The Application of DNA Fingerprinting to the Conservation of Threatened Species

by

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ABSTRACT

The human polycore minisatellite probes 33.6 and 33.15 developed by Prof. Alec Jeffreys and colleagues have been shown to detect hypervariable minisatellites in many taxonomically dispersed species. The mRNA derivatives of these two probes, pSPT19.6 and pSPT18.15, have here been used to probe the genomes of four species currently maintained in captivity. The wild populations of these species, Rothschild's mynah, the Rodrigues fruit bat, the British Merlin and the New Zealand falcon, are threatened with extinction to varying degrees. By using the technique of DNA fingerprinting, it has been possible to assess the levels of minisatellite variation remaining in these stocks, to confirm or refute the parent/offspring allocations made within, and in the case of Rothschild's mynah, to demonstrate that at least two of the founders of the stock were closely related. In addition, it has been possible to show that there is a significant positive relationship between the similarity coefficient calculated between two adults and the inbreeding coefficient calculated for their offspring.

CONTENTS

CHAPTER 2: MATERIALS AND METHODS

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 7

LIST OF TABLES

CHAPTER 5

CHAPTER 6

CHAPTER 7

CHAPTER 1

INTRODUCTION

1.1 EXTINCTION OF SPECIES

1.1.1 General Introduction

It has been estimated that there are somewhere between 5 and 30 million living species on the planet Earth, with a conservative consensus that there are at least 10 million (Holden, 1974; Myers, 1979; Erwin, 1982; Soulé, 1991). Of these, only a small proportion (about 1.7 million) have actually been identified by science (Wilson, 1989), and even then their full geographic range and ecological peculiarities have not been recorded (Lovejoy, 1986). As Woodruff (1989) points out, "fewer than 10,000 (animal) species have been characterised eco-behaviourally, and closer to 1,000 of these have been examined genetically." He further points out that "we are presently capable of conserving the evolutionary potential of (only) a few more than 100 species" due to our limited knowledge of their genetics, ecology and behaviour.

1.1.2 Extinction Rates and Reasons

> "Why do populations go extinct? Sometimes the answer is obvious - the individuals are all shot. More often the issue is not that simple."
>
> M.E. Soulé (1989)

Extinction can be defined as the failure of a species or a population to maintain itself through reproduction, due to environmental change exceeding the adaptive capacity of the species (Frankel & Soulé, 1981; Vermeij, 1986; Wilcox, 1986). This environmental change can be caused by abiotic (e.g. weather, pollution, volcanos, etc.) and/or biotic factors (i.e. alteration of the selectional environment due to a change in the species composition of an ecosystem) (Strahan, 1975; Vermeij, 1986).

Extinction has been a natural feature of ecosystems since life first evolved, and, given infinite time, extinction can be shown to be a certainty for any species with an upper limit on its numbers (Hooper, 1971). In fact, at least 90% of all the species that have ever existed have gone extinct (Myers, 1979). However, as these prehistoric species disappeared, they were replaced by other forms which were better adapted to the changing environment and these successful species evolved and diversified into the available environmental niches. Frankel & Soulé (1981) make this point on the first page of their book "Conservation and Evolution", "the emergence of new forms is interwoven with the passing out of older ones. It is implicit in the concept of...evolution that it is a continuing process, with survival, adaptation and speciation balancing extinction."

The crucial difference between these natural extinctions and those caused by Man is that humans abruptly remove species from habitats, they do not replace them over time with other better-adapted ones, although this is not to say that a suitable species could not move into the niche left by an organism driven to extinction by Man. The point is that species are being driven to extinction so rapidly that large plants and animals cannot evolve fast enough to keep pace with the increasing rate of human-mediated change (Frankel & Soulé, 1981).

There have been a number of mass extinctions through geological history, most notably marking the end of the Ordovician, Permian, Triassic, and Cretaceous periods (Raup & Sepkoski, 1982). However, even these extinctions are several orders of magnitude less than modern-day estimates of the current and predicted rates of species extinction (see Table 1.1).

TABLE 1.1: Species extinction rates over time.

PERIOD	MEAN EXTINCTION RATE[1] (In species)
GDE[2]	1 per 1000 years (Myers, 1979)
1600-1950	1 per 10 years (Myers, 1976)
1950-2000	1 per 1 year (Wemmer, 1977)
	1 per 1 day (Myers, 1983)
2000-2050	1 per 1 hour (Wilcox, 1988; Wilson, 1989)

1 - This list is composed of a small selection of the many extinction rate estimates available and shows the trend found in the literature over the past two decades.

2 - GDE is an abbreviation used by Myers for the Great Dinosaur Extinction that occurred at the end of the Cretaceous Period 65 million years ago, marking the end of the Age of Reptiles.

Since 1600 A.D. (from which date reasonably accurate historical records of extinctions are available), humans are documented to have driven 63 mammalian and 83 avian species to extinction (Diamond & Case, 1986). This is in the context of estimated global totals for mammals and birds of 4,200 and 8,500 species, respectively. Undoubtedly, numerous species of plants, invertebrates, fish, amphibians and reptiles have also been lost, but no records are available to confirm this.

The distribution of these losses has not been uniform over time (about 50% of those species known to have become extinct were lost in the 300 years between 1600 and 1900, the other 50% having been lost in only the last 90 years), or across habitats (for example, only 20% of all bird species live on islands, but 90% of all those driven to extinction were island dwellers) (Fisher, Simon & Vincent, 1969; King, 1979). The increase in the extinction rate has been due extensively to human activity (see Section 1.1.3), but the uneven distribution of losses across habitats, i.e. skewed in favour of the loss of island species, has been due to the

peculiarities frequently associated with island-forms.

Islands feature large numbers of endemics (species which occur nowhere else) and this is because islands (either geographical or ecological) effectively seal off a portion of a species' gene pool, allowing adaptive radiation to occur with the possibility of natural selection producing new species. The classic examples of this phenomenon are, of course, Darwin's finches on the Galapagos Islands, and the radiation of the genus *Drosophila* on the Hawaiian archipelago (Berry, 1974).

Compared to continental species, island forms evolve in the presence of relatively few competitors and predators, thus they are frequently characterised by limited competitive ability and poor defences, e.g. the Dodo's complete lack of fear for Man. If continental species are introduced to an island, their superior aggression, hunting skills, camouflage, greater choice of food species, etc., evolved over millennia in the presence of many other species, allows them to out-compete the native forms. This can lead to the rapid decline and possibly loss of parts of the island's flora and fauna, a phenomenon that has been documented on many occasions (for example, see references in King, 1979 and Goodwin & Holloway, 1978).

1.1.3 Increases in the Extinction Rate

It has been estimated that 75% of the extinctions which have occurred since 1900 have been due to direct and indirect human activity (Fisher, Simon & Vincent, 1969). This represents a massive increase in the rate of species extinctions (see Table 1.1), and some of the reasons for it include:

1) killing for pleasure;
2) killing for food;
3) killing for hides, furs, feathers, tusks, etc.;
4) environmental pollution;
5) exploitation for the pet trade;
6) fragmentation and destruction of natural habitats;
7) introduction of non-native species and diseases.

These factors have also caused "secondary extinctions"; the extinction of one species due to the loss of another (see Section 1.2.4) (Diamond, 1989; Jablonski, 1991).

The fragmentation and destruction of natural habitats is considered to be the primary cause of 67% of continental species' extinctions, with human persecution and the taking of wildlife for food or other products being responsible for the endangerment of 37% of *all* threatened vertebrate species (IUCN, UNEP & WWF, 1980; Vermeij, 1986). The most important factor affecting island species is the introduction of non-native species (e.g. cats, rats, dogs, pigs, goats and rabbits) which compete with, prey upon and destroy the natural habitat of over 50% of those endemic species threatened with extinction (Johnson & Stattersfield, 1990).

Man has played, and continues to play, the major role in the extinction of species, and the situation is expected to worsen as the size of the human population (currently estimated at 5-6 billion) increases (Myers, 1979; Flannery & Conlon, 1989). If the current rate of human population growth was to continue, the Earth would hold over 50 billion people by the end of the next century (Flannery & Conlon, 1989; Westing, 1981). However, it is thought improbable that the population will reach this size as demographic studies have suggested that it will actually peak at around 10 billion (Tudge, 1988), although this figure is still almost double the size of the current world population.

It is extremely unlikely that a population of 10 billion humans can be supported by the current levels of resource consumption, thus the latter will have to increase, inevitably leading to a rapid depletion of the Earth's finite resources (Flannery & Conlon, 1989). The rate of population growth is highest in the underdeveloped areas of the world where there is intensive exploitation of the natural resource base (Wilson, 1989). This resource base includes the tropical rainforests, where 40-50% of all species are thought to exist and which have suffered extensive logging to provide export income (WWF, 1991). Indeed, 25% of *all* extant species are threatened by the loss of wild habitats (Tudge, 1988).

Originally thought to cover about 16 million km^2 and to have existed continuously for 60 million years (Richards, 1973), the world's rainforests have been reduced to a little over half this area (Gomez-Pompa et al., 1972; Myers, 1979). At the end of the 1970s, rainforest losses in Latin America, Southeast Asia and Africa were estimated at 37%, 38%, and 57%, respectively (Myers, 1979; IUCN, UNEP & WWF, 1980). These figures have undoubtedly increased in the last decade. In a recent study carried out by the International Timber Trade Organisation, not one of the countries in Southeast Asia could be identified as managing its rainforests in a sustainable manner (Bawa, Primack & Woodruff, 1990). The World Wide Fund for Nature (WWF) has stated that the rainforests are currently being destroyed at the rate of 40 hectares a minute (WWF, 1991) and figures released by Brazil's National Institute of Space Research estimate that 404,000 km^2 of the "legal" Amazon alone (an area about twice the size of Great Britain) have been lost to date (Bonalume, 1990a).

The degradation of the rainforests and the inevitable alteration in microclimate has almost certainly resulted in a concurrent loss of numerous plant and animal species (Soulé & Wilcox, 1980; Myers, 1989). Of the approximately 1.7 million species that have been described, about 90% are from the temperate regions of the Earth, and so the Tropics are severely under-represented in the taxonomic records (Wilcox, 1988). The estimates of extinction rates may, thus, be far lower than the true figures.

1.1.4 Summary

Every calculation of extinction rates so far performed has resulted in an alarmingly high figure (Lovejoy, 1986). Wilcox (1988) used three different approaches to calculate a global estimate for the number of threatened species and

he presented his findings in the Guest Essay of the "1988 IUCN Red List of Threatened Animals",

> "...at least several hundred vertebrates, hundreds of thousands of plants, and over a million species of insects...will go extinct within the next three to five decades."

1.2 CONSERVATION OF SPECIES

1.2.1 General Introduction

During the 1960s, there was a general heightening of environmental awareness (Temple, 1978a) and this has continued to increase to the present day. People have become more concerned about the long-term future of the world in which they and all other animals live (Warren & Goldsmith, 1974). The "Green" movement has brought the subject of conservation to the world's attention recently by highlighting the problems that the global environment now faces. These include acid rain, destruction of the rainforests, the greenhouse effect, global warming, the damage to the ozone layer and the loss of species.

The increase in the public's concern for "wildlife" can be gauged from the proliferation of zoological gardens around the world, from 260 in 1946 to just under 1,000 in 1985 (Fisher, Simon and Vincent, 1969; Rawlins, 1985), and by the sheer number of people visiting them. In Britain, over 7 million people visited zoos and wildlife parks during 1989 alone, and similar institutions in the United States attract 100-200 million visitors a year (Pilkington, 1991; Soulé, 1985).

A worldwide poll conducted by Gallup in 1976 indicated that 75% of all people would like to see more done for wildlife (Myers, 1979). Such an obvious show of awareness invariably results in a change in the attitudes of commercial enterprises and governments (IUCN, UNEP & WWF, 1980), and the term "environmentally-friendly" is presently being used to sell a wide range of products like washing-up liquid, nappies, hairspray, cosmetics, shaving foam, cars and even political parties.

1.2.2 Why Conserve Species?

Warren and Goldsmith (1983) pointed out that nature has real value to many different groups of people. It is valued "by countrymen as part of their livelihood; by scientists for research; by research conservationists for the future; by teachers for education; by naturalists to satisfy their curiosity; and by most of us for the opportunities it offers for recreation, for its beauty and for its very naturalness." Indeed, *many* reasons have been advanced as to why conservation (i.e. the continued existence of species and their habitats) is important, and these have been divided into seven groups (after Kellert, 1986).

1) *Naturalistic Value.* Many people derive enjoyment from a direct contact with

natural environments. Walking, climbing and camping are pursued because of the opportunities they provide for "getting away from it all into the great outdoors". A study by Lime (1976) concluded that the chance to encounter rare species in their natural environments was one criterion listed by people as an important part of a "satisfying outdoor experience."

2) *Existence Value.* "Conservationists sometimes protest that it should be as unthinkable to destroy a species for whatever passing benefits as it would be to burn a Rembrandt painting to keep warm for an hour." (Myers 1979). This quote encapsulates the idea put forward by many conservationists that we have not inherited the world from our grandparents, but have borrowed it from our grandchildren. Therefore, by destroying something of inherent aesthetic value, future generations will be deprived of its pleasure.

A number of religious and cultural traditions around the world embrace the concepts that all species have the inherent right to exist and that they possess their own spiritual importance (Fox, 1980). Included within these beliefs is the concept that Man has a divinely imposed duty to protect and preserve all lifeforms.

Also, there is benefit to be gained from knowing that a species simply exists, even though those deriving pleasure from this fact may never see the species in question (Kellert, 1986; Soulé, 1985). It is obvious, however, that all species are not considered equally important. Many people would be disappointed if the Blue whale *(Balaenoptera musculus)* was driven to extinction, but how many would be equally disappointed to see the last of the organisms responsible for influenza, herpes, or AIDS?

3) *Scientific Value.*

> "The great German zoologist Karl von Frisch once said that the honeybee is like a magic well; the more you draw from it, the more there is to draw."
>
> E. Wilson, 1981 Congressional Testimony (Kellert, 1986).

Having reviewed the literature, Lovejoy (1986) considered that "our knowledge of biological systems is so superficial that there is not a single species for which it can be said, with confidence, that we know it in its entirety and need not retain it for its contribution to biological knowledge." All species have the potential to increase our knowledge and understanding of the world in which we live, and as we are living organisms ourselves, we have a vested interest in trying to understand the many systems which control the continued existence of life on this planet. Until we have this knowledge, it would seem prudent to try and maintain as much diversity as possible.

4) *Aesthetic Value.* On the whole people enjoy the variety present in Nature (Soulé, 1985). With the exception of those with phobias, people like to see, smell, and touch plants and animals. Many rare species (such as birds of paradise, the

Giant panda, callitrichid monkeys, orchids, roses, etc.) are recognised as possessing great beauty and many people are willing to pay for the privilege of travelling to other countries to view the native flora and fauna (Jewell, 1974). Unfortunately, in many cases it is this beauty, provoking the human desire for possession, that has contributed to their rarity (Schomberg, 1974). There is a certain degree of overlap between this category and that of "Existence Value".

5) *Utilitarian Value.* Species of both plants and animals may at some time in the future furnish us with new fibres, foods, fuels, or medicines (Frankel & Soulé, 1981). Although this will not involve more than a small percentage of species, these natural populations could provide significant contributions to human well-being. If just the major medical benefits so far gained are considered: aspirin for pain relief, digoxin for heartbeat regulation, reserpine to alleviate high blood pressure, and diosgenin for the contraceptive pill, it is arguable that sufficient evidence is provided to support the contention that as much natural diversity as possible should be conserved (WWF, 1991). Living species can be thought of as one of the Earth's finite natural resources and when consumed, i.e. driven to extinction, they cannot be renewed. Once the decision is taken to allow a species to go extinct, any benefits it may have produced will be lost (Norton, 1986).

In addition, certain species can be extremely useful as indicators of general habitat stability, such species usually being the higher trophic level predators. Being at or near the top of local food chains means that anything that has an effect on the species at lower trophic levels has the potential to affect these animals (Cooper & Forbes, 1986). If these animals can be identified and monitored, they can be used as an "early warning system" to bring any habitat degradation to the attention of conservationists (Lovejoy, 1986).

One group of animals that has been valuable in this regard is the predatory birds. Due to their feeding habits, they tend to accumulate any toxins present in the environment and concentrate them in their tissues (Chancellor, 1977; Myers, 1983). This phenomenon was seen in the great declines of the British raptor populations during the 1950s and 1960s, due largely to pesticide poisoning (see Chapter 5, Section 5.1.4).

6) *Cultural Value.* Since prehistoric times, plants and animals have been used as tribal totems, motifs of group identity and records of social experiences (Kellert, 1986). Many cultures have worshipped animals in some shape or form; many of the American and Central American Indian, Ancient Greek, Chinese, Asian Indian and Ancient Egyptian gods were portrayed as having an animal's heads on an otherwise human body, demonstrating the close affinity between Man and Nature (Cotterell, 1986).

7) *Ecological Value.* This is by far the most important category. The loss of a few species out of the millions currently in existence is a seemingly negligible event, but in aggregate such losses can prove of major significance to the ecosystems formerly inhabited by those species. Particular species, by nature of their numbers, biomass, and/or contributions to ecosystem energy flow, are

acknowledged as being important to the continuity of local flora and fauna because of the "selectional environments" they create (Myers, 1979; Vermeij, 1986). The loss of such species will alter the environments of the organisms with which they used to interact. This may benefit competitors, but it would not be advantageous to a plant dependent upon the threatened species for pollination, or a predator dependent upon them for food. Too little is known about the roles that most species play in their ecosystems to foresee the damage that may result from their elimination (Ziswiler, 1967; Wood, 1983).

1.2.3 Diversity and Stability

As mentioned above, the "ecological value" of certain species is extremely high. Many plants are pollinated by insects, birds or bats, animals that Gilbert (1980) classified as "mobile links". These are species that are essential for the reproduction and seed dispersal of numerous plant species, and which "link" together and support otherwise separate food webs. A serious decline in the numbers of these organisms would result in the loss of numerous plant species from a number of different areas (ecosystems), especially the rainforests (Lord, 1991). Such losses could badly disrupt these ecosystems, the existence of which is necessary for the maintenance of natural biochemical processes (e.g. the water and nitrogen cycles). This could have disastrous results on a global scale due to the resulting severe changes in weather patterns and the potential reduction in the world's supply of oxygen (Richards, 1973; Chandler, 1974; Lande, 1988).

There has been much argument regarding the contention that increased diversity results in greater ecosystem stability (see references below). Elton (1958) first observed that species-rich communities were more resistant to the invasions of foreign species than were communities with fewer species. He concluded that diversity (i.e. a multiplicity of food-chains) led to stability and that one of the main aims of conservation should be the preservation of maximum species diversity. However, Elton's theory lost favour due to Connell & Orias' discovery (1964) that it was more likely that greater stability resulted in an increase in diversity and May's (1973) theoretical demonstration that stability is actually reduced by diversity rather than enhanced.

However, as pointed out by Diamond & Case (1986), Elton and May were interpreting the meaning of "stability" in two different ways. Elton used stability to mean "resistant to change" with regard to invasions by non-native species, whereas May used stability in the context of an ecosystem retaining all of its species over time. His demonstration conflicted with Elton's assessment of the stability because, for May, the more diversity within the ecosystem, the less likely that all the species would remain in the long term (cf., Walker, 1989).

The relationship between diversity and stability is not a simple one. Although diversity does not *promote* stability, it does appear that high stability allows a system to develop a high species diversity (Goodman, 1975). This, in turn, makes the ecosystem more "stable" in that it contains a greater number of inter-species interactions, making it more resistant to invasion by foreign species

and more able to recover after damage, e.g. species loss, fire, etc. (O'Connor, 1974; Myers, 1983). Pimm (1987) argues that because the disruption of ecological interactions and relationships leads to instability (and possibly extinctions), the more varied the organisms living within a habitat, the more complex the network of inter-species interactions, and the less susceptible that biome to invasion or biotic collapse.

This can be illustrated if one considers the relationship between a primary producer (plant), a primary consumer (herbivore) and a secondary consumer (carnivore). In such a simple system, the organism at the highest trophic level (carnivore) is obligately dependent on one prey species (herbivore) which, in turn, feeds on only one species of plant. The depletion or loss of either of the organisms at the lower trophic levels, due to a disease epidemic for instance, could result in the decline or extinction of the predator in that area. These circumstances could be avoided if the primary and secondary consumers were polyphagous (see below).

1.2.4 Species Interdependence

> "In most communities, species are a significant part of one another's environment." (Soulé, 1985).

Species have evolved into their present niches over millions of years. Certain species have evolved very close relationships, known as mutualism, in which both species gain some benefit from their interaction. Examples of such mutualisms include the phenomenon of pollination, and the interaction of clownfish with anenomes (Cushman & Beattie, 1991). In some cases, one or other of the species may be so adapted as to be unable to withstand the selection pressures put upon it by the decline or extinction of the other. Thus, mutualism can lead to extreme, or possibly obligate, dependency (see below), leaving species extremely vulnerable to extinction if the co-evolved partner is lost (cf., Cushman & Beattie, 1991, for a discussion of the actual evidence available for obligate mutualism).

Temple (1977) presented an example of what he proposed to be obligate dependency, demonstrating how the loss of one species from a co-evolved pair can affect not only the partner species, but also a number of others in the same ecosystem. Certain seeds are unable to germinate unless they have passed through the digestive system of a particular animal. The very hard seeds of the tree *Calvaria major* require abrasion of the outer coat in order to germinate. The tree is endemic to Mauritius and none of the island's extant fauna are capable of softening the seeds sufficiently. Temple suggested that the only creature capable of performing this function was the Dodo, *Raphus cucullatus*, which was driven to extinction by Man in 1681. If this hypothesis is correct, *Calvaria major* is destined to go extinct when the few ageing individuals that still survive on the island die. Thus, the loss of the Dodo will probably lead to the loss of this tree, and could, thus, affect other island fauna that live upon, feed off, or otherwise interact with *Calvaria*.

A more complex ecological system with many food-chains and polyphagous primary and secondary consumers, avoids the problems associated with obligate dependency. This means that the loss of just one of a number of "food" species will have less impact on the "feeders" than it would in a simple system. This is not to say that the impact would always be unimportant, only that it will be less likely to lead to the extinction of any of the species at the higher trophic levels (see Section 1.2.3). In such a case, the "ecological pyramid" has a wider base and so the removal of one of the lower "supporting species" will be less likely to cause the food-chain or, by extension, the ecosystem to collapse (Pimm, 1987).

1.2.5 Summary

It should be pointed out that some of the values listed in Section 1.2.2 are only applicable to the more developed countries of the world. Since conservation often requires the use of resources and areas used by humans, sacrifices have to be made. On many occasions, it is only the wealthy nations of the world that can afford the "luxury" of conservation (IUCN, UNEP & WWF, 1980).

In order to encourage some of the poorer countries to devote resources to conservation, the requirements and aspirations of the local communities need to be incorporated into any proposed preservation schemes. The uniqueness of indigenous flora and fauna is one of the distinguishing features of a country (Kellert, 1986), and such factors have been stressed in recent conservation attempts with, for example, the Javan rhino (Seal, 1991a), the Bali starling (see Chapter 3) (PHPA, ICBP & AAZPA, 1987), and the Rodrigues fruit bat (see Chapter 4) (Carroll, 1981).

1.3 CONSERVATION WITHIN NATURAL HABITATS

1.3.1 General Introduction

The "1980 Global Report to the President of the United States" (Barney, 1980) estimated that between 500,000 and 2,000,000 species could be driven to extinction by the end of this century. Although direct exploitation and pollution of the environment are contributing elements, the destruction of wild habitat will continue to be the most significant factor in the decline of species diversity (Shaffer, 1987). It is obvious, therefore, that the preservation of natural habitats will play a major role in any conservation attempt, be it for the maintenance of general diversity or for the preservation of a particular species.

1.3.2 Conservation in Reserves

In crisis situations, a species-specific strategy is the most appropriate (see Section 1.4), but the conservation of ecosystems by the formation of reserves is preferable in all non-crisis situations (Norton, 1986). Conserving a species in its natural habitat circumvents some of the problems associated with captive breeding

(see Section 1.5). For example, a lack of knowledge regarding some of the species' essential requirements (light, minerals, water, temperature, etc.) may result in them being neglected when the species is held in captivity and this could have deleterious effects on that species (see Section 1.3.3). This has been a major consideration for the Specialist Groups of the IUCN Species Survival Commission, which, to date, have only recommended captive breeding in 4.5% of their Action Plans for the conservation of threatened species (Stuart, 1991). The remaining Action Plans recommend habitat conservation as the most efficient method of protecting the particular species in question, acknowledging that there are several reasons why this kind of conservation is important.

Many millions of years of evolution have adapted species to the selection pressures present in their environments. Each species has its own particular requirements and it therefore follows that the habitat in which a species is naturally found is the one in which it is best able to satisfy these requirements. Provided that human access is restricted, the largely undisturbed nature of a reserve offers species the chance to increase their numbers in the most favourable surroundings. In addition, wild populations are subject to natural selection and will, thus, maintain a semblance of their specific genetic make-up (Foose & Ballou, 1988).

A reserve can potentially conserve not only the particular species of interest, but also all the other species which live within its boundaries (Lovejoy, 1986). Ideally, reserves are chosen on the basis that they encompass functioning natural ecosystems, composed of many species of plants, animals, fungi, bacteria and protozoa, a number of which will benefit from the conservation measures applied to saving the threatened species in question (Jewell, 1974; Lande, 1988). Because this kind of conservation can encompass a great deal of natural habitat, and covers the preservation of many other forms of life, the term "umbrella species" has been coined (Wilcox, 1986) to describe those threatened animals with particularly large habitat requirements, e.g. the Mountain gorilla, (*Gorilla gorilla*) and the African elephant, (*Loxodonta africana*). Measures aimed at conserving the natural habitats of these "charismatic megavertebrates" help to conserve many other species for which the acquisition of funds for conservation schemes would be comparatively difficult (Foose et al., 1986).

Reserves provide scientists with the opportunity to study animals in their native habitats (Jewell, 1974). Data regarding the natural behaviour and demography of a species could be vital to the success of a captive breeding and reintroduction programme, should conservation of the habitat not prevent the species' decline. In addition, reserves can provide a source of income as tourist attractions. "Eco-tourism" and safaris allow habitat managers to gain (additional) funds from the public. This income can then be used for the upkeep of the reserve (paying perimeter guards, etc.) and the protection of the species within it. This is especially important in some of the underdeveloped countries where there may be limited financial resources available (Parker & Graham, 1971; Ayers, Bodmer & Mittermeier, 1991).

Obviously, reserves are necessary for reintroduction programmes. One or more species living within the boundaries of a reserve may become endangered and require captive breeding to prevent its extinction. If no former habitat remains intact, reintroduction ceases to be a feasible option and the species may be forced to remain in captivity for many generations (see below) (van Helvoort & Hartojo, unpublished).

1.3.3 Importance of Reserves

The overall genetic composition of a species is a result of the influence of all the environments that the species has experienced (Berry, 1971). Thus, a long period in captivity could result in the evolution of adverse behavioural and/or morphological adaptations (for example, see Moss, 1972), possibly leading to the domestication of the species (Stanley Price, 1989a). Indeed, it has been pointed out that breeding species *in* captivity can often mean breeding them *for* captivity (Myers, 1979). Unfortunately, a long period in captivity is exactly what will be necessary for many species as their reintroduction remains a remote possibility (Mace, 1986).

In addition to species developing deleterious traits in captivity, further complications are presented by the fact that the ecosystems from which the species are taken are *themselves* continually evolving. If taken out of the natural habitat for too great a length of time, a species may gain adaptations in captivity that are inappropriate to the changed conditions prevalent in the ecosystem to which it is returned (Anderegg, Frey & Muller, 1983; Slobodkin, 1986).

Many species learn their behaviours from their parents or from other adults with whom they are raised, a phenomenon known as cultural transmission (Slater, 1986). This can present problems because offspring will learn their parents' altered behaviours. This can leave captively bred individuals severely disadvantaged in the wild since they may, for example, be unable to mate with wild individuals, unable to rear young, unable to hide or escape from predators, etc. (Kleiman, 1980). With this in mind, Foose & Ballou (1988) asserted that the reintroduction of invertebrates and lower vertebrates will probably be more successful since a greater proportion of their behaviour is "hard-wired".

The problems mentioned above have been encountered in the attempts to reintroduce the Orangutan (*Pongo pygmaeus*) and the Golden lion tamarin (*Leontopithicus rosalia*) (Stanley Price & Gordon, 1989; Beck, 1991; Kleiman et al., 1986). Captive bred members of both species suffered high mortality rates (due to predation) when re-released into the wild because they foraged on the forest floor instead of through the trees. This inappropriate behaviour was learned in captivity and measures have since been taken to prevent a recurrence of these events (see references above).

In a crisis situation, where a species has to be taken into captivity and a breeding programme initiated, it is of the utmost importance that at least some of its former habitat remains intact. The process of searching for alternative habitat,

coupled with the transportation costs to the new location, could prove expensive, not only financially, but also in terms of individual mortality in transit. In addition, it is probable that due to the inherent complexities of ecosystems, the selection pressures acting in the new environment will not be completely understood by wildlife managers, and thus that almost nothing will be known about the effects of the species on the ecosystem to which it is introduced (Wood, 1983). This could be disastrous for resident species in the ecosystem, as demonstrated by the extinction of endemic cichlids in Lake Victoria (Africa) since the introduction of the Nile perch (*Lates niloticus*) (Miller, 1989). Therefore, the conservation of natural habitats should go hand-in-hand with any captive breeding attempts. If no suitable habitat can be found, the species may have to remain in captivity for a number of generations with no representatives extant in the wild, as has been the case with Przewalski's horse (*Equus przewalski*), the Black-footed ferret (*Mustela nigripes*) and the Red wolf (*Canis rufus*) (Seal, 1991b).

In contrast to conservation in reserves, emergency attempts to save species are both difficult to organise (often projects are directed from, and take place in, countries far removed from the species' country of origin) and *extremely* expensive (Gordon, 1991). For example, the plan to save the Sumatran rhino (*Dicerorhinus sumatrensis*) will cost more than $4 million (Maguire, Seal & Brussard, 1987), the ongoing programme to save the Golden lion tamarin (*Leontopithecus rosalia*) has cost over $10 million to date (Kleiman & Beck, 1991), and the recent success in the captive breeding and reintroduction of the Arabian oryx (*Oryx leucoryx*) cost an estimated $40 million (Pilkington, 1991). To put this into perspective, it has been estimated that the cost, in 1986, of maintaining the entire Serengeti ecosystem was $0.5 million (Woodruff, 1989).

In order to finance such species-specific conservation, money frequently has to be raised from the general public. Zoos have an extremely important role to play in this context by educating the public and promoting conservation schemes (Stuart, 1991). To appeal to people's hearts, minds and pockets, zoos emphasise those schemes which involve so-called "flagship species" (Seal, 1991b). "Flagships" are threatened species that are easily recognisable, usually aesthetically pleasing and of such character that they can act as a focus for conservation plans (Norton, 1986). Typical flagships include the Giant panda, the African elephant, the Indian rhinoceros and the Blue whale (Western, 1987). (See Section 1.4 for information regarding species conservation prioritization systems.)

1.3.4 Caveat

It must be pointed out that reserves cannot be established and then left unattended. They require intense management so that the effects of natural disturbances such as drought, fire, flood, etc. can be controlled and so that those species for which the reserve was established gain the appropriate level of benefit (Foster, 1980). It is possible that a predator, or one of the threatened species' competitors, could benefit more from being in the reserve, with the result that the population of the species to be saved actually goes down (Ziswiler, 1967). Also,

without proper protection, a reserve, which by its very nature is a haven for rare and potentially valuable species, could attract poachers looking for a quick profit. Adequate security is necessary if the reserve is not to become a "supermarket" for animal traffickers.

1.3.5 What Size Should Nature Reserves Be?

As mentioned previously, the establishment of a reserve is the most straightforward way of conserving natural habitats and species diversity, but there has been some debate over the ideal size for such reserves (Diamond, 1976; Terborgh, 1976; Simberloff & Abele, 1976a, 1976b; Higgs & Usher, 1980). The argument is centred on the question of whether conservationists should favour the establishment of one large reserve or a number of smaller ones of equal size. This contention also has implications for the establishment of captively breeding populations for reintroduction; should all the representatives of a species be kept at one breeding centre or should they be dispersed as smaller groups in a number of locations? (see Section 1.4.1).

In general, a large reserve can hold more species than a smaller reserve and the extinction rate of those species is lower (Hooper, 1971; Diamond, 1975; Diamond & May, 1981). Indeed, empirical studies of islands (both geographical and ecological) have shown that the number of species in a habitat increases by an average of about 25% for every 100% increase in area (Gilpin & Diamond, 1980). However, given certain assumptions, there are situations where a number of smaller reserves can hold a greater number of species, especially if the two are established in different habitats (Gilpin & Diamond, 1980; Higgs & Usher, 1980) (see Cousins, 1991 for a review of current methods of calculating diversity indices).

However, the size of a reserve should not be calculated only in relation to overall species numbers (Diamond, 1976). Diversity cannot be interpreted simply as the numbers of different species within an area, the species present should be weighted as well as counted, e.g. a pair of Peregrine falcons nesting in a reserve have greater value than a pair of House sparrows. Since reserves are often established to save the remaining members of a particular species and not species diversity in general, single large reserves have been the usual option (Goodman, 1987).

Economic pressures and the demands of an increasing human population will almost certainly lead to reductions in the size of reserves over time and so the larger the size of the reserve when first established the better. Vermeij (1986), among others, suggests that a larger reserve provides better insurance against extinction as species are less susceptible to local bad weather conditions or sudden epidemics. The counter argument is that such an epidemic can travel through a large reserve unhindered, but is less likely to move through an entire collection of smaller reserves as each is surrounded by inappropriate habitat (Simberloff & Abele, 1976a). Also, if a species is wiped out in one of a number of small reserves, the area can be recolonized with specimens from other areas, whereas if

all representatives of a species are in the same location a disease could wipe out the entire population *permanently*. Obviously, these arguments are equally applicable to species being held in breeding centres; should all specimens be held at one location or should they be dispersed? (Neesham, 1990).

Genetics is also important when considering the size of a nature reserve. The amount of genetic variation in a population is affected by a number of factors: immigration, recombination and mutation act to increase variation while emigration, natural selection and genetic drift reduce it (Berry, 1983). Calculations have shown that the forces which act to reduce variation in a large population can be countered by the immigration of a relatively small number of individuals (Wright, 1951; Lewontin & Hubby, 1966; Lacy 1987). However, the loss of variation in a dwindling population can be so rapid that the immigration rate would have to increase many times to reach a level high enough to counter it. Such a situation is compounded by the fact that when a population is small, the amount of variation produced by recombination and mutation is negligible. In addition, small populations rapidly lose genetic variability, including extremely important elements of their gene pool, such as disease-resistance genes (Soulé & Wilcox, 1980).

When a reserve is established it effectively becomes an island surrounded by a sea of potentially rapidly changing environment (Myers, 1979). The change is due to human alteration of the unprotected land outside the reserve and as the degree of change increases, the rate of immigration (gene-flow) into the habitat will be reduced. Ultimately, if alterations are sufficiently great, immigration will stop completely (Berry, 1983). Obviously, the *natural* gene-flow into an institutionalized, captive population is non-existent, as immigration rates are controlled by the human-mediated movement of animals. A decrease in the immigration rate caused by moving a species into captivity results in a loss of genetic variation because the numbers of individuals able to breed together will be limited (Mace, 1986). In addition, average mutation rates are extremely low and, even with the additional variation produced by recombination, will probably not be sufficient to counter such loss (Berry, 1974).

The decline in the amount of genetic variation can be slowed, however, by ensuring that there is sufficient variation within the population at the time it is first isolated (see references in Mace, 1986 for a discussion of these factors). The exact amount of variation necessary will depend upon a number of factors, such as the species' genetic load, its generation time and its natural method of reproduction (assuming this is not deliberately disrupted by the zoo-managers), but the greater the initial size of a reserve, the larger the population that it can hold and, therefore, the greater the chance that sufficient variation will remain in the relict population to prevent its rapid decline. If the reserve or the population is too small, then the loss of variation could result in the extinction of the species through inbreeding (see Section 1.6.2).

In order to maintain maximum possible variation in the world population of a threatened species, it has been suggested that captive-bred individuals should

be regularly exchanged with animals in the wild, if such animals are available (Foose & Ballou, 1988; Neesham, 1990). This concept, termed the "megazoo" (individuals in the wild and in captivity being managed essentially as one population), has been used to conserve both the Red wolf (*Canis rufus*) (Mace & Ballou, 1990) and the Black-footed ferret (*Mustela nigripes*) in North America (Thorne & Oakleaf, 1991). Unfortunately, the idea is complicated by the difficulties involved in the selection and transportation of appropriate animals, the possibility of mortality during transit and the financial costs involved.

When reserves are to be established for the conservation of a particular animal, the species' behaviour must be taken into account. The World Conservation Strategy, Section 6, Part 8 (IUCN, UNEP & WWF, 1980) states that "Whenever feasible, each protected area should safeguard all the critical habitats (the feeding, breeding, nursery and resting areas) of the species concerned." Some species have seasonally or spatially patchy food supplies, necessitating a large area to supply their daily nutritional requirements, e.g. wolves (*Canis* spp.) and bears (*Ursus* spp.) (Diamond, 1976). If the size of the reserve is to be limited, it is important that at least some of the animal's major feeding areas be included.

Similarly, certain species live at low population densities and rely on particular areas within their habitats to encounter conspecifics. For example, the highly endangered Sumatran rhino (*Dicerorhinus sumatrensis*) depend on traditional communication points within their ranges (Lang, 1977). If prevented from reaching these communal meeting places they lose contact with each other and no reproduction can take place. To ensure the continued existence of such species, the absolute size of the reserve is secondary to the requirement that the animals have access to these important places.

1.3.6 Summary

To date, the answer to the question of whether to favour single large or multiple small reserves has largely depended upon the availability of financial resources and not upon considerations of species genetics or demography. The main problem with multiple reserves is that they cost more to establish than a single reserve of equal size because they require more human resources to administer (O'Connor, 1974), and since many rare species are found in underdeveloped countries, money for reserves of any kind is often scarce (Ayers, Bodmer & Mittermeier, 1991). The debate in the literature over the best size for a reserve has now subsided and, as Wilcox (1986) pointed out, the question is no longer "whether bigger is better, but how big is big enough?"

1.4 CAPTIVE PROPAGATION

1.4.1 General Introduction

If the status of an animal population is changing rapidly, insufficient time may be available to assess the change before the species dwindles to extinction

(Wood, 1983). In such a crisis situation, where reserves cannot provide the level of intense management that a threatened species requires, conservationists have to intervene and a captive propagation programme may be the only answer (Temple, 1978b). However, as pointed out by many authors (see Section 1.3.2), such programmes should never be undertaken in isolation as, if there is to be any long term hope for the survival of the species, there must be an area of protected habitat into which it can eventually be re-released.

If captive propagation is deemed necessary, it has been suggested that the breeding be undertaken in a number of institutions (Lacy, 1987). The small numbers of animals usually involved in captive breeding programmes means that there is a danger of genetic drift occurring in the stock (see Section 1.6.2). The greater the number of institutions involved in the project, the greater the chance that the drift occurring in one breeding centre will be countered (to some degree) by that occurring in another (Lacy, 1987). This is in line with Drury's assessment (1974) that because different populations of a species often differ in genetic composition, the number of sub-populations is probably of greater importance for the species' security than the total population size.

The use of multiple reserves for breeding is thought to be particularly important if the period of captivity is to be long, i.e. 30 or more generations (Lacy, 1987). Fortunately, the financial constraints that have prevented the establishment of multiple nature reserves (see Section 1.3.5) have been less of a problem for captive breeding programmes, since a species in crisis provokes a more generous response from funding bodies and the public than does the idea of general habitat conservation (see Section 1.3.3).

1.4.2 Which Species Should Be Conserved?

Since it appears probable that hundreds of thousands to millions of species will be lost over the next century and that conservation budgets will remain limited (IUCN, UNEP & WWF, 1980; Wilson, 1989), it is important to determine which species are in the greatest danger of extinction, and which of *these* it is the most important to conserve. In order to do this, it is necessary to have standard definitions of species' frequency and a system that allows conservationists to prioritise such conservation.

A number of attempts have been made to provide working definitions of rarity (see references below). Mayr (1963) proposed that a species be considered rare if it is highly localized, highly specialised, or both, while Drury (1974) has produced a more detailed definition suggesting that a species is rare if it exhibits one of three major types of distribution:

1) the species occurs as a few individuals, or small groups, scattered widely over suitable habitats;

2) members of the species are found in very small numbers widely dispersed in each community, but they occur in many suitable areas;

3) the species occurs in large numbers at very few localities.

Obviously, this list is too small to cover all possibilities and, indeed, Drury points out that the Northern fur seal (*Callorhinus ursinus*), which fulfils the criteria for category 3 (it only breeds off Pribilof and Commander Islands in the Bering Sea), is not considered rare because individual members of the species are spread widely over the world's oceans during the non-breeding season.

In 1966, the International Union for the Conservation of Nature and Natural Resources (IUCN) produced the first Red Data Book. This listed all the animals known to be extinct, or threatened with extinction, at that time and included a list of "statuses" defining seven levels of species' endangerment. The IUCN's list, updated in 1972, has been routinely used for the assignment of rarity since that time (see International Zoo Yearbook, 10-25, but cf., Mace & Lande, 1991). The IUCN system for assessing the degree to which populations are in danger of extinction was reviewed by Brambell (1985), who added an eighth status to the seven designated. (For a complete explanation of the IUCN's statuses see King, 1979 or Goodwin & Holloway, 1978). Briefly, the statuses are:

1) *Extinct*: the species has not been located in the wild for 50 years.

2) *Endangered*: the species is in danger of extinction and will not survive if current trends persist.

3) *Vulnerable*: the species is severely exploited at the present time, or is known to inhabit areas of major environmental disturbance.

4) *Rare*: the species has a small world population located within restricted geographical areas or habitats and is at risk of moving into one of the higher categories if current trends persist.

5) *Indeterminate*: the species is known to be of status 2, 3, or 4, but exact information is not available at the present time.

6) *Out of Danger*: the species was formally of status 2, 3, or 4, but is now out of danger due to its response to conservation measures, or the removal of the threats to its survival.

7) *Insufficiently Known*: the species is thought to be of status 2, 3, or 4, but insufficient data are currently available for confident assignment.

8) *Abundant*: this is Brambell's category and is intended to cover species which are not, nor ever have been, in danger of extinction.

A lack of financial resources prevents conservationists from simply saving all those species which, for example, qualify for (Red Data Book) status 2. Further complications can also arise if the species only qualifies for status 3 or 4,

but its conservation is considered absolutely essential. To resolve this problem a system of priorities had to be developed for use in conjunction with the rarity assignments. To this end, Myers (1979) proposed that species qualifying for statuses 2-5 should be further assigned to one of four priority categories depending on their assessed ecological importance:

1) the species must be saved at all costs;

2) loss of the species would almost definitely precipitate "ecological breakdown";

3) loss of the species would leave ecosystems severely damaged;

4) the species can be allowed to disappear.

In addition to these ecological considerations, the IUCN have proposed a more esoteric priority system. Section 6.1 of their "World Conservation Strategy" (IUCN, 1980) states that a species would be prioritised if it was the sole representative of its taxonomic category, the prioritization increasing with the level of the taxon, i.e. a species which is the sole representative of its family would be prioritised over one that is the sole representative of its genus.

Obviously, there are problems with these systems of categorisation and prioritization. There have been difficulties in obtaining information about species, especially rare ones, that is sufficiently detailed to allow them to be allocated to categories (i.e. endangered, vulnerable, rare, etc.) within such systems (Shaffer, 1981). Also, the systems of categorization previously available were themselves somewhat subjective as they were not numerically based (see Section 1.4.3). Myers (1983) pointed out that conflicts of interest can result from subscription to the differing systems of prioritization. For example, the conservation of a subspecies of tiger is likely to attract more attention and resources than the conservation of a beetle, even though the latter is the sole representative of its taxonomic family.

To resolve the problems caused by these different categories, a numerically based system was required. This had to be applicable to all species and had to make the assessment of the degree of species' endangerment more accurate. The prioritization system presented above by Myers (see Section 1.4.2), for example, could then be applied to these categories, aiding conservationists in their decisions as to which species to conserve. The system which was proposed, and has since been developed, is a method for calculating the "minimum viable population" for a species, a technique known as population viability analysis (see below).

Given that some conservation *has* to be carried out on an individual species level, it is important to target species with a potentially high economic return (Soulé & Simberloff, 1986). Such species have, for example, important roles in the local food webs (keystone mutualists), or comprehensive area requirements (umbrella species) (Gilbert, 1980; Wilcox, 1986; Lande, 1988). By judicious

selection of the species to be conserved, the funds generated by widely publicising a particular animal's plight could then be used not only for *its* captive propagation programme, but also to cover the cost of the conservation of its natural habitat (see Section 1.3.3).

Because many such species are at the top of their local food chains (usually, but not always, predators) and are of a large size, the preservation of sufficient land to save them will simultaneously be of benefit to the other species that share their environment. Thus, the conservation of one species that is "aesthetically-pleasing" to the public will result in the preservation of a great many far less "attractive" organisms (Kellert, 1986). This may appear to be an unscientific method of prioritising the conservation of a species, but it must be remembered that the natural habitats of many threatened species are found in underdeveloped countries where the cost of such conservation programmes could represent a substantial proportion of the gross national product. These considerations present conservationists with yet another set of priorities which, due to the shortage of funds available for conservation, must be taken into account.

