Offspring.

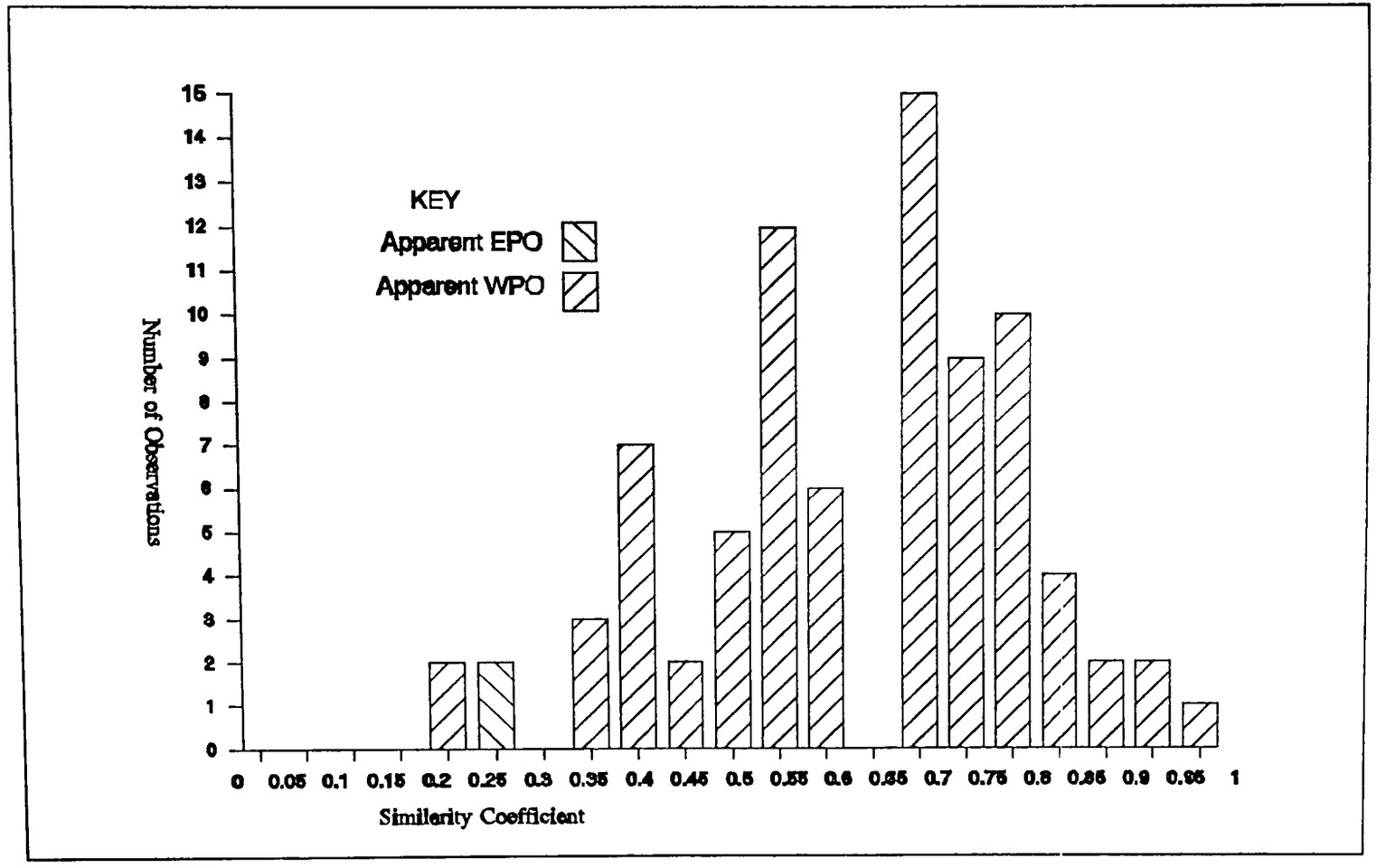


Figure 7.10: Distribution of similarity coefficients between offspring and mother.