1.4.3 Population Viability Analysis

The system of definitions presented above outline the criteria conservationists have used to determine whether a species was rare, but apart from the problems already mentioned, there have been difficulties in relating such numerically imprecise definitions to species in the wild (Mace & Lande, 1991). Population viability analysis (PVA) and the development of the concept of a "minimum viable population" (MVP) has solved this problem. Defined as the smallest number of individuals that must be maintained to provide a probability greater than 90% of the species persisting for the next 200 years, the MVP calculation has provided a far more precise measure for assessing species' vulnerability (Foose et al., 1986; Harris, Shaffer & Maguire, 1987; Foose & Ballou, 1988).

Population viability analysis, as the calculation of a MVP has been termed, has been extended by Mace & Lande (1991), so that the specific level of endangerment of a plant or animal can be measured as a probability of survival, i.e. 50%, 70%, 90%, etc., over a given time period. For example, a population with a probability of surviving for the next 200 years of only 20% would be "critically endangered". The working value of "200 years" was set because it was thought that a greater period of time was unrealistic in human terms and beyond the scope of current legislative systems (Mace & Lande, 1991).

The concept of the MVP was first described 30 years ago by Moore (1962), but it was almost 20 years before Franklin (1980) provided any numerical information which wildlife managers could use to determine the minimum size for their captive or reserve held populations (Denniston, 1978). Franklin combined data on the effects of inbreeding with Wright's theoretical expression relating inbreeding coefficients (F) to effective population size (N_e), the latter being the size of an ideal population undergoing the same amount of random genetic drift

as the population in question. As a result of this work, he proposed that a minimum population of around 50 individuals was required to maintain short-term fitness and to prevent inbreeding (see Section 1.6.2), and that to maintain sufficient genetic variation to allow for adaptation into a changing environment, a minimum population of 500 would be needed.

However, a number of authors (for example, Wilcox, 1986) have warned that the figure of 50 is probably the *absolute* minimum for short-term conservation (a population of this size would lose 50% of its genetic variation in only 20-30 generations). Also, Soulé (1987) has suggested that since N_e is calculated with reference to an "ideal" population (i.e. randomly-mating, equal sex-ratios, non-overlapping generations, etc.), to ensure a species' long-term evolutionary potential, a number of populations, each substantially larger than the "magic" 500, will probably be required. In addition, it should be pointed out that the numbers "50" and "500" were based on estimates derived from experiments conducted on model genetic systems, e.g. *Drosophila*, a highly polymorphic species, thus their applicability to the conservation of exotic, rare, genetically depauperate species is debatable (Benirschke, 1985; Lande, 1988).

The fact that Franklin's figures were, and in some cases still are, being used to determine the size of the populations maintained in captive propagation programmes, led some conservationists to develop the concept of population viability analysis (PVA) (see references in this section). Since Franklin's "50/500 rule" was being used as a standard for many different species, problems were foreseen due to the fact that this rule does not take any species-specific factors into account.

PVA is more accurate because this method of calculating a MVP takes into account a great deal of information specific to the species being analyzed, including the degree of genetic variability, rate of reproduction, density and type of distribution within its habitat, generation time, fecundity, viability, the age and breeding structure of the species, its present and future locations in time and space, and the time-frame for which the species is required to persist (Gilpin & Soulé, 1986; Schonewald-Cox et al., 1983; Shaffer, 1981). Thus, unlike Franklin's "50/500 rule", there is no single value for a MVP that would be applicable to all species; that calculated for a herd of African elephants, for example, will be different to that calculated for an ant colony or a tiger population (Soulé, 1987).

PVA can be used to determine whether a species is in danger of extinction, as in the case of the Yellowstone Park Grizzly bear (*Ursus arctos*) population (Suchy et al., 1985; Allendorf & Servheen, 1986). Also, by extension, such analyses can be used to calculate the ideal size for a proposed captive population, allowing zoo managers to assess the ideal size for a breeding stock (Shaffer, 1981). This has been done with a number of threatened species, including the Javan rhino, the Puerto Rican parrot, the Hawaiian crow, and Rothschild's mynah (see Chapter 3) (Seal, 1991b).

1.4.4 Summary

Systems of categorisation and prioritization allow those species that are in the greatest danger, and/or those which are essential to their ecosystems, to be identified. However, although such systems have been available for over a decade, the overwhelming majority of conservation efforts and captive breeding programmes have concentrated on vertebrates (the emphasis being on mammals and birds) (Shaffer, 1987). Innumerable species of plants, invertebrates, soil-bacteria and algae, which are vital for the maintenance of natural habitats, are likely to become extinct without any attempt having been made to save them (Slobodkin, 1986). Shaffer (1987) has suggested that until far more is understood about which species are crucial for the "continuation of basic life-sustaining processes", all phylogenetic groups should be given equal attention. Unfortunately, the implementation of Shaffer's ideal is currently impossible since zoological parks and reserves have neither the finances (see Section 1.3.3) nor the space (see Section 1.5.6) available to achieve it.

1.5 CAPTIVE BREEDING PROGRAMMES

1.5.1 General Introduction

Man has been breeding animals in captivity for millennia. It is known, for example, that the Ancient Egyptians kept numerous forms of African wildlife including leopards, giraffes, monkeys, many forms of ungulates, and numerous species of birds (Pilkington, 1991; Ryder et al., 1981). The reasons for captively breeding animals have changed over time, but this has always been due to the addition of new reasons for breeding, rather than the old reasons being superseded:

1) *Convenience*: Higgs and Jarman (1972) have suggested that the domestication of species began over 15,000 years ago in the Pleistocene epoch. This was probably the *first* reason that Man bred animals in captivity and was presumably done to reduce the time spent on hunting and following herds of herbivores as they migrated. This is still the primary reason why human societies across the world maintain stocks of animals.

2) *Education and amusement*: One of the first proper zoos known was founded in China around 1,000 B.C., and was designed as a place of "education and amusement" for the Emperor and his friends (Pinder & Barkham, 1978). Modern captive bred stocks can serve as substitutes for wild populations, allowing basic research into such areas as population biology, sociobiology, and the development of care and management techniques, e.g. the parental substitution programme that successfully used Gyr falcons (*Falco rusticolus*) to rear Peregrine falcon chicks (*Falco perengrinus*) (Conway, 1989). Education and amusement are still important driving forces for zoos today, although education has now grown to serious scientific study, and the amusement factor has been converted into financial gain (see below).

3) *Financial Gain*: Breeding species in captivity can provide monetary income in two ways: 1) visitors can be charged for the privilege of looking at the animals held, which is the way that zoos and wildlife reserves raise at least some of the income they need to support the animals they hold; and 2) the offspring of captive individuals can be sold to interested parties. Horse breeders and falconers have been indulging in this latter activity for centuries.

4) *Conservation and gene reservoirs*: In the field of conservation, captive breeding has only become an important tool during the last 20-30 years (Wayre, 1969; de Boer, 1992). However, in that time a number of species that have lost their wild habitats have been saved from extinction, e.g. the European bison (*Bison bonasus*), Père David's deer (*Elaphurus davidianus*), and Przewalski's horse (*Equus przewalskii*) (Myers, 1989).

Perry, Bridgwater & Horsemen (1972) argue that the idea of breeding animals in captivity as a "gene reservoir" was first proposed in 1889, by Smithsonian Secretary Samuel P. Langly when he said that a new zoo was to be the "home and a city of refuge for the vanishing races of the continent". Populations of rare species held in captivity represent "genetic reservoirs" from which infusions of "new blood" may be obtained to maintain variation in a captive breeding stock, or from which individuals can be drawn to establish new populations. In fact, it is now possible to preserve species in the form of frozen sperm, eggs, and/or embryos; a concept known as the "frozen zoo" (Durrell, 1975). Freezing tissues in this way preserves the genomes of the species without the necessity for holding live specimens and considerably reduces the cost to the zoo manager. In addition, the periodic exchange of gametes with wild populations or captive stocks can potentially extend the reproductive life of an individual after its death, a consideration which becomes increasingly important with declining population size (Ballou & Cooper, 1992).

Of these reasons for captive breeding, the one to which this thesis is addressed is conservation. As pointed out above, breeding animals in captivity for the purposes of conservation is a technique used only recently, in addition to which its use has been almost exclusively as a "last resort". Captive breeding/reintroduction programmes are only attempted after the standard techniques of reserve establishment, public education, and legislative action have failed to reverse the decline of a species (Scott & Carpenter, 1987). Once it has been determined that a species is in need of assistance, a Species Survival Plan (SSP) is drawn up by one of the conservation bodies, e.g. the American Association of Zoological Parks and Aquariums (AAZPA). This is done in conjunction with one or more of the institutions whose role it is to organise the captive breeding required for the project. The necessary financial resources are acquired by organisations such as the World Wide Fund for Nature (WWF) which also co-ordinate such plans on a global basis (Ziswiler, 1967), a necessary role as an SSP consists of many parts, all of which have to be carefully negotiated through any difficulties (Chivers, 1991).

1.5.2 Quantification and Qualification

Field personnel should be able to give an initial assessment of the status of the major species living within the ecosystem they are studying (Jungius, 1985). If a species is thought to be threatened and formal quantification has not already been used to determine the degree of endangerment faced by the species, then a population census should be conducted with a population viability analysis being performed if deemed necessary (see Section 1.4.3). The information thus obtained can be used to determine if the species is genuinely threatened and, if so, how great the threat.

During this initial stage, it is essential to identify the cause(s) of the species' decline so that counter-measures can be taken against any ongoing threats in the natural environment (Temple, 1978b; Plunkett, 1978). If the species is threatened by human persecution, plans must be made to stop this either by public education or legal enforcement. If the species is threatened by competition or predation from an introduced species, that threat must be removed. For example, before the captive-bred stocks of the snail *Partula* can be reintroduced to the Pacific island of Moorea, it is imperative that the predatory mollusc *Euglandina*, which caused the former's extinction, is removed (Wells, 1988).

The taxonomic status of a species thought to be in danger must be confirmed. Many species were given taxonomic assignments before the beginning of this century, with such classifications often being based on morphological traits alone, the genetic foundations of which are largely uncertain or unknown (Avise, 1989 and 1992; Ryder, 1987). These traits can be misleading and molecular genetic techniques should be used to secure accurate taxonomic information if limited funds are not to be wasted on simple polymorphisms (Conway, 1989). For example, there is currently argument regarding the Red wolf (*Canis rufus*) which is being conserved in North America by the creation of a new reserve, even though it is not morphologically distinct from the coyote (Moore & Smith, 1991; Gittleman & Pimm, 1991). There is also the consideration of "cryptic" species which, if not identified, could cause problems within breeding programmes (see below).

There has been some debate as to whether recognised infra-specific taxa should be considered for conservation (Lyles & May, 1987). Frankel & Soulé (1981) pointed out that since the criteria used to differentiate between subspecies is frequently "quite arbitrary", conserving subspecies individually is of dubious merit, and so the limited funds available for conservation should be concentrated on fully confirmed species. However, Sir Peter Scott (1975), among others, supported the conservation of subspecies since they represent "the birth of...new species" and, as such, should be "respected". In addition, because subspecies only differ slightly from one another, and the data obtained from their comparisons are often the easiest to interpret, they are considered by some biologists as ideal material for the study of evolution (Kear, 1977).

Sometimes infra-specific distinctions have to be ignored in captive

propagation programmes due to the extremely small number of animals available and the need to maintain as variable a gene pool as possible (Conway, 1967). In addition, the proposed cost of a project must be considered. For example, the Spider monkey (*Ateles spp.*) has 17 subspecies, all of them endangered, and to conserve all 17 separately would be extremely expensive (Birchall, 1990). It is therefore likely that the individual subspecies will have to be "combined" whilst in captivity.

Where subspecies have to be mixed (for example, in an attempt to balance saving at least part of the species group against the most economical use of the funds available), it is hoped that after release natural selection will act on the hybrids to produce the correct "locally-adapted" genotype (Lyles & May, 1987). Such a philosophy has successfully been employed to save the European bison (*Bison bonasus*) from extinction, the current population being descended from 16 Polish animals (*B.b.bonasus*) and one Caucasian male (*B.b.caucasius*) (Kear, 1977).

Unfortunately, the hybridisation of subspecies is not always successful. Hybrid offspring can exhibit deleterious characteristics such as reduced fertility, sterility, etc. (see references below). This can be caused by any one of a number of factors, such as differences in the chromosome number (Benirschke, Lasley & Ryder, 1980) and/or the presence of incompatible chromosome inversions in the parent subspecies (Benirschke, 1983), disrupted genetic control of breeding cycles, again due to differences between the parent subspecies (Greig, 1979), and reduced fitness caused by the disruption of advantageous gene-complexes, known as "outbreeding depression" (Ralls & Ballou, 1983).

1.5.3 Logistics

Political decisions need to be taken over the acquisition and housing of the wild and/or captive individuals around which a breeding programme is to be developed (Stanley Price, 1991). The appropriate government departments in the species' country of origin, or current captive-location, need to be approached and the requisite licences obtained. Such contacts need to be as "friendly" as possible since it may be necessary to acquire further animals in the future by capture, trade, or purchase, to supplement the breeding stock. Obviously, similar authorizations are also necessary from the country in which the breeding is to take place. The complicated matters of travel, health checks, customs and quarantine need to be addressed so that losses in transit are kept to a minimum. It is necessary to ensure that a suitable natural site is available into which the animals can be re-released and firm commitments (legal protection) need to be obtained for the conservation and the sanctity of the re-release area, since the breeding programme may take a number of years (see Section 1.5.5).

1.5.4 Breeding Programmes

The establishment of a successful breeding programme is dependent upon a knowledge of the species' natural needs and an understanding of how those

needs relate to its requirements in captivity (Plunkett, 1978). The effects of activity cycles, food type and availability, population density, sex-ratio, age of first breeding, duration of reproductive capacity, mean number of offspring produced, genetic background of the individuals, etc., all need to be understood if a *sustained* production is to be achieved (Eisenberg & Kleiman, 1977; Lande, 1988).

It is often the case that a breeding programme commences with a limited understanding of the species and acquires additional information as the animal is studied in captivity (Myers, 1979; O'Brien et al., 1985). A lack of information about the genetic composition of a captively held species and the relationships between breeding individuals can result in genetic drift, a loss of genetic variation and inbreeding (see Section 1.6.2). To try and alleviate some of these problems, it should be ensured that the founder members of a captive stock are a large (if possible) and varied sample of the remaining natural population (Griffith et al., 1989). Also, it should be ensured that any additional individuals acquired for the programme, either from other institutions or from the wild, are as genetically diverse as possible.

Past experience has shown that it is important to keep accurate records of the composition of breeding units, so that individual fertility can be monitored (for example, see Fisher, 1991). The recording of reproductive success for all individuals at all stages can allow problems such as diminished viability, low growth rate, or general failure to thrive (all of which can be evidence of inbreeding depression) to be detected (Falconer, 1981).

The measured fertility of an individual consists of two components: 1) fecundity (the number of offspring produced) and; 2) viability (the number of offspring that survive to breeding age). The technique of DNA fingerprinting (see Section 1.7) can be used for accurately monitoring fecundity because it allows individuals to be assigned to parents, whether the individual in question is alive or dead. If declines in quantitative characters, as described above, are noted, they should be countered either with new breeding strategies or, if available, freshly acquired stock.

A species in decline may often have additional problems other than inbreeding depression, such as the Allee effect, i.e. non-genetic declines in viability and reproductive success (Lande, 1988). These are caused by the number of individuals in the population dropping below a threshold density, affecting a species which, for example, modifies its environment to promote its own reproduction, or depends upon social interactions to efficiently reproduce. If these interactions are limited or non-existent, the species numbers will decline. These additional problems, encountered by small groups of species held in captivity, are essentially beyond a zoo manager's ability to control. Given this, it would be irresponsible to allow *preventable* losses in genetic variability (viability) to occur within a managed captive stock.

Seventy years ago, the major animals exhibited by zoos still existed as wild natural populations, thus losses were easily replaced and major institutions had

little incentive to maintain viable breeding stock (Wayre, 1969). This is no longer the case; many wild populations have declined drastically and the presence of strict legislation governing the sale and transport of wild animals, e.g. the Convention of International Trade in Endangered Species (CITES), has forced zoos to breed their own exhibits. In addition, the increasing public awareness of environmental conservation has meant that the right of zoological parks to exist at all is being challenged (Slobodkin, 1986; Pilkington, 1991).

During the past decade, zoos have been transferring resources to captive breeding projects and attempting to distance themselves from the speculative collecting of endangered wildlife which, unfortunately, still occurs (Brambell, 1985; BBC et al., 1991). The claim that zoos have an important role to play in educating the public in support of conservation, and in actual research, is becoming increasingly valid (Benirschke, 1983; Rawlins, 1985). Data obtained from the study and breeding of captive animals (life span, age of sexual maturity, embryonic development, clutch or litter size, etc.) can be of great value for future reintroduction programmes involving related but more threatened species (Martin, 1975; Conway, 1989). Added to this are the zoos' roles as refugia for living specimens of endangered taxa, as gene banks for the storage of frozen gametes and as international fund-raisers (Foose, 1983; Holt, 1992).

There have been a number of studies assessing the contribution of zoological parks to conservation (for example, see Perry, Bridgwater & Horsemen, 1972; Pinder & Barkham, 1978). In a recent study, Rawlins (1985) assessed the breeding success of 20 major zoos and found that across all vertebrate species, the percentage of the stock that had been captively bred increased from 8.3% to 22.1% between 1964 and 1982. If only those species listed in the IUCN Red Data Books as "rare" to "endangered" were considered, the results for the same period show an increase from 36% to 71%.

This information is good news for two reasons: 1) the breeding of rare species makes them less rare; and, 2) if zoos are able to maintain or exceed their chosen quotas of rare species, they can sell or exchange the excess with other institutions, removing the need to acquire further animals from the wild (Rawlins, 1985). As emphasised by Brambell (1985), "There is no reason why in future the responsible zoos...should not be stocked with zoo bred animals".

1.5.5 Reintroduction

It is obvious that the original cause of a species' decline must be identified and removed as a precondition for any reintroduction attempt (Jungius, 1985). Assuming that a reserve has been established, there will be logistic and political problems, as mentioned above, associated with maintaining a certain level of security for the habitat to which the species is reintroduced (Plunkett, 1978).

When reintroduction goes ahead, it is important that the animals chosen for the project are a varied sample of the captive stock, that the sex and age ratios are appropriate to the species in question, and that the release is performed in a

controlled and systematic manner with due regard for the demography of the species being released, e.g. that reintroduction is timed to the appropriate season (Lyles & May, 1987).

For any reintroduction programme to succeed the local community must be informed and educated about the project. If a reintroduction project is not made acceptable to the people living in the release area, it may fail because of local human animosity/attraction to the species being reintroduced. Education of the local peoples is particularly important when potentially dangerous animals, e.g. the tiger (*Panthera tigris*), are to be released and/or where the project is expected to take a number of years to complete (Stanley Price, 1989a). LaBastille (1978) believed this kind of information distribution to have been crucial to the successful reintroduction of the Giant Pied-billed grebe to Lake Atillán in Guatemala.

The importance of post-release monitoring has been stressed by a number of authors (Stanley Price, 1989a; Kear & Berger, 1980; Berger, 1978). Such monitoring means that data can be collected regarding the success of a reintroduction programme, i.e. the numbers of individuals, the sex and age ratios, etc., and allows conservationists to make informed decisions regarding the amount of further species' management required. Obviously, since the ultimate aim of a reintroduction programme is the establishment of a self-sustaining wild population, data on the status of the population at various stages after its release would be required to prove the success of a project and to make such claims acceptable to the scientific community (Scott & Carpenter, 1987). In addition, if evidence is provided for the failure of a project, the causes can be analyzed and the "mistakes" avoided by future project managers.

1.5.6 Summary

In the absence of knowledge regarding the true biological relationships of the founders for a captive propagation programme, there should be a regular programme of mixing and exchange between breeding units, as this can help to alleviate inbreeding and deleterious adaptations (Bennett, 1990). Individuals can be exchanged between captive stocks, or between captive stocks and wild populations, a concept known as the "megazoo" (Neesham, 1990). For this management method to work, there needs to be consultation and agreement between the relevant bodies, i.e. governments and managers, as the distinction between management of captive stocks and management of natural reserves breaks down (Mace & Ballou, 1990).

Population analyses provide zoo/reserve managers with a basis for formulating both the goals of population management and the specific breeding recommendations for each individual in the population (Ballou & Cooper, 1992). Such recommendations specify which animals are to breed and with whom, and how many offspring they should be allowed to produce. The accurate recording of information regarding relationships is extremely important as these analyses are complicated when critical pedigree information is missing or incomplete. The acquisition of such data should be ameliorated by the use of molecular techniques,

such as DNA fingerprinting, which allow individuals to be unambiguously identified and aid in the construction of family trees (see Chapter 3, Section 3.3).

The increase in the numbers of rare species bred in zoos means that larger viable stocks of threatened animal species are being maintained than ever before (see International Zoo Yearbook, 13-27). The hope is that, ultimately, these captive populations can be used as a source of founder individuals for introduction, reintroduction and restocking programmes. Some of the reintroductions already attempted, with variable success, include the Hawaiian goose (*Branta sandvicensis*) (Wayre, 1969), the Trumpeter swan (*Olor buccinator*) (Denson, 1970), the Orangutan (*Pongo pygmeaus*) (Aveling & Mitchel, 1980), the Spider monkey (*Ateles belzebuth*) (Konstant & Mittermeier, 1982), the Indian rhino (*Rhinoceros unicornis*) (Sale & Singh, 1987), and the Arabian oryx (*Oryx leucoryx*) (Stanley Price & Gordon, 1989).

The fact that more species have not been reintroduced is largely due to the political and economic circumstances that persist in some parts of the world. Zoos around the world hold sufficient individual representatives of a number of threatened species to start reintroduction schemes (Rawlins, 1985), but it would not be prudent to attempt such reintroductions in, for instance, a country racked by war and famine. This means that many species will have to remain in captivity for the foreseeable future, making the role of zoos as sanctuaries for the remaining members of threatened species very important.

Apart from the inherent difficulties that maintaining species in captivity for long periods of time presents, there are additional problems:

1) if 75% of the space currently available in zoos around the world was devoted solely to conservation, then only 800 of the estimated 2,000 threatened species could be accommodated (Tudge, 1988).

2) many rare species have had poor breeding records in captivity. Animals such as the large felids, penguins, condors, and fruit bats have failed to thrive under captive propagation conditions (O'Brien et al., 1985; Myers, 1979; Carroll, 1979a and b). Even if the species that are endangered could be accommodated, it is by no means certain that they would survive in captivity.

Current demographic theory predicts that the human population will rise to 10 billion by the middle of the 21st century and, barring a major disaster, will not start to decline for 500 to 1,000 years (Tudge, 1988). During this period it is the task of the breeding institutions to maintain their captive stocks and the genetic variation within them so that when species are reintroduced, they retain sufficient variation to allow for their evolution.

The sheer number of species in need of assistance is already beyond our capacity to deal with and the situation will get worse as the human population grows. The task of preserving animals for a better future has given zoos the

mantle of the Biblical ark, but this time, before the animals are released, it is the tide of humanity that must subside.

1.6 MAINTENANCE OF GENETIC VARIATION IN CAPTIVE STOCKS

1.6.1 General Introduction

Since genetic plasticity is crucial for the evolution of a species in a changing environment, it is important that zoos maintain as much genetic variation as possible in their stocks. To do this, zoo managers must be able to monitor the levels of this variation in some way. Wayne et al. (1986) have suggested that three approaches be used:

1) collection and analysis of pedigree data;

2) molecular resolution of protein and DNA variation, and;

3) analysis of morphological variation.

This thesis is concerned with the relationship between the first two of these three.

Monitoring genetic variation can be crucial for the success of a reintroduction programme utilising captive stocks. Research has shown that high heterozygosity in natural populations (the percentage of observed genotypes at which the average individual is heterozygous) is positively correlated with an individual's rate of development, adult body size, social dominance and fecundity (for example, see Cothran et al., 1983; Baker & Fox, 1978). In addition, direct links have been found between a paucity of such variation and impairment of reproductive function, e.g. an increase in the production of abnormal spermatozoa (Wildt et al., 1987).

Recently, molecular monitoring has been facilitated by the development of a number of techniques (see Section 1.6.3), the most recent being DNA fingerprinting (see Section 1.7). This technique is extremely powerful as it allows specific individuals to be identified from a sample of almost any tissue, at any time of life (Jeffreys, Wilson & Thein, 1985a). In addition, DNA fingerprinting can allow the allocation of offspring to parents or the confirmation of such parentage allocations presented in pedigrees and stud books (see Chapters 3-6).

The potential value of this technique to captive breeding programmes is obvious; the confirmation/refutation of parent/offspring assignments allows the construction of accurate family trees and the compilation of stud books. Since pairings based on incorrect assignments can lead to unforeseen increases in inbreeding (see Section 1.6.2), the mating of individuals previously thought to be unrelated can be avoided (see Chapter 3). In addition, by identifying the genetic similarities between individuals, the most dissimilar animals can be paired. The success of such an approach is, of course, dependent upon the differences in the

minisatellites detected by DNA fingerprinting being a good reflection of the general degree of genetic differentiation between two organisms.

Less inbreeding means that a population will retain a greater level of genetic variability over time. This improves the species' chance of successfully evolving in response to selection pressures present in the environment into which it is re-released. By the time a species has declined sufficiently to warrant attention, the level of genetic variation in the population is usually low, and it is therefore important to emphasise the retention of what little variation remains (Denniston, 1978).

1.6.2 Declines in Variation

Zoo managers have to solve many problems when establishing captive-breeding programmes: demographic problems including unexpected failures in reproduction, distortions of the age and sex ratios, and severe fluctuations in births or deaths can reduce the chance of a breeding programme being successful (Foose & Ballou, 1988). There are also the problems associated with small/relict populations, namely random genetic drift and inbreeding, both of which have been extensively studied in the wild (Greenwood, Harvey & Perrins, 1978; Ralls & Ballou, 1983; Allendorf & Serveen, 1986; Gilpin, 1987; Packer et al., 1991b) and in captivity (Ralls, Brugger & Ballou, 1979; Ballou & Ralls, 1982; Ralls & Ballou, 1986; Laikre & Ryman, 1991). The chance of accidental inbreeding within captive stocks is increased because the genetic background and relationships of individuals are frequently unknown (Foose & Soulé, 1981).

The paucity of genetic variability in many captive stocks is due to zoo managers making relatively little attempt to avoid inbreeding, maintaining small effective population sizes, and being reluctant to separate well established/productive pairings, even though the members of the pair are closely related (Ryder et al., 1981; Foose & Soulé, 1981). As a consequence, considerable variation has undoubtably been lost from zoo-maintained species. Unfortunately, the exact extent to which variation has been lost is difficult to assess due to a lack of knowledge regarding the degree of variability present in the founders at the start of a captive propagation programme, and the subsequent incomplete nature of many breeding records and stud books.

There are three main methods by which reductions in the number of alleles within a population, and hence a decline in the amount of genetic variation exhibited by that population, can occur. These are genetic bottlenecks, genetic drift and inbreeding. Presented below are details of these phenomena and their effects on populations:

1) *Genetic bottlenecks*. If the size of a population is suddenly reduced, by disease for instance, and then the cause of the reduction is removed allowing the remaining individuals to reproduce and increase the size of the population again, that population is said to have been through a "genetic bottleneck". Since by definition, only a relatively small number of individuals pass through a bottleneck

(the minimum population size that a species has experienced), a species emerging from one is equivalent to an equal number of individuals founding a new population either in captivity or on an island (Frankel & Soulé, 1981). In both cases, genetic (allelic) variation is usually lost as the individuals surviving the population constriction are unlikely to contain the full range of alleles previously present in that population (Nei, Maruyama and Chakraborty, 1975). This loss of alleles, particularly the rarer ones, may be advantageous if those lost are deleterious, or disadvantageous if those alleles produced phenotypic characters that would help a species adapt in the event of environmental change.

A number of species are either known or thought to have survived genetic bottlenecks in their recent evolutionary past. The Elephant seal (*Mirounga angustirostris*) experienced a bottleneck at the end of the last century, when censuses show that the population was reduced to just 20 individuals (Bonnell & Selander, 1974). The cheetah (*Acinonyx jubatus*) has such a lack of variation that reciprocal skin-grafts between unrelated individuals are not rejected (O'Brien et al., 1985). This apparent uniformity in their major histocompatibility complex is attributed to a previous severe bottleneck in the species' evolutionary past. The Arabian oryx (*Oryx leucoryx*) was hunted to extinction in the wild during the 1960s (Dolan, 1976), but, fortunately, the species survived in captivity. Today, the entire world population can be traced back to approximately 13 individuals (Ryder et al., 1981).

2) *Genetic drift.* Animal populations usually produce more progeny than will survive to reproductive age with the alleles present in each offspring varying from individual to individual. If the mortality within the offspring is not uniform across all genotypes, those surviving will carry an array of alleles with frequencies different to those in the parent generation (Foose & Ballou, 1988). This can result in the progressive, random loss of variation (heterozygosity) occurring due to a limited, and therefore incomplete, sample of a species' allele compliment being transmitted to the next generation (Denniston, 1978). The frequencies of the alleles within the population can therefore be said to have "drifted" from one set of values to another, giving rise to the phenomenon known as "genetic drift".

Because offspring inherit approximately half their genetic material from each of their parents, half of each parent's genome is not transmitted to a single offspring. If those parents do not produce any further offspring, alleles within the half of the genome that is not inherited will be lost (Lacy, 1987). Obviously, only alleles unique to the parents involved would be irretrievably lost from the population (notwithstanding mutation), but if a species was to pass through a number of generations producing few offspring, many low frequency alleles could be lost. For example, the proportion of heterozygosity that remains when a population passes through a bottleneck can be approximated with the equation $(1 - {}^{1}/_{2N})$, where N = the number of individuals remaining in the population. The expected proportion of heterozygosity remaining after t generations is therefore $(1 - {}^{1}/_{2N})^{t}$. If a population remained at approximately 30 individuals for 10 generations, a plausible scenario for Rothschild's mynah (see Chapter 3), then that population would lose approximately 15% of its genetic variation.

A species with a small population size (i.e. less than a few hundred individuals) may lose considerable allelic variation as the result of such drift. Maintaining heterozygosity is important in outbreeding populations as any loss of variation reduces that population's fitness, and as Frankel & Soulé (1981) point out, there is no "safe" level of fitness detriment for a normally outbreeding population.

Without strict management, the general loss of variability results in a reduction in the number of alleles present in the population, leading to the expression of deleterious, normally recessive alleles through the production of homozygous individuals (Soulé, 1985). Theoretical analyses have shown that this loss will be more rapid in smaller populations and that the rarer the allele the more quickly it will be lost (Falconer, 1981; Lande & Barrowclough, 1987).

If the reductions in allelic diversity are sufficiently great, and the deleterious recessives borne by the species are not lethal when homozygous, alleles can become fixed (no other alleles exist at that locus) in the population, reducing the fitness of the species. Because the efficacy of natural selection is a function of the effective population size, when this is low (less than 100 individuals) natural selection is almost powerless to counteract the fixation of these non-lethal, deleterious alleles (Lacy, 1987). However, when $2N_e$ is greater than 1000, genetic drift is negligible, and selection can "cleanse" a population of such genes (Frankel & Soulé, 1981).

If a population of a species is "fixed" for a particular gene, then every individual in the population has two copies of the same allele at that locus and there is thus no variation present in that gene. This means that there no "choice" for selection to make and the species cannot evolve with respect to that gene (unless, of course, mutation produces a new allele). The greater the number of homozygous loci, the less opportunity the species has to evolve. This is why it is important for a captive species to produce many offspring during the early stages of a propagation programme, so that as large a proportion of the population's allele complement as possible is transmitted to the next generation.

3) *Inbreeding*. Another problem faced by captive stocks is inbreeding. The term inbreeding is generally used to describe matings between individuals related at the level of first cousins or higher (Blouin & Blouin, 1988). Such matings can result in the homozygous expression of alleles which are "identical by descent"; that is, both alleles at a locus can be traced back to one allele on one chromosome of a recent progenitor/ancestor. Inbreeding has three major consequences (Hooper, 1971):

1) differentiation of populations;

2) genetic uniformity within populations, and;

3) an increase in the frequency of homozygous genotypes.

This last factor is especially important for threatened species as almost every species carries a number of alleles, held as recessives and/or in epistasis, which would be seriously deleterious or lethal if homozygous (Lovejoy, 1978).

Inbreeding results in the gradual accumulation of homozygous deleterious alleles in offspring, a reduction in the expression of heterozygous superiority, and the disruption of polygenic characters, a combination of which cause a drop in the overall fitness of the population (Falconer, 1981). Increased levels of homozygosity can expose deleterious recessive alleles to the action of natural selection resulting in inbreeding depression which is detrimental to normal development (Foose & Ballou, 1988). Inbreeding depression is characteristically manifested as general decreases in body size, fecundity and viability, although there are some apparently species-specific effects, e.g. blindness in Rothschild's mynah (*Leucopsar rothschildi*) (Schmidt, 1983). If these decreases in fitness are sufficiently severe, they can lead to the elimination of the population (Falconer, 1981; O'Brien et al., 1985; Wildt et al., 1987).

Different species have different tolerances to various levels of inbreeding before inbreeding depression (the fitness cost of inbreeding) is manifest (Avise, 1989; Baverstock et al., in press). The breeding biology of the Naked mole rat shows that inbreeding is not always deleterious (Young, 1990). Susceptibility to inbreeding depression is related to the amount of inbreeding with which a population has evolved. It is also dependent upon the proportion of lethal and deleterious alleles within the population. If this is high, then the effects of inbreeding can be disastrous. If the number of such alleles is low, as it is in the cheetah (*Acinonyx jubatus*), then inbreeding has little effect (O'Brien et al., 1985). However, given that there is wide variation among species, small populations will be more likely to exhibit inbreeding depression than large populations. This is because individuals within a small population are more likely to be related and there is an increased likelihood of a loss of allelic variability due to the action of random genetic drift.

The degree of inbreeding in a group of individuals can be measured by calculation of the inbreeding coefficient, F, the probability that two alleles present in one individual are identical by descent (Wright, 1921). F ranges from 0-1, 0 representing no inbreeding, and 1 representing an individual who is homozygous for every allele, each of which can be traced back to the same recent progenitor/ancestor. However, since all individuals will have a common ancestor if their lineage is traced back far enough, F is always defined with reference to some base population and is therefore a relative rather than an absolute measure (Ralls & Ballou, 1983). For example, if the mean F = 0.25 for a population, then 25% of the variation present in the original animals has been lost.

For captive stocks, the base population for inbreeding coefficient calculations is usually defined as those individuals in the pedigree beyond which no further information is available (Ralls & Ballou, 1983). These may be wild-caught founders or those individuals that existed in a population when the first pedigree was recorded. Unfortunately, such calculations can often underestimate

F, since such individuals are assumed to be unrelated and this is not always so (see Chapter 3).

1.6.3 Monitoring and Maintenance of Variation

One method of reducing the rate at which genetic variation is lost in captive animal stocks was suggested by Wright (1921), and is known as the "maximum avoidance of inbreeding". This method involves mating those individuals that are the least related in each succeeding generation, and it has been estimated that for a captive stock of 50-100 animals, this breeding scheme would preserve about 50% of the genetic variation over 100 generations (Flesness, 1977). Such a period of time may be sufficient to allow our descendants to recreate natural habitats into which such captively bred species can be reintroduced.

Unfortunately, there is a problem with this scheme in that it relies on the relationships between the animals to be mated being known (so that the least related can be determined), and unfortunately, for a large proportion of captive zoo stocks, this information has not been available (O'Brien et al., 1985). Improvements in the situation have, however, been made as a consequence of zoos using a variety of molecular techniques to monitor their stocks (Ryder et al., 1981; Wayne et al., 1986). These have included blood typing studies, protein gel electrophoresis, chromosome banding analysis (karyotyping), and the use of restriction fragment length polymorphisms (RFLPs) (Benirschke et al., 1980; Ryder et al., 1981; Wayne et al., 1986; Mace & Ballou, 1990; Wolfes et al., 1991). Data obtained from such studies have been used for parentage determination, heterozygosity analyses, evolutionary assessment and systematic assignments (Avise, 1989). However, some of these techniques have failed to produce adequate data when used to deduce the structure of small, highly inbred populations (see Section 1.6.4).

The standard way to present the relationships in a population is to construct a pedigree or family tree. This allows relationships to be seen "at a glance", eliminating the need to read through pages of breeding records, and making the monitoring of proposed or established relationships far easier. Traditionally, pedigree data for wild populations, especially birds, have been determined by field observations of matings between individually-marked specimens (Baverstock et al., in press). Unfortunately, the observer is unlikely to be able to monitor all the individuals in a population for 24 hours a day, and the accuracy of parentage assignments derived from such studies has been found to be adversely affected by phenomena occurring out of sight of the observer, e.g. extra-pair copulation (mating outside an established pair).

This problem can also occur in those zoo stocks where a number of individuals are allowed to freely interbreed. Obviously, where a single pair of individuals is retained in accommodation separate from others of the same species, the chance of an extra-pair copulation occurring is zero, and any subsequent failings in the pedigree or studbook data must be due to human error (see Chapter 3, Section 3.3).

1.6.4 Protein Analysis

Until the middle of the 1980s, one of the most frequently used techniques for assessing the relationships between individuals was protein gel electrophoresis. Electrophoresis works on the principle that proteins will migrate through a (starch) gel under the force of an applied electric current (Hubby & Lewontin, 1966). The distance that the individual proteins move (relative to each other) over time is determined by the protein's charge which is, in turn, determined by its amino acid composition. When the electrophoresis is complete, the positions to which the allozymes (different allelic forms of the protein) have moved can be detected by staining. Because differences in the electric charge of proteins are the result of amino acid differences between them, they in turn reflect the nucleotide sequence of the DNA encoding them. However, proteins allow only an indirect method of studying the genome (Berry, 1974) because the coding system for the DNA template from which they are produced contains redundancies (King & Jukes, 1969).

The nucleotide sequence of DNA is transcribed into mRNA which is then "read" by ribosomes, in sets of three bases known as codons. Since there are four bases in RNA, there are 64 possible codons. Three of these are "stop" codons and the remaining 61 are used to correctly arrange the transfer RNAs to produce a protein. Since there are only 21 amino acids, all except two (tryptophan and methionine) are coded for by more than one codon, hence the redundancy or degeneracy of the code. This redundancy means that some changes in the nucleotide sequence, specifically those at position 3 (the "wobble" position) in the codon, are not translated into differences in the amino acid sequence of a protein. For example, the codons GCA and GCG both code for the amino acid arginine. The proteins produced by two alleles whose DNA sequences differed only in one base pair at the third position of this particular codon would be electrophoretically indistinguishable. Thus, levels of genetic variation tend to be underestimated by this technique as only 25% of nucleotide substitutions are detectable (Flavell, 1991).

In addition, the data derived from enzyme loci are further weakened by other factors. Many mammal and bird species show electrophoretic variation at only about 10% of their loci (Berry, 1974) and the allele frequencies at these polymorphic loci are sufficiently skewed that the majority of individuals within a population are of one or two genotypes (Wetton et al., 1987). A number of enzyme systems can only be examined post-mortem so that these loci are of little use if the animals to be studied are to be left in the wild (Wetton et al., 1987). Bands that migrate to the same position on a starch gel may represent more than one allele with the same electric charge, but differing biochemical properties (Aquadro & Avise, 1982). The majority of proteins studied are soluble, since these are the easiest to work with, but these represent a biased sample of structural proteins and are not representative of other loci. Because of these problems, allozyme electrophoresis is restricted as a tool for studies of behaviour and parentage assignments (Quinn et al., 1987).

These problems are exacerbated when protein gel electrophoresis is applied to conservation genetics, as the populations involved tend to have a paucity of allozyme variation (Powell, 1983). For example, it is known that the cheetah (*Acinonyx jubatus*) (O'Brien et al., 1985), the lion (*Panthera leo*) (Wildt et al., 1987), and Rodrigues fruit bat (*Pteropus rodricensis*) (see Chapter 4) exhibit low allozyme variability. The Elephant seal (*Mirounga angustirostris*), which was close to extinction at the start of this century, also shows a very low level of genetic variation (Bonnell & Selander, 1974). Of 21 proteins examined in 159 seals, all were found to be monomorphic.

Electrophoretic studies on allozymes do not have sufficient resolution to confidently exclude individuals from the possible paternity of offspring and this failure to accurately allocate offspring to parents can hamper some of the methods used to study populations, such as heritability analyses and estimates of the effective population size (Burke, 1989). Over the last 25 years, an array of techniques has become available that by-pass these problems by detecting variation in the DNA itself. These include restriction fragment length polymorphisms, DNA sequencing and DNA fingerprinting (see Sections 1.6.5 and 1.7).

1.6.5 DNA Analysis

Studies on restriction fragment length polymorphisms (RFLPs) began at the end of the 1960s when restriction enzymes were first recognised (Meselson & Yuan, 1968) and, since then, several hundred RFLPs have been discovered, both in humans and other species (Lewin, 1986). RFLPs are analyzed by cleaving whole, genomic DNA with one or more restriction enzymes to produce DNA fragments of various sizes. These fragments are then separated by gel electrophoresis and detected using radio-labelled probes.

Obviously, if the individuals tested were genetically identical, the same pattern would be produced, but because restriction sites can be created or destroyed by mutation (addition, deletion, point-mutation, etc.), individuals have different sets of fragments, hence the name (Lewin, 1986). Quinn et al. (1987) found that RFLPs were inherited as co-dominant, Mendelian alleles, but that reliable parentage assignments required many probes and digests making the technique expensive and tedious. Also, RFLPs suffer from some of the same drawbacks as allozyme studies, the most important of which is that the majority of polymorphisms come in just two forms, limiting the usefulness of RFLPs for genetic analysis (Jeffreys, 1979; Jeffreys, Wilson & Thein, 1985a).

DNA sequencing, as the name suggests, is a method of determining the exact nucleotide sequence of a fragment of DNA. The two techniques currently used were developed by Sanger et al. (1977) and Maxam & Gilbert (1977). Both techniques generate radio-labelled oligonucleotides which start from a fixed point, but which terminate randomly at a specific base determined by the contents of the reaction mixture. As there are four bases (adenine, thymine, guanine and cytosine), a sample from each reaction vessel is loaded into one of four separate, but adjacent wells in a polyacrylamide gel. The length differences between the

fragments are resolved by electrophoresis under conditions which allow discrimination at the level of one nucleotide. The positions to which the oligonucleotides migrate are detected by autoradiography and the resulting ladders can be read giving the exact sequence of nucleotides in the fragment of DNA tested. This type of sequencing allows the variation between individuals to be assessed directly from the DNA, but, unfortunately, the technique is time-consuming and complex, and only short sequences of base pairs (300-400 base pairs) can be analyzed at one time (Sambrook, Fritsch & Maniatis, 1989).

The technique of DNA fingerprinting was first reported by Jeffreys, Wilson & Thein (1985a) and uses human derived probes to simultaneously detect a number of loci within a genome. This provides a faster and more effective alternative than RFLPs and sequencing, and can be applied to almost any species (see refs. in Section 1.7). The effective use of DNA fingerprinting in birds (Burke & Bruford, 1987; Wetton et al., 1987) for the confirmation of parent/offspring allocations and the detection of cases of non-paternity, has led to the technique being used in several long-term population studies. The background to the technique is covered in greater detail in Section 1.7 and the materials and methods involved are presented in Chapter 2.

1.6.6 Summary

The foundations of major conservation programmes are dependent on accurate information about species' genetic composition, demography, frequency, ecology, and behaviour. Data regarding the genetic composition of a species have traditionally been acquired using protein gel electrophoresis and certain forms of DNA analysis. However, these techniques have certain severe limitations and the recent discovery of the far more powerful technique of DNA fingerprinting has enabled researchers to gather such data with much greater accuracy.

1.7 DNA FINGERPRINTING

1.7.1 General Introduction

DNA fingerprinting has revolutionised the determination of individual identity and the assessment of relationships between individuals. The technique is based on the use of radioactively-labelled DNA probes (derived from the human genome) to simultaneously detect many other highly polymorphic loci throughout the subject genome. The genetic material used for DNA fingerprinting can be extracted from any tissue composed of nucleated cells, and an individual's pattern of minisatellites remains constant from tissue to tissue, and from birth to death.

The length of the sequences at these polymorphic loci, or minisatellites as they have become known, are extremely variable, leading to a remarkably high degree of heterozygosity (>95% in humans) (Jeffreys, Wilson & Thein, 1985a and b). The probability of two unrelated individuals sharing a common banding pattern for a single probe is thus extremely small, and this allows highly

significant statistical probabilities to be calculated when confirming individual identity or assessing likelihoods of paternity or maternity (Wong et al., 1987; Jeffreys, Brookfield & Semeonoff, 1985). It is this extreme variability in the pattern of minisatellites, together with their Mendelian mode of inheritance that makes DNA fingerprinting such a powerful technique (Dodd, 1985).

1.7.2 Discovery of Minisatellites and the Development of DNA Fingerprinting

The families of sequences which were to become known as "minisatellites" were initially discovered by chance at a number of locations within the human genome (see references in Jeffreys, Wilson & Thein, 1985a). The term "minisatellites" was given to these families of sequences because of their similarity to the satellite DNA that is found when a digested genome is spun in a density gradient (Kit, 1961). The organisation of the nucleotide pairs in a minisatellite is similar to that of satellite DNA, in that both are composed of repeat regions and both are non-coding, although some minisatellites are known to form parts of coding sequences (Swallow et al., 1988).

One such minisatellite was discovered by Weller et al. (1984) whilst investigating the structure of the first intron of the human myoglobin gene. This minisatellite was structurally similar to the ones discovered previously, in that it was composed of a small number of tandemly repeated G≡C rich "units". In this case, the minisatellite was composed of four tandem repeats of a 33 base pair (bp) unit, the whole flanked by a 9-bp direct repeat. It was this flanking DNA, similar to sequences associated with transposable elements, that led Jeffreys and his colleagues to initially believe that minisatellites were related by transposition (Jeffreys, Wilson & Thein, 1985a); an idea since rejected (see Section 1.7.4).

In 1985, Jeffreys, Wilson & Thein (1985a) published the results of work they had carried out using a probe "comprised almost entirely of 23 repeats of the 33-bp sequence" found in the myoglobin intron, to investigate the distribution of other similar sequences throughout the rest of the human genome. They screened a human genomic library using this probe and detected a number of plaques to which the probe hybridised. The DNA from eight of these plaques was purified and sequenced, and was found to contain minisatellites composed of 3 to 29 tandem repeats of units between 16-bp and 64-bp in length. Two of these minisatellites are the sources of the polycore probes now known as 33.6 and 33.15. By making radioactive copies of these minisatellites, Jeffreys, Wilson & Thein (1985a) were able to probe a nylon filter to which digested DNA fragments had been previously transferred, in an attempt to detect cross-hybridising sequences. The results were spectacular. Indeed, they discovered so many sequences that they coined the term "hypervariable minisatellite" to describe the extraordinary allelic variation found at these loci. The exact location of these sequences in the genome was not known at that time, but subsequent work established that the loci from which these alleles came were spread throughout the genome, with concentrations at chromosomal centromeres and telomeres (Miklos, 1985; Royle et al., 1988).

In order to determine which part of the minisatellite was responsible for

binding to such a large number of other sequences, the internal structures of the eight "probe" minisatellites were investigated by DNA sequencing. Jeffreys and his colleagues established that the repeat units from which the minisatellite alleles were composed were between 17-bp and 64-bp in length and had a very constant nucleotide sequence. Specifically, each unit had a highly conserved, G≡C rich internal "core" sequence of 10-bp to 15-bp flanked by more variable DNA (Jeffreys, Wilson & Thein, 1985a).

Of the minisatellites detected using these probes, it was found that there were between 6 and 80 alleles occurring at every locus and that most of these alleles were heterozygous at their loci (Jeffreys, Wilson & Thein, 1985a). The great variability seen in minisatellite alleles is due to the individual units being tandemly repeated from one to thousands of times, and this has given rise to the alternative name for these sequences, "variable number of tandem repeats" or VNTRs (Nakamura et al., 1987). The patterns of minisatellites produced by using these probes were originally thought to be as unique as the patterns of arches, loops and whorls which form the dermal ridges of conventional fingerprints (Holt, 1961), and this is why the technique was given the name "DNA fingerprinting".

In their second paper, published only months after the first announcement of their discoveries, Jeffreys, Wilson & Thein (1985b) showed that the patterns of minisatellites they had discovered were individual-specific, with the chance of two unrelated individuals sharing the same banding pattern (when probed with 33.6 and 33.15) being $< 5 \times 10^{-19}$. They also showed that each band seen in an individual's DNA fingerprint could be traced back to either one or both of that individual's parents, and thus that minisatellite bands were inherited in a Mendelian manner. The majority of bands were inherited independently, but those that were not were found to be linked or allelic (Jeffreys, Wilson & Thein, 1985b). Minisatellite linkage can occur in one of two ways:

1) when two minisatellite loci with different repeat unit structures are located in close proximity on the same chromosome (Royle et al. ,1988); or

2) when the nucleotide sequence of one of the units in a minisatellite changes so that a restriction enzyme recognition site is created. The enzyme will thus cleave *within* the minisatellite, creating two bands were there was previously only one.

Linkage could be detected on a DNA fingerprint by the appearance of one or two bands in an offspring which were not present in either of the parents. If only one of these bands was seen, the other having been so small that it was lost from the end of the gel during electrophoresis (see Chapter 2, Section 2.1.3), it would not be possible to prove linkage. Also, if the cleavage site was present in each repeat unit, the enzyme would digest the minisatellite into fragments so small that they would all be lost from the end of the gel and thus not scored. If more than one mutation event was to occur in the sequence of one minisatellite allele, a pattern would be produced showing linkage between more than two bands, given that they were sufficiently large to be scored.

Allelism may also occur, but like linkage is a rare phenomenon, suggesting that alleles at the same locus are not constrained to being of similar size (Jeffreys, Wilson & Thein, 1985b). The majority of loci thus have one allele that is of a size that can be scored on a DNA fingerprint (between 4,000-bp and 20,000-bp), and another that is of such low molecular weight that it runs off the end of the gel. Linkage and allelism are important phenomena as, in addition to the chance association of alleles derived from different loci, they will reduce the number of independently segregating bands that can be used for analysis.

Minisatellites are inherited in a Mendelian manner, meaning that on average one would expect an offspring to inherit half of its minisatellites from its mother and half from its father. Also, one would expect an individual to share approximately half its minisatellite bands with any other offspring produced by its parents. However for unrelated individuals, the probability that they share all of their bands is given by x^n, where x is the probability that a band present in individual A is also present in individual B, and n is the number of independently segregating bands scored in their DNA fingerprints (Jeffreys, Wilson & Thein, 1985b). As mentioned above, this probability for humans is $< 5 \times 10^{-19}$. This means that a set of minisatellites can be used to confidently characterise an individual.

1.7.3 Probes 33.6 and 33.15

The minisatellite probes used in this study are two of the eight originally sequenced by Jeffreys and his colleagues (designated 33.15 and 33.6), and are described in detail elsewhere (Jeffreys, Wilson & Thein, 1985a and b). The original sequences from which the probes were derived are located on chromosomes 7 (q31.3-terminus) and 1 (centromere-q24), respectively (Jeffreys, Wilson & Thein, 1985a). The probe 33.15 is composed of 29 repeats of a 16-bp variant of the consensus "core" sequence, and probe 33.6 consists of 18 repeats of a diverged trimer of the most highly conserved 3' 11-bp of the "core" sequence (see Table 1.2).

TABLE 1.2: 5' to 3' consensus sequence of the minisatellite "core" and two of the probes developed by Jeffreys et al. (see text for details).

"Core" sequence	$GGAGGTGGGCAGGA^{G}/_{A}G$
Probe 33.15	$(AGAGGTGGGCAGGTGG)_{29}$
Probe 33.6	$[(AGGGCTGGAGG)_3]_{18}$

The above probes detect many hypervariable loci and, as less than 1% of the sequences in the human genome to which 33.15 hybridises are also hybridised by 33.6, each subset is essentially completely different. The sequences detected by these probes can be released from the genome by digestion with a number of

endonucleases. Enzymes that are 4-bp "cutters" are preferred over those with 5 or 6-bp target sequences because the recognition site of the former occurs often enough in non-satellite DNA to reduce this part of the genome to fragments so small that they are lost off the end of the agarose gel during electrophoresis. This effectively releases the long minisatellites, which do not contain the recognition site of the restriction enzyme used, with a little flanking DNA (Jeffreys, Wilson, Thein et al., 1986).

1.7.4 Generation of Minisatellites

The extreme variability of minisatellite alleles and the high rate at which new alleles are generated are connected to the presence of the "core" sequence within the minisatellite repeat units (Jeffreys, Royle et al., 1988). Jeffreys, Wilson & Thein (1985a) pointed out that the "core" sequence was similar in both length and base pair composition to the χ-sequence responsible for generalized recombination in the bacterium *E. coli*. This gave rise to the hypothesis that minisatellite loci could be recombination "hot spots" that generated new minisatellite alleles by the unequal exchange of DNA between homologous chromosomes (Jeffreys, Wilson & Thein, 1985a).

However, more recent evidence (reviewed by Pemberton & Amos, 1990) downgrades this hypothesis. Studies of minisatellites linked closely to marker loci have shown that the size variation in these alleles is mostly due to the loss or gain of a small number of repeat units (Jarman & Wells, 1989). It is still not clear whether inter-allelic unequal exchange through meiotic or mitotic recombination is involved in generating these changes, or whether other processes such as unequal sister-chromatid exchange (which has been shown to be the sole method of new allele generation at one locus) or replication slippage are the primary source of minisatellite mutation (Jeffreys, Neumann & Wilson, 1990).

The frequency with which mutations occur at minisatellite loci has been found to be the same in both sperm and oocytes (Jeffreys, Royle et al., 1988), with estimates of the rate at which new variants are created clustering around $2\text{-}4 \times 10^{-3}$ (Jeffreys, Wilson & Thein, 1985a; Wong et al., 1986; Burke & Bruford, 1987). The heterozygosity at these minisatellite loci also varies, but is highly correlated with the mutation rate, and the relationship between them has been shown to closely match results obtained from a model based on neutral mutation and random genetic drift (Jeffreys, Royle et al., 1988). However, Kelly et al. (1989) have detected new alleles early in the developmental period of mice, indicating that mutation events are not restricted to the germline as originally thought (Jeffreys, Royle et al., 1988), but can also occur in the soma (see also Jeffreys, Neumann & Wilson, 1990).

Sequence analysis of alleles cloned from the human minisatellite locus MS32 has shown that the repeat units are seldom all identical (Jeffreys, Neumann & Wilson, 1990). However, it seems that the occurrence of sequence variation between the units is not uniform across the length of the allele. It appears that the 3'-ends of alleles at this particular locus show greater inter-allelic variability than

the 5'-ends. This gradient in variability is thought to arise because the turnover processes which alter allele length are preferentially affecting the 3'-end of the alleles (Jeffreys, Neumann & Wilson, 1990). Unfortunately, the reason for this phenomenon is as yet unknown.

1.7.5 Applications of DNA Fingerprinting

Jeffreys, Wilson & Thein (1985b) originally used DNA fingerprinting to determine relatedness in humans, but it was not long before the scientific, medical, and legal communities "discovered" the technique and applied it to a wide variety of species and situations. In humans, the technique has been used to determine familial relationships (Jeffreys, Brookfield & Semeonoff, 1985 and 1986), for the identification of specific individuals in forensic science (Gill, Jeffreys and Werret, 1985; Wong et al., 1987), for linkage analysis (Nakamura et al., 1987), monitoring of bone marrow transplants (Jeffreys, 1987), and studies of tumorous tissue (Armour et al., 1989).

Shortly after the publication of the papers by Jeffreys, Wilson & Thein (1985a and b), the technique of DNA fingerprinting was shown to work equally well in other mammalian species, including domestic cats and dogs (Jeffreys & Morton, 1987), and mice (Jeffreys, Wilson, Kelly et al., 1987). Since this demonstration, a large number of papers have appeared in the scientific literature, detailing the manifold species upon which the technique has been found to work and the different uses to which it has been put.

In addition to cats, dogs and mice, DNA fingerprinting has been found to work in a number of other mammals, e.g. marmosets (Dixson et al., 1988), cattle (Buitkamp et al., 1991) and cetaceans (Amos & Dover, 1990; Hoelzel & Dover, 1990); in all the great apes (see references in Ely, Alford & Ferrell, 1991); a variety of birds (Wetton et al., 1987; Burke et al., 1989; Kuhnlein et al., 1989; Anming, Carter & Parkin, 1990; Gyllensten, Jakobsson and Temmin, 1990; Westneat, 1990); a number of plant species, e.g. rice (Dallas, 1988); some insects (Ryskov et al., 1988; Amichot, Fournier & Berge, 1989; Carvalho et al. 1991), and *Caenorhabditis elegans* (Uitterlinden et al., 1989). Where the technique has worked it has been used for determining genetic distances (Kuhnlein et al., 1989), investigating genetic variation and taxonomic affinities (Anming, Carter & Parkin, 1990), demonstrating that aggression in House mice is a function of genetic relatedness (Everitt et al,. 1991), determining parentage (Wetton et al., 1987; Burke et al., 1989; Westneat, 1990; Rabenold et al., 1990), and monitoring captive stocks of threatened species (Ashworth & Parkin, 1992), to name but a few of the applications.

The widespread conservation of minisatellites among species suggests that these sequences appeared in the genomes of some of the first living organisms on Earth. Jeffreys, Royle et al. (1988) proposed that minisatellites are selectively neutral, at least in multicellular organisms, because they have persisted for so long in so many different taxonomic categories. However, this fact indicates to the writer, at least, that minisatellites are *positively* selected for.

1.7.6 Summary

DNA fingerprinting is a powerful method for determining paternity and for resolving genetic differences between closely related individuals in a large number of taxonomically diverse species. However, for the technique to be used to its greatest efficiency, the system to which it is applied has to be suitable. It is not as strong a technique when applied to promiscuous populations, since in order to determine a particular offspring's paternity, the mother/offspring pair would have to be compared with all the males in the study population (Pemberton, Bancroft & Amos, 1991). This constraint on the technique is demonstrated by the fact that within the field of behavioural ecology, the majority of papers published have applied the technique to monogamous or weakly polygamous populations (Pemberton, Bancroft & Amos, 1991).

In addition, there are problems in applying the technique to the determination of specific relationships between individuals. This is because the proportion of bands shared by even unrelated individuals is sometimes large (10 - 30%), and will thus be higher than their coefficient of relatedness (Burke, 1989). Also, comparisons between parents and offspring, and between siblings will show that they share approximately the same number of bands, and therefore if nothing is known about the ages of the individuals compared, only the fact that they are related to the same degree will be discerned. These constraints and the technical difficulties in analysing a DNA fingerprint, i.e. scoring highly complex profiles, within which individual bands lie effectively on a continuum of both intensity and position (Amos, 1992), have somewhat reduced the original expectations that researchers had for the technique. However, DNA fingerprinting is still a powerful and widely applicable technique which, provided its limitations are acknowledged, can produce excellent data obtainable in no other way.

1.8 SPECIES CONSIDERED AND AIMS OF THIS STUDY

In 1987, Wetton et al. demonstrated that DNA fingerprinting could be used to investigate the demographic structure of a natural House sparrow population. The success of this work led to the establishment of this project which has been aimed at examining the possible role that DNA fingerprinting can play in the monitoring of captive breeding programmes. Two of the captive stocks considered in this study (Rothschild's mynah and the Rodrigues fruit bat) were held in zoological parks (see Chapters 3 & 4, respectively), and two stocks (the British Merlin and the New Zealand falcon) were held by private individuals (see Chapters 5 & 6, respectively).

The amount of information available for these stocks, i.e. relationships between individuals, breeding success, etc., varied greatly between species. A regularly updated stud book is maintained for Rothschild's mynah, but some of the information contained within is educated guesswork (G. Mace, pers. comm., 1989). The details for the Rodrigues fruit bats are contained in a breeding record, but this only lists details of which females are (thought to be) the mothers of which

offspring, and is not complete. The Merlins sampled for this study came from a number of different breeders and their relationships were, for the most part, unknown. The work on the New Zealand falcons was done in collaboration with Dr. Nick Fox, who has maintained detailed records of their breeding programme.

The captive breeding programmes for Rothschild's mynah, the Rodrigues fruit bat, and the New Zealand falcon are all aimed at producing a number of viable individuals for reintroduction to the wild. The success of such programmes depends upon having genetically variable stocks from which individuals for reintroduction can be selected. The genetic backgrounds of individuals taken from the wild for use in breeding programmes are usually unknown, and DNA fingerprinting can be used to determine which individuals are the least related so that they can be paired. Also, DNA fingerprinting is an extremely powerful technique for detecting parent/offspring misallocations within a stock and for providing evidence for their correction. In this way, the managers of a stock can pair individuals so as to avoid inbreeding (Wright, 1921), and maintain as much variation as possible within the stock.

The research presented here on the variability and individual specificity of DNA fingerprints within Merlins has a potential legal application. Under the Wildlife and Countryside Act 1983, certain birds of prey must be registered with the Department of the Environment if held in captivity, and one species listed is the Merlin. At registration, each bird is given a unique number which is stamped on a metal ring and affixed to the bird's leg. Details such as origin, parentage and date of birth are recorded and stored with the ring number. Unfortunately, it is possible to remove these rings, as unscrupulous dealers have been known to do. The ring can then be transferred to another bird, possibly one taken illegally from the wild, which is then passed off as captively bred. However, if a blood sample was taken at registration, a DNA fingerprint for that individual could be produced and this would be a permanent record of that particular bird's identity. The data presented in Chapter 5 indicates that a registration scheme of this kind is indeed possible.

CHAPTER 2

MATERIALS AND METHODS

2.1 DNA FINGERPRINTING

Presented below are details of the methods I routinely employed. On occasion, it was necessary to make adjustments to these techniques depending on the species being studied. Details of such alterations are given in the sections dealing with the individual species. The whole process, from tissue sample to completed DNA fingerprint, took from two to three weeks.

2.1.1 DNA Extraction

2.1.1.1 General Introduction

The technique of DNA fingerprinting relies on the use of high molecular weight, protein-free, genomic DNA. Such DNA can be prepared from the majority of tissues: the ones used most frequently being blood from live specimens, or liver from post-mortem corpses. Liver was used in preference to other tissues because of its high DNA content.

Whole blood was used fresh as the cells within it were well dispersed, but if solid tissue was used the cells had first to be dissociated. This was done by immersing a tissue sample (about 50mg) in liquid nitrogen (-195°C) and grinding the frozen specimen to a fine powder with a pestle and mortar. Several volumes of nitrogen were required due to the rapid evaporation of the gas, each volume being replaced as necessary. Typically, a 50mg piece of tissue required the use of 200ml of liquid nitrogen. This treatment rendered the cells more accessible to the chemicals used in the process of lysis (see Section 2.1.1.2).

The tissue preferred for analysis was to some extent dependent on the taxonomic Class to which the donor species belonged. For avian studies involving live specimens, erythrocytes were used because of their ease of collection and the fact that they contain nuclei. However, for live mammalian studies, I used isolated leucocytes, since mammalian erythrocytes contain no genomic DNA. If the mammalian specimens were not live, then a number of tissues could be used, although liver was preferred.

2.1.1.2 Lysis of Cells

Approximately 15-25µl of whole blood (or an equivalent volume of dissociated tissue) was suspended in 550µl of 1xSET isotonic buffer (see p.47) in a 1.5ml eppendorf tube. The cells were lysed by the addition of 7.5µl of 25% w/v sodium dodecyl sulphate (SDS) solution, an anionic detergent which disrupts both the cellular and nuclear membranes releasing the genomic DNA. This treatment

renders the DNA accessible to cellular nucleases which were denatured by the addition of 15µl of Proteinase K solution (10mg/ml) to prevent the degradation of the genetic material. The mixture was then incubated overnight at 55°C.

(1xSET; 0.15M NaCl, 1mM EDTA, 50mM TRIS, pH8.0.
EDTA; Ethylene Diamine Tetra Acetic acid, disodium salt.
TRIS; Tris Hydroxymethylamino Methane.)

2.1.1.3 Cleaning of DNA

Proteins, lipids and other cellular debris were removed from the mixture by washing with organic solvents. Initially, each sample was washed with 500µl of buffered phenol (pH8.0). To improve the efficiency of washing, the samples were agitated by repeated inversion on a rotating mixer (30revs/min) for 20-30 minutes. After mixing, the samples were centrifuged at 8000g for five minutes. As the organic solvent and the buffered DNA solution were immiscible, they formed distinct layers. Cellular debris either dissolved in the lower organic solvent layer or collected at the interface between the two. The upper aqueous layer, which contained the DNA, was transferred to a clean eppendorf tube using a 1ml pipette fitted with a disposable tip from which the end had been removed (to reduced the shearing forces on the DNA). It was ensured that none of the interface was carried over during transfer.

Further washes were performed, typically twice with phenol and twice with phenol/chloroform/isoamyl alcohol (24:23:1 v/v). If, however, any precipitation was noted at the interface, further washes were performed with phenol/chloroform/isoamyl alcohol (24:23:1 v/v). The organic solvents removed cellular debris and stripped histone proteins from the DNA molecule. Any traces of phenol remaining in the samples were removed by a final wash with chloroform/isoamyl alcohol (23:1 v/v). This was important as phenol can interfere with the action of restriction enzymes (see Section 2.1.2).

2.1.1.4 Precipitation and Recovery of DNA

After the final wash, the aqueous layer was transferred to a clean eppendorf tube and the DNA recovered from solution by the addition of two volumes of cold (-20°C) absolute ethanol. The ethanol was added slowly to the tube, allowing the DNA to precipitate out at the interface. Each sample tube was swirled vigorously by hand until a clump of DNA precipitate appeared, and was then stored at -20°C for 30 minutes to allow complete precipitation to occur.

The DNA was pelleted by centrifugation at 8000g for five minutes. The ethanol in the tube was removed by pipette, and the tube then placed in an oven at 37°C for 15-30 minutes to dry the DNA. The dried pellet was resuspended in 100-150µl of TE buffer at 55°C overnight. This solution formed the stock from which aliquots were taken for restriction (see Section 2.1.2).

(1xTE; 10mM TRIS, 1mM EDTA, pH8.0.)

2.1.2 DNA Restriction

2.1.2.1 General Introduction

The stock solution contained protein-free, double-stranded genomic DNA, this being a suitable substrate for digestion using restriction enzymes. The particular enzyme used was chosen on the basis that it cut frequently within most genomic DNA, but not within the minisatellite sequences. The suitability of the enzyme could also depend upon the peculiarities of the species, but I found the "four base-pair cutter" *HaeIII* to be reliable and cost effective for those species presented in this volume.

HaeIII recognises the target sequence GG/CC (G is the purine base guanine and C the pyrimidine base cytosine) within the DNA molecule. In the reaction mixture, the enzyme "finds" this target sequence and cleaves the DNA molecule between the central G and C bases. The genomic DNA is thus cut into a number of "blunt-ended" fragments of different lengths.

2.1.2.2 Digestion of DNA

A 15µl aliquot of the stock DNA solution was digested to completion with 1µl (approximately 10 Units) of *HaeIII*, in the presence of 2µl of the appropriate buffer (depending on the manufacturer's instructions) and 2µl of 4mM Spermidine HCl (2µl of a 40mM stock solution). Digestion was carried out in an eppendorf tube incubated in a waterbath at 37°C, usually overnight, although a shorter time was occasionally allowed (minimum four hours).

2.1.2.3 Qualitative and Quantitative Assays

The digestion was assayed both qualitatively and quantitatively. The qualitative analysis was performed to check that the enzyme was working and that the digestion was proceeding correctly. A 0.8% agarose gel was prepared for this purpose by dissolving 0.24g LE agarose in 30ml of 1xTBE buffer (see p.49) and heating the mixture in a microwave oven. When all the agarose had dissolved, the mixture was poured into a Cambridge Bioscience minigel apparatus and allowed to set. A plastic comb penetrated the gel as it set, and on removal left wells into which the samples could be introduced.

After about two hours, 2µl of each sample digest was mixed with an equal volume of 2xBPB buffer (see p.49), loaded into an empty well in the gel and electrophoresed at 70-80 volts for one hour. The gel was then stained with ethidium bromide which intercalates into the DNA molecules, fluorescing orange in the presence of 354nm ultra-violet light. This allowed visualisation of the DNA and the resulting smear within the gel could be checked to see whether the enzyme had worked effectively.

An excess of high molecular weight DNA in the gel indicated that the sample was only partially digested. At this stage, an extra 2µl of enzyme was

added and the sample again incubated in the waterbath for a minimum of four hours. After this time, the sample was tested again. If there was still an excess of high molecular weight DNA, the sample was discarded and a new digest set up. A paucity of high molecular weight DNA indicated the presence of a contaminant in the sample. Such samples were discarded immediately and fresh digests prepared, as contaminated samples could produce spurious bands in fingerprint patterns, affecting the analysis.

The quantitative assay was performed to assess the concentration of DNA in each digest. A 2µl aliquot was suspended in 2ml of 1xTNE buffer, in the presence of 0.1µg/ml Hoechst 33258 dye. This dye binds to the DNA and emits light at a wavelength of 458nm when excited by 365nm ultra-violet light. The amount of light produced is directly proportional to the amount of DNA (within certain extremes), and was assayed using a calibrated Hoefer TK-100 fluorometer. After assessment, all the samples were adjusted to a concentration of 0.15µg/µl using 2xBPB buffer.

(5xTBE; 0.445M TRIS, 0.445M Boric Acid, 0.002M EDTA, pH8.0.
10xBPB; 0.25% w/v Bromophenol blue, 20% w/v Ficoll®400, 0.2M EDTA, 0.25% Xylene cyanol FF.
10xTNE; 100mM TRIS, 1M NaCl, 10mM EDTA, pH7.4.)

2.1.3 DNA Electrophoresis

2.1.3.1 General Introduction

The DNA fragments were separated according to size by electrophoresis through an agarose gel. The gel acts as a molecular sieve forcing the molecules that pass through it to move at speeds related to their sizes. This means that the smallest fragments move the furthest and the largest fragments the least, in a given time. Although different concentrations were tested, the concentration of agarose used in all the gels detailed in this study was 0.7% (see below).

2.1.3.2 Gel Preparation

Three litres of 1xTAE buffer were prepared and 375ml measured into a screw top 500ml Duran bottle. LE agarose was added (2.62g for a 0.7% gel) and the mixture heated in a microwave oven until all the agarose had dissolved. The bottle was then transferred to a 55°C waterbath where it remained for one hour. After this time, the agarose was cool enough to pour into a 24cm by 29cm perspex gel mould. This mould contained a 16-tooth comb, 2cm from one end. The gel was allowed to set for approximately two hours before being placed in a LKB H4 horizontal electrophoresis tank and immersed in the remaining 1xTAE buffer solution.

(1xTAE; 0.04M Tris Acetate, 1mM EDTA, pH8.0.)

2.1.3.3 Loading and Running

The restriction enzyme produced a number of DNA fragments all of which had the same cohesive ends. These ends can rejoin and produce increases in the size of certain fragments, altering the fingerprint pattern. To dissociate those fragments that had become joined, the digested samples were incubated in a 65°C waterbath for ten minutes. Before loading, the samples were quenched on ice for a further ten minutes, to ensure that they remained separate as they were loaded into the gel. The plastic comb was removed and 40µl of each sample loaded into individual wells. Because all the samples were adjusted to the same concentration after the assay (see Section 2.1.2.3), the same quantity of DNA was loaded into each well. A clean, disposable pipette tip was used for each sample to avoid cross-contamination. In addition to the digests, a 5µl sample of restricted λ-DNA was loaded into the gel. This contained fragments of known length and allowed bands to be cross-identified on different autoradiographs.

The length of time taken for electrophoresis depended on the running conditions, which in turn were dependent on the species to be analyzed. Typically, a voltage of 40-50 volts was used, and the samples run for 36-48 hours (1440-2400Vh). Depending on the conditions used, at the end of the separation, fragments smaller than 3.0-4.5kb had run off the end of the gel into the buffer.

2.1.4 Blotting

2.1.4.1 General Introduction

When electrophoretic separation was complete, the individual DNA fragments had migrated to specific positions in the gel. The DNA was transferred out of the agarose by capillary blotting onto a nylon or nitrocellulose membrane, in such a way that the fragments retained their relative positions. This was important for the accurate analysis of the data. The exact method of blotting depended on the membrane being used. Southern blotting (Southern, 1975; Sambrook, Fritsch & Maniatis, 1989) was employed for both the nylon and the nitrocellulose membranes. "Alkali transfer" (see Sambrook et al., 1989) was more rapid, but would only work with some types of nylon. Both methods are described below.

2.1.4.2 Southern Blotting

The gel was removed from the electrophoresis tank, inverted and immersed in 0.2M hydrochloric acid (HCl) for 10-20 minutes. HCl hydrolyses the purine bases in the DNA and, at this concentration, produces single stranded nicks in the molecule, every 200 base pairs on average. This process is known as depurination and it reduces the size of the larger DNA fragments so that their movement out of the gel is facilitated.

The acid was poured away and the gel soaked in 1.5M NaCl, 0.5M NaOH

for 45 minutes to denature the double stranded DNA. To restore chemical equilibrium, the gel was neutralised by immersion in 3M NaCl, 0.5M TRIS, pH8.0 for 45 minutes. This procedure was repeated.

The DNA was transferred to a membrane by blotting. The blot was constructed so that a perspex sheet sat above a tray containing 20xSSC, a high ionic strength buffer. A wick made from Whatman 3MM paper was soaked in 20xSSC and placed on the perspex sheet, ensuring that there were no bubbles underneath it and that the ends dipped into the buffer in the tray. The gel was placed on top of the filter paper and a 20cm by 20cm membrane was then pressed on top of the gel, again ensuring that there was a close contact. Two sheets of filter paper were cut to the correct size (20cm by 20cm) and placed on top of the membrane, followed by a stack (about two inches high) of bronze towels (Kimberly-Clark, UK). The fragments of DNA moved from the gel onto the membrane under the force of capillary action, as the towels absorbed the 20xSSC.

(TRIS; Tris Hydroxymethylamino Methane.
20xSSC; 3M NaCl, 0.3M Sodium Citrate, pH7.0.)

2.1.4.3 Alkali Transfer

The depurination and denaturation steps were carried out as above, but the neutralisation step was omitted. The blot was constructed in the same way as for Southern blotting, but the tray was filled with 1.5M NaCl, 0.25M NaOH. The rest of the technique was the same.

2.1.4.4 Fixation of DNA

After an overnight blot, the apparatus was dismantled and the membrane immersed in 2xSSC. This was done twice, the previous SSC solution being discarded, to remove traces of agarose and neutralising solution. The membrane was air-dried for about ten minutes and then baked in a vacuum at 80°C for two hours. This bound the DNA fragments to the membrane in such a way that the molecules remained free to hybridize to the probe (see Section 2.1.6), while ensuring that they were secure enough not to be lost during the rest of the technique.

(20xSSC; 3M NaCl, 0.3M Sodium Citrate, pH7.0.)

2.1.5 Probe Preparation

2.1.5.1 General Introduction

The probe used is a piece of nucleic acid, either DNA or RNA, with a specific internal sequence that allows it to hybridize to those DNA fragments that possess sufficient homology. The probe was radioactively labelled with α^{32}P-CTP (cytosine triphosphate) to allow its autoradiographic detection after hybridization.

Early in this study, the Jeffreys' DNA probes (33.6 and 33.15) were replaced by Carter's RNA derivatives (pSPT 19.6 and pSPT 18.15, respectively) (Jeffreys, Wilson & Thein, 1985; Carter, Wetton & Parkin, 1989). Presented below is the protocol for the Carter probes only, as these were by far the most frequently used during this study.

2.1.5.2 Labelling the Probe

The cloning of the minisatellite regions of 33.6 and 33.15 into the pSPT vectors, and their linearization prior to labelling, was carried out by R.E. Carter.

The labelling of the probe was carried out in a sterile eppendorf tube using the following recipe of ingredients from a BRL labelling kit:

1.0µl ATP (10mM)
1.0µl GTP (10mM)
1.0µl UTP (10mM)
1.0µl RNAase inhibitor (25 units)
1.0µl T7 RNA polymerase
2.0µl DL-Dithiothreitol (DTT) (100mM)
4.4µl 4xTranscription buffer
5.0µl α^{32}P-CTP (400Ci/mmol)
5.5µl Restricted pSPT

Each ingredient was added to the inside wall of an eppendorf tube using a fresh, sterile pipette tip. The ingredients were mixed by a brief pulse in a centrifuge and then incubated in a 38°C waterbath for one hour. After this time, the reaction was stopped by the addition of 20µl Nick-stop mix.

(Nick-stop; 0.9% Blue dextran, 0.03% Bromocresol purple, 20mM EDTA.)

2.1.5.3 Separation of Probe

Spun column chromatography was used to separate the unincorporated nucleotides from the completed probe. The end of a 1ml plastic syringe was plugged with a small piece of glass wool, inserted with a Pasteur pipette, to prevent the contents of the syringe from falling out. The barrel was filled with TE-buffered Biogel P60 granules and then the whole centrifuged at 2000g for five seconds to pack the column. 150µl TE were added and a further spin of five seconds performed. This last procedure was repeated.

The probe was loaded into the top of the column using a sterile pipette tip and the column spun at 2000g for five seconds. 50µl TE was then added and the column again spun at 2000g for a further five seconds to wash through the probe. Unincorporated nucleotides adhered to the Biogel granules leaving almost pure probe in the eluted TE buffer.

To determine the efficiency of the incorporation, a 1µl sample of the

reaction mixture was taken before chromatography, and an equal volume of probe taken after elution from the column. The two were separately mixed with Ecoscint (Emulsifier-Safe) scintillation fluid and assayed in a scintillation counter. The remaining probe solution was transferred to a clean eppendorf tube and stored in a lead container until required (see Section 2.1.6.3).

(1xTE; 10mM TRIS, 1mM EDTA, pH8.0.)

2.1.6 Prehybridization and Hybridization

2.1.6.1 General Introduction

The membranes were washed with the probe which bound to the minisatellites sequences within the DNA fragments. The fragments were fixed to the membrane by one end only and so were easily accessible to the probe. By altering the temperature and/or the ionic strength of the solutions (the stringency) during hybridization, the degree to which the probe bound to the minisatellites could be controlled. All the hybridizations used in this study were conducted at the same stringency; 1xSSC at 65°C (see below).

2.1.6.2 Prehybridization of Membranes

The nylon or nitrocellulose membranes were immersed in 500ml of "prehybe" solution (see below) in either a 22cm by 22cm plastic box or a screw-top glass bottle, depending on how many membranes were to be processed. The former method allowed the simultaneous treatment of up to 15 membranes, the latter up to five. Both treatments were conducted at 65°C for eight hours, during which time the membranes were regularly agitated to ensure even distribution of the prehybe. This process blocked the positively charged sites on the membranes' surfaces so that non-specific hybridization of the probe was minimised.

(Prehybe; 1xSSC, 0.1% Sodium Dodecyl Sulphate, 1% BLOTTO.
20xSSC; 3M NaCl, 0.3M Sodium Citrate, pH7.0.
10% BLOTTO; 10% non-fat powdered milk, 0.2% Sodium Azide, 10µl Diethylpyrocarbonate; see Johnson, Gautsch, Sportsman & Elder, 1984.)

2.1.6.3 Hybridization

After eight hours, the membranes were removed from the box or bottle and $6x10^7$cpm of the probe added. The prehybe solution was stirred briefly to distribute the probe and then the membranes were reintroduced to the container, ensuring that no bubbles were trapped between them. The hybridization was allowed to proceed at 65°C overnight.

2.1.6.4 Post-Hybridization Washing

After the overnight hybridization, the membranes were removed from their container and the prehybe solution discarded. To remove unbound probe, the membranes were washed in 1xSSC, 0.1% Sodium Dodecyl Sulphate (wash solution) at 65°C. The membranes were returned to the container ensuring that each was thoroughly immersed in the wash solution and that no bubbles were trapped. Several washes were performed, each for 45 minutes, with the previous wash solution being discarded each time. After washing, the membranes were air-dried for about ten minutes and then wrapped in Saran Wrap.

(20xSSC; 3M NaCl, 0.3M Sodium Citrate, pH7.0.)

2.1.7 Autoradiography

2.1.7.1 General Introduction

Once the radioactively-labelled probe has hybridized to the DNA fragments, its position on the membrane can be detected by autoradiography as the radiation (β-emission) from the probe exposes X-ray film. Intensifying screens were used to amplify the signal from the membrane, but this lead to blurring of the bands on the fingerprint. A sharp image could be produced without the use of screens, but this required a longer exposure (see Section 2.1.7.2).

2.1.7.2 Exposure

The membranes were placed in contact with a sheet of pre-flashed Fuji RX film in a lead-lined cassette, the whole operation being conducted in a darkroom. If necessary, calcium tungstate intensifying screens were used. If screens were used, the exposure was performed at -80°C to improve efficiency. An exposure of this kind would typically take 4-24 hours, depending on the intensity of the radioactivity. Without-screen exposures were performed at room temperature and took from 3-10 days.

2.1.7.3 Developing and Fixing

Developing and fixing were conducted in a darkroom. The film was removed from the cassette, immersed in Kodak X-ray developer and agitated for four minutes. It was then transferred to Hypam fixer (Ilford) for 2-4 minutes, ensuring that all parts of the film were immersed. The film was removed from the fixer and washed in clean tap water before being hung in a drying cabinet. The resulting dry autoradiograph formed the DNA fingerprint which could then be analysed.

2.1.8 Analysis of DNA Fingerprints

All the fingerprints used in this study were scored by eye while being

illuminated using a transmission light-box. If possible, individual samples were repeated within and between fingerprints to improve the accuracy of scoring. Distortion was observed in some fingerprint patterns, but it was usually possible to correct for this when it occurred.

The fingerprints were scored by noting the positions of all the bands in a particular molecular weight range within each individual. The range for each species analyzed is given in the section dealing with that species (see Chapters 3-6). Bands which migrated to the same position in the gel were assumed to be the same allele, but this is not necessarily always the case. Due to the fortuitous co-migration of different minisatellite fragments, the "similarity coefficient", D (see below), between two individuals will be overestimated (Jeffreys & Morton, 1987). Similarity coefficients were calculated using the equation given below (from Lansman, Shade, Shapira & Avise, 1981 and Wetton, Carter, Parkin & Walters, 1987);

$$D = \frac{2N_{ab}}{N_a + N_b}$$

D = similarity coefficient,
N_a = total number of bands scored in individual A,
N_b = total number of bands scored in individual B,
N_{ab} = total number of bands that occurred in both individuals.

2.2 CONSTRUCTION OF FAMILY TREES

2.2.1 General Introduction

The breeding histories of the individuals within captive stocks are held in breeding records or in formal studbooks, if they are kept at all. Of the species presented in this volume, studbooks were available for Rothschild's mynah (see Chapter 3) and the Rodrigues fruit bat (see Chapter 4), and breeding records were available for some of the Merlins sampled (see Chapter 5) and for the New Zealand falcons (see Chapter 6). The information for the mynahs and the falcons, though extensive, contained some calculated guesses (G. Mace, pers. comm., 1988; N. Fox, pers. comm., 1989), and one of the aims of this study was to clarify the parent/offspring allocations of those individuals for which such information was unavailable or unreliable. The bat and Merlin data were, unfortunately, very sketchy, making the resolution of such allocations difficult (see Chapters 4 & 5, respectively).

I decided that the easiest way to make use of the information contained in the records I was able to obtain, would be to display the data in the form of family trees. This would allow parent/offspring allocations and familial relationships to be seen, without having to search through pages of data. This made the process

of checking the fingerprinting results against the recorded relationships much easier.

The first family tree constructed was that for the British Rothschild's mynah population. Although breeding records for the mynahs had been available since 1971 (Lucas, 1971), and a complete studbook had been in annual production since 1984 (PHPA, ICBP & AAZPA, 1987), no family tree existed. A number of workers had tried to construct a pedigree from these data, but all attempts had been abandoned due to the complexity of the mate allocations in the captive stock (G. Mace, pers. comm., 1988).

A version of the tree appeared in the 1988 Rothschild's Mynah Studbook (Mace, unpublished a) (see Figure 2.1). The lineage presented contained all the individuals listed in the studbook at that time, although later versions of the tree contained only those individuals that were alive or that had produced offspring that were alive at the time (see Chapter 3).

The success of the mynah family tree encouraged me to display the data for the other species analyzed in a similar manner. These lineages are presented in the relevant chapters, although the only other major tree assembled was that for Dr. N. Fox's New Zealand falcons (see Chapter 5).

2.2.2 Equipment

The "Dazzle Draw" graphics package written by David Snider (part of the Creative Workshop series copyrighted by Broderbund Software Inc.) was used to construct the trees. A "mouse" was used as the input device on an Apple IIe computer fitted with a Keyzone Spectragram RGB video card.

The nature of the software meant that much of the information for the trees had to be assembled on the computer at the level of individual pixels. This proved very time-consuming even though the package has a number of functions designed to assist with the reproduction of specific shapes and their movement around the screen.

Due to the size of some of the trees they had to be constructed in sections on the computer, each section being printed out separately. These printouts were then cut to size and glued together. The trees produced in this way were too large for direct inclusion in papers or this thesis (the mynahs' tree was 40cm by 40cm), and so they were converted to A4 size by reduction-photocopying on a Canon NP-4540 RDF photocopier.

2.2.3 Rules for the Construction of Family Trees

Below are the rules developed for the construction of the Rothschild's mynah family trees presented in this volume, and below these are the modified rules used for the New Zealand falcons. Modification was necessary because of the length of the identity numbers for the latter species:

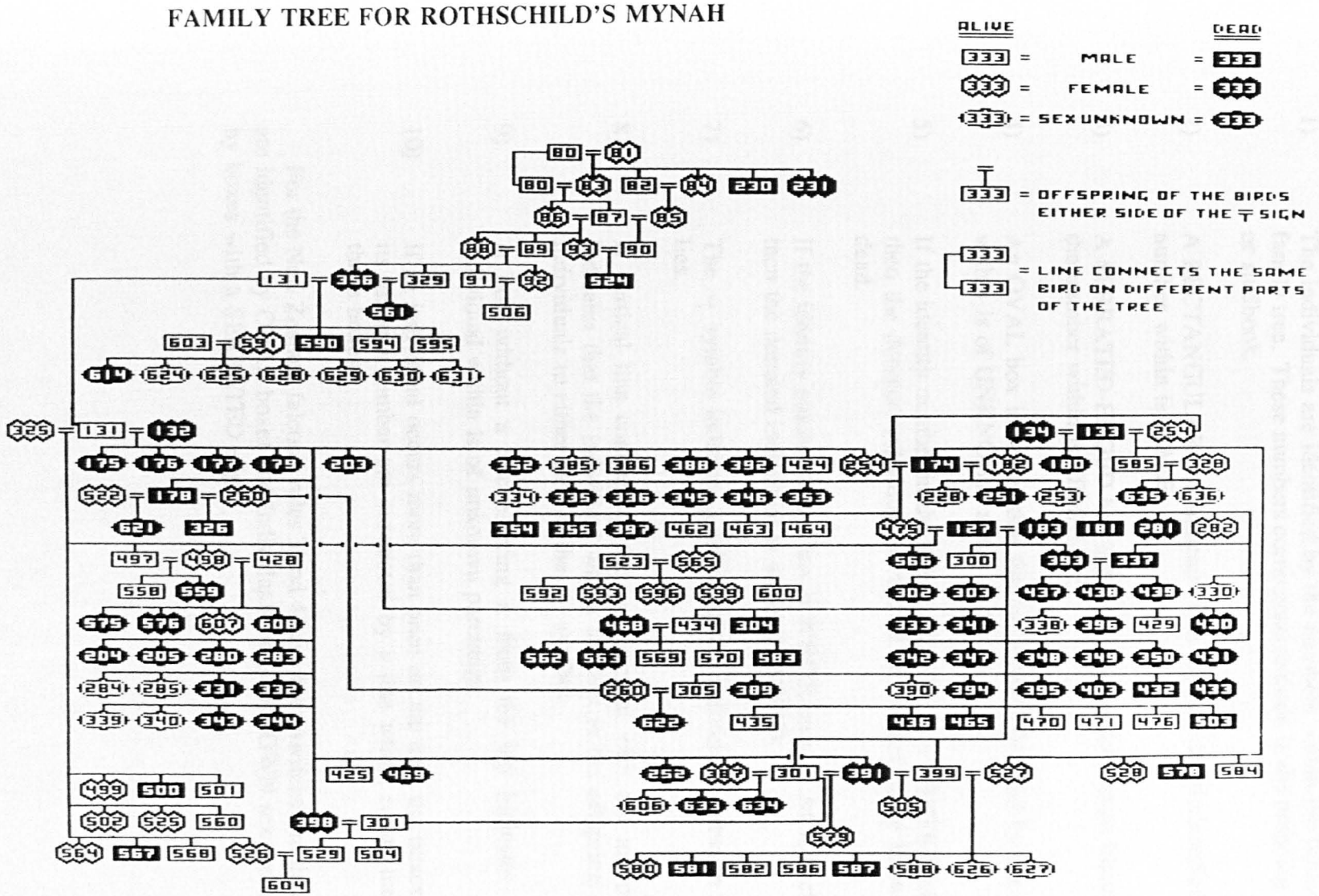


FIGURE 2.1: Family tree for the British captive stock of Rothschild's mynahs, including all individuals listed in the stud book for 1988 (Mace, unpublished a).

1) The individuals are identified by the numbers within the boxes on the family tree. These numbers correspond to those in the breeding records or studbook.

2) A RECTANGULAR box indicates that the individual identified by the number within is MALE.

3) A SERRATED-EDGED box indicates that the individual identified by the number within is FEMALE.

4) An OVAL box indicates that the individual identified by the number within is of UNKNOWN sex.

5) If the identity number in the box is BLACK on a WHITE background then the denoted individual is ALIVE or not definitely known to be dead.

6) If the identity number in the box is WHITE on a BLACK background then the denoted individual is definitely DEAD.

7) The = symbol indicates a pairing of the individuals between which it lies.

8) A vertical line connecting the = symbol with the top of a box indicates that the individual within the box is the offspring of those individuals to either side of the = symbol.

9) A box without a line entering it from the top indicates that the individual within is of unknown parentage.

10) If an individual occurs more than once on the tree, the boxes holding its identity number are connected by a line which enters the side of those boxes.

For the New Zealand falcons, rules 3 and 4 have been switched so that females are identified by OVAL boxes and individuals of UNKNOWN sex are identified by boxes with a SERRATED edge.

CHAPTER 3

ROTHSCHILD'S MYNAH

(*Leucopsar rothschildi* Stresemann, 1912)

3.1 INTRODUCTION

3.1.1 General Introduction

It is possible for a bird to be rare simply because the habitat into which it has evolved is in a restricted area, such as an island, a mountain top or an isolated valley. Further localization of the species may occur if it has evolved with some particular ecological requirement, such as a narrow food range or a limited temperature tolerance. A rare species, therefore, is not necessarily a threatened one, although it may be said to be more vulnerable to extinction than one which is naturally more abundant.

When those involved in the international cage-bird trade become aware of the existence of a rare bird, especially an aesthetically pleasing one that might command high prices, they can quickly obtain specimens to satisfy a newly created demand (Fisher, Simon & Vincent, 1969). This demand can increase rapidly and, due to the heavy losses that occur in the capture, transportation and storage of the species, the natural stocks can decline sharply. If such persecution continues unabated, the species will rapidly move towards extinction. This appears to be precisely what has happened to Rothschild's mynah, a bird endemic to the island of Bali, whose numbers in the wild have declined to between 30 and 40 individuals, about 3% of their total when originally discovered (Seibels, 1991).