Comparison	Number of Families	Number of Offspring	Mean Similarity Coefficient ± s.e.
Mother/Offspring	14	82	0.612 ± 0.019
Father/Offspring	20	106	0.629 ± 0.018
Offspring/Offspring	21	283	0.627 ± 0.091

Table 7.8: Summary of coefficients of similarity between related birds. Results calculated using probe pSPT 19.6.

The Relationship Between Extra-pair Mating and Breeding Biology

It seems probable that extra-pair mating accounts for the four mis-matching offspring of 112 who were compared with their fathers, a frequency of 3.5%. Although the sample size precludes any formal comparison between the breeding biology of nests with and without EPO's, I present anecdotal evidence relating to the three nests in Table 7.9. The clutches are all earlier than the median, and are smaller than the mean clutch size. The clutches are also smaller than the expected clutch size, given the laying date.

Box	No. EPO's	Laying Date	CS	BSF	Age of Male	Age of Female
K06	2	6th May	4 (7.1)	3	Ringed before this study	Ringed in 1988
K07	1	9th May	6 (6.9)	6	Ringed before this study	Unknown
K14	1	9th May	5 (6.9)	5	Ringed as adult in 1988	Ringed in 1988
Population Statistics	4	Median L.D.= 12 May	Mean CS= 6.42±0.11 (115)	Mean BSF= 5.83±0.15 (108)	Ringed before this study 42% Ringed in 1988 25% Ringed in 1989 31%	Ringed before this study 42% Ringed in 1988 22% Ringed in 1989 36%

Table 7.9: Breeding data for the 3 broods in which EPO's were found. Laying Date is the date on which the first egg was laid. CS= Clutch size (figure in brackets is the predicted clutch size given the laying date). BSF is the brood size at fledging.

CHAPTER 8

Discussion and Conclusion

Introduction

In this chapter I discuss the findings of the study; that polygamy and cuckoldry are rare in this population, as revealed by a DNA fingerprint survey, and that their prevalence would be erroneously estimated by analysis of tarsus length heritability. I relate these findings to other work on the Pied Flycatcher, especially in Scandinavia. The compelling conclusion is that Pied Flycatcher mating habits are variable across their geographical range and that hypotheses concerning their evolution should incorporate this. Alternatively, it should be clearly stated that hypotheses relate only to populations with common ecological and environmental conditions.

Polygyny and Deception

Observational results and DNA fingerprint analysis reveal that polygyny and cuckoldry were rare in this population. Only 2.5% of males were polygynous and approximately 3.5% of offspring resulted from EPC's. In previous studies, particularly of a population near Uppsala in Sweden (Alatalo *et al.* 1981), both mating strategies were found to be more common.

There was also evidence of a difference between the territorial nature of polygyny in this study and in Uppsala. The results suggested that there were two reasons why a male could be caught in more than one box. Males whose first nest is abandoned, or who are driven away, tend to move to a relatively distant box. Males who are polyterritorial defend boxes that are near to each other. Of those, the ones who become polygynous tend to have the closest

territories of all. Perhaps several different selective pressures favour polyterritoriality, some may even be unrelated to polygyny.

The findings support those of Alatalo *et al.* (1981) and Stenmark *et al.* (1988), who describe evidence that females are, or could easily become, aware of the mating status of the male, and that deception in the literal sense is not ubiquitous. This does not mean that the generalities of the Deception Hypothesis are incorrect. Deception *per se* might occur in some populations, or, alternatively, female knowledge of male mating status may not be important. If the costs of searching for a mate are sufficiently high, then the best option for a female may still be to become the secondary mate of an already-mated male.

Cuckoldry and the Heritability of Tarsus Length

It has already been established (Wetton *et al.* 1987), that large errors can be found in estimates of reproductive output if they do not account for extra-pair mating and intraspecific brood parasitism. In this study, although the effects of cuckoldry upon the fitness of the birds involved was high, its frequency was low. Intraspecific brood parasitism was not found, so neither behaviour had a large effect upon overall measures of reproductive success. However, workers should not assume that this will always be the case; genetic analysis should become a routine part of studying reproductive success.