3.1.2 The Island of Bali

Bali is one of 13,600 islands which make up Indonesia, about 3,000 of which are inhabited (Myers, 1979; ICBP, 1990). Situated off the southwest coast of Java in the Indian Ocean, Bali is 144km long and up to 80km wide, covering an area of about 5,200km^2 (see Figure 3.1) (Klapste, 1984). The island is one of the most densely populated regions on Earth; with nearly three million inhabitants, it has a population density as high as that of Britain or Japan.

Land is obviously at a premium on such a populous island and competition with Man for this land has been one of the factors that has contributed to the decline of the island's many species. Of the 32 native mammalian species originally thought to have existed on Bali, only four remain (Soulé, Wilcox & Holtby, 1979). Man has had a major impact on the natural range of Rothschild's mynah (see Section 3.1.5) and it is now limited to a small area on Bali's northwestern peninsula, within the Bali Barat National Park (Harrison, 1968; PHPA, ICBP & AAZPA, 1987).

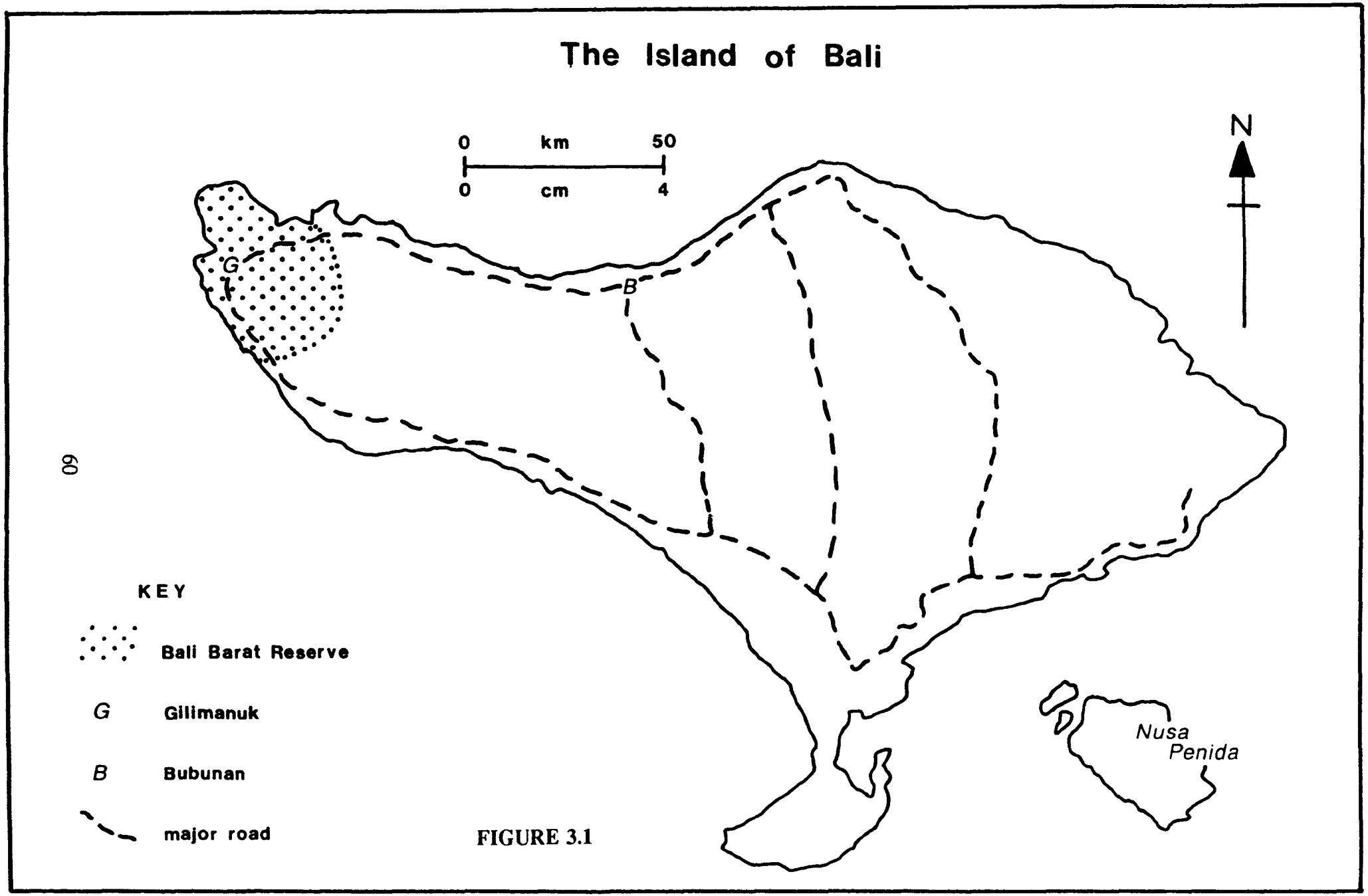

The Island of Bali
0 km 50
0 cm 4
N
G
B
Nusa
Penida
KEY
Bali Barat Reserve
G Gilimanuk
B Bubunan
major road

FIGURE 3.1

The Bali Barat was established as a reserve in 1947 (de Iongh, 1983b) and given National Park status in 1984 (Anon., unpublished a). It is about 70,000ha in area and is an arid region of scrub and low monsoon forest, with an undergrowth of grasses and thorny shrubs (Anon., unpublished a; Hughes & Turner, 1975; de Iongh, 1983b). This is the only area of natural habitat for the mynahs that remains on the island.

3.1.3 Taxonomy

KINGDOM	- Animalia
PHYLUM	- Chordata
SUBPHYLUM	- Vertebrata
CLASS	- Aves
ORDER	- Passeriformes
SUBORDER	- Passeres (Oscines)
FAMILY	- Sturnidae
SUBFAMILY	- Sturninae
GENUS	- Leucopsar
SPECIES	- rothschildi

The family Sturnidae contains about 110 species grouped in 25 genera (King, 1979). Starlings are restricted to the Old World, except where they have been introduced by Man (Campbell & Lack, 1985). *Leucopsar rothschildi* is also known as Rothschild's grackle, the Bali starling, the Bali Star, and the Bali mynah, and is the only representative of its genus (King, 1979).

3.1.4 Discovery of Rothschild's Mynah

Rothschild's mynah was discovered on the 24th of March, 1911, when the ornithologist Erwin Stresemann caught a female specimen in the forest at Bubunan on the island of Bali (see Figure 3.1) (Klapste, 1984). Stresemann described the bird, which is the only endemic vertebrate on Bali, in a letter to the Bulletin of the British Ornithologist's Club a year later (Stresemann, 1912). He named the bird *Leucopsar rothschildi*; the genus means "white starling", and the species name was given in honour of Lord Rothschild (Klapste, 1984).

3.1.5 General Biology and Behaviour

Leucopsar rothschildi is a white starling, about 10 inches long (see Figure 3.2). It has a long crest, the primary wing feathers have black tips, and there is a terminal black band to all the tail feathers. The iris is dark brown, contrasting markedly with the bright blue skin of the bare orbital region. Its main food in the wild consists of insects, seeds and fruit, and it also occasionally eats small reptiles (Stresemann, 1912; de Iongh, 1983b; Klapste, 1984). In 1980, van der Zon reported that the birds lived in flocks of up to 40 for six months of the year, with these groups breaking down from September to March when pairs formed for the breeding season.

FIGURE 3.2: Rothschild's mynah, *Leucopsar rothschildi*. Photograph by Robert Dawson.

Rothschild's mynah is sexually dimorphic, although the dimorphism is not extensive. Both sexes possess crests, but on average they are longer in the males. Unfortunately, these longer crest plumes do not appear until the second post-juvenile moult, and so sexing is usually not possible until after this time (Hughes & Turner, 1975). Also, there is an overlap in the variation found in the crest sizes of each sex. Some adult males have crests which are shorter than those of some of the females, making the sexing of even fully adult birds difficult without surgical intervention (Jeggo, 1981).

As mentioned previously (see Chapter 1, Section 1.3), the retention of animals in captivity for prolonged periods of time can lead to deleterious changes in behaviour. One such change found in Rothschild's mynah is the phenomenon of feather-plucking. This is related to allopreening, a normal behaviour which is seen both in captivity and in the wild (Hughes & Turner, 1975). Allopreening usually occurs between members of a mated pair and involves one bird grooming the crest and neck feathers of the other. However, under conditions of stress, such as aviary overcrowding, this behaviour can become exaggerated to actual plucking of the feathers. Feather-plucking removes the feathers of the throat and crest, and is a phenomenon that has only been observed in captivity (Hughes & Turner, 1975). Plucking damages the crest, compounding the difficulties in sexing the birds, and it produces unsightly areas of bare, blue-black skin on the throat and neck (pers. obs., 1988). This problem is currently estimated to affect about 20% of captive individuals (Seibels, 1991).

There are some sex-specific vocalizations and behaviour patterns that can be used to assist sexing, but these are characteristic of pair-formation, and if pairs are to be housed separately from other birds, as they are at Jersey (see Section 3.1.9), then their sex obviously needs to be determined before pairing. Since non-invasive sexing of this species is made difficult by the factors outlined above, it was hoped that this study could improve the accuracy of male/female determination by using either sex-specific DNA probes or sex-specific bands found in a normal multi-locus DNA fingerprint (see Section 3.3).

3.1.6 Decline of Rothschild's Mynah

When Stresemann discovered Rothschild's mynah in 1911, it was distributed across an area extending from the western most tip of Bali to Bubunan, about 50km to the east of the present Bali Barat National Park (see Figure 3.1) (van der Zon, 1980; de Iongh, 1983a). In 1925, van der Paardt (1929) reported that he saw "hundreds" in the areas of greatest concentration. Unfortunately, however, all the estimates of the mynahs' numbers in recent years have indicated a steep decline (see Table 3.1 and Figure 3.3).

The decline of Rothschild's mynah has been due to the combined effects of four factors:

1) *Interspecific competition from the Black-winged starling Sturnus melanopterus tertius.* This subspecies of starling exists only on the islands of Bali and Lombok

TABLE 3.1: Estimates of the wild Rothschild's mynah population

YEAR	ESTIMATE	REFERENCE
1975	1000	Pranoto, 1975
1976	500-1000	Sieber, 1978
1976	300-400	van der Zon, 1980
1977	1000	Alikodra et al., unpublished
1978	95-217	Suwelo, 1976
1978	550	Taynton & Jeggo, 1988
1979	200	van der Zon, 1980
1980	≤200	Morrison, 1980
1980	≤200	van der Zon, 1980
1982	≤200	de Iongh et al., 1982
1983	230-280	de Iongh, 1983b
1984	200-250	Klapste, 1984
1984	125-180	van Helvoort & Hartojo, unpub.
1987	≤150	PHPA, ICBP & AAZPA, 1987
1989	24-31	Taynton & Jeggo, 1988
1990	≤30	ICBP, 1990
1991	36-39	Seibels, 1991

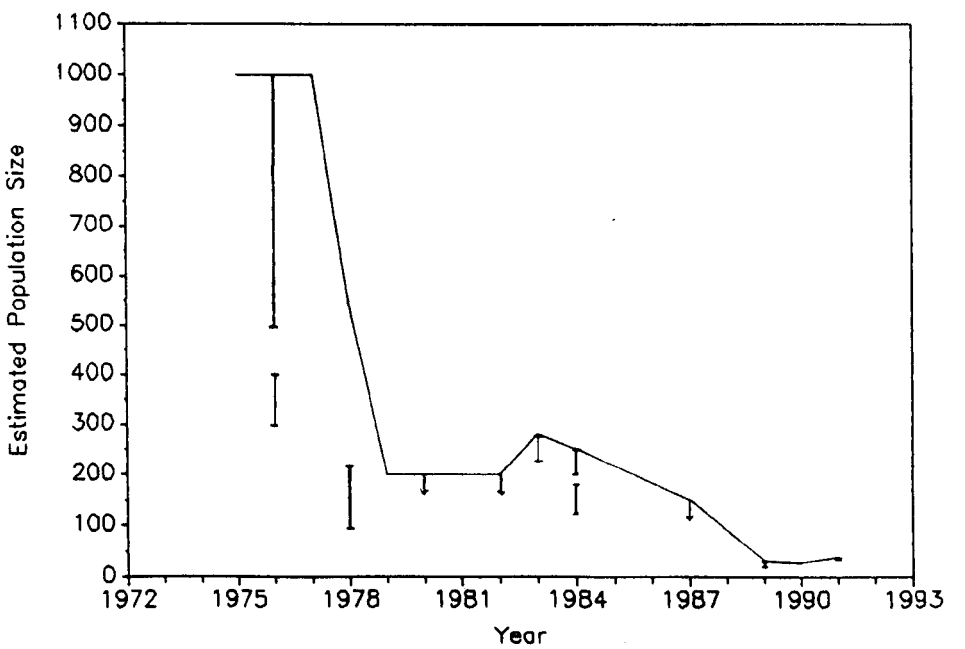


FIGURE 3.3: Graph showing the reduction in the numbers of wild Rothschild's mynahs over the last 20 years.

(Howard & Moore, 1980) and, although it did not exist within the mynahs' territory at the start of this century, its numbers increased until it was more than three times as abundant as the mynah (van der Paardt, 1929; King, 1979). The starling does not tolerate the presence of the mynah, and competes with it for nesting space (de Iongh, 1983a);

2) *Human disturbance by firewood collectors and farmers.* The peninsula where the Bali Barat National Park is now located was uninhabited before 1970 (Hughes & Turner, 1975). However, by 1984 over 4,500 people had moved into the reserve, making a living by planting crops and collecting and selling firewood (de Iongh, 1983b). These people had to be relocated by the Balinese government before the Bali Barat reserve could be granted National Park status (King, 1979).

3) *Destruction of its limited habitat.* The felling of trees for firewood resulted in the loss of the mynah's nest sites and feeding grounds. This problem was compounded by the activities of the Balinese Forestry Service, which cleared large areas of forest to develop teak and coconut plantations (de Iongh, 1983b). Unfortunately, the mynahs do not feed or breed within these plantations.

4) *Exploitation in the form of poaching to supply the pet-trade.* This is the most important cause of the decline in the wild mynah population. During the early 1960s, major importations of Rothschild's mynahs into Europe began, and by 1965 there were reported to be exactly 100 individuals in zoos around the world (Harrison, 1968). Due to the political situation in Indonesia at that time, it was impossible to find out what these importations were doing to the native stock and no data are available as to how many mynahs died during capture, storage or transportation in this period (Harrison, 1968).

Although such details were not available, it was known that the population was in serious decline, and in 1965 the mynah first appeared in the IUCN's Red Data Book, where it was classified as "Rare" (Jarvis, 1965) (see Chapter 1, Section 1.4.2 for an explanation of the IUCN's classifications). In 1979, the mynah was reclassified by the IUCN as "Endangered", there being thought to be only 200 individuals remaining on Bali (King, 1979; van der Zon, 1980).

The decline continued with birds regularly appearing in the markets of Java, Bangkok and Singapore (Morrison, 1980). In 1984, the remaining wild population was estimated at between 125-180, in sharp contrast with the approximately 1,000 birds then held in captivity worldwide (van Helvoort & Hartojo, unpublished). Recent estimates put the wild stock at just over 30 individuals (Seibels, 1991).

3.1.7 Conservation Measures Taken

Rothschild's mynah has been protected in Indonesia since 1957, when the Nature Protection Ordinance came into force, but it was not until 1971 that its capture, shooting and export were all expressly prohibited (King, 1979). The Bali Barat National Park, the last refuge of the mynah population, is now protected

from poaching and fire-wood collecting by a system of patrol trails, guard-posts and wardens (Anon., unpublished a). Unfortunately, this has failed to stop the mynah's decline since poachers simply disregard the law. In response, the International Council for Bird Preservation (ICBP) has formulated a series of proposals to safeguard and increase the size of the remaining wild mynah population (van Helvoort & Hartojo, unpublished).

All the major components of the plan are presented below for completeness, although this project has only been concerned with part 1):

1) *Increasing the size of the wild population to a minimum of 500 individuals through the reintroduction of captive birds* (see below).

2) *Installation of 100-150 nest boxes.* Unfortunately, these have gone largely unused by the mynahs due to them being usurped by the Black-winged starlings (van Helvoort & Hartojo, unpublished).

3) *Planting and propagating native fruit-bearing trees and to institute other habitat improvement measures.* It is acknowledged that this part of the plan will take some time to produce anything of value to the mynahs.

4) *Continuation of the public awareness exercises.*

5) *Involvement of local people in appropriate aspects of the project.*

The ICBP, who are coordinating the programme, have conducted surveys of Nusa Penida Island (South East of Bali) and the Bubunan area (North Bali) (see Figure 3.1) to locate potential sites for the reintroduction of mynahs captively bred in Indonesia. There has been only one previous introduction attempt using this species and that occurred in 1975, when four pairs were released on to the 40ha Lokrum Island, off the coast of Yugoslavia (King, 1979). Unfortunately, I have been unable to find any further reference to this programme in the literature, and so have no information regarding the success or otherwise of this project.

3.1.8 Current Threats to the Mynahs

The Black-winged starling continues to affect the mynah population by usurping its nest sites and the nest-boxes placed by the ICBP. The possibility of reducing or eliminating the starling population within the area of the National Park has been discussed, but there has been no action so far (G. Mace, pers. comm., 1989).

The destruction of the mynah's habitat has effectively ceased, and the threat presented by this is now negligible (Johnson & Stattersfield, 1990). Unfortunately, the persistent threat from poachers remains, and is currently the most important challenge to the continued existence of the wild population (de Iongh, 1983b; ICBP, 1990).

3.1.9 Captive Breeding

Up until the late 1960s, no Rothschild's mynahs had ever been bred from two previously captive bred birds (Partridge, 1969), even though the first live mynahs were brought to Europe in 1928, and the first successful captive breeding was in 1931 (Klapste, 1984; Taynton & Jeggo, 1988). The first individual bred from two captive bred parents was hatched in the early 1970s at Zurich Zoo and the first similar birth in Britain was at Jersey in 1975 (Fisher, unpublished).

In 1964, the International Zoo Yearbook listed 50 Rothschild's mynahs being held in 17 collections around the world. However, only five of these birds (10%) had been captively bred, and so a programme was initiated to increase the size of the captive stock (Jarvis, 1965). The mynah was found to breed well, producing a maximum of four offspring each year compared with one in the wild (PHPA, ICBP & AAZPA, 1987), and by 1969 there were 180 birds in captivity (Duplaix-Hall, 1973; Campbell, 1974). By 1973, there were 498, of which 27% were captive-bred (Duplaix-Hall, 1974), and there were 526 individuals in 1974, with the proportion bred in captivity having increased to 36% (Duplaix-Hall, 1975). By 1980, a total of 109 zoos had bred an estimated 612 Rothschild's mynahs (de Iongh, 1983b), and by the mid-1980s, the total had risen to over 1,000 (van Helvoort & Hartojo, unpublished).

The marked difference between the number of mynahs held in captivity and the number remaining in the wild meant that the "raw material" for a reintroduction programme was available. In 1987, the ICBP, in collaboration with the Indonesian Government and American and British zoos, launched a project to save Rothschild's mynah. The aim of the project was to secure the existence of the remaining individuals in the Bali Barat National Park, and to boost their numbers by restocking with mynahs bred in captivity (PHPA, ICBP & AAZPA, 1987; Taynton & Jeggo, 1988; ICBP, 1990).

The plans for the captive breeding and reintroduction programme involve sending birds bred in the USA and Britain to a breeding centre at Surabaya on Java. The first consignment, comprising 38 individuals from the American Association of Zoological Parks and Aquaria (AAZPA) and four from the Jersey Wildlife Preservation Trust (JWPT), arrived there in 1987, marking the beginning of a five year project. The ultimate target of the breeding programme is a minimum wild population of 500 birds, and the cost has been estimated at about $200,000 over the five years to 1992 (PHPA, ICBP & AAZPA, 1987). The projects target was revised in 1988 to a minimum viable population of 600 individuals, allowing 90% of the original genetic variation to be maintained over the next 200 years (Seibels, 1991).

3.1.10 The British Captive Stock

In 1971, the Jersey Zoological Park, later to become the Jersey Wildlife Preservation Trust, obtained eight Rothschild's mynahs to establish a captive population. The first successful breeding was in 1973 and, since then, in excess

of 100 individuals have been reared (Jeggo, 1981). The birds are housed in pairs in outdoor aviaries and, although they never occupy adjacent cages (they fight through the wire), they are in sight and sound of others. In 1975, a stock was also established at London Zoo using individuals bred at Jersey (Fisher, unpublished). It is from these two institutions that most of the mynah samples used in this study came.

The British breeding programme is currently in its eighth year, with the population at the end of 1991 reaching 110 individuals (50 males, 35 females and 25 of undetermined sex). Unfortunately, although the population is stable it is not growing (Fisher, unpublished).

According to the studbook, which was started in 1985, the captive British population was originally founded by 8 individuals, imported in two separate batches:

1) 4 birds from the wild were transported to a stockist in Vogel, Germany, and then to Jersey (studbook nos. 131, 132, 133, 134),

2) 4 birds were imported to Jersey from Bali via Keston Bird Farm in England (studbook nos. 125, 126, 127, 128).

The majority (93%) of the birds in the current population are descended from just five of these eight founders: the four "German" individuals and one of the wild specimens (127). I was able to analyse blood samples from only three of these birds, those being 131, 133 and 127, and the results have serious implications for the British stock (see Section 3.3.2).

Over the past few years, a number of birds have been imported from the continent in an attempt to reduce the levels of inbreeding within the stock (Mace, unpublished a). The genetic background of these birds, and their relationships to each other or those birds already in this country, is unknown. It is hoped that the influx of birds from external sources will increase the genetic diversity within the British stock (providing they breed successfully) and stabilise the base for population growth, increasing the long-term viability of the stock (Fisher, unpublished).

3.1.11 Aims of this Study

The problems caused by declining diversity within the British captive stock have, in some cases, been exacerbated by the pairing of unsuitable, i.e. related, individuals. This has been due to a lack of information regarding the parentage and relatedness of certain birds. Some of these inappropriate pairings occurred because it was necessary during the development of the studbook to adopt a policy that if a bird's parentage was unknown, that individual would be allocated to the most productive pair then breeding at the location where it was born (G. Mace, pers. comm., 1988). Obviously, this could lead to birds being assigned to the wrong parents and, eventually, to the pairing of birds which, although apparently

unrelated "on paper", were in fact relatives.

The aim of this study was to use DNA fingerprinting in an attempt to identify the birds which had been misassigned and to try and allocate them to the correct parents. In addition, an attempt was to be made to determine which birds were unrelated (or distantly related) so that they could be paired, thus retaining as much genetic diversity in the stock as possible. To this end, the relationships between the founders of the British stock were investigated.

3.2 MATERIALS AND METHODS

3.2.1 General Introduction

The information presented in this section outlines the alterations which were made to the materials and methods presented in Chapter 2 in order to produce a DNA fingerprint for Rothschild's mynah. The numbers of the specific sections in Chapter 2 to which these amendments and relevant comments apply are given in parentheses.

3.2.2 Comments on Tissue (2.1.1)

The amount of tissue available for the study of the mynahs was limited. In the case of blood samples, this was due to the fact that the birds were only bled at specific times of the year, and in the case of post-mortem tissue, because the veterinarians at the zoos supplying the samples (i.e. London and Jersey), disposed of individuals shortly after sampling. In order to conserve the limited tissue stocks at my disposal, the initial analyses to determine the best enzyme to use and the most appropriate running conditions were done on blood samples from a related species, the Common starling, *Sturnus vulgaris* (data not shown).

Some blood samples were found to have congealed when they were defrosted for use. In such cases, a sterile tooth-pick was used to obtain a sample of clot approximately equal to the volume of liquid blood normally used, i.e. 25µl. This method is imprecise and occasionally an excess of tissue was taken from some of the samples. This made extraction of DNA more difficult necessitating the division of the sample (after lysis of the cells (2.1.1.2)) into two or more eppendorf tubes. The sample could then be diluted with the appropriate volume of 1xSET to return the total sample volume to 0.5ml. After extraction (2.1.1.3) and resuspension (2.1.1.4) the samples were recombined.

For solid tissues, e.g. liver, brain and muscle, the volume used for analysis was slightly larger than the equivalent amount of blood as some tissue was lost during processing, i.e. tissue adhered to the sides of the pestle after the sample had been ground in liquid nitrogen, and could not be recovered.

3.2.3 DNA Restriction (2.1.2)

As mentioned above, at the start of this study the amount of tissue available was limited and so *HaeIII*, the enzyme that the laboratory used as a standard, was used. This produced good results, but later in the study when more tissue became available (specifically a whole liver), other enzymes were tested. These included the "four base-pair cutters" *AluI* (recognition sequence AG/CT), *TaqI* (T/CGA), *MaeI* (A/CGT) and *MspI* (C/CGG), and the "five base-pair cutters" *HinfI* (G/ANTC) and *DdeI* (C/TNAG). The results of restricting the mynahs' DNA with the latter two enzymes were poor, but the data obtained from the "four base-pair cutters" were good, although they were certainly no better than those produced by *HaeIII* (data not shown). If additional tests were to be conducted, *AluI* would be the best enzyme to use, but for this first study of the mynahs, *HaeIII* was used exclusively.

3.2.4 Gel Electrophoresis (2.1.3)

The restricted mynah DNA samples were initially run through the agarose gel for around 1,600Vh, but it was found that when filters prepared from these gels were probed with pSPT19.6, they revealed an aggregation of high molecular weight minisatellites. These were poorly resolved after a run of this length and so later gels were run for an average of 2,000Vh, allowing better resolution of this region.

3.2.5 Probe Usage (2.1.5)

Probe pSPT19.6 was the one used extensively for this analysis, although pSPT18.15 was used to probe one filter. Probe pSPT18.15 does not detect the aggregation of high molecular weight minisatellites hybridized by pSPT19.6, and for this reason would have been the one chosen for this study. However, because of certain technical difficulties associated with this probe and problems of availability, pPST18.15 had to be abandoned during the early stages of this study. Unfortunately, by the time these problems had been solved, there was insufficient time to re-probe all the filters.

3.2.6 Analysis of DNA Fingerprints (2.1.8)

After electrophoresis, the bands remaining in the agarose matrix were approximately 30,000 to 3,000 base pairs (30kb - 3kb) in length. Of these bands, it was not normally possible to score those below about 5kb in length due to their "fuzziness" on the autoradiograph. In addition, even after an electrophoresis of 2,000Vh, the very heaviest minisatellites remained unresolved and unscorable, and these are excluded from this analysis.

3.3 RESULTS AND CONCLUSIONS

3.3.1 Initial Analysis

The mean number of bands scored per individual mynah in this study, using the Carter derivative of probe 33.6, pSPT19.6 (Carter, Wetton & Parkin, 1989), was 16.1 (σ = 3.01) for males, and 16.3 (σ = 2.36) for females; the combined mean, weighted for the numbers of each sex, is 16.15. The lack of a significant difference between these numbers indicates that the probe does not hybridise to any sex-specific minisatellites, thus it is not possible to sex the birds using this method. This was an unfortunate discovery as the possibility of non-invasive sexing of young birds was to be a point in favour of this survey when approaching private breeders for their assistance in this study.

The distribution of all the similarity coefficients (D) obtained for comparisons between the mynahs fingerprinted with the probe pSPT19.6 is shown in Figure 3.4.

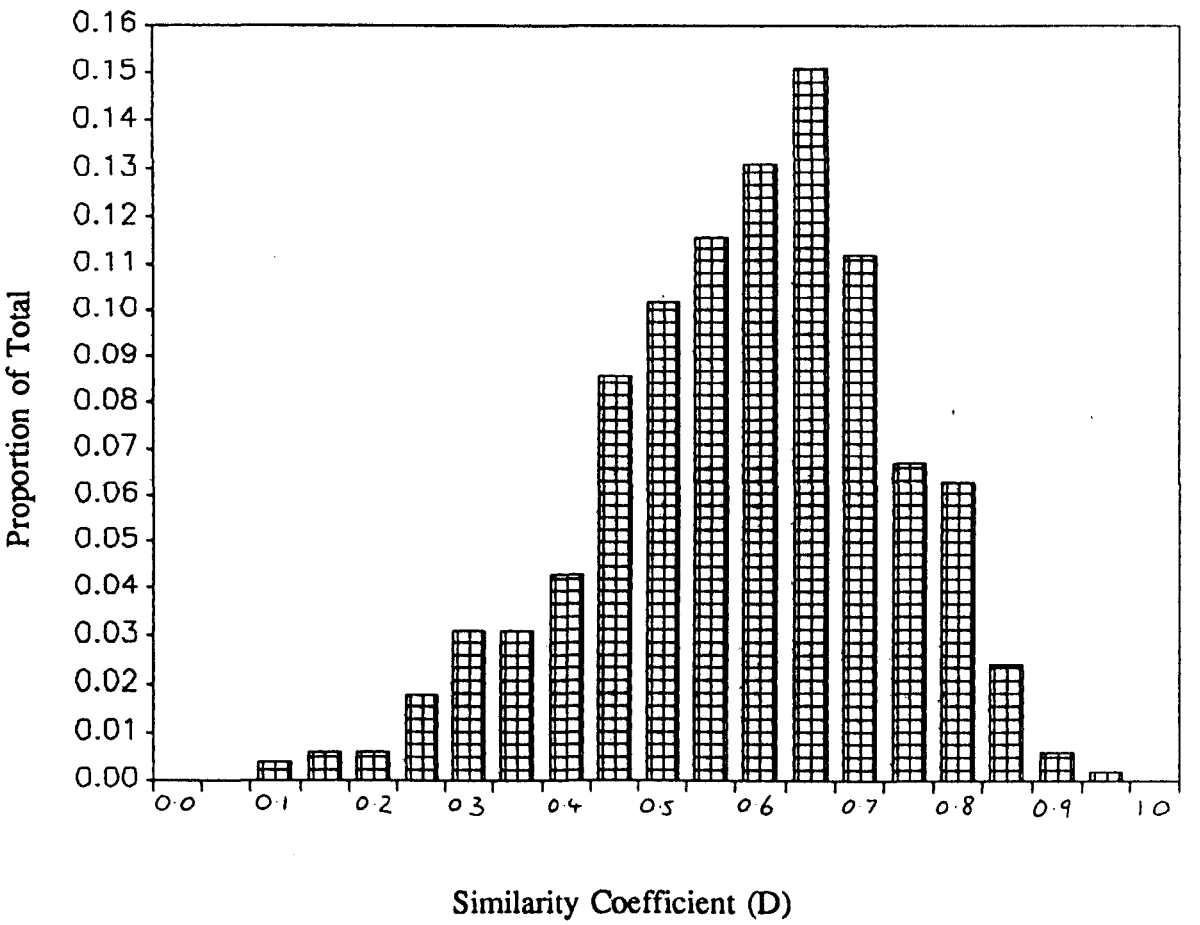


FIGURE 3.4: Graph showing the distribution of all mynah similarity coefficients (D) generated in this study using the probe pSPT19.6.

In an ideal population, a graph of the kind shown in Figure 3.4, would be expected to display a number of distinct peaks, each of which could be attributed to the modal value of D for a particular degree of relatedness. However, it can be seen that the distribution of the graph for the mynahs appears unimodal (mean = 0.592, σ = 0.144).

In an attempt to decipher these data, comparisons were made between the distributions of D for three separate relationship categories: unrelated individuals, 2nd-degree relatives, and 1st-degree relatives (see Figure 3.5). It should be pointed out that the allocation of relationships to individuals was based on data from the stud book and, as such, was non-independent and subject to any errors that might be present in the breeding records.

Table 3.2 shows the means and the standard deviations for the graphs presented in Figure 3.5 and as can be seen, the difference between the distributions of D for unrelated individuals, 2nd-degree relatives and 1st-degree relatives is not dramatic. This would explain why the graph in Figure 3.4 is unimodal.

TABLE 3.2: Comparison of the means and standard deviations for the distribution of similarity coefficients (D) produced by comparisons between birds of "known" relationship.

Statistic \ Relationship	Unrelated	2nd-degree	1st-degree
No. of comparisons	177	69	61
Mean	0.495	0.624	0.699
Standard deviation (σ)	0.153	0.137	0.144

In order to use DNA fingerprint data to give an indication of relationships in a situation where allele frequencies are unknown, the background level of band-sharing must be known. A natural population of the House sparrow (*Passer domesticus*) has been studied by members of the Genetics Department at the University of Nottingham for over ten years, the last seven of which have involved DNA fingerprint analysis (Wetton et al., 1987). The results have shown that even in this large, outbred population the background level of band-sharing, as revealed by similarity coefficients, is well above zero (see Table 3.3).

Among unrelated sparrows, D was found to be 0.150, and that between known 1st-degree relatives was 0.575 (Wetton, Parkin & Carter, in press). Table 3.3 also shows the results of the same analysis performed on the British stock of Rothschild's mynah and shows that known 1st-degree relatives (i.e. individuals whose relationship as listed in the stud book is not directly contradicted by fingerprint data) have a mean similarity coefficient of 0.699. This is 0.124 higher than that obtained for the sparrows and can be explained by the greater degree of inbreeding in the mynah stock.

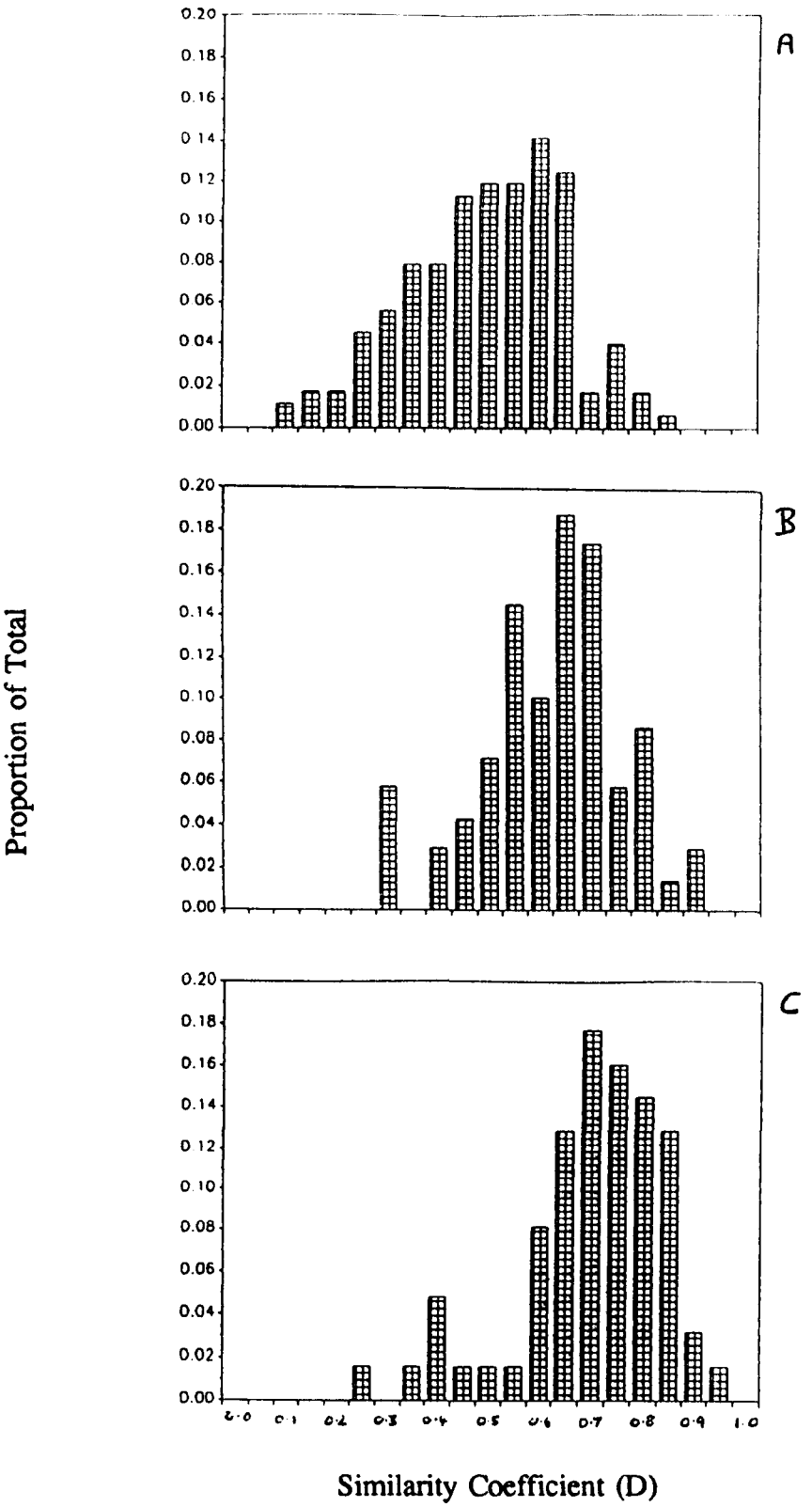


FIGURE 3.5: Distribution of similarity coefficients (D) for three categories of relationship; a = comparisons between unrelated mynahs, b = comparisons between 2nd-degree relatives, c = comparisons between 1st-degree relatives.

TABLE 3.3: Comparison of mean similarity coefficients for House sparrows and Rothschild's mynahs. Numbers of comparisons used shown in parentheses.

Species \ Relationship	Unrelated	2nd-degree	1st-degree
Sparrows	0.150 (51)	-	0.575 (833)
Mynahs	0.495 (177)	0.624 (69)	0.699 (61)

However, the mean D for unrelated mynahs, which represents the background level of band-sharing, was 0.495. This is only 0.129 lower than that for 2nd-degree relatives and 0.204 lower than the estimate for 1st-degree relatives. This means that when determining relatedness, the sharing of just two extra bands could be the difference between a pair of individuals assigned to the "unrelated" category and a pair assigned as "2nd-degree relatives". Furthermore, the sharing of just one additional band could be the difference between "2nd-degree relatives" and "1st-degree relatives". In sparrows, the difference between "unrelated" and "1st-degree relatives" is 0.425 for a mean number of bands scored per individual of approximately 14 and the distinction is more clear; the sharing of 6 extra bands is required to move a pair of individuals from "unrelated" to "1st-degree relatives". One way to explain these data is to assume that some of the information in the Rothschild's mynah stud book is wrong, resulting in the incorrect assignment of relatedness.

3.3.2 Relationships of the Founders

Given the data in Section 3.3.1, it would not be possible to confidently allocate a relationship to a pair of birds based solely on the similarity coefficient between them, as has been discussed in the scientific literature (Lynch, 1988; Westneat, 1990; Morton, Forman & Braun, 1990). As mentioned above, the failure to adequately distinguish between these three degrees of relatedness is probably caused by incorrect relationship allocations given in the stud book. Indeed, during the construction of the mynah stud book, there was a policy of allocating birds of unknown parentage to the breeding pair that were the most productive in that particular location at that time (G. Mace, pers. comm., 1989). This system would almost definitely have resulted in some birds being incorrectly allocated.

Another cause of error was the absence of information regarding the relationships of five of the founders of the British stock. The founders (stud book nos. 127, 131, 132, 133 and 134) were of particular importance because the majority of the birds in the current stock are descended from one or more of these five individuals, and so the genetic constitution of the population depends greatly on the alleles possessed by these birds. Three have been analyzed here (see Figure 3.6), but, unfortunately, the other two died some years before this project began,

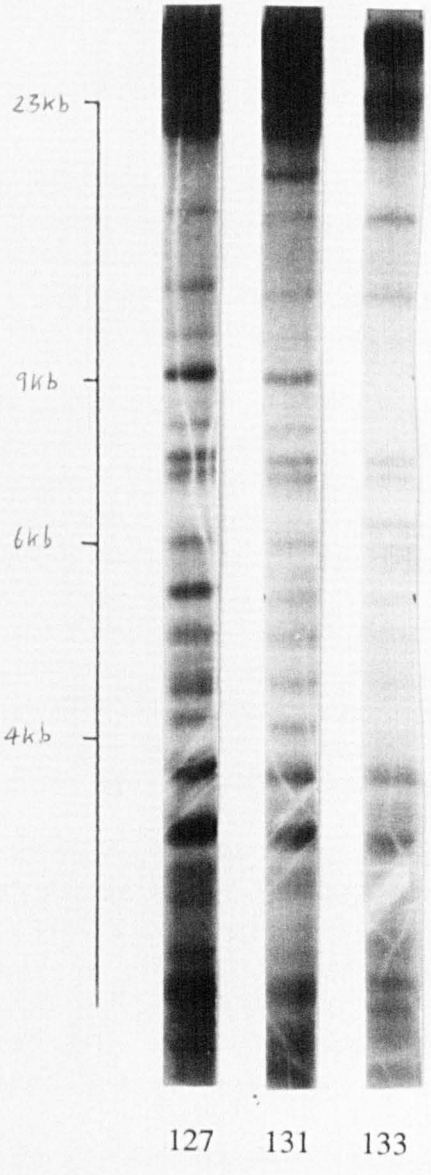


FIGURE 3.6: DNA fingerprint of the three British stock founders available for analysis: individuals 127, 131 and 133. The white "cracks" on the photograph were caused by a fault on the intensifying screen used (see Chapter 2, Section 2.1.7.2).

and now all these five founders are dead. The similarity coefficients between those available for analysis are shown in Table 3.4.

TABLE 3.4: Similarity coefficients (D) between three British captive stock founders.

Stud Book No.	133	131
127	0.658	0.800
131	0.640	/

As can be seen from a comparison of the results in Table 3.4 with those in Table 3.2, the similarity coefficients between these three founders are all higher than the mean obtained for mynah 2nd-degree relatives, and one is greater than that for 1st-degree relatives. The results, therefore, point to the possibility that at least three of the five founders of the British stock were close relatives.

One of the reasons that the mean D for unrelated mynahs (shown in Table 3.2) was high could be that the calculations included results from the comparisons between the founders, and between these individuals and their immediate offspring. If the founders *were* related, this would result in a number of high similarity coefficients being used in the analysis of unrelated individuals, increasing the mean value of D for that group. Also, data were included from comparisons between birds listed as the offspring of pair 435/565 in the stud book, but which were later found to be misallocated (see Section 3.3.3). In addition, further data were included from comparisons between birds considered "unrelated", although they were later found to be related, and at least one of them was probably a 3rd-degree relative of one of the founders (see Section 3.3.4).

Although the founders share a high proportion of their bands, this does not prove that they are closely related. Because there are inaccuracies in the use of single similarity coefficients (Lynch, 1988), it was decided that the founders should be compared with as many other individuals as possible, and indeed that as many comparisons between members of the British stock as possible be made and D for these calculated. This would hopefully give a more accurate overall picture of the relationships of the founders.

Five hypotheses were formulated to explain the similarity coefficient data, assuming that these data indicated the possibility of 1st-degree relationships among the founders (see Table 3.5). The 1st-degree relationship "siblings" was chosen because all the birds came from Bali at much the same time, although 127 arrived before 131 and 133 (see Section 3.1.10). Although there were thought to be over 1,000 birds in the wild at the time the founders were imported to Britain (see Table 3.1), the methods employed to gather wild specimens does not rule out the possibility that the founders were related. It is known that, when a bird gatherer

on Bali found a nest, all the eggs and young within would be taken and sent to Java for distribution around the world (Morrison, 1980; D.J. Jeggo, pers. comm., 1989).

TABLE 3.5: Five hypotheses for the relationships between the founders 127, 131 and 133.

Model	Hypothetical relationship
A	None of the three founders are related.
B	Individuals 131 and 133 are siblings.
C	Individuals 127 and 131 are siblings.
D	Individuals 127 and 133 are siblings.
E	All three founders are siblings.

If the 1st-degree relationship "parent/offspring", or 2nd-degree relationships are also considered, many other hypotheses can be formed for the relationships between the founders, but for the purposes of this analysis, only the possibility of the founders being siblings was considered.

Approximately 500 similarity coefficients (D) were calculated between members of the British stock. Similarly, for each hypothetical relationship, the corresponding expected proportion of genes shared (a statistic I have called G) was estimated between individuals. G was obtained directly from the family tree (see Figure 3.7) and so was estimated under the assumption that the stud book data were correct.

The statistic G was calculated by determining how many different paths there were between two individuals on the family tree, given that the specific hypothesis being tested (model A, B, C, D or E) was correct, and how many steps there were in each path. For example, parent to offspring is one step, cousin to cousin is three. One step means that G is 0.500; three steps equates to 0.125 (0.5^3). The scores for each path were added together to give a final overall estimate of G (see Appendix 1). D and G were then plotted and a regression analysis performed to determine to which of the five hypotheses the similarity coefficient data fitted best. Figures 3.8a, b, c, d and e show scatter graphs of the similarity coefficients obtained (D) plotted against the appropriate proportion of genes shared (G) for the model being tested.