Estimates of the frequency of cuckoldry based upon the heritability of tarsus length do not agree with those from the DNA fingerprint survey. Birkhead *et al.* (1990) suggested that variation between estimates derived using different techniques might be due to sampling errors, variation between samples and biases inherent in the less accurate techniques. In this case, the last alternative seems most likely.

Using the heritability method on the 20 males and 18 females whose offspring were used in the fingerprint survey reveals a male/offspring heritability of 0.65±0.22 (n_{pulli}=118), and a female/offspring estimate of 0.55±0.452 (n_{pulli}=95). Only the male/offspring regression is significant. This method would predict that females rather than males, mis-matched with their offspring.

It seems that comparisons of heritability are not an accurate means of estimating the rate of extra-pair paternity. Better alternatives using DNA techniques are now widely available. However, important questions regarding the heritability studies remain, primarily concerning the reason why the similarity of offspring and fathers might differ from that between young and their mothers.

The analysis of pooled data from published and unpublished studies found no evidence of an overall difference between the paternal and maternal heritabilities (Chapter 6). A flaw in this analysis was that it treated each record as an equal independent observation, without weighting for sample size. Alatalo and Lundberg (1989) had by far the largest sample, containing 607 families. They found similar results in a sample of 1044 families of the Collared Flycatcher (*Ficedula albicollis*). Both analyses found a significant difference between the male/offspring and female /offspring heritabilities. While an overall difference might be in doubt, there is strong evidence that in particular studies, at particular sites, the two values do differ. There are two explanations for this.

Firstly, it may be due to random variation. As the simulations in Chapter 6 show, the regressions from which heritabilities are calculated, have large standard errors and are sensitive to small perturbations in the dataset. Small variations in the slopes of parent/offspring regressions are magnified by the cuckoldry estimation equation. Perhaps the significant differences reflect such errors and there is no underlying difference in the resemblance of offspring to their parents.

Alternatively, there may be genuine genetic or environmental reasons why the two heritabilities should differ. It now seems likely that the explanation is not extra-pair fertilisation; so, what could it be? A skewed sex ratio would produce such results. If more than half the offspring were females, then the overall resemblance of pulli to females would exceed that of the offspring and fathers. Sex-related differences in growth patterns might cause a similar pattern. If, when offspring are measured, males and females are at a different stage in their growth curve, then the heritability estimates would be distorted. Falconer (1981) stresses that separate heritability estimates should be calculated for male and female offspring. When a suitable means of sexing pulli is found, this could easily be included in such studies as this. If the technique is DNA-based, then existing measurements and blood samples could be used.

Flexibility and Variation in Mating Strategies

Geographical and temporal variation in the frequency of mating strategies has been described in other species. Some of these cases are more fully discussed in Chapter 1.

Differences in polygyny rates between areas are found in species such as the Hen Harrier (Balfour and Cadbury 1979) and the Pied Flycatcher (Røskaft *et al.* 1986). A change in food availability caused a change in the breeding patterns of Foxes (*Vulpes vulpes*) in the Alaskan arctic (Zabel *et al.* 1989). There is evidence from several sources that in colonial species, the frequency of extra-pair mating is related to the breeding density (Møller 1987, Wellbourn 1993). Quinn *et al.* (1987) found that in Lesser Snow Geese (*Anser caerulescens caerulescens*), there was evidence for large differences in the frequency of extra-pair mating between different areas of a colony.

Such effects are probably direct or indirect consequences of environmental variations, and may be responsible for some dissent among workers whose

results do not concur. There is widespread evidence that area-to-area and seasonal variations are responsible for differences in the breeding success of many species, and that these are factors that also affect the distribution of species. It seems only reasonable to expect that these also affect the frequency and relative success of alternative mating strategies.

Some aspects of breeding success that were measured in this study suggest that life for a Pied Flycatcher in the Gwenffrwd may differ from that in, for example, Uppsala. Mean clutch sizes differ among several studies throughout Europe (Table 8.1). Birds in more northerly areas lay fewer eggs (Spearman Rank R_S=-0.59, n=27, P<0.025), although this is not significant if the Finnish samples are removed (R_S=-0.19, n=20, P<0.4). The median laying dates also differ; northern areas being later (Table 8.2). Regression coefficients for the decline in clutch size during the season are shown in Table 8.3. At least in 1988, it seems that the decline is shallower in Wales than in Sweden. It is possible that, with a more detailed study, a consistent relationship might be found between latitude and the rate of decline of clutch size. (However, statistical comparisons of these are likely to be complicated by departures from normality of clutch size data, and by the sensitivity of regression to outliers and sampling variation). The cost to a female of changing her mate should be less where the slope is shallow. Thus the selective pressures upon female choosiness would also differ. This might help explain some disparity among the results of different workers.

Other factors, perhaps meteorological in origin, may affect not only the quality but the predictability of breeding conditions. Predation rates are likely to differ between the large long-established forests of Scandinavia and the heavily-managed woods of the Gwenffrwd. Thus, there is probably an environmental cause for several different features of Pied Flycatcher breeding biology found to differ among studies. These may also affect the frequency of the various mating strategies. It is noteworthy that a very similar situation is found in the Swallow (*Hirundo rustica*), in which differences in mating behaviour between

Scandinavian and British populations may be related to the different environmental conditions in their breeding habitat (Wellbourn 1993, see also Møller 1987).

Mean Clutch Size	Notes
6.43 6.53 6.79 6.87	Different ages of adults Harvey *et al.* (1984) Southern Britain 52°N
6.87 5.99 6.25	3 Years in the Gwenffrwd Stowe (1985) Southern Britain 53°N
6.61 6.42	2 Years in the Gwenffrwd This Study Southern Britain 53°N
6.47	Källander *pers. comm.* in Askenmo (1982) Southern Sweden 55°N
6.35 6.48 6.57 7.00	Various styles of nestbox. Gustaffson and Nillson (1985) Southern Sweden 57°N
6.06	Askenmo (1982) Southwest Sweden 57°N
6.12	Askenmo (1982) Southwest Sweden 57°N
6.67 6.50	Different breeding densities Alatalo and Lundberg (1984a) Central Sweden 59°N
6.20	Johansson (1972) in Askenmo (1982) Central Sweden 59°N
6.33	Johansson *pers. comm.* in Askenmo (1982) Central Sweden 59°N
5.20 5.20 5.23 5.38 6.13 6.13 6.24	Järvinen A. and Väisänen R.A. (1984) Finnish Lapland 69°N

Table 8.1: Mean clutch sizes of Pied Flycatchers from a number of studies in Scandinavia and Britain, showing lower clutch size in more northerly regions. The results are ordered according to latitude.

Median Laying Date	Notes
13 May 12 May	This Study Southern Britain
23 May	Stowe (1985) Southern Britain
27 May	Källander (1975) in Askenmo (1982) Southern Sweden
23 May 27 May	Alatalo and Lundberg (1984a) Central Sweden
2nd June to 19th June	Järvinen and Väisanen (1984) Finnish Lapland

Table 8.2: Median Laying Dates from studies in Britain and Scandinavia, showing that breeding is later in the higher latitudes.

Regression Coefficient	Notes
-0.085 -0.129	High and low breeding density Alatalo and Lundberg (1984) Central Sweden
-0.080	Källander (1975) in Askenmo (1982) Southern Sweden
-0.058 -0.087	1988 and 1989 This Study Southern Britain

Table 8.3: Decline in clutch size with laying date in Sweden and Southern Britain.

The two Scandinavian groups, in Oslo and Uppsala, found differences between the breeding and mating biology of their populations (see Chapter 2). These led one group to favour a model based on male or territory quality for the evolution of polygyny, and the other to propose the Deception hypothesis. Their results suggest considerable difference in mating behaviour between the two study populations, which are, in turn, likely to differ from the Welsh one.