Table 3.6 shows the results of regression analysis of the data presented in Figures 3.8a, b, c, d and e. As can be seen, the regression for model C (founders 127 and 131 are 1st-degree relatives) is the only one which produces a significant fit, $t = 3.376$ for 490 d.o.f). However, because all the relationship data used in this analysis came from a family tree which was based on entries in the Rothschild's mynah stud book, it was decided to check these data more carefully

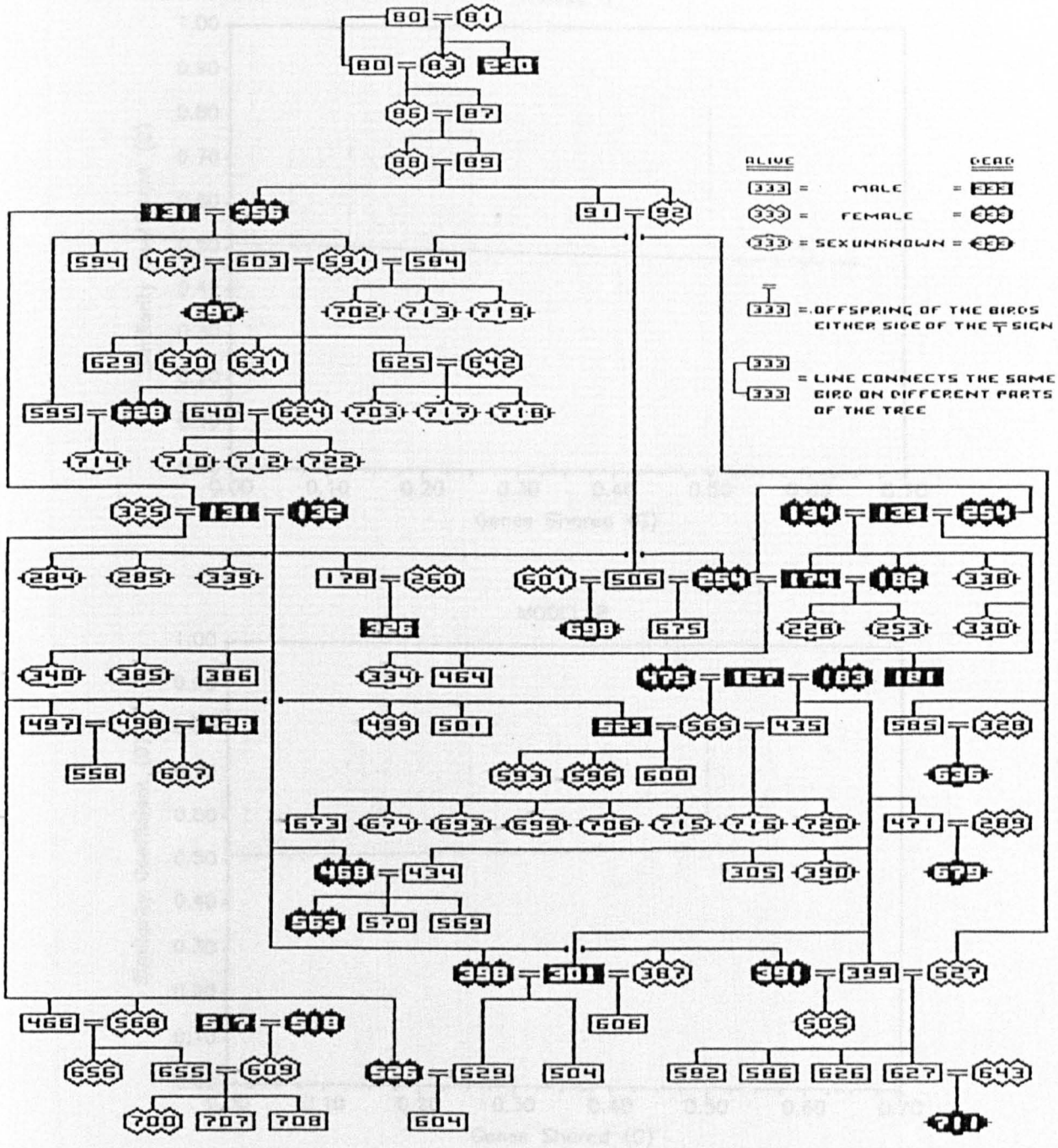
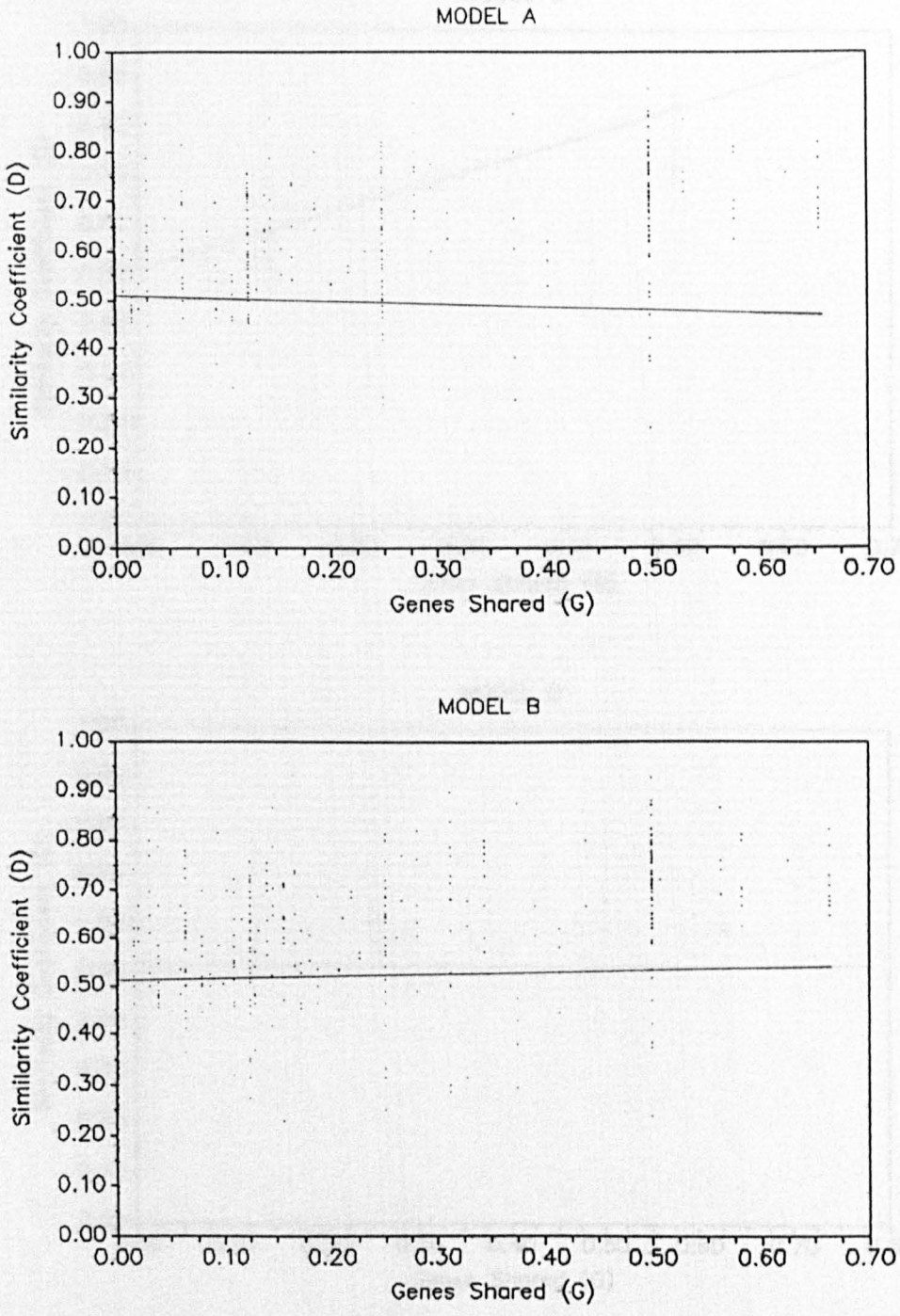
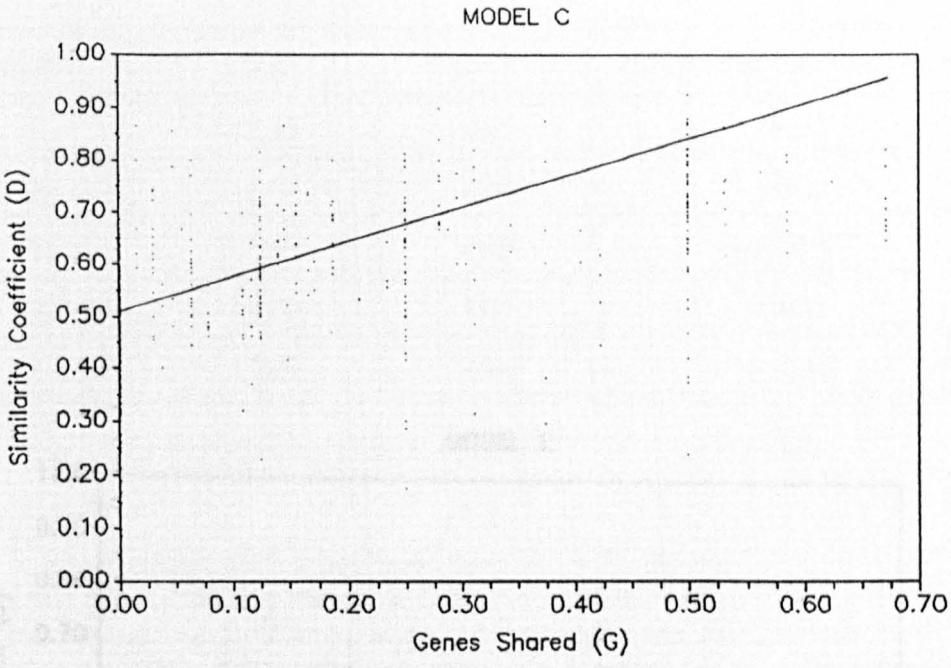
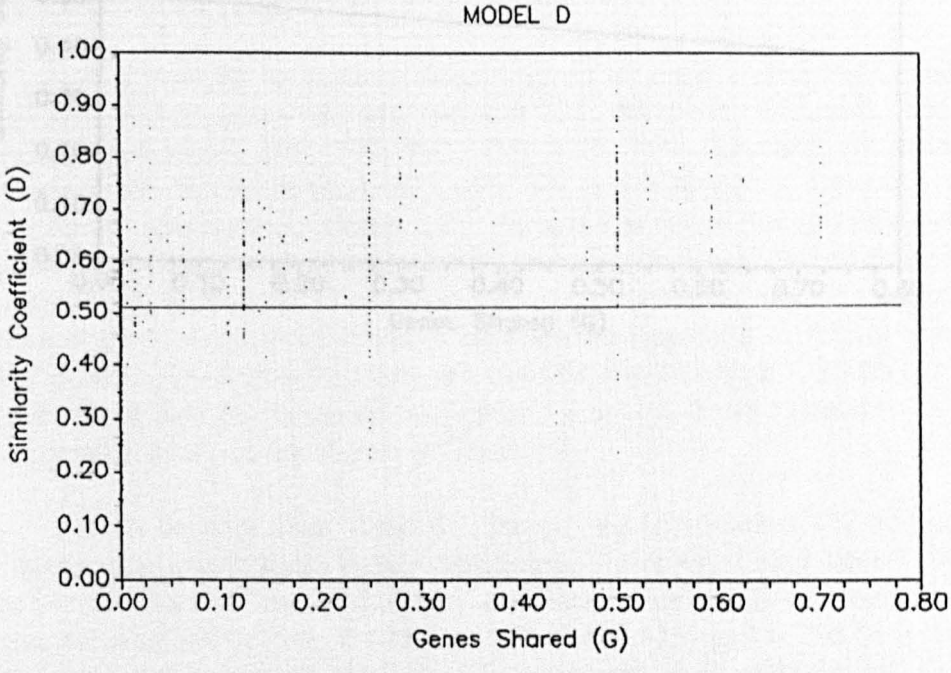


FIGURE 3.7: Family tree of the British captive stock of Rothschild's mynah, *Leucopsar rothschildi*, compiled from information in the stud book for 1989.



FIGURES 3.8a and b: Graphs showing the results of plotting the similarity coefficient data (D) obtained from comparisons between members of the British captive stock of Rothschild's mynah against the proportion of genes shared (G) between them given founder relationship hypotheses A and B. Regression lines shown.





FIGURES 3.8c and d: Graphs showing the results of plotting the similarity coefficient data (D) obtained from comparisons between members of the British captive stock of Rothschild's mynah against the proportion of genes shared (G) between them given founder relationship hypotheses C and D. Regression lines shown.

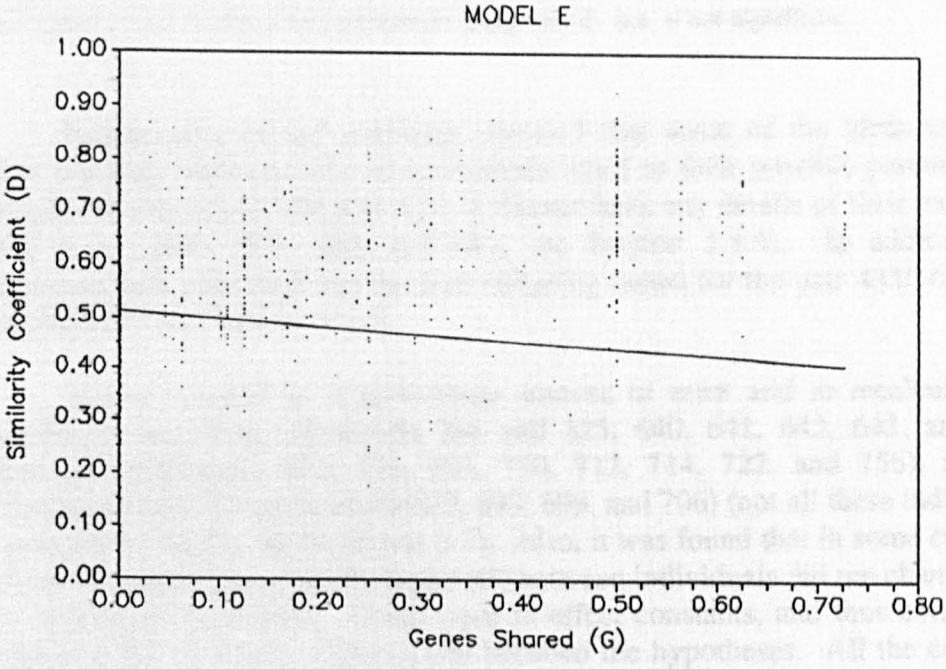


FIGURE 3.8e: Graph showing the result of plotting the similarity coefficient data (D) obtained from comparisons between members of the British captive stock of Rothschild's mynah against the proportion of genes shared (G) between them given founder relationship hypothesis E. Regression line shown.

to ensure that errors in the stud book had not affected the analysis.

TABLE 3.6: Regression analysis of fitting similarity coefficient data to models for hypotheses A-E.

Model[a]	t-test	d.o.f.	Probability and significance
A	-0.339	490	n.s.
B	0.117	490	n.s.
C	3.376	490	<0.001***
D	0.016	490	n.s.
E	-1.209	490	n.s.

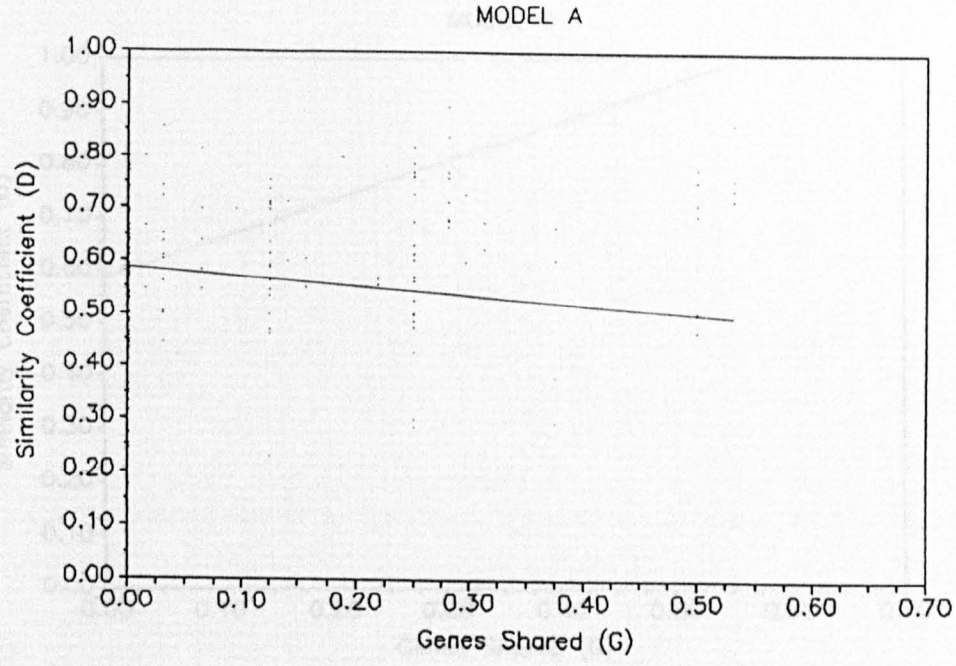
[a] See Table 3.5 for details of the hypothesis being tested. n.s. = not significant.

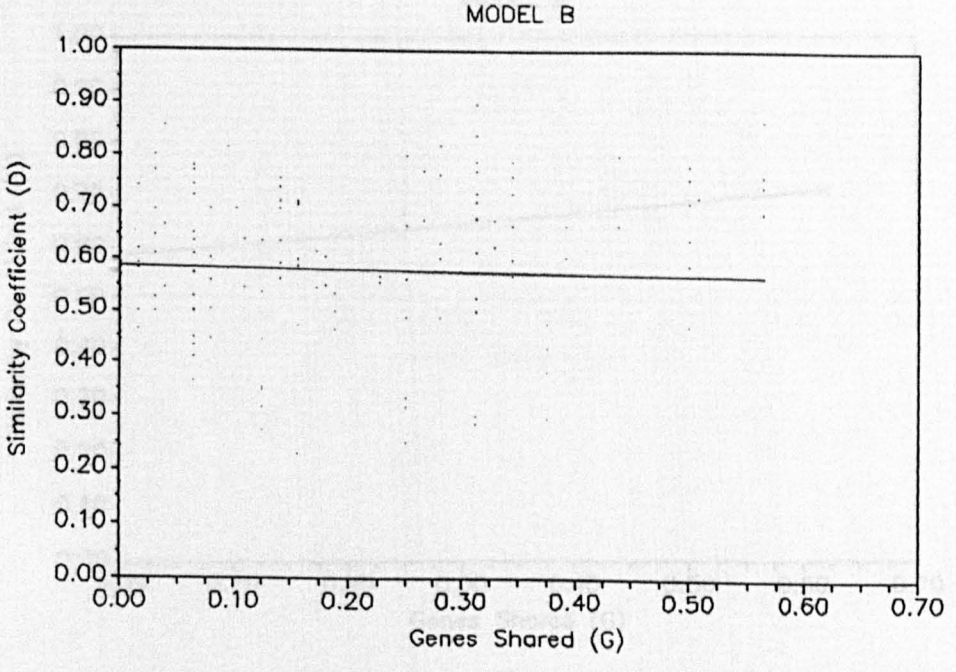
Examination of the studbook revealed that some of the birds analyzed either had more than one pair of individuals listed as their possible parents (as is the case for individuals 296 and 325) or did not have any details of their parentage listed at all (640, 641, 642, and 643; see Section 3.3.4). In addition, the fingerprint data indicated that the four offspring tested for the pair 435/565, were misallocated (see Section 3.3.3).

It was decided to remove these sources of error and to recalculate the regressions excluding individuals 296 and 325; 640, 641, 642, 643, and their offspring (individuals 692, 701, 703, 710, 712, 714, 722, and 756), and the offspring of 435/565 (individuals 673, 693, 699, and 706) (not all these individuals appear on the family tree in Figure 3.7). Also, it was found that in some cases the calculated proportion of genes shared (G) between individuals did not change from one hypothesis to another. These were in effect constants, and thus contributed nothing to the attempt to differentiate between the hypotheses. All the constants were therefore also removed before the analysis was repeated. The scatter graph plots for these data are presented in Figures 3.9 a, b, c, d and e, and the results of the regression analysis are shown in Table 3.7.

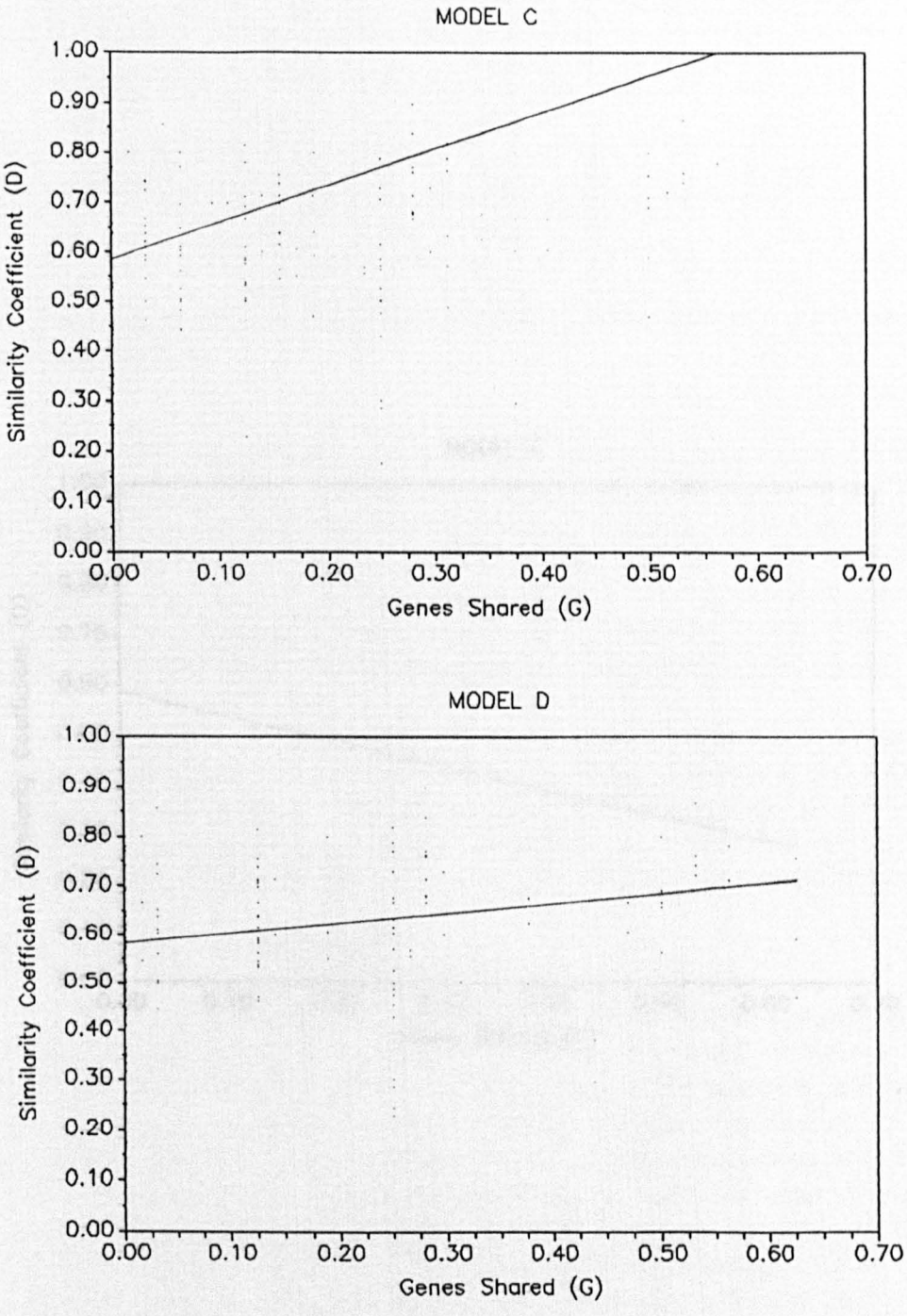
As can be seen from Table 3.7, hypothesis C (founders 127 and 131 are 1st-degree relatives) remains highly significant. However, if hypothesis C is taken to be correct, then the mean similarity coefficients for unrelated individuals and 1st-degree relatives become 0.425 ($n = 40$, $\sigma = 0.155$) and 0.706 ($n = 54$, $\sigma = 0.141$), respectively (cf., Table 3.2). Unfortunately, it is unlikely that a clearer picture will emerge from the data currently available, as the rather random appearance of the points on the scatter graphs would seem to confirm.

Checking these findings with information from the stud book (and G. Mace pers. comm.) revealed that the first four mynahs imported to Jersey, which included





FIGURES 3.9a and b: Graphs showing the results of plotting the adjusted similarity coefficient data (D) obtained from comparisons between members of the British captive stock of Rothschild's mynah against the proportion of genes shared (G) between them, given founder relationship hypotheses A and B. Regression lines shown.



FIGURES 3.9c and d: Graphs showing the results of plotting the adjusted similarity coefficient data (D) obtained from comparisons between members of the British captive stock of Rothschild's mynah against the proportion of genes shared (G) between them, given founder relationship hypotheses C and D. Regression lines shown.

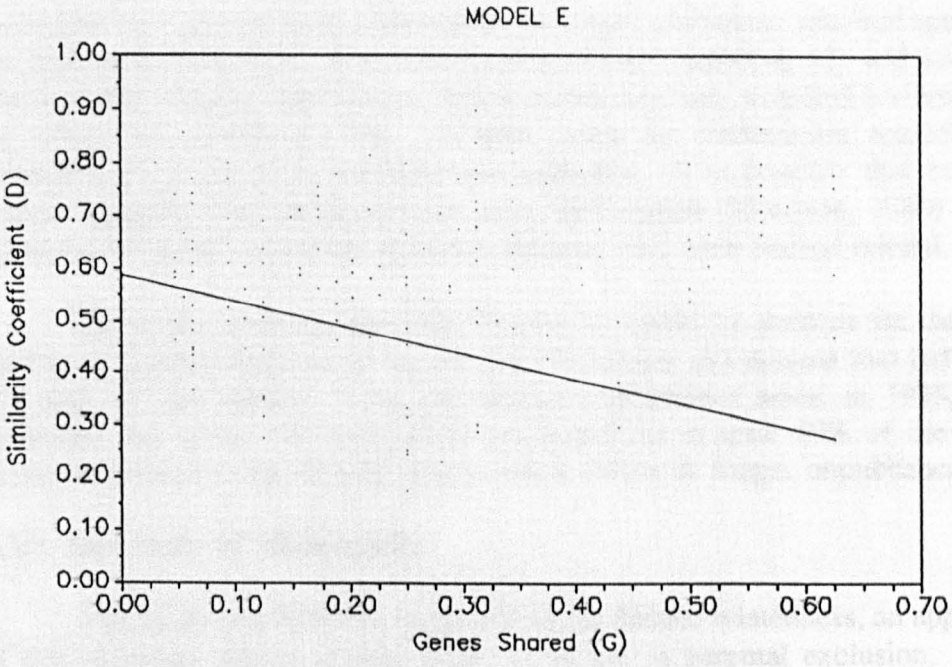


FIGURE 3.9e: Graph showing the result of plotting the adjusted similarity coefficient data (D) obtained from comparisons between members of the British captive stock of Rothschild's mynah against the proportion of genes shared (G) between them, given founder relationship hypothesis E. Regression line shown.

TABLE 3.7: Regression analysis of fitting the modified similarity coefficient data to models for hypotheses A-E.

Model	t-test	d.o.f.	Probability and significance
A	-0.596	151	n.s.
B	-0.187	151	n.s.
C	3.674	151	$<0.001^{***}$
D	1.073	151	n.s.
E	-2.397	151	n.s.

n.s. = not significant.

individual 127, arrived from a bird dealer in Vogel, Germany, who had apparently obtained them from Bali. The second batch of four, including 131 and 133, were obtained five months later from a British dealer who also acquired his birds from the wild (see Section 3.1.10). In both cases, no information regarding the relationships of the birds supplied was available. It is possible that both bird dealers acquired their birds from the same bird market (Morrison, 1980) so that, although the birds came from different dealers, they were indeed related.

Given the evidence presented above, it would be prudent for the future pairing of captive birds, to err on the side of caution and assume that individuals 127 and 131 are related. This has serious implications since, in 1985, it was estimated that these two birds alone accounted for at least 40% of the genetic variation present in the British captive stock (Mace & Jeggo, unpublished).

3.3.3 Discovery of Mismatches

Unlike the use of DNA fingerprinting to deduce relatedness, an application of the technique that is beyond doubt is its use in parental exclusion. This is important for certain population studies, e.g. heritability analyses, and to ensure the accuracy of a captive stock's breeding records. In studies of natural populations, if the identity of either of an offspring's parents is known, it is most often the mother's and so this technique can be used for paternal exclusion. Conversely, if the father is known, the technique can be used for maternal exclusion. Because of the way that minisatellites are inherited, an offspring will share approximately half its bands with its mother and half with its father. By comparing the DNA fingerprint of the known parent with that of the offspring, the bands which came from the other parent can be determined. It follows, that if a putative parent does not possess all these bands then he/she cannot have produced the offspring in question. Mutation events can complicate this procedure, but they are rare and are dealt with elsewhere (see Section 1.7).

In certain cases, it is not possible to exclude only one of the parents. If neither parent has been positively identified, there is no parental DNA fingerprint with which the offspring can be compared. In such a situation, the number of bands shared with each of the putative parents becomes important. If it is the case that the offspring shares a significantly greater number of bands with one of the parents, the latter can be excluded and the true parent sought.

In studies of wild bird populations, the discovery that an offspring shares very few bands with either of its parents is indicative of a mismatch. In such a case, the offspring is not directly related to either parent and its presence in the nest can be attributed to cuckoldry or egg-dumping (Wetton et al., 1987). During this study, a group of mismatches came to light in the British stock of Rothschild's mynah, but since the parents in question are in captivity, these mismatches are most likely to be due to incorrect parental assignment.

Individuals 673, 693, 699 and 706 are listed in the stud book as being the offspring of male 435 and female 565, the most productive pair currently breeding in Britain (Fisher, unpublished). When the DNA fingerprints of these six birds were examined, it was discovered that the offspring possessed a number of minisatellite bands which did not occur in either of the putative parents (see Figure 3.10). This indicates that at least one of the "parent" birds (435/565) is not the true parent of these offspring. Similarity coefficients (D) calculated between the "parents" and their "offspring" are uninformative in this case, as D is purely a measure of the number of bands that two individuals have in common. Instead, the number of bands shared between the "offspring" and their "parents" was assessed to determine whether any of the "offspring" shared a significantly greater number of their bands with either "parent" bird. The results of this analysis are shown in Table 3.8.

TABLE 3.8: Number of minisatellite bands shared between individuals 673, 693, 699, and 706 and their "parents" 435 and 565. Figures in square parentheses are the number of bands scored per individual and the figures in round parentheses are the number of bands shared which are unique to each of the "parents".

Stud Book No.	673 [17]	693 [15]	699 [20]	706 [15]
435 [19]	13 (3)	11 (3)	16 (3)	12 (2)
565 [18]	12 (2)	10 (3)	15 (2)	12 (2)

Unfortunately, both 435 and 565 share approximately the same number of bands with each of the "offspring", so one cannot be excluded in favour of the other. Because the "parents" share over 70% of their bands themselves, the analysis was repeated, this time taking into account only those bands unique to each of the parents. The results, as shown in round parentheses in Table 3.8, were

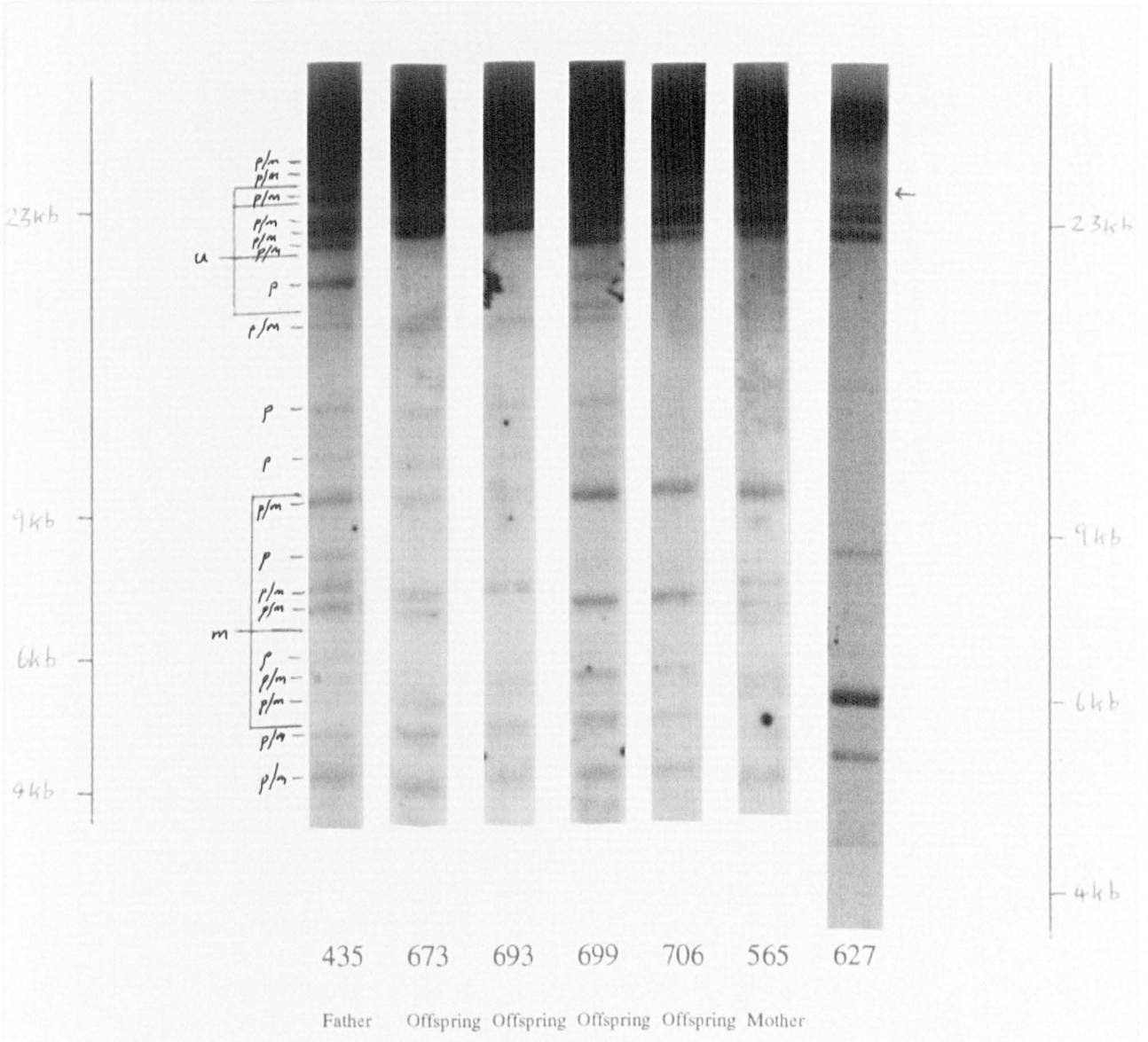


FIGURE 3.10: DNA fingerprint of individuals 435, 565 and their "offspring". The lines to the left side of the figure indicate the positions of the bands present in the "offspring" which are shared with 435 alone (p), 565 alone (m), both "parents" (p/m), or with neither individual (u). Also, shown is the DNA fingerprint of individual 627 (this differs from those of the other birds due the sample having been electrophoresed for a greater length of time). The arrow to the right of the figure indicates the position of the band shared by 693, 699, 706 and 627, that is not present in either 435 or 565.

again inconclusive, with neither "parent" obviously sharing a greater number of each "offspring's" bands than the other.

If there had only been one band unique to one "offspring", then a mutation event could be invoked to explain the results. However, the fact that all four "offspring" have at least one unassigned band and, indeed, if the time allowed for electrophoresis is reduced, individual 673 can be scored as having three unassigned bands (Ashworth & Parkin, 1992), this means that a number of separate mutation events would have to have occurred, and this is extremely unlikely.

If mutation is ruled out, there are two other possible explanations for these results:

1) the "offspring" were incorrectly assigned to parents 435 and 565;

2) the blood samples taken were incorrectly labelled or the birds identification numbers were incorrectly read.

The latter possibility has been investigated from the DNA fingerprinting end and the samples were correctly labelled, i.e. they match the numbers on the sample vessels from Jersey Wildlife Preservation Trust (JWPT), and they were correctly loaded on the gel. Therefore, either explanation 2) is correct with respect to the sampling location or explanation 1) must be accepted.

If the mismatching bands seen in Figure 3.10 can be identified in the fingerprint of another individual breeding at JWPT between 1988 and 1989, when individuals 673, 693, 699 and 706 were all hatched, it should be possible to identify their nearest relatives. Examination of the DNA fingerprint data obtained during this study shows that it is possible to identify at least that mismatching band shared by individuals 693, 699 and 706, in the fingerprint of individual 627, and possibly one of the mismatching bands in individual 673. It should be pointed out that the photographs in Figure 3.10 do not convey as much information as the original autoradiographs.

According to the stud books (Mace, unpublished a and b, Fisher, unpublished), individual 673 was hatched at Jersey Wildlife Preservation Trust in September 1988 and individuals 693, 699 and 706 were hatched in May and June 1989. Since individual 627 was hatched in July 1987, he is probably excluded from being the sire of 673, as he was only 14 months old at the time 673 was hatched, but it is possible that he is the sire of individuals 693, 699 and 706 as he was almost two years old by the time they were hatched. However, if individual 627 was paired later than May 1989, he cannot have sired these offspring, and so other candidates must be sought for the parentage of individuals 673, 693, 699, and 706.

If individual 627 is excluded from being the sire of individuals 673, 693, 699 and 706, it is probable that the true parents of these birds are related to 627

because his DNA fingerprint contains at least one the "mismatching" bands found in the fingerprints of individuals 673, 693, 699 and 706, and 627 must have inherited this band from at least one of his parents. The parents of 627, individuals 399 and 527, who have been paired since 1985, are therefore possible parents of 673, 693, 699 and 706.

As can be seen from the family tree (see Figure 3.7), individual 399 is a sibling of 435, and a half-sibling of 565, and the sire of 527 is the "grandfather" of 435, which would explain why individuals 673, 693, 699, and 706 share so many bands with 435 and 565. Unfortunately, no samples could be obtained from individuals 399 and 527, and the samples provided from their respective parents (127/183 and 133/254) were exhausted by this stage of the study. It therefore appears that the hypothesis that 399 and 527 are the parents of individuals 693, 699, and 706 will have to remain untested.

3.3.4 The Origins of the 640 Subgroup

It is known, although not documented in the stud book, that JWPT sent some birds (number unknown) to Hong Kong in March 1976, and that they were the offspring of either founder pair 131/132 or 133/134 (David Jeggo, pers. comm., 1989). In Hong Kong, these birds were interbred to produce offspring, one or more of which were then crossed with one or more wild birds imported directly from Bali, also producing offspring. These latter offspring are the birds 640, 641, 642 and 643, and they were sent back to JWPT in 1988.

In an attempt to determine which birds were originally sent to Hong Kong, I consulted the stud books (Mace, unpublished a and b; Fisher, unpublished). These records list 23 offspring of either 131/132 or 133/134 that were hatched before the end of 1976. Of these, the best two candidates for the birds sent to Hong Kong are, in my opinion, 175 and 179. This is because these individuals are the only ones listed as being of "unknown" sex, they are the only ones that have no transfer information, they are the only ones that have "hatch dates" (~1973) but no "death dates", and assuming that this means they are still alive, they are the only ones not present in any British breeding centre.

Assuming that the data from David Jeggo is correct, at least one of the founder sires (131 and 133) of the British captive stock is the "great grandfather" of these birds. Three of the birds sent from Hong Kong (individuals 640, 641 and 642) have been DNA fingerprinted (see Figure 3.11), and the similarity coefficients (D) calculated for comparisons between them are presented in Table 3.9. Also presented in this table are the similarity coefficients calculated between these three birds and the two founders 131 and 133. It should be pointed out that some of these latter similarity coefficients were calculated between individuals whose samples were run on different gels. For example, 131 and 641 have never been run on the same gel, but by comparing the DNA fingerprints of 641 with 640, and then 640 with 131, it is possible to compare 641 with 131 and calculate a similarity coefficient between them (see Figure 3.11). However, even though these gels have internal controls (samples from the same birds present on different

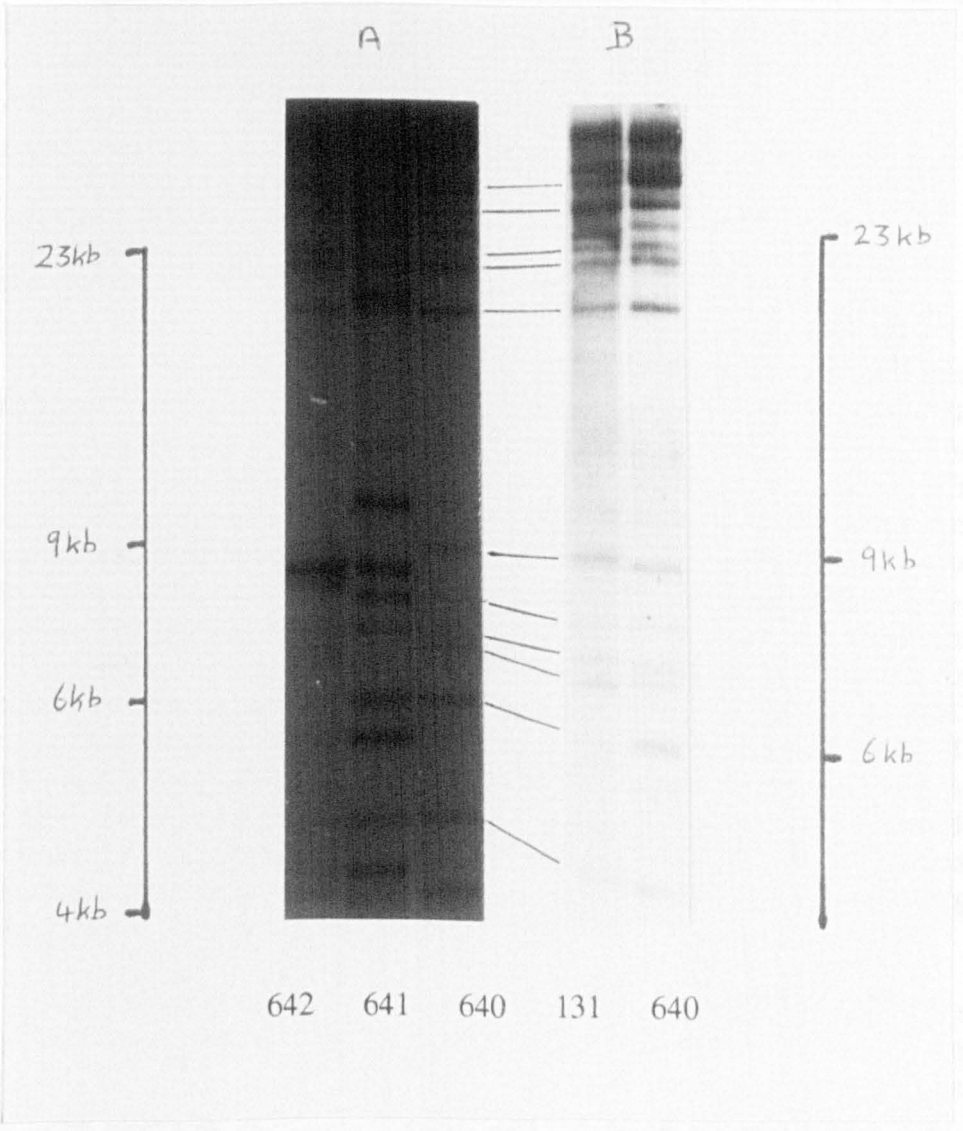


FIGURE 3.11: DNA fingerprints of a) three of the birds that arrived from Hong Kong in 1987, and b) 131 and 640 run in adjacent lanes on the same gel. The lines connecting the figures link matching bands on the two autoradiographs. (See text for details.)

gels) and molecular weight marker ladders, it is possible that some bands were erroneously scored, i.e. incorrectly assigned as identical or non-identical, and so the significance of these data should be treated with appropriate caution.

TABLE 3.9: Similarity coefficients (D) calculated between the two founders 131 and 133, and the three birds from Hong Kong.

Stud book No.	642	641	640
131	0.564	0.857	0.786
133	0.512	0.538	0.485
640	0.750	0.759	
641	0.483	/	

The results presented in Table 3.9 show that the similarity coefficients (D) calculated between two of the birds from Hong Kong (640 and 641) and individual 131 are higher than D between 640, 641 and 642, and *these* birds are thought to be siblings or half-siblings (David Jeggo, pers. comm., 1989). It is difficult to explain these results, so in an attempt to clarify these relationships the DNA fingerprints of the birds from Hong Kong were compared with those of as many direct descendants of individual 131 and individual 133 as possible. Comparisons between the importees from Hong Kong and birds that were descended from *both* 131 and 133 were avoided, as they would not have helped to differentiate between the two hypotheses, namely that either 131 or 133 was the "great grandfather" of the importees. If the birds originally sent to Hong Kong (i.e. individuals 175 and 179) were indeed offspring of just one of these two founders, the importees would be expected to share more bands with the direct descendants of that founder than they shared with the descendants of the other. Table 3.10 shows the results of calculating similarity coefficients (D) between the three birds from Hong Kong and 11 birds "known" to be descended from either founder 131 or 133.

A U-test comparing the similarity coefficients calculated between each of the individuals 640, 641 and 642, and the two founders (131 and 133) produced z-values of -1.795, -1.604 and -0.066, respectively. The associated probabilities for these values are 0.073, 0.109 and 0.508 for m and n values of 7/4, 6/4 and 7/4, respectively. These non-significant results mean that it has not been possible to confidently determine which of the founders was the sire of the birds that originally went to Hong Kong. This is not really surprising, as it was probably overly optimistic to expect that a 3rd-degree relationship could be determined from these data, especially in view of the facts that the relationship between 131 and 133 is unknown, although possibly close (see Section 3.3.2), the number of wild birds used in the breeding programme in Hong Kong is unknown, and the mates of 131 and 133 (individuals 132 and 134, respectively) were not available for analysis.

TABLE 3.10: Similarity coefficients (D) calculated between three of the birds imported from Hong Kong (640, 641 and 642) and 11 direct descendants of founder 131 or 133 (7 descendants of the former and 4 descendants of the latter).