The cost of rejecting a male, to find and assess another, could be highly influential on the mating behaviour of a female Pied Flycatcher. It depends partly upon the decline in breeding success through the season and the availability of suitable males with territories. When males are readily available and delayed breeding costs little, then females might be expected to be discriminating in their selection of a mate. Theories such as the Polygyny Threshold model and the Sexy Son hypothesis predict some of the ways in which secondary females might benefit from this mating decision. Should the breeding situation be right, females will chose to mate polygynously to enhance their reproductive fitness. In the opposite conditions, when suitable mates are rare, or the costs of choosiness are high, a "Deception"-like system might be found; polygyny might still occur. It is quite possible that two females of the same species in the same area during the same season might experience different levels of male availability or costs of choosiness. Both models might apply. In different years or areas this is even more likely.

Variation in the rate of predation might affect the costs and benefits of polyterritoriality and polygyny. Orians (1969) suggested that low predation rates would favour polygyny. von Haartman (1956) suggested that polyterritoriality might benefit males whose original nest was predated. The foraging patterns of predators, and their abundance might have an effect upon the number of males with multiple territories and mates.

In future studies, it seems useful to compare the different areas more thoroughly. This might reveal the links between habitat quality, food availability, predation and weather and the mating strategies adopted by the Pied Flycatchers. It also seems that female mate choice and sampling behaviour might be central to the distinction of "Deception"-type and male/territory quality models.

The apparent flexibility in mating strategies also suggests that populations nesting in natural nest cavities might experience different conditions and

behave differently from those in artificial boxes. Work by Nillson (1984) has already revealed that this is true for clutch size and breeding success.

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APPENDIX

List of reagents and their abbreviations:

BPB	= BromoPhenol Blue (loading Buffer)
EDTA	= Ethylene Diamino Tetra-acetic Acid
SDS	= Sodium Dodecyle Sulphate
SDW	= Sterile Distilled Water
SET	= Sodium, EDTA, Tris
SSC	= Sodium Chloride, Sodium Citrate
Tris	= Tris (Hydroxymethyl) Amino Methane
TAE	= Tris Acetate, EDTA
TBE	= Tris Borate, EDTA
TE	= Tris, EDTA
TNA	= Tris, Sodium, EDTA

Solutions required for various stages of DNA fingerprinting:-

DNA extraction (solutions made up in sterile distilled water = SDW)

20x SET	= 3M NaCl, 1M Tris, 20mM EDTA Na_2, pH to 8.0 with HCl
Proteinase K	= 10mg/ml Proteinase K
SDS	= 25% w/v
TE	= 10mM Tris, 1mM EDTA Na_2 pH to 8.0 with HCl

DNA Electrophoresis (solutions made up in distilled water)

5x TBE	= 0.445M Tris, 0.445M Boric Acid, 0.002M EDTA (pH 8.0)
50x TAE	= 2M Tris, 2M Glacial Acetic Acid, 0.05M EDTA (pH 8.0)
BPB	= 20% Ficol, 0.2M EDTA Na_2, 0.25% Bromophenol blue, 0.25% Xylene cyanol

Blotting (solutions in distilled water)

Depurination solution	= 0.2M HCl
Denaturing solution	= 1.5M NaCl, 0.5M NaOH
Alkaline Transfer solution	= 1.5M NaCl, 0.25M NaOH
Neutralising solution	= 3M NaCl, 0.5M Tris, pH to 7.0 with conc. HCl
20x SSC	= 3M NaCl, 0.3M Sodium citrate

Hybridization

Nick-stop mix	= 0.9% Blue dextran, 0.03% Bromocresol purple, 20mM EDTA solution in TE
10x Blotto	= 10% skimmed milk powder, 0.2% sodium azide, in SDW
5x Denhardts	= 0.5gm Polyvinylpyrrolidone, 0.5gm BSA, 0.5gm Ficoll in 500ml SDW

Fluorometric Assaying

10x TNE	= 100mM Tris, 10mM EDTA, 1M NaCl, pH 7.4 with HCl

Agarose gels

A 1% solution	= 3.75g in 375ml 1x TAE