Stud book No.	Comparisons with descendants of founder 131			Comparisons with descendants of founder 133			Stud book No.
	640	641	642	640	641	642	
624	0.540	0.533	0.642	0.550	0.387	0.524	183
625	0.621	0.646	0.502	0.413	0.461	0.190	626
628	0.584	0.516	0.361	0.417	0.480	0.167	627
629	0.578	0.533	0.411	0.553	0.575	0.505	636
630	0.651	/	0.531				
631	0.637	0.647	0.493				
675	0.437	0.514	0.408				

Nevertheless, in view of the fact that D calculated between 131 and 641 is higher than D for comparisons between 131 and his "known" offspring (see Appendix 1), and that the U-test results for individuals 640 and 641 are close to significance, it would probably be advisable for the managers of the captive stock to be cautious about pairing at least 640 and 641 with the descendants of 131. Unfortunately, examination of the family tree (see Figure 3.7) reveals that 640 and 642 have already been paired with 2nd-degree relatives of individual 131 and have produced offspring. If 131 is indeed the sire of the birds originally sent to Hong Kong, the inbreeding coefficients (see Section 3.3.5) of 640 and 642's offspring would be higher than calculated in the stud book (see Table 6, Fisher, unpublished) and these birds should therefore be carefully monitored so that the effects of possible inbreeding depression can be quickly detected and counter-measures taken.

3.3.5 Relationship Between Similarity Coefficients and Inbreeding

The Rothschild's mynah stud book lists the inbreeding coefficients (F) calculated for offspring of all possible matings between living birds in the British stock (see Table 6, Fisher, unpublished). Of these pairings, I was able to obtain similarity coefficients (D) between the prospective sires and dams in 70 cases (see Appendix 2). Figure 3.12 shows the results of plotting D against F for these 70 pairings. A regression analysis of these data gives a t-value of 5.261, which is highly significant ($p < 0.001$, 69 d.o.f).

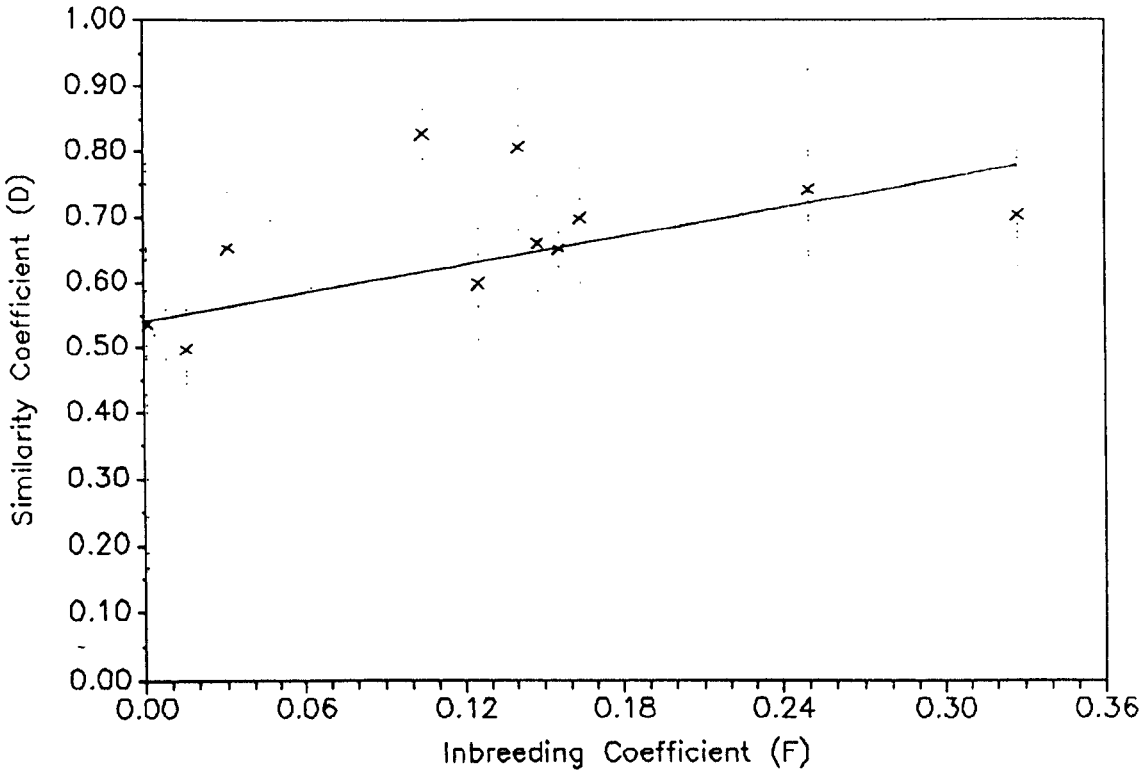


FIGURE 3.12: Graph showing the relationship of similarity coefficients (D) between pairs of Rothschild's mynahs plotted against the inbreeding coefficients (F) of their potential offspring, calculated using data from the stud book. X = mean values for specific inbreeding coefficients. Regression line shown.

The inbreeding coefficients used here were calculated by Ian Fisher (see reference above) under the assumption that all the data in the stud book were correct, however, as has been shown in the previous sections (3.3.3 and 3.3.4), there is cause to doubt whether this is so. Notwithstanding these results, recalculation of the inbreeding coefficients was not undertaken because it is not possible to be confident about the significance of some of the amended relationship data provided by this study. If the results presented in the previous sections are correct, then a number of the F-values attached to the points plotted on the graph in Figure 3.12 are too low. For example, given that the amended relationship data are correct, the points plotted for the following pairs would move to the right on the graph due to an increase in the F-value: 468/434 because they are 3rd-degree relatives, 565/575 because they are 4th-degree relatives, 624/606 because they are related through the 1st-degree relationship between the founders 127 and 131, as well as through the descendants of 131. In addition, many of the points plotted for relationships between the 640 subgroup and birds in the British stock would move to the right because the inbreeding coefficients (F) for their potential offspring were calculated under the assumption that the importees were unrelated to any members of the captive stock, and this does not appear to be so (see Section 3.3.4).

According to the stud book for 1989 (Mace, unpublished b), one of the

policies adopted to maintain the levels of genetic variation in the captive stock, was to not establish pairs if their potential offspring had an inbreeding coefficient (F) in excess of 0.125. The regression line shown in Figure 3.12, intercepts the F = 0.125 value at a similarity coefficient (D) of approximately 0.63. It may thus be prudent, in the absence of accurate information regarding the true relationships of the founders of the stock (see Section 3.3.2), and certain other individuals within it (see Sections 3.3.3 and 3.3.4), to avoid pairing birds between which D $\geq$ 0.60, a figure just below the mean similarity coefficient calculated for 2nd-degree relatives in the British captive stock (see Table 3.2).

The policy mentioned above was introduced about four years ago, but the general level of inbreeding in the stock has probably continued to increase due to the fact that the policy has not always been strictly observed. Productive pairs have been maintained regardless of the value of F for their offspring rather than risk pairing two "untried" birds (D. Jeggo, pers. comm., 1989), and certain pairings have simply overlooked the policy; witness the pairing of male 595 and female 628, an "uncle/niece" association (see upper left of Figure 3.7).

Figure 3.13 shows the results of plotting D (between parents and their offspring, and between the siblings) against the year that the eldest sibling was hatched (F for a pair's offspring will not change regardless of how long the pair remain productive), and it can be seen that there has been a significant increase in the mean similarity coefficient between 1st-degree relatives over the period 1973 to 1990 (data presented in Appendix 3). A regression analysis of these data produces a highly significant t-value of 7.327 ($p < 0.001$, 61 d.o.f.). This indicates that the general degree of relatedness between the individuals paired to produce these offspring has increased over time.

The results presented in Figure 3.12 show that there is a very significant positive relationship between similarity coefficients (D) for parental comparisons and the inbreeding coefficients (F) of their potential offspring. A high similarity coefficient between two parents indicates that they share a large proportion of their minisatellite alleles. Given that these minisatellites will be transferred to their offspring in a Mendelian manner, it would be expected that a high D between the parents will result in high similarity coefficients between the parents and their offspring, and between the offspring themselves. Given this assumption, it is possible that the increase in the mean similarity coefficients obtained for offspring groups over time (as presented in Figure 3.13) could indicate a corresponding increase in the general levels of inbreeding in the stock. It should be pointed out, however, that these data are biased in that the analysis was only performed on birds whose samples were sent for study. It is therefore possible that other offspring groups, hatched over this period, have lower similarity coefficients (D) and hence a lower level of inbreeding than these data indicate, although examination of the family tree (see Figure 3.7) makes this seem doubtful.

A further analysis was performed on the data in Appendix 3 to determine whether the distributions of D calculated between parents and their offspring, and among the siblings themselves, showed any appreciable difference. Figure 3.14a

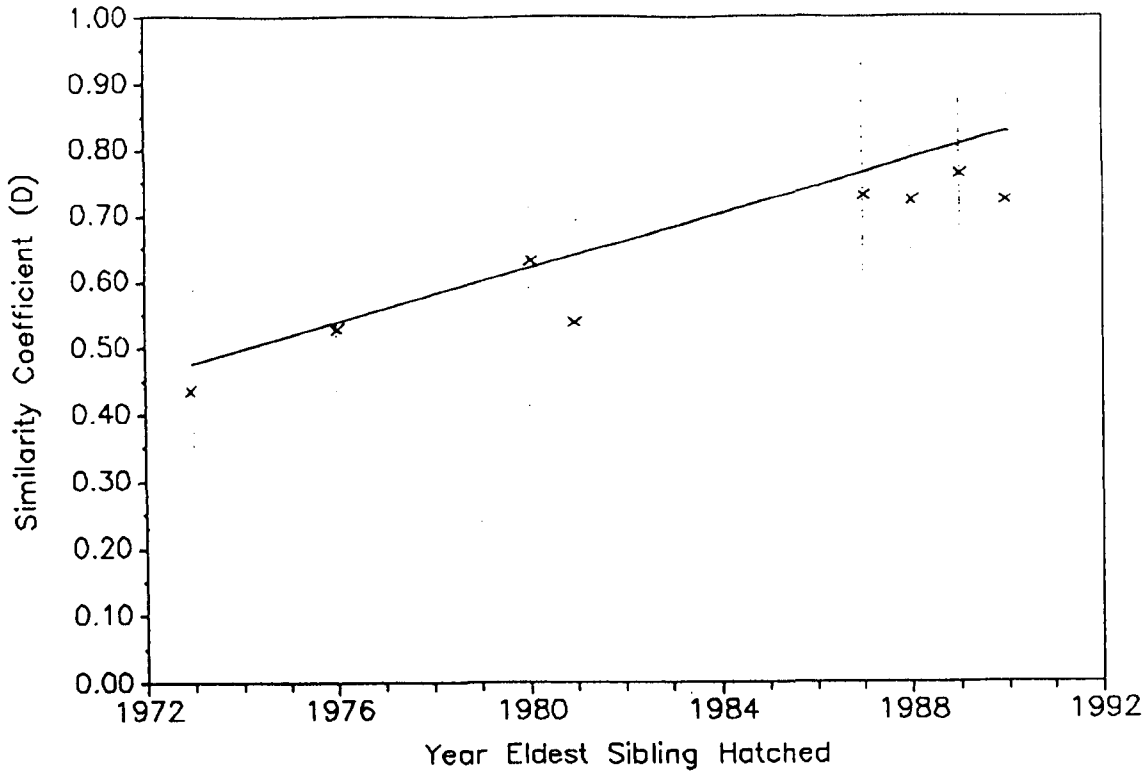


FIGURE 3.13: Graph showing the increase in similarity coefficients (D) calculated between 1st-degree relatives in the British Rothschild's mynah stock over the period 1973 to 1990. See Appendix 3 for the similarity coefficient data used for this analysis. X = mean values for specific years. Regression line shown.

shows the distribution of D for all 1st-degree relative comparisons used here, Figure 3.14b the distribution of D for parent/offspring comparisons only, and Figure 3.14c the distribution of D for intra-sibling comparisons only, and as can be seen, there is little difference between the distributions for the two types of 1st-degree relative comparisons.

3.3.6 Linkage and Allelism

The occurrence of linkage and allelism has been difficult to assess in the mynahs due to the absence of data from extended families. The DNA fingerprints of the largest family group available in this study are shown in Figure 3.15. This group consists of founder 131, five of his "grandchildren" (individuals 624, 625, 629, 630 and 631), two individuals from Hong Kong (individuals 640 and 642) whose relationships are discussed in Section 3.3.4, and six of individual 131's "great grandchildren" (individuals 692, 701, 703, 710, 712 and 722). As can be seen from the figure, all the bands present in the "great grandchildren" are also present in one or both of their parents. This contrasts with the results shown in

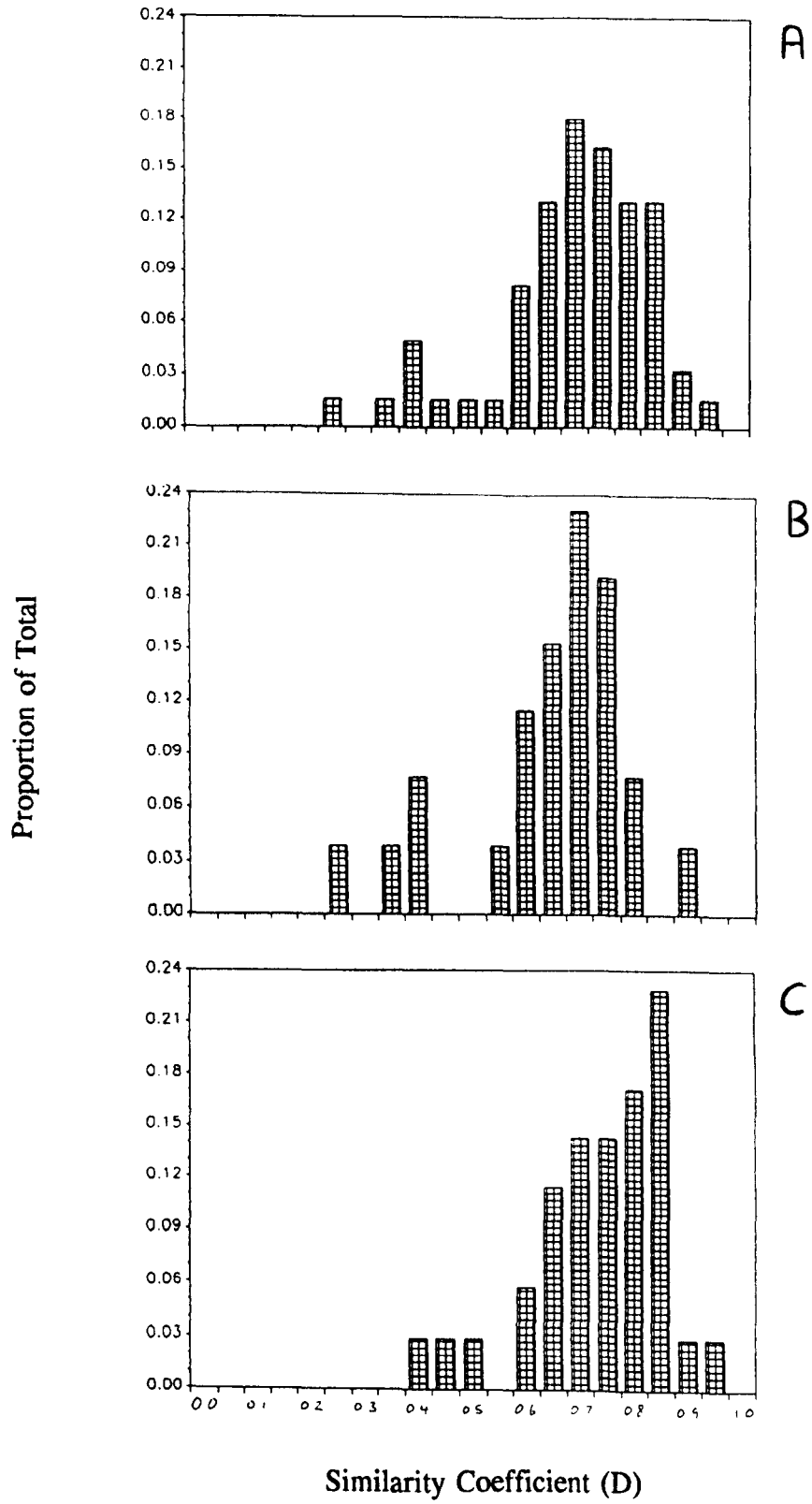


FIGURE 3.14: Graphs showing a) the distribution of similarity coefficients (D) for comparisons between all 1st-degree relatives, b) the distribution of D for parent/offspring comparisons only, and c) the distribution of D for intra-sibling comparisons only.

Figure 3.10, where the presence of bands in the offspring's DNA fingerprints that are not present in either of the parent's indicates a mismatch.

By studying the DNA fingerprints of the individuals in Figure 3.15, it is possible to identify bands which are inherited together, i.e. if one is present in an individual the other(s) is (are), and which are thus possibly linked. As explained in Chapter 1, Section 1.7, linkage occurs when the sequence of a single minisatellite allele contains one or more enzymatic restriction sites. This means that during digestion (see Chapter 2, Section 2.1.2), the enzyme cleaves within the minisatellite producing one or more band from one distinct locus. Also, conventional linkage is possible when two minisatellite loci are in close proximity on the same chromosome (Royle et al., 1988).

As can be seen from the solid lines on the left of Figure 3.15, there are two bands present in female 624 that are either both present or both absent in her five offspring 692, 701, 710, 712 and 722. In addition, this linkage appears to continue in the siblings of 624 (individuals 625, 629, 630 and 631), and in the offspring of sibling 625 (individual 703). The probability that these bands are actually unlinked and have just fortuitously co-segregated in the siblings and offspring of 624, and in the offspring of 625, is 0.5^{10} (1 - the number of individuals in which both minisatellite alleles/bands are present or absent = 11) or 0.001 (Jeffreys and Morton, 1987).

If the bands inherited from the sire (640) are examined it can be seen that a block of five bands are either all inherited or none are inherited by his offspring (dashed and dotted lines on the right of Figure 3.15). However, if 640 and 642 are indeed siblings (see Section 3.3.4), it would appear that only four of these bands are linked, as one is not present in individual 642. Furthermore, only two of these four bands co-segregate into the offspring of 642, individual 703. It therefore appears unlikely that these all five of these bands are linked, although further investigation would be required to clarify the situation.

Unfortunately, all the inheritance patterns investigated in this study appear to be produced by alleles that come from autosomes, and, as mentioned in Section 3.3.1, no sex-linked alleles could be found in the mynahs. There is thus currently no evidence that any of the minisatellites detected by probe pSPT19.6 are located exclusively on the W or Z-chromosome.

If two bands are present exclusively in the DNA fingerprint of one parent, and only one of the two is seen in the DNA fingerprints of that individual's offspring, then it is possible that the two bands in question are alleles. A possible example of allelism has also been detected in the family group of mynahs presented in Figure 3.15.

All the offspring of individuals 624 and 640 can be seen to inherit band B from their mother, where none inherited band A. It is possible, therefore, that bands A and B are alleles at the same locus and thus, that only one of them can be inherited by the offspring. However, it is also possible that 624 is heterozygous

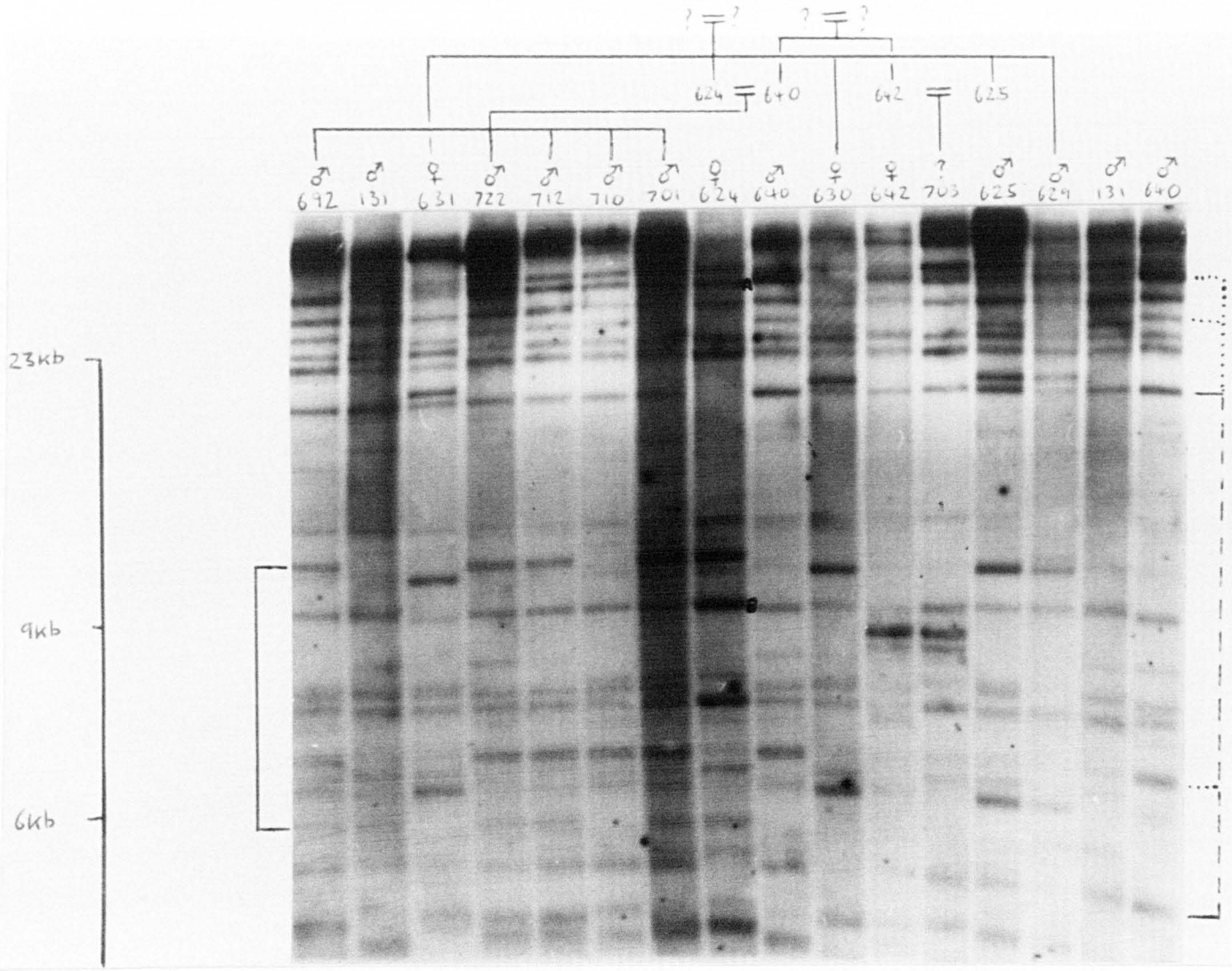


FIGURE 3.15: DNA fingerprints of the largest family group of mynahs analyzed in this study. The relationships between and sex of the individuals presented is indicated at the top of the figure. The solid lines on the left connect the bands thought to be inherited as a linked group from dam 624. The dashed and dotted lines on the right connect those bands that appear to be inherited as a linked group from sire 640; the dashed lines connect the two bands which co-segregate in 640, 642 and 703 (see text for details). The potentially allelic bands A and B are marked.

at the locus from which band A is derived and that the other allele at this locus is either so small that it has been lost from the gel, or so large that it is located in the poorly resolved region at the heavy molecular weight end of the DNA fingerprint. Another possibility is that individual 624 is homozygous for band B and so all her offspring would be expected to inherited this band. Unfortunately, it is not possible using these data to determine which of these hypotheses is correct and, as with the phenomenon of linkage, further investigations will be necessary before further conclusions can be drawn.

Knowledge of the occurrence of linkage and allelism is important because these phenomena affect the way that a DNA fingerprint is scored, and can alter the calculations of proportion of bands shared and similarity coefficients (D). In an extreme example, if two individuals each have 20 bands and they share ten of them, the similarity coefficient (D) calculated between them would be 0.50 (see equation at the end of Chapter 2, Section 2.1.8). However, if all ten of those bands were linked, the two individuals would only have eleven bands each, and would share only one, since all ten bands were from the same allele at one locus. The similarity coefficient calculated in this case would be 0.091, considerably lower than that calculated in the first case.

It should be pointed out that the calculations presented in this study have been based on the assumption that none of the bands scored were linked, and so some of the calculated similarity coefficients (D) will change if the linkage and allelism presented in this section are confirmed. However, the changes produced in D would be small if based on the data in this section. Table 3.11 shows a comparison of the similarity coefficients (D) obtained between individual 624 and her offspring assuming no linkage, and those obtained with this phenomenon considered. Only the bands linked in dam 624 were considered, as the evidence for linkage of the sire's (640) bands is not good. In addition, allelism was not considered as the evidence for this was not significant. As can be seen in Table 3.11, there is little change in the values of the coefficients calculated in these two different ways.

3.3.7 Comparison of the Results Obtained Using pSPT19.6 and pSPT18.15

As mentioned in the methods to this chapter (see Section 3.2.5), the multilocus probe used for this study of the mynahs was pSPT19.6. However, one filter was also probed with the probe pSPT18.15, allowing a comparison to be made between the hypervariable minisatellites detected by each probe. Figure 3.16 shows the DNA fingerprints produced by separately hybridising the same filter with these two probes.

Probe pSPT19.6 was the first probe hybridised to the filter and, after production of an autoradiograph, this was stripped and the filter re-probed with pSPT18.15. To ensure that the former probe had been completely removed from the filter before the latter was applied, the "stripped" filter was used to expose an X-ray film overnight. The resulting autoradiograph showed no visible bands (data not shown) therefore one can be confident that the bands present on the DNA

fingerprint produced using probe pSPT18.15 were all genuinely detected by that probe, and were not due to exposure of the film by residual pSPT19.6.

TABLE 3.11: Comparison of similarity coefficients (D) calculated between individual 624 and her offspring under the assumptions that the bands indicated on the Figure 3.15 are or are not linked.

Stud book No.	624	692	701	710	712	722
624	-	0.703	0.757	0.722	0.649	0.595
692	0.686	-	0.850	0.821	0.850	0.800
701	0.743	0.842	-	0.872	0.900	0.800
710	0.743	0.842	0.895	-	0.769	0.718
712	0.629	0.842	0.895	0.789	-	0.850
722	0.571	0.842	0.789	0.737	0.842	-

UNLINKED

LINKED

Table 3.12 lists the number of bands scored in each individual bird sampled, using the two probes. Examination of the two DNA fingerprints presented in Figure 3.16 reveals that there are a number of minisatellites to which both probes hybridise, and Table 3.12 also shows the number of these bands in each individual. It is possible that these bands are produced by the probes binding to the same minisatellite allele, but the bands could also be different alleles from different loci that, by coincidence, have similar migration rates, and are thus located in the same position on the filter. Unfortunately, it is not possible to tell which of these hypotheses is correct without sequencing the minisatellites in question (see Chapter 1, Section 1.6).

As shown in Table 3.12, approximately 20% of minisatellites detected by probe pSPT19.6 are also detected by pSPT18.15 (as compared with 1% in humans (Jeffreys, Wilson & Thein, 1985a)), and so for the most part the two probes detect different minisatellite alleles. This means that the data from the two probes could be combined, with the apparently analogous bands only being scored once. This would allow a mean of approximately 21.1 (σ = 3.0) bands to be scored per individual compared with a mean of 13.4 (σ = 2.8) for probe pSPT19.6 only, and 10.7 (σ = 2.3) for pSPT18.15. This would hopefully increase the accuracy with which similarity coefficients (D) calculated between two individuals reflected the degree of relatedness between them.

Similarity coefficients (D) were calculated between all the individuals whose

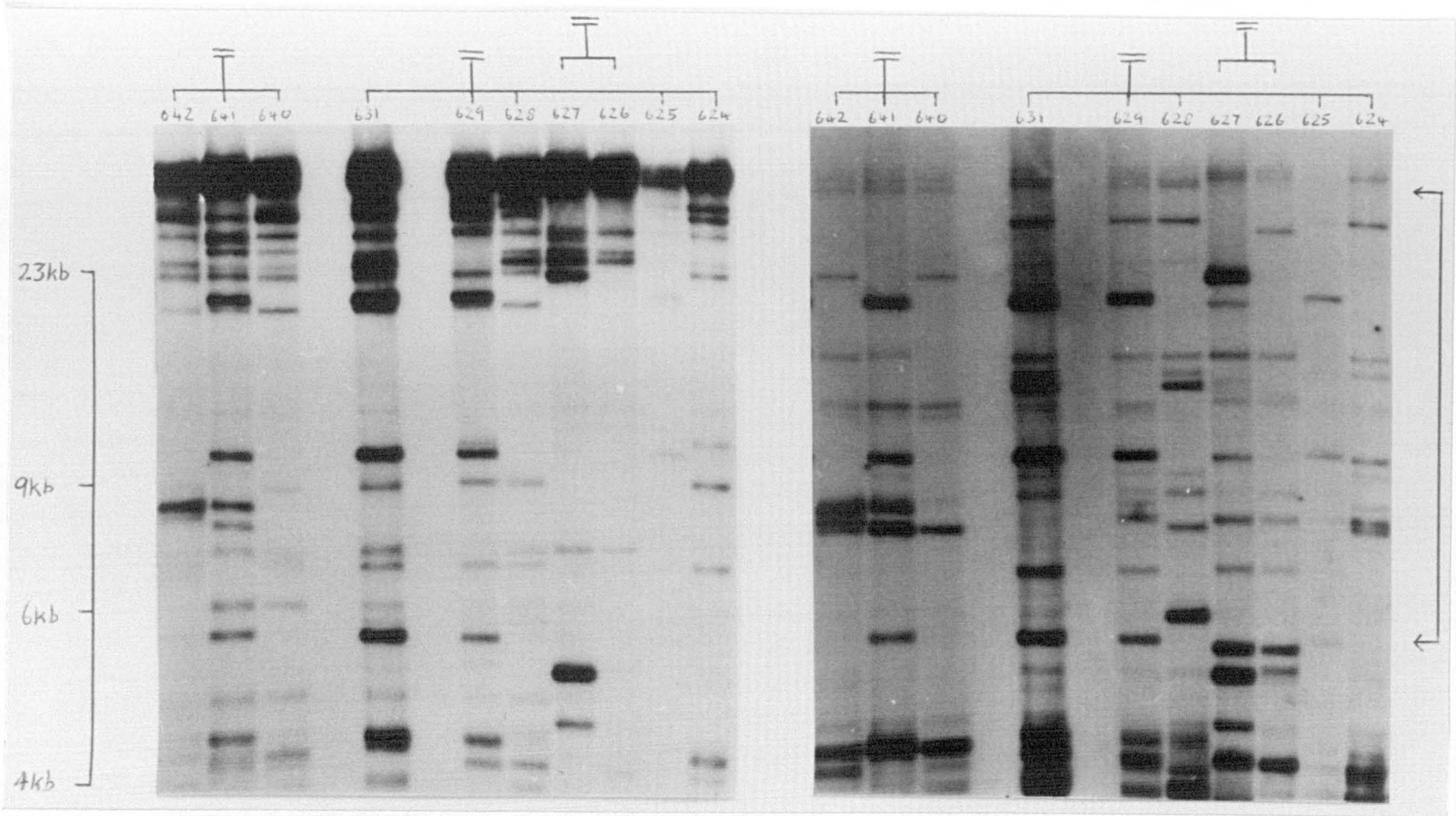


FIGURE 3.16: DNA fingerprints produced by hybridising the same filter first with probe pSPT19.6 and then with pSPT18.15 (the former probe was stripped from the filter before the second was applied). The relationships of the individuals whose samples appear on the DNA fingerprints are indicated at the top of the figure (see Figure 3.7 for a complete illustration of their relationships). The arrows on the right of the figure mark the borders of the region of the DNA fingerprints across which bands were scored.

TABLE 3.12: Number of bands scored for each individual using the multilocus probes pSPT19.6 and pSPT18.15. The bottom row of the table shows the number of apparently analogous bands in each individual.

Probe \ Bird	624	625	626	627	628	629	631	640	641	642
19.6	14	12	8	11	15	14	18	13	16	13
19.15	11	8	11	11	11	14	14	7	11	9
Both	2	3	2	2	3	4	6	1	5	2

DNA fingerprints are shown in Figure 3.16, initially using those bands detected by probe pSPT19.6, and then using those detected by pSPT18.15 (these calculations included all bands scored, and thus no allowance was made for the possibility that identical alleles were scored twice). D for each dyad using each probe were plotted against one another and the results are shown in Figure 3.17. A regression analysis of these data, with the results from pSPT19.6 arbitrarily chosen as the dependent variable, produced a significant t-value of 1.826 ($p < 0.05$, 44 d.o.f.) (see Appendix 4 for raw data).

However, this result is only just significant and an examination of the graph in Figure 3.17 shows that the points are widely scattered, the most extreme differences between the values of D obtained using the two probes being 0.667/0.118 and 0.167/0.500 (D calculated using bands detected by pSPT19.6 listed first). Also, of the 45 points plotted, in only 13 cases are the similarity coefficients (D) obtained using probe pPST19.6 exceeded by those obtained using pSPT18.15. One would have expected a more significantly positive relationship between similarity coefficients calculated using different sets of minisatellites than the one observed, although it is possible that the significance of the regression would increase if there were more data points.

There are a number of conclusions that can be drawn from these observations. Assuming that the two probes detect approximately the same number of minisatellite loci, as seems to be the case in humans (Jeffreys, Wilson et al., 1987), those detected by pSPT18.15 have a complement of alleles that are, on average, smaller than those at the loci detected by pSPT19.6. This would account for the smaller number of bands detected using the former probe. Given this, the length variation of the alleles in the size range indicated in Figure 3.16 is greater in pSPT18.15, leading to less band sharing, and the generally lower similarity coefficients calculated using this probe. It is possible that this difference in variation is caused by the fact that the size range across which the minisatellites are scored samples different parts of the allelic distribution for the loci detected by the probes. In other words, those bands scored using pSPT19.6 may represent 50% of all the alleles present at the particular loci detected by this probe, whereas those scored using pSPT18.15 only represent, for example, 25% of the allelic variation present. If the length of time allowed for electrophoresis of the restricted

DNA fragments was reduced, it may be possible to also score 50% of the alleles present at loci detected by pSPT18.15, and the variation of alleles across the increased size range would be closer to the variation originally observed for alleles detected by pSPT19.6. It is clear that these data require further investigation.

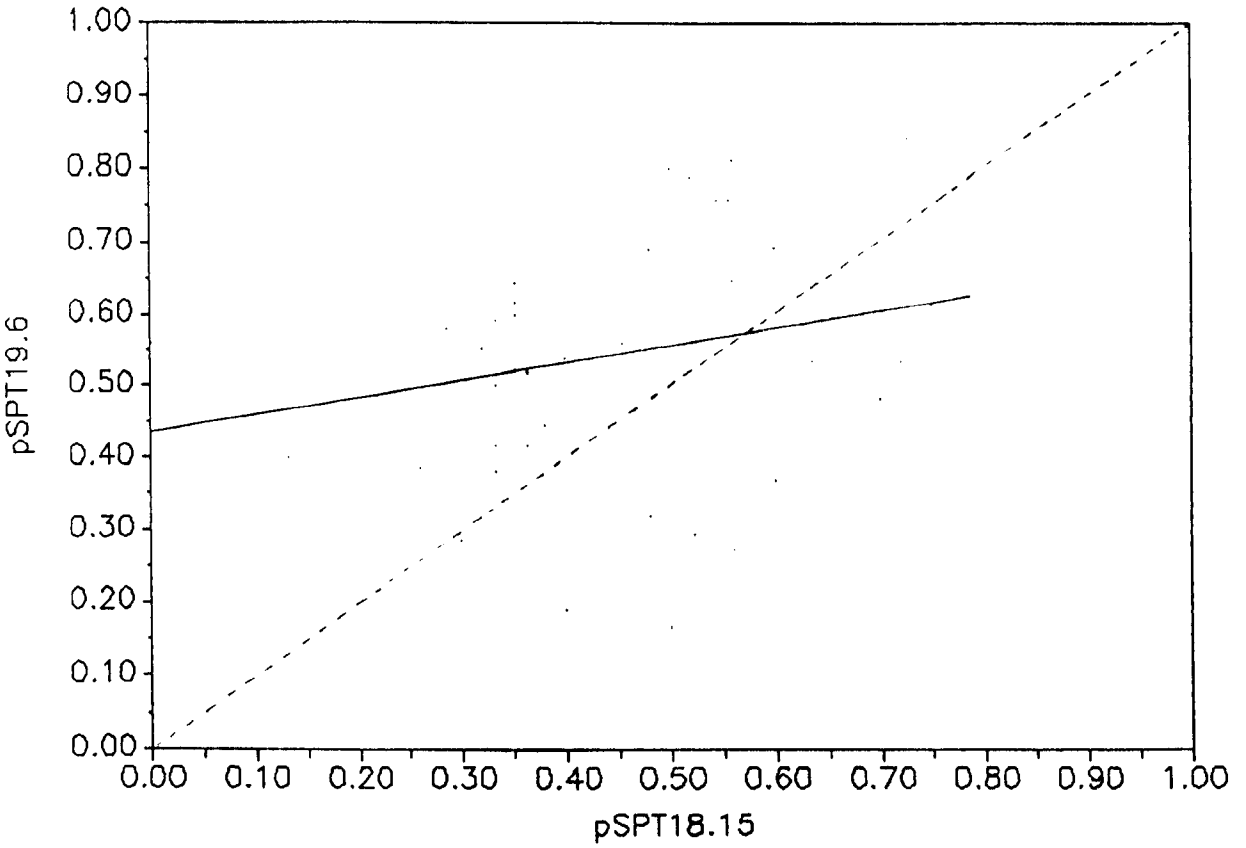


FIGURE 3.17: Graph showing the results of plotting similarity coefficients (D) calculated between individuals using bands detected by the two probes pSPT19.6 and pSPT18.15. The solid line is the regression line and the dashed line follows a plot of $D_{pSPT19.6} = D_{pSPT18.15}$

3.4 DISCUSSION

The continued existence of Rothschild's mynah in the wild is seriously threatened. Its precipitous decline in numbers during the 1970s and early 1980s, has resulted in the population now numbering only a few dozen individuals (see Figure 3.3), and it appears likely that if the species is to survive, it must be given considerable assistance. The measures currently being employed to save the species from extinction are based on a captive breeding/reintroduction programme, there being over 1,000 mynahs in captivity around the world. However, the reservoir of captive individuals, outnumbering those in the wild by around thirty to one, may not be as reassuring as it at first appeared. Some, if not all, of the stocks are known to be suffering from inbreeding depression leading to poor fertility, high mortality rates, etc. (see Table 3.13), reducing the potential of those

stocks to contribute genetically variable specimens to a reintroduction programme. In addition, as conservation bodies recommend that reintroduction should only be attempted with individuals from the F_1 generation after captive breeding or the F_3 generation at the latest, a large number of the Rothschild's mynahs currently held in captive stocks would be ineligible for the programme; in the USA some stocks are already up to F_{10} (Seal, 1991b).

TABLE 3.13: Number of offspring hatched in the British captive stock between 1988 and 1991, and the number of these offspring that died in the first 12 months. The final column of the table shows the mortality rate based on the data in the preceding two columns. The figure in the "Number Dead" column for 1991 represents only those individuals that had died before the end of that year, and so the mortality rate calculated for this year is probably an underestimate (Data from the stud books).

YEAR\CATEGORY	Number Hatched	Number Dead	Mortality Rate
1988	30	24	80.0%
1989	33	20	60.6%
1990	54	39	72.2%
1991	35	23	65.7%

This study was undertaken to assist in the monitoring of the British captive stock of Rothschild's mynahs, which currently numbers approximately 120 individuals. The aims were to determine the relationships of as many individuals as possible within the stock or to confirm those already "known", and to assess the ways in which the information obtained through DNA fingerprinting could be used. There has been discussion in the scientific literature concerning the accuracy of estimates of relatedness derived from DNA fingerprint data (Westneat, 1990; Morton, Forman & Braun, 1990). Lynch (1988) showed that unbiased estimates of relatedness cannot be obtained without prior knowledge of the distribution of minisatellite alleles in the population. Accurate estimates of relatedness can only therefore be taken directly from DNA fingerprints if the allelic variation in the population is extremely high, so that the number of alleles shared between unrelated individuals is close to zero. Since the background level of band sharing is increased by inbreeding, one has to be cautious when using the technique to determine relationships within a captive zoo stock like Rothschild's mynah.

At the start of the study, samples were provided from three of the founders of the British stock. It was important to establish the relationships between these birds as they, along with four others, were solely responsible for the first decade of captive breeding in this country, and thus had contributed a large proportion of the allelic variation present in the stock. By comparing the similarity coefficients (D) calculated between individuals with the proportion of genes they would be

expected to share given a particular hypothesis regarding the relationships between the three founders that were tested, it was possible to establish that at least two of the founders were very closely related, probably at 1st-degree level. This was an unfortunate discovery as these two individuals are estimated to have contributed approximately 40% of the genetic variation present in the British stock (Mace & Jeggo, unpublished).

There is an annually updated stud book for the mynahs produced by the Institute of Zoology, London, which is compiled from the breeding records of all institutes and individuals holding this species. However, it was possible to show that errors have probably been made with some of the parent/offspring allocations. It was discovered that the most productive pair of birds in the British captive stock were not actually the parents of the four individuals with which they were submitted for testing. Had this not been detected, the "offspring" may have been paired with individuals to whom they appeared, on paper, to be unrelated, but with whom they in fact shared close relatives. Unfortunately, it was not possible to precisely establish which birds were the true biological parents of these individuals as not all the members of the stock were tested. However, it was possible to identify at least one band, common to three of the "offsprings'" DNA fingerprints, that was present in the offspring of a pair of birds that were known to be breeding at Jersey at the time these birds were hatched. Samples from this breeding pair were not available during this study, but analysis of DNA fingerprints from them should reveal if they are or are not the true parents.

Other discrepancies were discovered regarding the relationships between the 640 subgroup (hatched in Hong Kong) and the birds in captivity in Britain. The stud book lists the group from Hong Kong as being of unknown relationship to both each other and the British birds. In addition, the inbreeding coefficients calculated for members of the stock assumed that "unknown relationship" meant "no relationship". However, it was possible to show, using DNA fingerprinting, that at least some of the importees are in fact more closely related than previously thought. Indeed, it appears that at least one of the birds is a 2nd-degree relative of one of the British stock's founders (individual 131). Given this information, it should be possible to re-pair the birds with individuals to whom they are least related, and so minimise inbreeding and the loss of genetic variation.

A potentially extremely useful discovery was made during this study regarding the relationship between similarity coefficients (D) and inbreeding coefficients (F). A regression analysis of a comparison between D for parents and F in their (potential) offspring produced a very significant positive relationship. This means that it may be possible to use DNA fingerprinting to assess the suitability of selected individuals for breeding. Managers of the British stock follow a policy of not establishing pairs if F for their offspring will be greater than 0.125. In the regression analysis, this corresponds to a similarity coefficient (D) of approximately 0.630. It is therefore proposed that in the interests of maintaining as much genetic variation as possible within the stock, the pairing of individuals between which there is a calculated D of 0.600 or greater should be avoided.

Dr. Georgina Mace at the Institute of Zoology in London has calculated founder genome equivalents (Fge) for the mynahs held in this country (Mace, unpublished b). *Fge* is a measure of the number of unrelated wild individuals that would show the same level of genetic variation as the sample group. In the case of the British stock, *Fge* lies between 5.89 and 9.87, indicating that ten unrelated wild birds would show greater genetic variability than the 120 individuals in the captive stock. This is not surprising as there are only thirteen founders with descendants in the current stock, and their contributions have not been equal. This information, in addition to the results presented in Section 3.3.5, indicates that the British captive stock is very inbred, and that the assessment of the degree of this inbreeding presented in the stud books is probably below the true figure. It is therefore important to ensure that the relationships between the individuals within the stock are known, and that the birds are all paired in a manner that will reduce the rate at which inbreeding is increasing.

CHAPTER 4

RODRIGUES FRUIT BAT
(*Pteropus rodricensis* Dobson, 1878)

4.1 INTRODUCTION

4.1.1 General Introduction

Man has played a major role in the decline of the Rodrigues fruit bat, a species endemic to the Mascarene island of Rodrigues. Human activities such as hunting, habitat destruction and the introduction of non-native species, have compounded the problems caused by the frequent cyclonic storms which affect the island (Diamond, 1987). Still abundant at the beginning of this century, the population declined to less than 100 individuals and, although there has been some recovery in numbers, Rodrigues fruit bat is still considered to be one of the rarest bats in the world (Carroll, 1981; Gade, 1985).

4.1.2 The Mascarene Islands

The Mascarene islands, comprising Mauritius, Réunion and Rodrigues, are of volcanic origin, having risen from the ocean floor about 1.5 million years ago (Gade, 1985). Rodrigues is the smallest of the three islands, having an area of only 110km^2 (Goodwin & Holloway, 1978), and it is also one of the most remote islands in the world. The nearest land is Mauritius 574km to the west; to the east there is only ocean until the coast of Australia, and the nearest land to the north and south is several thousand kilometres away (Strahm, 1983).

When the Mascarene islands were first discovered, the endemic flora and fauna were extremely diverse (Carroll, 1981). The most famous native vertebrate of the islands is the Dodo, *Raphus cucullatus*, which lived on Mauritius and Réunion, but this was just one of over 40 different species of birds endemic to the islands, of which 14 existed on Rodrigues alone (Carroll, 1981). Of the three islands, Rodrigues is the most ecologically devastated with less than 2% of its natural habitat remaining (see Figure 4.1) (Goodwin & Holloway, 1978; Cheke, 1987).

Unfortunately, of the 14 original endemic bird species on Rodrigues, only two survive today: the Rodrigues fody, *Foudia flavicans*, and the Brush warbler, *Bebrornis rodericana*. The only other endemic vertebrate is *Pteropus rodricensis* which is the only land mammal to have colonised Rodrigues naturally, the other mammals present (i.e. cats, rats, pigs, goats, etc.) having been subsequently introduced by Man (Carroll, 1981; Atkinson, 1989).

The Island of Rodrigues

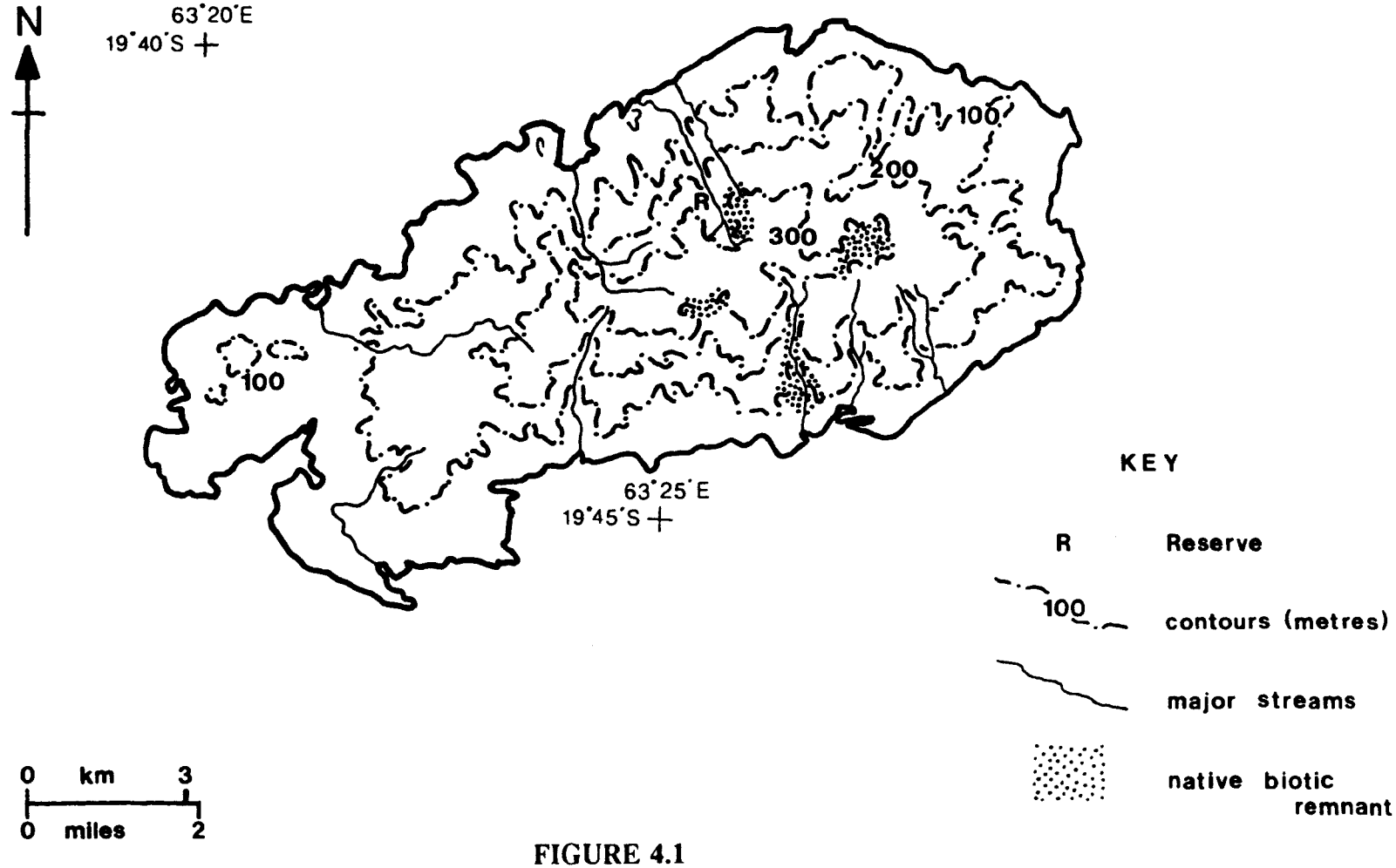


FIGURE 4.1

4.1.3 Taxonomy

KINGDOM	- Animalia
PHYLUM	- Chordata
SUBPHYLUM	- Vertebrata
CLASS	- Mammalia
SUBCLASS	- Theria
INFRACLASS	- Eutheria
ORDER	- Chiroptera
SUBORDER	- Megachiroptera
FAMILY	- Pteropodidae
SUBFAMILY	- Pteropodinae
GENUS	- Pteropus
SPECIES	- rodricensis

There are more than 900 chiropteran species, of which 174 belong to the frugivorous Megachiroptera (flying-foxes) of the Old World tropics and sub-tropics (Neuweiler, 1989). The suborder Megachiroptera, with the single family Pteropodidae, consists of 43 genera and 174 species (Marshall, 1985). About 79% (34) of these genera and 84% (146) of the species are contained in the sub-family Pteropodinae, of which the largest genus is *Pteropus*.

First described by Erxleben in 1777, the genus *Pteropus* ("winged foot") is now recognised to contain 66 species. It is a widely distributed genus with representatives in Madagascar, India, Southeast Asia, Australia, Fiji and Samoa (Pook, 1978). *Pteropus* has colonized all the major islands in the western Indian Ocean, but has failed to establish itself on the African continent (Cheke & Dahl, 1981).

The species *rodricensis* is endemic to the island of Rodrigues and, although discussed in some detail by Leguat (1708), it was not formally described and catalogued until 1878 (Dobson, 1878; Cheke & Dahl, 1981). Rodrigues fruit bat is also known as the Rodrigues flying-fox and is called the Rousette de Rodrigue by native islanders (Goodwin & Holloway, 1978).

4.1.4 General Biology and Behaviour

The Rodrigues fruit bat weighs about 300g and has a dark chestnut brown coat with a mantle of golden brown fur that covers the head, neck and shoulders (Pook, 1978; Durrell & McGeorge Durrell, 1980). There is considerable variation between adult individuals, both in the shade of colour and size of the mantle (Pook, 1978; pers. obs., 1989). Apart from the obvious differences one would expect to find between mammalian sexes, i.e. genitalia, the only physical dimorphism is that adult male specimens are slightly larger than the females. This obviously makes sexing from a distance difficult. Behaviourally, only the males hold territories, but both sexes fight ferociously during antagonistic encounters, be this over territory, perches or food (Carroll, 1979 a and b; pers. obs., 1989).

In the wild, the bats usually roost during the day, using the same trees year by year (Durrell & McGeorge Durrell, 1980). In captivity, they either congregate in harem groups (one male associating with up to eight females), or roost individually. However, during their active nocturnal phase, they feed in different groups to the harem groups, and so an individual female may associate with a number of males during any one 24-hour period (Carroll & Mace, 1988).

In the wild, the bats forage for food at dusk, flying to their favourite trees. Their principal food is fruit which they chew to extract the juices. They usually spit out the fruit pulp and seeds, unless the pulp is soft, e.g. bananas, in which case they swallow it. They have also been seen to chew eucalyptus, tamarind and some other flowers to obtain the juices and pollen (Walker, 1975). In captivity, their favourite food includes bananas, melon, pears and grapes (Pook, 1978).

4.1.5 Decline of the Rodrigues Fruit Bat

As recently as the early 1900s, the Rodrigues fruit bat was reported as abundant, and it is thought that the massive reduction in numbers that occurred between 1955 and 1965 (see Table 4.1 and Figure 4.2) was primarily due to the effects of severe storms and droughts, exacerbated by habitat destruction which reduced the amount of natural vegetation (Cheke & Dahl, 1981).

TABLE 4.1: Estimates of the wild Rodrigues fruit bat population between 1955 and 1979.

YEAR	ESTIMATE	REFERENCE
1955	>1000	Cheke & Dahl, 1981
1965	190	Carroll & Mace, 1988
1974	<100	Cheke & Dahl, 1981
1974	75-80	Anon., 1977
1976	120-130	Durrell, 1976
1977	120	Cheke & Dahl, 1981
1978	150	Cheke & Dahl, 1981
1978	120-125	Goodwin & Holloway, 1978
1979	70	Durrell & McGeorge Durrell, 1980

The decline of the Rodrigues fruit bat was due to the interaction of three factors:

1) *Weather.* Rodrigues is regularly hit by cyclones, with winds gusting to over

100 mph (Cheke, 1987). This can be devastating to the bat population as *Pteropus rodricensis* is a poor flyer (Strahm, 1983). The bats prefer to shelter in the branches of trees during a storm, but sometimes the winds are strong enough to tear the bats off their perches and sweep them out to sea, where they drown (Durrell, 1976). When the wind speeds are high, the bats are unable to feed since they are prevented from flying, and even after a storm has passed, the survivors face starvation because the trees are stripped of fruits and flowers (Durrell, 1976).

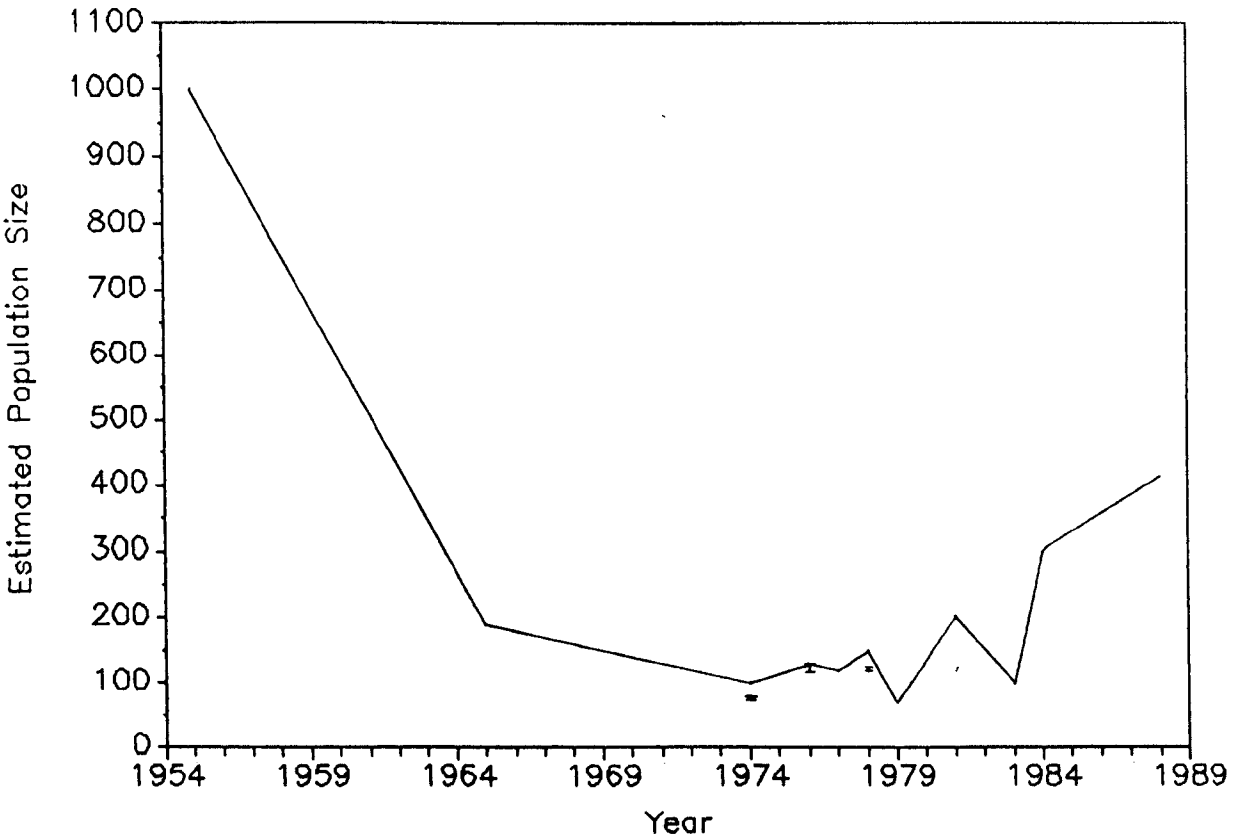


FIGURE 4.2: Estimated size of the wild Rodrigues fruit bat population between 1954 and 1988.

2) *Habitat Destruction.* When Rodrigues was first discovered, it was covered in lush, evergreen vegetation (Gade, 1985), but by the mid-1800s the forests had largely been destroyed (Higgin, 1849). The practice of deforestation has been continued in more recent years. Between 1955 and 1968, the Mauritian Forestry Commission removed extensive areas of native forest and the removal of so many trees, especially the tamarind, *Tamarindus indicus*, deprived the bats of both food and shelter (Cheke & Dahl, 1981). This loss of habitat was made worse by the fact that the islanders' traditional agricultural practice is to "slash and burn", a method of farming that strips the soil of its vegetation cover, leaving the torrential rains which accompany storms to wash large quantities of soil into the sea (Durrell & McGeorge Durrell, 1980).

The effect of this deforestation has been to reduce the natural vegetation to only 2% of its former extent, and this small area is where the remaining population of bats roost (Cheke, 1987). Unfortunately, because of persistent trampling and grazing by introduced animal species, i.e. pigs and goats, and the inability of native plants to compete with introduced continental species, there has been little ecosystem regeneration (Cheke & Dahl, 1981; Gade, 1985).

3) *Human Persecution.* Hunting has also played a part in the decline of *Pteropus rodricensis*. The bat is apparently very tasty, and the native islanders believe that its fat is helpful for relieving rheumatism and curing baldness (Goodwin & Holloway, 1978).

The combination of extensive habitat destruction and the almost yearly storms affected the bat population so badly that by 1974 it was considered to be one of the rarest bats in the world (Carroll, 1981). With little upturn in the population's fortunes over the following 4 years, the IUCN declared *Pteropus rodricensis* "Endangered" (Goodwin & Holloway, 1978).

4.1.6 Conservation Measures Taken

In 1976, Gerald Durrell and his team surveyed the wild bat population on Rodrigues to assess its chances of survival given the removal of individuals for the establishment of a breeding programme (Durrell, 1976; Carroll, 1979a). Having determined that the wild population would remain secure, 12 females and 6 males were caught and used to established two breeding colonies: one with 5 females and 3 males in the Mauritian Government aviaries, and the other with 7 females and 3 males at the Jersey Wildlife Preservation Trust (Pook, 1978). This captive breeding programme has been very successful and it is currently one of the most important chiropteran breeding projects in operation (Carroll & Mace, 1988).

In 1983, continued pressure from concerned wildlife welfare bodies resulted in the Mauritian Government passing the Wildlife Act which made it illegal to harm any of Rodrigues' endemic vertebrates, i.e. the fruit bat, the fody and the warbler (Diamond, 1987). In addition, the area where most of the fruit bats roost has been fenced-off and declared a reserve (Young, unpublished) (see Figure 4.1).

4.1.7 Current Threats to the Fruit Bat Population

Cyclones and deforestation continue to be major threats to the fruit bats' existence, and the problems of introduced species and the lack of tree cover remain to be solved. Fortunately, the islanders have stopped eating the bat since being made aware of its rarity, but human persecution continues because of illegal grazing, mango farming and firewood collection within the bats' fenced reserve (Carroll, 1981; Young, unpublished). In addition, there exist potential genetic problems associated with reductions in population size and loss of genetic diversity (see Chapter 1, Section 1.6.2), although these have not as yet been studied.

However, notwithstanding the continuing problems, the conservation

measures so far taken do appear to have helped the fruit bats recover some of their numbers. From the nadir of 1979, when only 70 individuals could be located in the wild, the population has grown to over 400 individuals (see Table 4.2 and Figure 4.2)

TABLE 4.2: Estimates of the wild Rodrigues fruit bat population between 1981 and 1988.

YEAR	ESTIMATE	REFERENCE
1981	200	Carroll & Mace, 1988
1983	>100	Gade, 1985
1984	>300	Carroll & Mace, 1988
1988	412	Carroll & Mace, 1988

4.1.8 Captive Breeding

The ten founders of the British captive stock arrived in Jersey in May 1976, and the first successful birth occurred 2 years later (Carroll, 1979b). The colony bred successfully, reaching 38 individuals by the end of 1981 (Carroll, 1981). The captive stock continued to grow and was split into two separate groups about five years ago. One group is housed in a three-sided glass cage built into an archway next to the Trust's Headquarters, and the other is housed in a specially built enclosure with a reverse day/night regime (pers. obs., 1989).

The captive population had reached over 120 individuals by 1988, with representatives in five different locations: Mauritius, Jersey Wildlife Preservation Trust, Chester Zoo, Bronx Zoo, New York, and Brookfield Zoo, Chicago. The stocks held at the latter three zoos were all captively-bred at Mauritius and Jersey. Indeed of the present population at Jersey, 57 individuals have been captively bred with only 8 having originated in the wild (Anon., unpublished b).

4.1.9 Aims of this Study

When the bats first arrived at Jersey in 1976, they were not marked in any way, and the relationships between the founders were unknown (J.B. Carroll, pers. comm., 1989). A ringing system was not brought into operation until 1982, and so the parentage of the individuals born before this time is unknown (Carroll & Mace, 1988). To complicate the problem, the species is promiscuous, although the extent of this behaviour is being re-evaluated (Young & Carroll, unpublished), and so the presence of a ringing system has not been of great help in the study of the population's breeding structure.

The aim of this study was to determine if sufficient minisatellite variation

remained in the stock to allow the allocation of maternity and paternity for those individuals for which such information was unavailable (the majority of the colony), and to thereby construct a family tree. The information gained could then be used in conjunction with the colony records and behavioural data to assess individual breeding success, and by appropriate management to keep the loss of genetic diversity to a minimum.

4.2 MATERIALS AND METHODS

4.2.1 General Introduction

The information presented in this section outlines the alterations which were made to the materials and methods presented in Chapter 2 in order to produce a DNA fingerprint for Rodrigues fruit bat. The numbers of the specific sections in Chapter 2 to which these amendments and relevant comments apply are given in parentheses. Also presented are the methods used to investigate some aspects of allozyme variation in the bats.

4.2.2 Comments on Tissues (2.1.1)

All the samples of Rodrigues fruit bat analyzed in this study were provided by Dr. J.E. Cooper of the Royal College of Surgeons, London, and Mr. J.B. Carroll of Jersey Wildlife Preservation Trust (JWPT), to whom I am most grateful. The first samples received for this study were from the collection of Dr. J.E. Cooper. This collection is of post-mortem individuals and their organs, all of which are preserved in formalin. Unfortunately, information regarding the relationships between the individuals stored was not available.

A number of different tissue samples were obtained from the collection including heart, liver, muscle, lung, skin, tongue, brain and placenta. In order to extract DNA from these tissues, they were first ground in liquid nitrogen (2.1.1.1), then washed twice in 500µl TE buffer to remove the formalin, accompanied by repeated inversion on a mixing board and centrifugation to pellet the tissue. Unfortunately, after phenol extraction (2.1.1.3), the precipitation step (2.1.1.4) revealed very little DNA, certainly far less than would be expected from tissue samples of 50mg, and so the DNA pellets were resuspended in only 50µl of TE buffer and restriction digestion (2.1.2) carried out in the same tube, thus utilising all the available DNA. When the samples were qualitatively assessed on a minigel (2.1.2.3), they all showed severe degradation of the DNA with the most metabolically active tissues showing the greatest degradation (data not shown). Liver and brain samples produced no detectable DNA at all, with skin showing the least degradation. These extractions were repeated with identical results, indicating that the collection at the Royal College of Surgeons was, disappointingly, of no use in this study.

The second set of samples received were from Mr. J.B. Carroll. This set consisted of blood samples and one liver from a neonate that had recently died.

This liver, unlike the preserved specimens, was removed from the bat within 12 hours of its death, and so it was possible to extract good quality DNA. This allowed me to experiment and determine the most appropriate restriction enzyme to use (2.1.2) and the best running conditions for electrophoresis (2.1.3).

The blood samples proved to be very difficult to process because most were very small, typically 250-500µl. In addition, since mammalian erythrocytes contain no genomic DNA, nucleic acid extracted from such samples originates in the leucocytes, and is thus in very low concentration.

A number of methods were examined to separate the leucocytes from the blood sample before DNA extraction. Those presented by Reymond (1987), and Potter and Potter (1988), were deemed inappropriate since they both required more than 5ml of blood and the samples received from Jersey were in the order of 0.25-0.50ml.

The method that I decided to try was a modified version of that detailed by Signer et al. (1988a), which was specifically developed for small blood volumes. Approximately, 250µl of bat blood was mixed with 500µl of distilled water (AnalaR) in a 1.5ml eppendorf tube. This was then centrifuged at 4°C and 10,000g for 20 minutes and the supernatant discarded. The resulting pellet was resuspended in 500µl 0.1% (v/v) Nonidet P40 and re-spun as above. Again, the supernatant was discarded and the pellet resuspended in 1ml of a specially prepared lysis mix (see below), and incubated at 37°C for 16 hours.

After incubation, the samples were centrifuged at 20°C and 1,000g for 15 minutes, and then the supernatant was transferred to a new tube. At this point I diverged from the Signer et al. method, and washed the supernatant once with phenol and once with phenol/chloroform. A final wash with chloroform was then performed and the DNA precipitated and recovered (2.1.1.4). The DNA yield was very small, but no degradation was seen, and the DNA was sufficiently clean to restrict with *HaeIII* (2.1.2).

(Lysis mix: 0.01M Tris-HCl, 0.4M NaCl, 2mM EDTA pH8.2, 0.5% w/v sodium dodecyl sulphate, 125µl/ml Proteinase K.)

4.2.3 Electrophoresis and Probing (2.1.3 & 2.1.5)

The restricted bat DNA samples were electrophoresed for approximately 1600Vh, allowing minisatellite fragments of less than 3,000 base pairs (bp) in length to migrate off the end of the gel. All the filters holding bat samples were probed with pSPT19.6 (see Chapter 3, Section 3.2.5 for the reasons why this probe was used), which hybridised to very few high molecular weight fragments, i.e. those minisatellites more than 20,000bp in size.

4.2.4 Allozyme Analysis

A brief study of protein polymorphism in the bats was undertaken with the

assistance of Ian Wilson to whom I am most grateful, and followed the methods outlined in Selander et al., 1971. The systems established by Ian Wilson were to test for enzymes present in the livers of gastropods and so were not selected specifically for use with the bats. Notwithstanding this caveat, results were obtained for all the allozymes tested, although these results were not encouraging.

Small samples of blood (approximately 100µl) were mixed thoroughly with cold distilled water (AnalaR) to lyse the cells and then centrifuged at 4°C and 10,000g for 45 minutes. After this time, the supernatant was transferred to a separate tube and the samples stored in a 4°C fridge until required. Prior to electrophoresis, the samples were mixed with a solution of DTT (see below) in a 1:4 ratio and incubated in darkness for 45 minutes at 37°C. This procedure ensured that the sulphydryl groups in the proteins were evenly reduced, eliminating errors in the subsequent running and staining steps.

(DTT soln.; 5mg dithiothreitol, 1ml distilled water.)

Whilst the samples were incubating, the starch gel in which they were to be run was prepared. For each enzyme to be tested, an appropriate buffer system had to be used and this is detailed below. Each gel consisted of 24g of potato starch in 200ml of buffer. The two were mixed in a round bottomed flask and heated in a bunsen flame. During the heating, the flask was held with a heat-resistant glove and the contents constantly swirled until they boiled vigorously. This procedure polymerised the starch causing the gel to thicken. At this point, the flask was removed from the flame and degassed using a vacuum pump secured into the neck of the flask. The contents were swirled continuously during degassing until no further bubbles formed in the gel. It was then poured into a mould approximately 180mm long, 100mm wide and 6mm thick, covered with a glass plate and allowed to set.

When the gel had set, the glass plate was removed and a steel comb with a number of teeth about 3.5mm wide was pushed into the gel to produce a line of slots 20mm away from one of the long edges of the gel. Pieces of chromatography paper were cut to a size of 5mm x 3mm and approximately 10µl of sample applied to an individual piece. When the chromatography paper was seen to lose its immediate wetness, the pieces of paper were pushed into the slots in the gel.

The gel was placed in standard electrophoresis apparatus with each electrode consisting of a platinum wire in a perspex container, each of which held 600ml of the appropriate buffer. "J-Cloth" wicks connected the buffer containers to the gel, overlapping the latter by about 1cm. The gel itself was covered in a single sheet of Saran Wrap and then sandwich between two continually running cooling plates, which held the temperature at around 4°C during the run.

The gels were run at between 170 and 200 volts (direct current) for 3-4 hours. After this time, the electrophoresis apparatus was dismantled and the chromatography paper removed from the gel slots. The gel was then cut along its

length into two slices, each about 3mm thick, with a 0.5mm fishing wire. These slices were peeled apart revealing their inner surfaces and it was to these that the appropriate stains were applied.

4.2.5 Allozyme Buffers and Stains

Table 4.3 lists the enzymes which were examined for variation in the Rodrigues fruit bat. Details of the compositions of the buffer systems used, the stains required to detect the enzymes and the incubation times for the stains, are given in the notes below the table and are referred to within it.

TABLE 4.3: Enzymes investigated for variation in the Rodrigues fruit bat.

ENZYME	BUFFER SYSTEM	STAIN
Malate dehydrogenases (MDH)	Continuous tris-citrate[1]	See note 3
Diaphorases (Dia)	As above	See note 4
Phosphoglucose isomerase (PGI)	As above	See note 5
6-Phosphogluconate dehydrogenase (PGD)	As above	See note 6
Xanthine dehydrogenase (XDH)	Tris-versene borate[2]	See note 7
Esterases (Est)	As above	See note 8

1) *Continuous tris-citrate*: 27g Tris and 18.07g monohydrate citric acid were dissolved in 0.5 litres of distilled water. When all the solute had dissolved the solution was brought to pH6.3 using 1M NaOH and then made up to 1 litre with distilled water.

2) *Tris-versene borate*: 60.6g Tris, 40g boric acid and 6g EDTA were dissolved in 0.75 litres of distilled water. When all the solute had dissolved, the solution was made up to 1 litre with distilled water.

3) The stain used to detect MDH was made using the following recipe:

> 30mg L-malic acid, 55mg Tris, 1ml 1M magnesium chloride ($MgCl_2$), 1ml MTT tetrazolium (MTT), 5mg phenazine metho-sulphate solution (PMS), 8ml 0.2M Tris/HCl pH8.0, 1ml 1M β-nicotinamide adenosine diphosphate (NAD) and 12ml (2%) agar. Incubation was in the dark at 37°C for 1-2 hours.

4) The stain used to detect Dia was made using the following recipe:

10mg reduced nicotinamide adenosine dinucleotide (NADH), 1ml 1M $MgCl_2$, 1ml MTT, 8ml 0.2M Tris/HCl pH8.0, 1ml DCPIP and 11ml (2%) agar. Incubation was in the dark at 37°C for 90 minutes.

5) The stain used to detect PGI was made using the following recipe:

20mg D-fructose-6-phosphate, 1ml 1M $MgCl_2$, 1ml MTT, 1ml nicotinamide adenosine dinucleotide phosphate (NADP), 1ml PMS, 8ml 0.2M Tris/HCl pH8.0, 20µl glucose-6-phosphate dehydrogenase and 12ml (2%) agar. Incubation was in the dark at 37°C for 30-60 minutes.

6) The stain used to detect PGD was made using the following recipe:

10mg 6-phospho-gluconic acid, 1ml 1M $MgCl_2$, 1ml MTT, 1mg NADP, 1mg PMS, 8ml 0.5M Tris/HCL pH8.0 and 12ml (2%) agar. Incubation was in the dark at 20°C for 1 hour with stain being reapplied after 30 minutes.

7) The stain used to detect XDH was made using the following recipe:

25mg hypoxanthine, 10mg NAD, 10mg nitro blue tetrazolium (NBT), 10mg MTT, 5mg PMS, 50ml 0.2M Tris/HCL pH8.0 and 11ml (2%) agar. Incubation was in the dark at 37°C for 3 hours.

8) The stain used to detect Est was made using the following recipe:

30ml sodium phosphate buffer (4:1 mixture of 0.2M dibasic sodium phosphate and 0.2M monobasic sodium phosphate), 1ml α-naphthyl propionate solution (1g α-naphthyl propionate in 100ml acetone), 25mg Fast Garnet GBC and 12ml (2%) agar. Incubation was in the dark at 37°C for 1-2 hours.

4.3 RESULTS AND CONCLUSIONS

4.3.1 Initial Analysis

The probe used for this analysis of the Rodrigues fruit bat was pSPT19.6. After an electrophoresis of approximately 1350Vh, the mean number of bands that could be scored per individual male was 13.4 (σ = 3.1), and the mean for females was 18.0 (σ = 2.2). Females thus have approximately five more bands in their DNA fingerprints than do males. It is possible that this is due to the presence of one or more minisatellite loci on the X-chromosome, in which case a male would tend to display half as many X-chromosome alleles as a female. Unfortunately, it was not possible to determine if this hypothesis was correct as there were no

extended families available in which to study the inheritance patterns of these alleles. Indeed, there were no families at all. The breeding record (Anon., unpublished b) lists only mother/offspring relationships, the fathers of these offspring being unknown, largely due to the promiscuous behaviour of the species. In addition, it was not possible to assess the occurrence of linkage and allelism within the species because of this absence of family groups.

The distribution of the similarity coefficients (D) calculated between pairs of bats is shown in Figure 4.3 (see Appendix 5 for raw data). A comparison of this graph that one produced for Rothschild's mynah (Figure 3.4) reveals that although both appear unimodal, the graph for the bats is shifted to the right relative that for the mynahs. The mean D for comparisons between members of the captive stock of Rodrigues fruit bat is 0.772 (σ = 0.127), indicating that the similarity coefficients calculated for the bat stock are, on the whole, higher than those calculated for the mynahs. The background level of band sharing in the bats is therefore greater than that in the mynahs, possibly due to the greater degree of inbreeding that the bats have undergone. This result is not unexpected given the fact that only ten individuals (the founders of the bat stock) appear to be responsible for all the breeding that has occurred during the past 17 years (Anon, unpublished b).

4.3.2 Determination of Relationships

As can be seen from Figure 4.4, the individual DNA fingerprints of the members of the captive bat stock submitted for analysis are all very similar, with many of the same bands being present in a large proportion of individuals. In addition, there appear to be a number of bands that are invariant in those individuals tested. This is probably due to the bands in question having become fixed in the population, i.e. the minisatellite loci from which these alleles are derived contain only the allele seen. This high level of band sharing makes it difficult to identify individuals from their DNA fingerprints and makes confident paternal assignment almost impossible.

Female bats and their offspring share such a large proportion of their bands that the number of non-maternal bands available for matching to a male's DNA fingerprint is small. The high degree of band sharing between the bats means that it has not been possible to identify any bands that are only present in the DNA fingerprint of one individual. Therefore, the best that can be done when using probe pSPT19.6 in an attempt to assign a father to an offspring, is to compile a list of possible sires which may then be compared with observations of mating behaviour, allowing the males to be ranked in order of probability that they are the father.

Of the samples submitted for analysis, there were only three mother/offspring pairs (breeding record numbers 1260/1347, 1263/1319 and 1314/1316, mother listed first) that produced DNA fingerprints of sufficient quality for analysis. These pairs were examined to see if it would be possible to determine the offsprings' paternity. The DNA fingerprints for the first pair listed

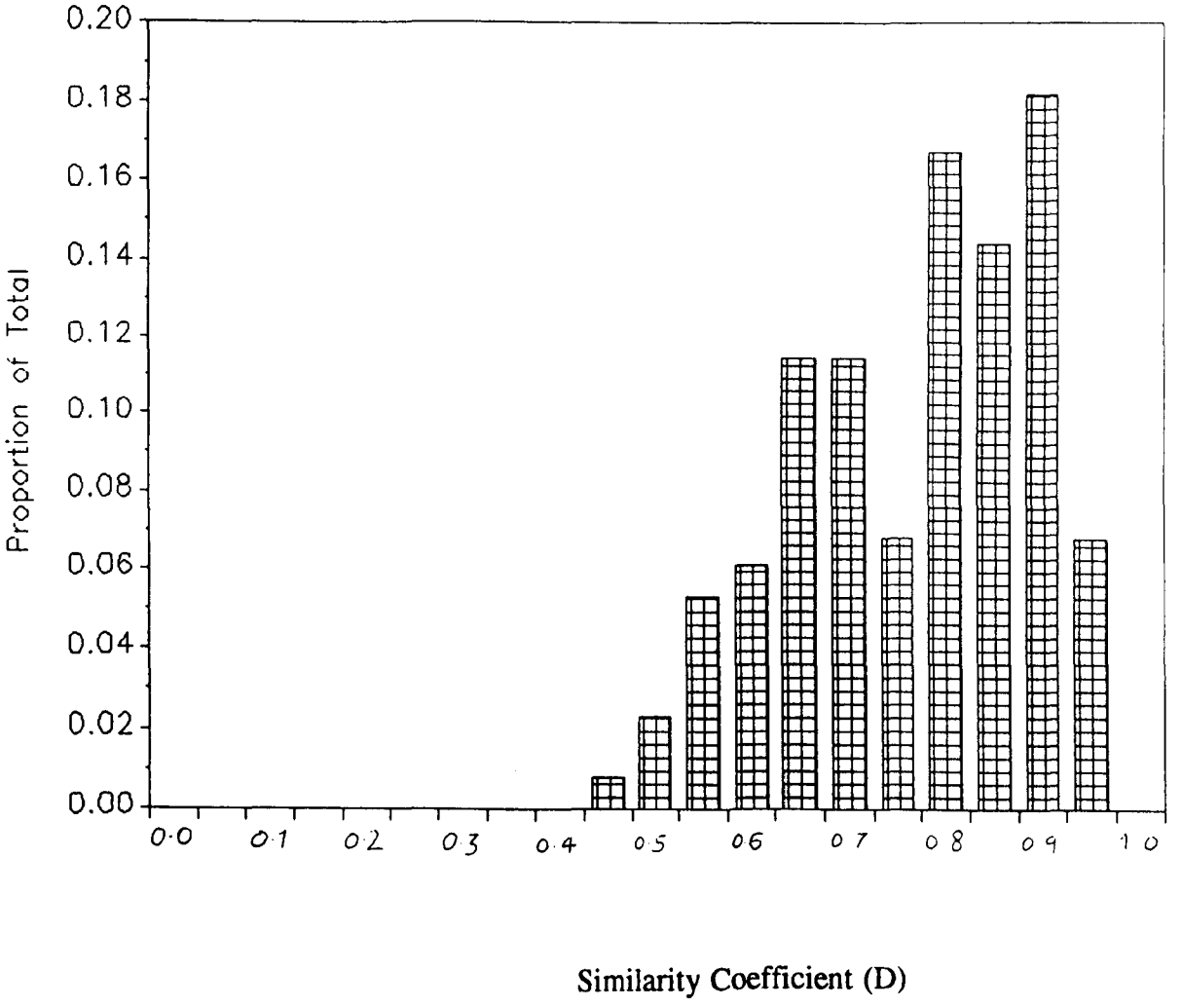


FIGURE 4.3: Graph showing the distribution of all the similarity coefficients (D) calculated for comparisons between members of the captive stock of Rodrigues fruit bat. Probe used: pSPT19.6

were compared and it was found that all the bands present in the DNA fingerprint of individual 1347 were also present in the fingerprint of its mother, 1260. Since there are no bands in the former individual's fingerprint that can be assigned as "non-maternal", it is not possible, on the basis of these results, to determine which of the males in the stock is the sire of this offspring. However, three bands were scored in the DNA fingerprint of individual 1319 that were not present in its mother's fingerprint, and one band was detected in 1316 that was not present in 1314. This allows some scope, albeit limited, for the identification of these offspring's fathers. Three males (individuals 1258, 1274 and 1277) have the non-maternal bands present in the fingerprint of individual 1319 in their fingerprints, and four males (individuals 1274, 1275, 1278 and 1310) have the non-maternal band identified in individual 1316.

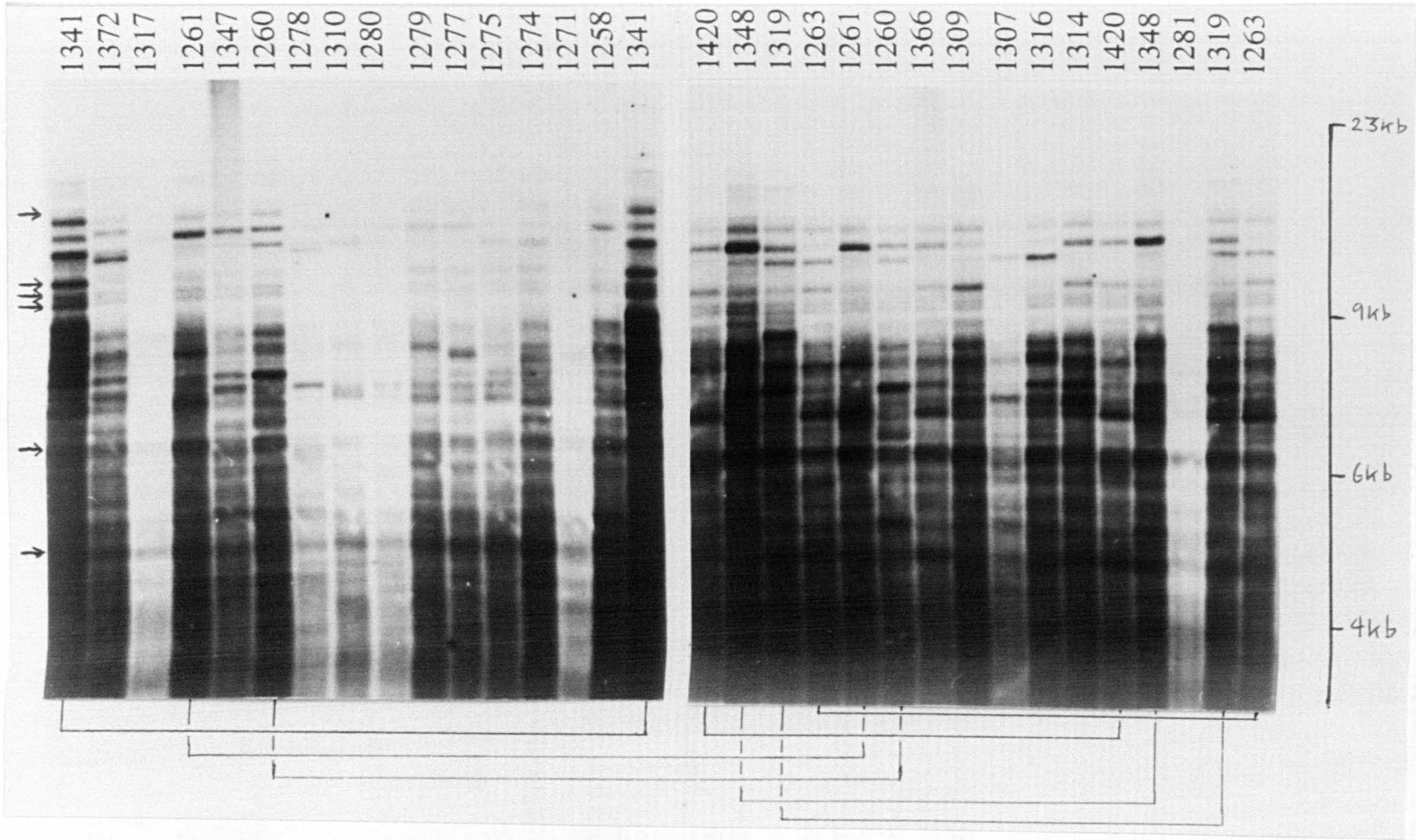


FIGURE 4.4: Two sets of DNA fingerprints produced using probe pSPT19.6 on samples from Rodrigues fruit bats. The arrows to the left of the figure indicate those bands that are invariant in the stock. Individuals are identified by their Taxon Report ID numbers (see text for details).

According to observations made by Jeanette Young at JWPT (Young & Carroll, unpublished), Rodrigues fruit bats are not as promiscuous as once thought. The five females that were the focus of Ms. Young's study showed a high degree of mate fidelity, being observed to copulate with only 1-3 males during observation periods of 18 hours (for each female) spread over 20 days. Of these five females, it was only possible to analyse one (1314) with an offspring (1316), and the three males with whom she had been seen to associate (individuals 1275, 1259 and 1278) (see Figure 4.4). The results from DNA fingerprinting given above show that, of these three males, only 1259 can be excluded from the possible paternity of individual 1316, leaving two possible sires, 1275 and 1278. Unfortunately, it was not possible during this study to distinguish which of these two individuals is the true biological father of 1316, even though additional analyses were undertaken (see Section 4.3.3), and further tests will therefore have to be performed to resolve this problem.

The three mother/offspring pairs listed above, in addition to one sibling pair (1348/1420), are the only examples of "known" 1st-degree relationships that were analyzed in this study. The mean similarity coefficient for comparisons between these individuals is 0.886 (σ = 0.058), compared with 0.772 (σ = 0.127) for the stock as a whole. There were also samples available from four of the stock's founders, individuals 1258, 1260 (also one of the mothers used for the 1st-degree relative comparisons), 1261 and 1263, and so it was possible to compare their mean similarity coefficient (0.746, σ = 0.110) with that of the "known" 1st-degree relatives. The difference between these two statistics is 0.140, which, based on the mean number of bands scored per bat, means that the sharing of just two extra bands would change the relationship category to which two individuals would be assigned from "unrelated" to "1st-degree relatives". Clearly, this result cannot be accorded great significance as the sample sizes are very small, but it does demonstrate that the lack of variation in the alleles detected and the high level of band sharing in the stock, make using probe pSPT19.6 to assign parentage and determine degrees of relatedness very difficult. However, the confidence with which these relationship criteria are assigned would be increased if additional multilocus probes, or even specially developed single locus probes (see Chapter 7) were used, and the resulting data pooled.

4.3.3 Allozyme Analysis

Because the data produced by DNA fingerprinting were not satisfactory, a brief study of allozyme variation was conducted. It was hoped that this, in combination with the DNA fingerprinting data, would allow the elimination of one or more of the possible sires in the groups listed above, allowing a single individual to be assigned to the paternity of the offspring in question. Figures 4.5, 4.6 and 4.7 show the results of testing members of the Rodrigues fruit bat captive stock for variation in the enzymes 6-phosphogluconate dehydrogenase (PGD), esterases (Est) and diaphorases (Dia), respectively. In all cases, the anode is to the top of the figure. The samples tested were selected from individuals held in both the Nocturnal house and the Archway cage at JWPT, the specific location being listed on the figures (see Appendix 6 for the ID numbers of the individuals from

which samples were obtained). As can be seen from the figures, no electrophoretic variation could be detected in these enzymes. Samples from individuals held in the Nocturnal house were also tested for the enzyme xanthine dehydrogenase (XDH), but again, no variation was detected (data not shown).

Figures 4.8 and 4.9 show the results of testing for variation in the enzymes phosphoglucose isomerase (PGI) and malate dehydrogenases (MDH), respectively (see Appendix 6 for the ID numbers of the individuals from which samples were obtained). As indicated by the arrows on these figures, a number of individuals do appear to have electrophoretic variants, although these are not very convincing. For PGI (Figure 4.8), five out of the 25 samples from individuals held in the Nocturnal house at JWPT displayed a "slower" variant than the rest and, with appropriate alterations to the pH and running time, it may prove possible to make the differences between the variants, if they are genuine, more pronounced.

Staining for MDH revealed no discernable variation in the samples from individuals kept in the Nocturnal house (data not shown), although a degree of variation was detected within the individuals maintained in the Archway cage. Individuals 3, 5, 10, 11 and 12 appear to lack the variant slightly anodal to the insert line, and both 3 and 5 appear to have "fast" alleles which are brighter than those of neighbouring samples.

As has been pointed out in the methods to this chapter (see Section 4.2), this analysis used the systems established by Ian Wilson to examine enzyme polymorphisms in snails. It is possible that the enzymes present in the blood of the Rodrigues fruit bat show little variation and that if liver or kidney samples had been used, more variation would have been detected. Indeed, Selander et al. (1971) suggest that liver and kidney are the best tissues to use for detecting variants in GPD, XDH, PGI and MDH, and blood is recommended for PGD and esterases. However, in this study variation appears to have been detected in PGI and MDH using blood samples, whereas no variation was detected in PGD and esterases. There are many other enzymes that could be examined for variation, but given that the bat stock has been inbreeding for the greater part of 20 years, that the results produced by DNA fingerprinting indicate that there is limited minisatellite allele variation, and that the allozymes tested here showed little variability, one would not expect there to be an abundance of variation in any enzyme system.

4.4 DISCUSSION

After the steep decline in the numbers of fruit bats located on Rodrigues during the late 1950s and early 1960s, the population has now increased in size to over 400 individuals (see Figure 4.2). The major causes of the species' decline were severe weather conditions and human interference/persecution. The captive breeding programme was initiated when the bats numbered only around 100 individuals and, due to the limited number of bats present in the wild and their protected status, no further specimens have since been acquired. This has resulted in there having been no influx of new genes from the wild since the ten original

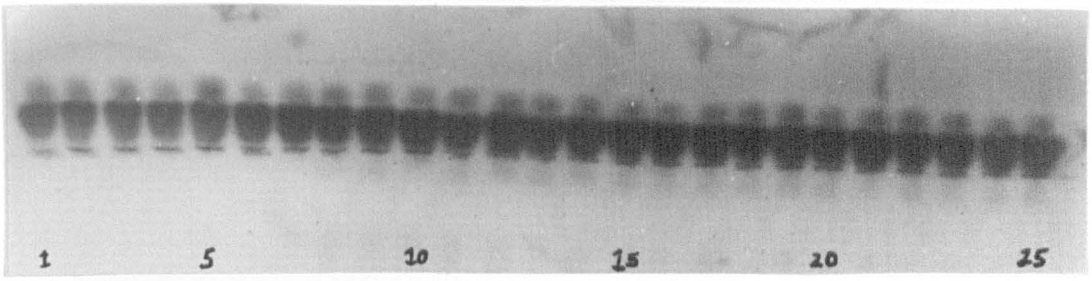


FIGURE 4.5: 25 Rodrigues fruit bat samples stained for 6-Phosphogluconate dehydrogenase (PGD). Samples from the Nocturnal house.

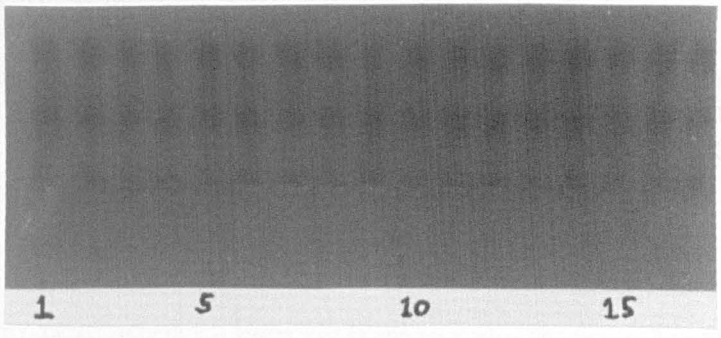


FIGURE 4.6: 17 Rodrigues fruit bat samples stained for Esterases (Est). Samples from the Archway cage.

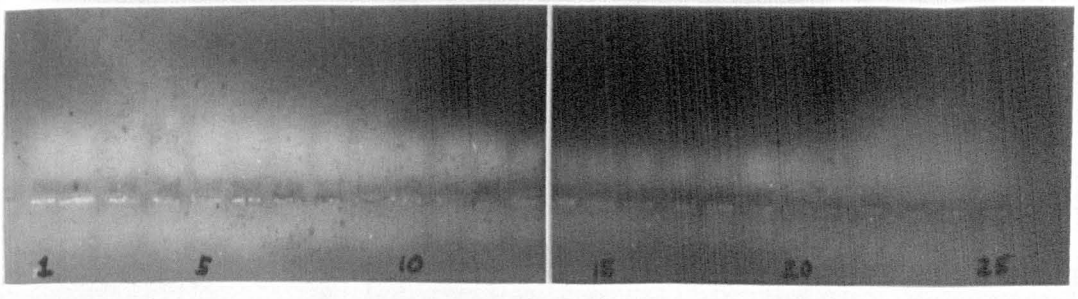


FIGURE 4.7: 25 Rodrigues fruit bat samples stained for Diaphorases (Dia). Samples from the Nocturnal house.

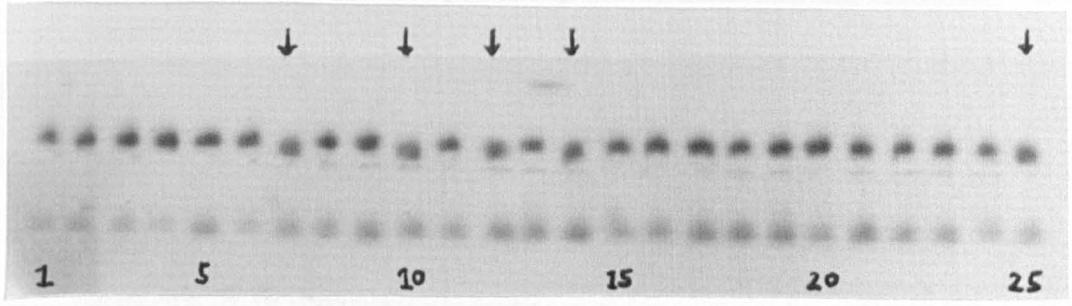


FIGURE 4.8: 25 Rodrigues fruit bat samples stained for Phosphoglucose isomerase (PGI). Samples from the Nocturnal house. Arrows above figure point to possible electrophoretic variants.

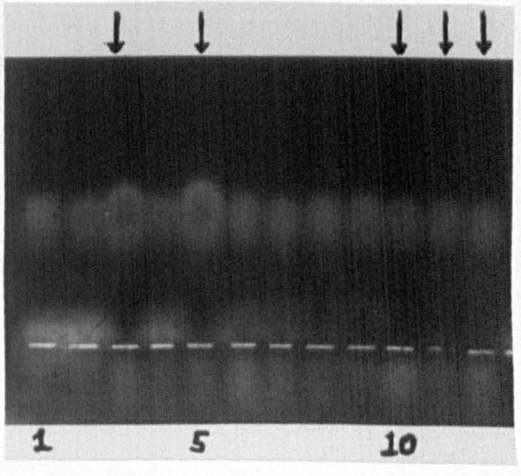


FIGURE 4.9: 12 Rodrigues fruit bat samples stained for Malate dehydrogenases (MDH). Samples from the Archway cage. Arrows above the figure point to possible electrophoretic variants.

founders of the colony were imported almost 20 years ago.

A ringing system for the stock was not established until six years after the bats arrived at JWPT and unfortunately, as they were maintained as a freely associating group in a large enclosure, there remained little possibility of monitoring the breeding of specific individuals even with the rings in place. This study has used DNA fingerprinting to assess the degree to which the technique can be used to monitor breeding and establish the relationships between the individuals already present in the stock. The results of this work show that if the technique is to be used, a number of multilocus or single locus probes will have to be employed (see Chapter 7). The variation of minisatellite alleles detected by the polycore probe pSPT19.6 is not sufficient to allow confident identification of individuals or allocation of all offspring to parents. Such assignments are crucial if the stock is to be managed in a way that maintains what little variation appears to remain, and if the stock managers are to be allowed to select genetically "fit" individuals for reintroduction into the wild should the population crash again.

It should be pointed out that, although the wild bat population was reduced to around 70 individuals at its lowest point in 1979 (not much greater than the number currently held in captivity at JWPT), population numbers have since climbed to more than 400 individuals. It is therefore possible that the species has been through one or more genetic bottlenecks in its evolutionary past, leaving it with a low genetic load (see Chapter 1, Section 1.6.2). It would thus be more able to survive large reductions in its population size and the inbreeding which accompanies this. As samples from individuals currently living on Rodrigues (who have never been in captivity) were not available for this study, it is not possible to assess the validity of this hypothesis. However, if the results from DNA fingerprinting of the four founders are any guide, the minisatellite variation was low when the captive breeding programme was initiated. Unfortunately, the relationships between the founder individuals are not known and it is therefore difficult to assess the significance of these results. If they were unrelated, then it appears that there was little allelic variation present in the population when the specimens were captured and thus their situation probably cannot be ameliorated by species translocation.

CHAPTER 5

THE BRITISH MERLIN
(*Falco columbarius* Linnaeus)

5.1 INTRODUCTION

5.1.1 General Introduction

Falconers have kept and trained birds of prey in captivity for over 3,000 years, but it is only in the last 30 years that coordinated efforts to breed them in captivity have been undertaken (Cade, 1982; 1986). The prime incentive for these efforts was the great decline in the size of the wild European raptor populations during the 1950s and 1960s (Cooper, 1977). Indeed, European raptors are reported to have lost up to 99% of their numbers since 1800 (Myers, 1979). These declines prompted governments and other bodies, i.e. the EEC (European Economic Community), to enact legislation to protect the raptor stocks in the countries concerned (see Section 5.1.5). This made the removal of birds and eggs from the wild illegal, forcing falconers to breed their own birds (Cooper, 1986).

Unfortunately, this new legislation did not deter some people from continuing to take raptors from the wild, and persecution by falconers and egg-collectors has been a factor in the continued decline (see Section 5.1.4) (Williams, 1981). However, it should be pointed out that the new laws did reduce the levels of persecution, and allowed prosecution of those removing birds from the wild (Cooper, 1986). In addition, the recovery of raptors was helped by the banning of certain agricultural pesticides resulting in a number of raptor populations recovering their numbers (Newton, Meek & Little, 1978). Unfortunately, the numbers of British Merlins have continued to decline despite these measures (Newton, Robson & Yalden, 1981).

5.1.2 Taxonomy

KINGDOM	- Animalia
PHYLUM	- Chordata
SUBPHYLUM	- Vertebrata
CLASS	- Aves
ORDER	- Falconiformes
SUBORDER	- Falcones
FAMILY	- Falconidae
SUBFAMILY	- Falconinae
GENUS	- Falco
SPECIES	- columbarius

The order Falconiformes, with the single suborder Falcones, contains four

families of which the largest is the Falconidae, containing about 60 species (Campbell & Lack, 1985). The subfamily Falconinae contains four genera, the largest of which is *Falco*, recognised as containing about 39 species. Indeed, *Falco* is the largest genus within the order Falconiformes (Thompson, 1964; Grzimek, 1972).

Falco evolved about 25 million years ago in the Miocene epoch (Grossman & Hamlett, 1965), and, with the range of morphological traits and habits of the birds it represents, is probably the most varied raptorial bird genus (Grzimek, 1972). Because of the extent of this variation, a number of species have been further classified into subspecies. The British Merlin, being one of these, is sometimes accorded the subspecific status of *subaesalon* (Howard and Moore, 1980).

5.1.3 General Biology and Behaviour

The Merlin is a small falcon 25-30cm in length with a wingspan of about 64cm (Grzimek, 1972; National Geographic Society, 1983). Like many raptor species the female is larger than the male, weighing on average 200g compared with the male's 170g (Campbell & Lack, 1985). The female also tends to be more aggressive and sometimes kills her mate in captivity (Campbell, 1979).

The species is sexually dimorphic with a number of plumage differences between the male and the female (Peterson, Mountfort & Hollom, 1983). The male has slate-blue upper-parts with creamy undersides showing rufous-striping. The most characteristic feature of the male's plumage is the broad terminal black band on the tail. In contrast, the female's upper-parts are dark-brown and the tail is creamy with a number of brown bars.

The Merlin's favoured habitat is open moorland, although they are also found in grasslands, forest bogs, and on the coast (Campbell, 1979). They hunt over territories of 10-20km^2, feeding mainly on other birds, although voles, bats and some insects are also taken (Haworth & Fielding, 1988; Newton, Meek & Little, 1978). The Merlin is a Holarctic species distributed across Canada, North America and Eurasia, that migrates in the winter either to more temperate southerly regions, or just to a lower altitude (Campbell & Lack, 1985).

Merlins nest on boulders, on crags, and in trees, occasionally utilizing the unoccupied nests of other birds, e.g. crows (Grossman & Hamlett, 1965). They also nest extensively on the ground, a habit that leaves them vulnerable to predation and disturbance by Man (Newton, Meek & Little, 1978; 1986). They usually lay clutches of 4-5 eggs that are incubated by both the male and female (Williams, 1981; Campbell, 1979). They hatch in 30-31 days, with an average of 3-4 chicks successfully fledging (Bibby, 1986).

5.1.4 Decline of the Merlin

Over the past 50 years, the numbers of Merlins have been declining in

many countries, with a notable loss of numbers having been recorded in North America, parts of northern and eastern Europe, and in Britain (Campbell & Lack, 1985; Cooper & Forbes, 1986; Chancellor, 1977). This decline has been mirrored by reductions in the sizes of other raptor populations (the best documented being those of the Peregrine falcon, *Falco perengrinus*, and the Sparrowhawk, *Accipiter nisus*) all of which were primarily caused by the widespread use of certain agricultural pesticides in the 1940s and 1950s (Newton, Bogan, Meek & Little, 1982).

The British Merlin population is thought to have been slowly declining since the beginning of this century (Roberts and Green, 1983). However, there was a major decline between 1955 and 1965, and the species is now mainly confined to Wales, northern England and Scotland (Bibby & Nattrass, 1986; Cadbury, Elliott & Harbard, 1988). However, unlike the British Peregrine and the Sparrowhawk whose populations have shown recoveries in the past two decades, the numbers of British Merlins remain low. Indeed, it is the only breeding British raptor whose population is still declining (Newton, Robson & Yalden, 1981; Bibby, 1986).

The British Merlin population is composed of a number of subpopulations and all have suffered declines of varying severity (Moore & Walker, 1964; Newton, Robson & Yalden, 1981). A study in Wales found that between the years 1970 and 1984, the Merlin population declined from an estimated 150 pairs (Williams 1981) to about 40-45 pairs (Bibby, 1986). In the Peak District, Merlins were cited as "quite common" during the 1950s, but by the late 1970s they had declined to just 10% of their former numbers, and by 1980 there were only one or two pairs (Newton, Robson & Yalden, 1981; Bibby & Nattrass, 1986). In addition, the species is almost extinct as a breeding bird in the southwest of England (Cadbury, Elliott & Harbard, 1988).

The decline of the British Merlin has been caused by the interactions of five factors:

1) *Pesticide Poisoning*. By far the most important factor has been extensive poisoning due to the widespread agricultural use of certain pesticides, specifically organochlorine insecticides, such as DDT (dichlorodiethyltoluene), cyclodiene compounds (dieldrin, aldrin, and heptachlor), and compounds containing the heavy metal mercury (see references below). These compounds are known to cause a number of sub-lethal effects in those birds not killed outright (Newton & Haas, 1988). For example, DDT affects the eggs and young, reducing the thickness of shells, addling the contents of eggs, decreasing hatchability, and reducing the viability of the chicks (Moore & Walker, 1964). Other pesticides, e.g. cyclodienes, affect the adult's behaviour causing them to break their eggs, fail to defend the nest and its contents against predators, and in some cases, to desert their offspring (Fox & Donald, 1980).

Of the pesticides shown to have deleterious effects on birds, the organochlorine insecticides are the ones with the most significant contribution to

raptorial declines (Ratcliffe, 1970). The Merlin is the British raptor most heavily contaminated with organochlorine insecticides (Newton, 1973b; Cadbury, Elliott & Harbard, 1988) and the decline in its reproductive success and population size has been found to correlate to both the areas and the times over which these chemicals were used (Moore & Walker, 1964).

One such insecticide, DDT, is extremely persistent in the environment. It has been found that Merlins absorb it through the wall of their gut from the prey they ingest, and that it is stored in the body fat of the birds (Cadbury, Elliott & Harbard, 1988; Ratcliffe, 1970; Newton, 1973b). This means that during periods of starvation, when the birds have to mobilise their fat reserves, they are exposed to the effects of the stored organochlorines (Newton, Meek & Little, 1978; Roberts & Green, 1983).

2) *Habitat Destruction.* Large areas of the Merlin's traditional nesting, breeding and hunting grounds on the uplands of Britain have been destroyed and replaced by extensive tree-plantations and pastureland (Newton, Meek & Little, 1978). At the beginning of this century, 171km^2 of the Peak District were dominated by heather, one of the Merlin's favourite nest sites, but now only 93km^2 of this habitat remains (Newton, Robson & Yalden, 1981). Similar losses have occurred in parts of Northumberland (Newton, Robson & Yalden, 1981) and in mid-Wales, where more than 12% of the moorland has been lost (Cadbury, Elliott & Harbard, 1988).

3) *Disturbance.* Throughout this century there has been an increase in the public's access to moorlands and this has resulted in an increase in the disturbance of the Merlin, especially in the area of the Peak District National Park, where the species is now almost extinct (Newton, Robson & Yalden, 1981). In addition, this increase in accessibility has been accompanied by an increase in the frequency of accidental and/or deliberate fires, which have serious consequences for the ground-nesting Merlin (PPJPB, 1981).

4) *Removal from the Wild.* Falconers and egg-collectors continue to illegally remove adults, chicks and eggs from the wild (Prestt, 1977). A study in Wales found that of the 25 known nest failures occurring between 1967 and 1978, 15 had failed due to the eggs or chicks being removed by humans (Williams, 1981).

5) *Competition and Predation.* A decrease in the number of gamekeepers employed on land known to support Merlins has led to an increase in predation by "pest" species, e.g. foxes and crows, whose population sizes were previously strictly regulated (Newton, Robson & Yalden, 1981; Bibby & Nattrass, 1986). These species have been known to take Merlin eggs and chicks, with the fox being a particular menace to those birds nesting on the ground.

5.1.5 Conservation Measures Taken

The British Merlins are regarded as internationally important because they represent a significant proportion of the total European Merlin population

(Cadbury, Elliott & Harbard, 1988), and it is because of this importance that the species is extensively protected by International, European and British law (Haworth & Fielding, 1988).

The Merlin is covered by the following legislation:

1) The Protection of Birds Act 1954;

2) The Endangered Species (Import & Export) Act 1976;

3) The Convention on International Trade in Endangered Species of Flora and Fauna (CITES);

4) "Special" Protection under Schedule 1 of the Wildlife and Countryside Act 1981;

5) The EEC Directive on Conservation of Wild Birds (Annex 1).

These laws mean that it is illegal to take, kill or injure a Merlin, take, damage or destroy its nest, take or destroy its eggs, possess any live, dead, part of, derivative of or egg of a Merlin, to disturb or be deliberately in the vicinity of a wild Merlin or its nest containing young, eggs or in the process of being built (EEC, 1985; Cooper, 1986; Cadbury, Elliott & Harbard, 1988).

5.1.6 Current Threats to the British Merlin Population

The recoveries in the Peregrine and Sparrowhawk populations seen in recent years have not occurred in the Merlin (Newton, 1973a; Newton, Robson & Yalden, 1981) and, although the pesticides responsible for the great declines of 30 years ago are no longer in use, the Merlin continues to experience high rates of breeding failure (Newton, Meek & Little, 1981; Newton & Haas, 1988). This is thought to be due to the fact that the thickness of its eggshells remains well below that of eggs measured before the use of DDT (Newton, Meek & Little, 1978) and, also, that there are still substantial quantities of a number of pesticides in the environment, especially around coasts where some Merlins overwinter (Newton, Robson & Yalden, 1981).

The extent of available breeding habitat, i.e. managed heather moor, continues to decline, primarily due to afforestation, and much of that which remains has deteriorated in quality (Newton, Meek & Little, 1986). In addition, further declines have resulted from the development of grazing pasture in some areas, made necessary by the increased size of the British sheep population; by 1980 this was three times as great as in the 1930s (Newton, Robson & Yalden, 1981).

It is thought that the sizes of some of the remaining subpopulations are being held down by persistent human persecution from gamekeepers who continue

to shoot, poison, and trap Merlins to protect gamebird stocks, and by falconers and collectors removing the birds and their eggs from the wild (Newton, Meek & Little, 1986; Newton, Robson & Yalden, 1981).

5.1.7 Current Status of the British Merlin Population

Bibby & Nattrass (1986) estimated the British Merlin population at 550-600 pairs, based on the number of birds known to be breeding at that time. Of this total, about half were in Scotland with the remainder distributed through Northern England (about 190 pairs), and Wales (about 60 pairs).

The Peak District and Welsh subpopulations, where breeding success remains low, are thought to be extremely vulnerable to extinction (Bibby, 1986; Roberts & Green, 1983). This is because they are at the extreme southwest limit of the species' European range and so the chances for immigration and recolonization are limited (Newton, Robson & Yalden, 1981; Roberts & Green, 1983). There is some hope, however, as slight increases in the size of some subpopulations have been documented, specifically in those areas where the use of organochlorines has been banned, e.g. Northumberland, but even here breeding success has remained persistently low with few nests (less than 33%) being successful (Newton, Meek & Little, 1986).

5.1.8 Aims of this Study

The laws enacted to protect the Merlin strictly define the term "captive-bred", so that a bird can only be registered as such if both the parents can be shown to have been legally in captivity at the time that an egg was laid (Cooper, 1986). The Department of the Environment is legally required to ring and register all such birds. However, it has proved difficult for the authorities to convince courts that a bird had been taken from the wild and not captively-bred if the owner a) protested his innocence; and, b) was in possession of two adults from which he claimed the suspect bird had been hatched.

This problem and the continuing decline of certain raptor stocks, e.g. the Merlin, has led to calls for the development of a better system of permanent marking for captively-bred raptors, so that they may be distinguished from ones removed from the wild (Cade, 1986). The advent of DNA fingerprinting has meant that individuals can now be positively identified, and will allow the authorities and falconers to prove the true parentage of a bird. This will protect the honest breeders and will help to convict those taking birds illegally from the wild.

The aim of this study was to analyze a cross-section of the British captive Merlin population by obtaining samples from a number of bird breeders, to determine if there was sufficient minisatellite variation in the stock to allow individuals to be uniquely identified.

5.2 MATERIALS AND METHODS

5.2.1 General Introduction

The information presented in this section outlines the alterations which were made to the materials and methods presented in Chapter 2 in order to produce a DNA fingerprint for the British Merlins. Also presented here are relevant comments, with the numbers of the specific sections in Chapter 2 to which these amendments and comments apply given in parentheses.

5.2.2 Comments on Tissue (2.1.1)

All the samples used in this analysis were kindly supplied by the following private breeders to whom I am most grateful: Mr. M. Fountain, Mr. T. I'Anson, Dr. N. Fox and Mrs. C. Scott. The majority of the samples were blood, but Dr. Fox supplied some other tissue samples.

It proved more difficult to extract and purify DNA from the blood samples drawn from the Merlins than from any other species detailed in this volume. Typically, the blood samples required one extra phenol and one extra phenol/chloroform wash to clean the DNA sufficiently to allow efficient enzymatic digestion. The reasons for these difficulties are unknown, although the more "solid" tissues presented no additional problems.

5.2.3 Electrophoresis and Probing (2.1.3 & 2.1.5)

The *HaeIII* restricted Merlin DNA samples were electrophoresed for an average of 1,600Vh, after which time minisatellites of less than approximately 2,500 base pairs (bp) in length had migrated off the end of the gel. Both probe pSPT19.6 and pSPT18.15 were used to hybridise to the filters produced from these gels and both produced good results, with the minisatellites appearing well spread along the length of the fingerprint (see Section 5.3). Probe pSPT19.6 hybridised to bands that were distributed in three clear regions of the fingerprint; > 20,000bp, 20,000bp to 7,500bp, <7,500bp to 5,000bp. Probe pSPT18.15 produced a fingerprint with a more regular distribution of minisatellite bands along its length, and those in the size range >20,000bp to 5,000bp were scored for the analysis.

5.3 RESULTS AND CONCLUSIONS

5.3.1 Initial Analysis

Of the 35 Merlin samples analyzed in this study, the sex of only 24 of the original donors was known. If these donors alone are considered, the mean number of bands scored per individual using probe pSPT19.6 is 16.25 (σ = 2.45) for males, and 14.08 (σ = 2.47) for females; the combined mean for equal numbers of each sex is 15.02 (σ = 2.48). If all 35 individuals sampled for this study are included, the mean number of bands scored is 15.83 (σ = 2.98).

As in the case of Rothschild's mynah (see Chapter 3, Section 3.3.1), the lack of a statistically significant difference between the mean numbers of bands scored for each sex (specifically that the mean for females, the hemizygous sex in avians, is not higher than that for males), indicates that the minisatellites detected by pSPT19.6 are probably autosomal, as has been found in a number of other species (Jeffreys, Wilson et al., 1986; Jeffreys & Morton, 1987; Anming et al., 1990).

Figure 5.1 shows an example of a DNA fingerprint produced by probing a number of Merlin samples with pSPT19.6. In this case, samples from 13 Merlins (donated by four different breeders) have been used and, as can be seen, sufficient variation is detected using just one multilocus probe to clearly identify each individual. Minisatellites detected by this probe have been grouped into three regions and these are shown on the figure. Region I covers minisatellite alleles greater than 20,000 base pairs (bp), region II covers alleles from ≈ 20,000bp to 7,000bp, and region III covers alleles from ≈ 7,000bp to ≈ 5,000bp. Even if the bands within each region are considered in isolation, it can still be seen that each Merlin has a unique pattern. This means that providing the part of the DNA fingerprint from which the minisatellites were scored is known, it should be possible to identify an individual from only four or five bands.

Table 5.1 lists the mean number of bands scored per individual Merlin (considering all 35 individuals analyzed in this study) in each of the regions I to III. As can be seen, in addition to the probe detecting approximately the same number of bands in each sex over all regions of the DNA fingerprint, it also detects approximately the same number within regions. Thus, there is no part of the DNA fingerprint detected by probe pSPT19.6 that can be used to sex the birds.

TABLE 5.1: Mean number of minisatellite bands (detected using probe pSPT19.6) scored per individual in each of the three "regions" of the DNA fingerprint (see text for details).

	REGION			
	I	II	III	ALL
Males only	3.67 (σ = 0.89)	4.17 (σ = 1.40)	8.42 (σ = 2.08)	16.25 (σ = 2.45)
Females only	3.83 (σ = 1.27)	3.33 (σ = 1.07)	6.92 (σ = 2.38)	14.08 (σ = 2.47)
All birds	3.66 (σ = 1.03)	3.74 (σ = 1.36)	8.14 (σ = 2.31)	15.98 (σ = 2.98)

The distribution of all the similarity coefficients (D) obtained for comparisons between the Merlins analyzed in this study is shown Figure 5.2 (see

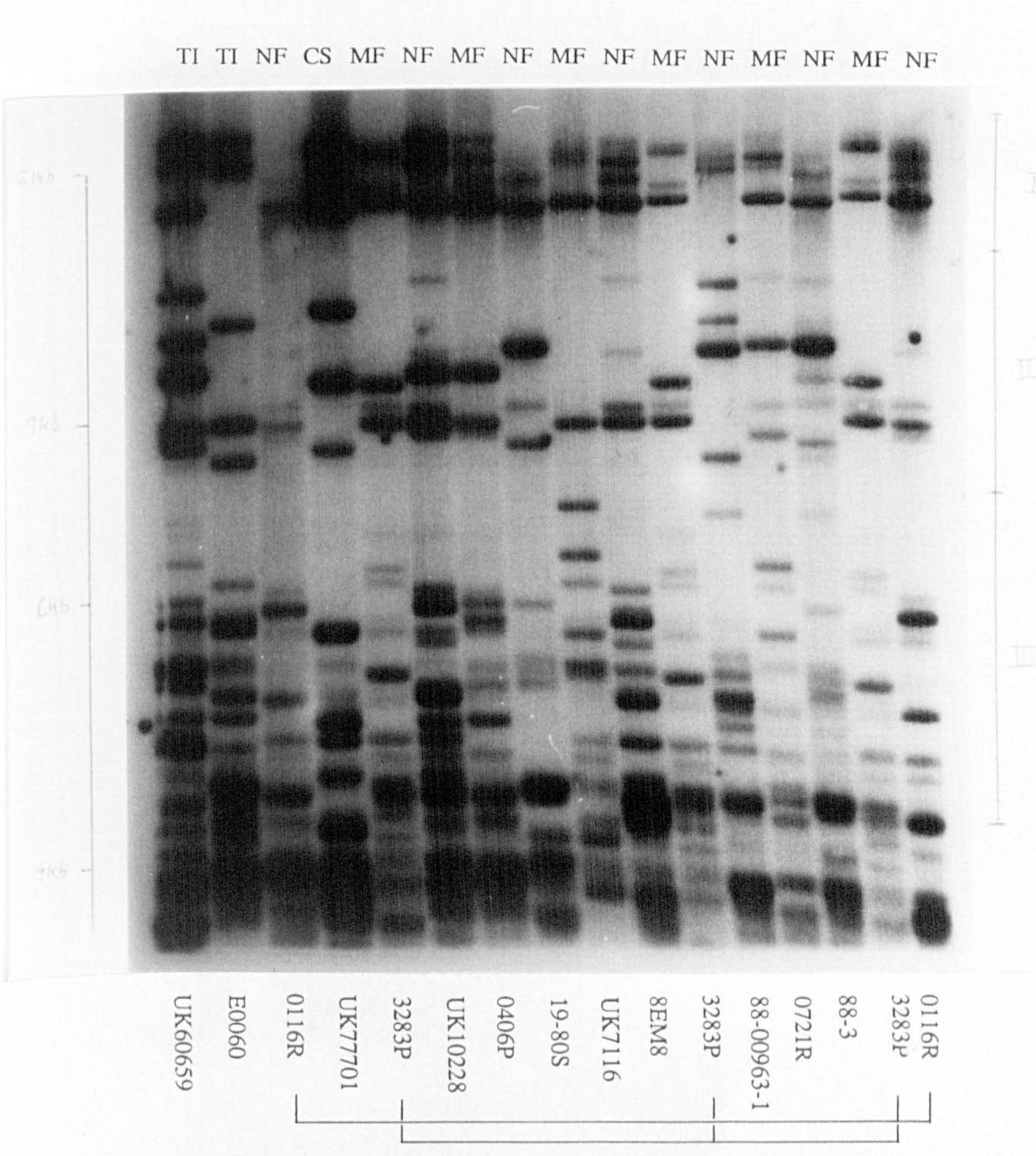


FIGURE 5.1: DNA fingerprint produced by hybridisation of 13 individual Merlin samples with probe pSPT19.6. The identity numbers of the individuals sampled are shown at the bottom of the figure and the lines below them connect samples from the same individuals present more than once on the fingerprint. To the right of the figure, the three regions into which the bands were grouped are indicated (see text for details). The letters at the top of the figure show which breeder supplied the samples; NF = Dr. Nick Fox, MF = Mr. Mike Fountain, CS = Mrs. Carol Scott, and TI = Mr Terry I'Anson.

Appendix 7 for raw data). A comparison of this graph with those obtained for Rothschild's mynah (Chapter 3, Figure 3.4) and Rodrigues fruit bat (Chapter 4, Figure 4.3) shows that there is far more allelic variation in the minisatellites detected in the Merlins than in those detected in the representatives of the two captive zoo stocks. Mean D for comparisons between these Merlins is 0.270 (σ = 167), compared with 0.592 (σ = 0.144) for the mynahs, and 0.772 (σ = 0.127) for the bats. These differences could be due to the fact that the level of inbreeding in the captive Merlin stock is lower than that in the mynahs and the bats, and that the specimens in the captive stocks of the latter two species have spent a greater number of generations in captivity.

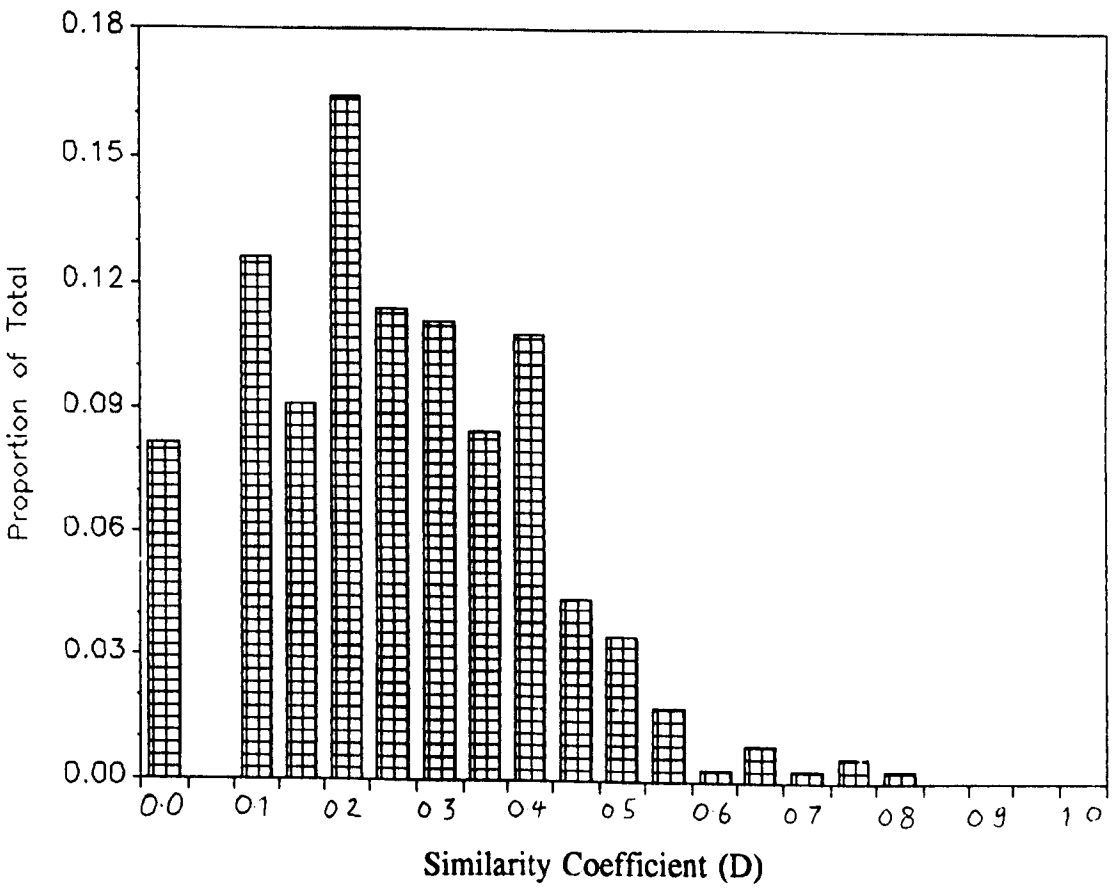


FIGURE 5.2: Graph showing the distribution of all the similarity coefficients (D) calculated for comparisons between 34 Merlins from four individual captive stocks (see Appendix 7).

5.3.2 Estimating Relatedness.

Information regarding the relationships between the Merlins within each breeder's stock were, for the majority of individuals, unavailable and the relationships between members of different stocks, non-existent. However,

samples from a family group of five birds was provided by Mr. T. I'Anson (individuals 0721P, E0060, 1277P, 1887P and 4134R), where the parents (0721P and E0060) were believed to be unrelated, allowing comparisons to be made between 1st-degree relatives. The mean similarity coefficient produced by comparisons between each parent and their offspring, and between the siblings, was 0.608 (σ = 0.139), more than twice the mean D calculated between all the Merlins in this study (D between the "unrelated" parents was 0.250).

If one assumes that the individuals within one of the four separate stocks from which samples were supplied are unlikely to be related to any of the members within another stock, an estimate of the background level of band sharing can be calculated. By taking a mean of only those similarity coefficients (D) calculated between individuals from different private stocks, it is possible to calculate a mean D for each pairwise group comparison. The results of this analysis are shown in Table 5.2. The approximate proportion of background band sharing between the Merlins tested in this analysis is therefore the mean of the results in Table 5.2, i.e. 0.25. This figure should be treated with caution because the individuals within each breeding group are probably related to different degrees, and the frequencies of the bands within each captive stock will be biased by the relationships within those particular stocks. However, if this caveat is borne in mind, this result indicates that the background level of band sharing is low in this species (compared to the other captive stocks considered herein) and in addition, 0.25 is most probably an overestimate of the background level of band sharing, so that two unrelated wild Merlins would be expected to share less than one quarter of their minisatellite alleles.

TABLE 5.2: Similarity coefficients (D) calculated for comparisons between individuals from different captive stocks. The letters MF, TI, NF and CS are the initials of the private breeders that own the stocks in question (see Section 5.2.2 for breeders full names). N is the number of comparisons between the members of the two stocks in question and not the number of individuals within either stock.

BREEDER	CS	NF	TI
MF	0.310 (σ = 0.118) N = 30	0.202 (σ = 0.139) N = 59	0.264 (σ = 0.121) N = 55
TI	0.272 (σ = 0.117) N = 20	0.205 (σ = 0.122) N = 62	
NF	0.250 (σ = 0.140) N = 33		

5.3.3 Comparison of Results from Probes pSPT19.6 and pSPT18.15.

As with the analysis of Rothschild's mynahs (see Chapter 3, Section 3.3.7), it proved possible towards the end of this study to probe some of the Merlin samples with the polycore probe pSPT18.15. The samples in question were bound to a filter that had already been probed with pSPT19.6 and the results for both probes are shown in Figure 5.3. As can be seen, the DNA fingerprints appear similar and a number of bands can be seen to have been hybridised by both probes (the same precautions were taken with the Merlins as with the mynahs, see Chapter 3, Section 3.3.7).

A regression analysis performed on the two sets of similarity coefficient data obtained (arbitrarily taking the data for pSPT19.6 as the dependent variable) produced a highly significant t-value of 12.517 ($p<0.001$, 90 d.o.f) (see Figure 5.4). Although the result of the regression analysis is more satisfactory than that obtained for the equivalent analysis in the mynahs, the similar fingerprint patterns cast doubt upon the independence of the data from each probe. Further examination of the two autoradiographs concerned reveals that a large proportion of the minisatellite alleles hybridised by pSPT19.6 are also hybridised by pSPT18.15 (see Figure 5.3). Indeed, the mean proportion of bands detected using pSPT19.6 per individual that are apparently also hybridised by pSPT18.15 is 0.632 ($\sigma = 0.161$) (see Table 5.3). As with the mynahs (see Chapter 3, Section 3.3.7), these bands could be analogous or they could be different alleles from different loci. However, given these results it would probably not be advisable to use data pooled from both of these probes for future analyses of the Merlins. A better alternative would be to use other multilocus probes or specially developed single locus probes, such as the one reported by Longmire et al. (1988).

5.4 DISCUSSION

The use of organochlorine insecticides for agriculture during the middle of this century caused a dramatic decline in the size of a number of native raptor populations. Fortunately, after the introduction of legislation to curb the use of these chemicals, many of the raptor stocks increased their numbers, in some cases to almost pre-2nd world war levels (Newton, Robson & Yalden, 1981). However, the Merlin population has remained low and so legislation has been enacted to ensure the protection of the remaining individuals (Cooper, 1986). Unfortunately, it has proved very difficult to prosecute individuals for taking Merlins from the wild as the burden of proof lies with the authorities not the breeder, and it is they who have to prove that a particular specimen has not been captively bred (K. Bradbury, RSPB, pers., comm., 1992).

This study has shown that Merlin minisatellite alleles detected by probe pPST19.6 are sufficiently variable to individually identify a specimen from its DNA fingerprint, and it is possible that future registration schemes will include this technique. Legitimate breeders will be safeguarded as an individual reared from a pair thus registered can easily be proved to be their offspring. Additionally,

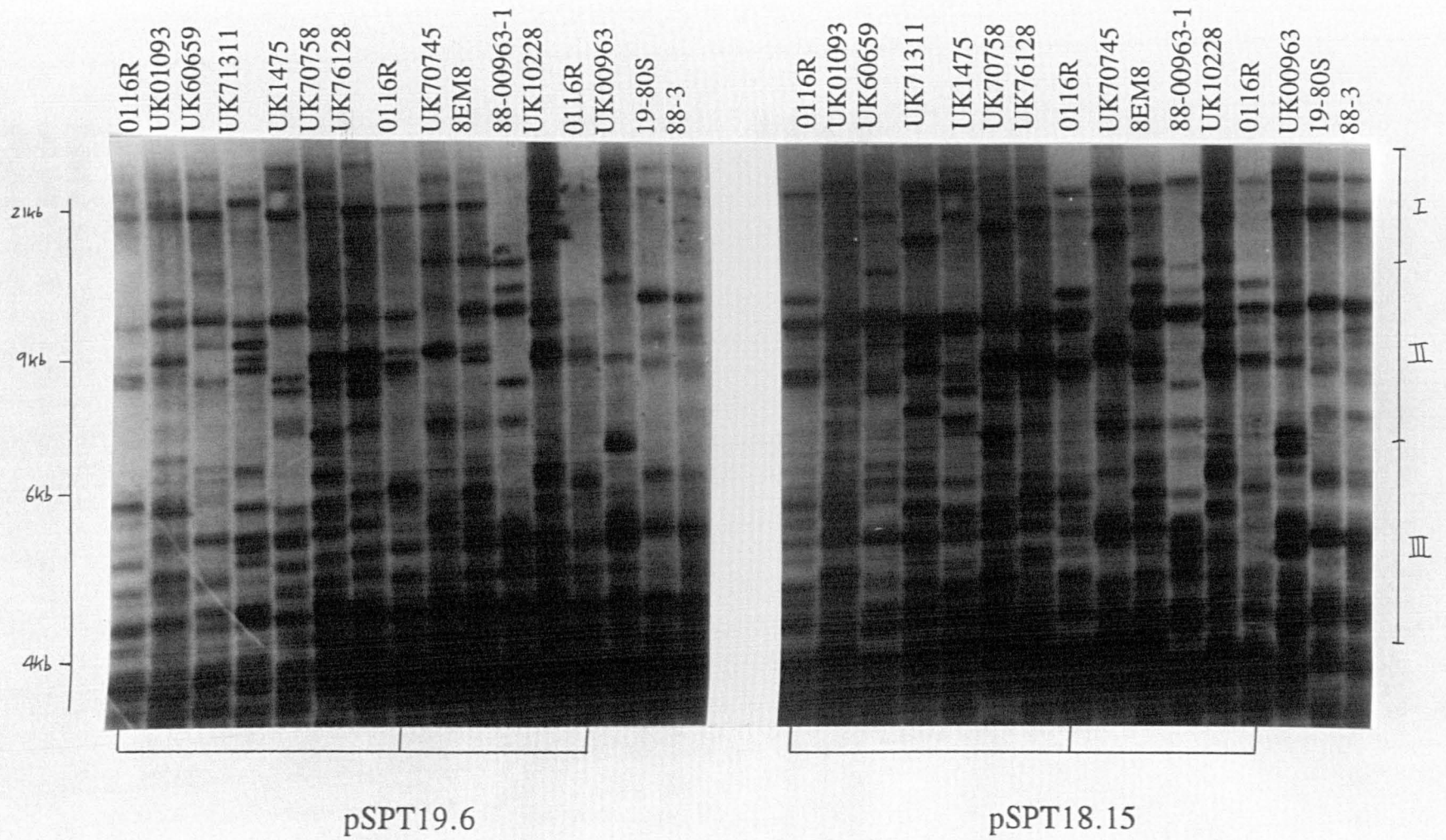


FIGURE 5.3: DNA fingerprints produced by hybridising the same filter with pSPT19.6 and pSPT18.15. The identification numbers of the individuals sampled are given at the top of the figure and the lines at the bottom connect samples from the same individual present more than once on the gel. The regions across which bands were scored are indicated to the right of the figure.

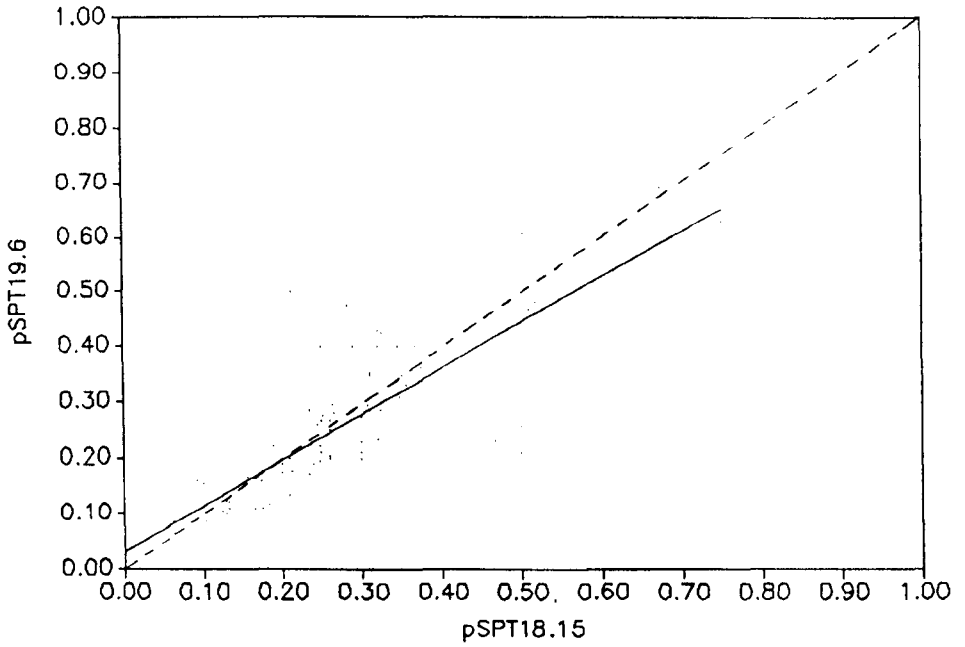


FIGURE 5.4: Graph showing the results of plotting similarity coefficients (D) calculated between individuals using bands detected by the two probes pSPT19.6 and pSPT18.15. The solid line is the regression line and the dashed line follows a plot of $D_{pSPT19.6} = D_{pSPT18.15}$

PROBE	pSPT19.6			pSPT18.15		
MERLIN \ REGION	I	II	III	I	II	III
88-3	2 (1)	7 (4)	7 (5)	2	5	8
19-80S	3 (1)	3 (2)	4 (2)	2	3	5
UK00963	3 (0)	2 (1)	6 (3)	3	4	8
0116R	3 (0)	7 (2)	4 (3)	2	5	7
UK10228	2 (1)	6 (4)	11 (7)	2	7	10
88-00963-1	2 (1)	4 (4)	9 (6)	1	4	8
8EM8	4 (2)	4 (4)	10 (7)	2	6	9
UK70745	2 (2)	2 (1)	10 (4)	3	2	8
UK76128	2 (1)	5 (5)	9 (6)	2	5	9
UK70758	3 (1)	6 (5)	10 (9)	2	6	11
UK1475	3 (2)	3 (3)	8 (6)	3	5	11
UK71311	2 (1)	4 (4)	11 (9)	3	5	11
UK60659	2 (1)	4 (3)	7 (6)	1	6	8
UK01093	5 (1)	6 (2)	5 (5)	2	4	9

TABLE 5.3: Comparison of the number of bands scored using probes pSPT19.6 and pSPT18.15, in each region of the DNA fingerprint for the individual Merlin samples shown in Figure 5.3. Numbers in parentheses in the column headed "pSPT19.6" are the numbers of bands that are hybridised by both probes.

in the event of a bird being stolen, a sample of blood from a specimen suspected to be the one taken will be all that is required to perform a DNA fingerprint analysis that will conclusively prove or refute an accusation.

During this study, a possible opportunity to put this legal aspect of DNA fingerprinting into practice arose when one of Mr. T. I'Anson's Merlins was stolen (individual 1736R). This bird has been DNA fingerprinted as part of this study (data not shown), and if another Merlin can be found that is suspected to be the missing bird, it should be possible to prove whether the two samples came from the same individual. At the time of writing, no candidate for the missing individual has been found and this crime remains unsolved.

CHAPTER 6

THE NEW ZEALAND FALCON
(*Falco novaeseelandiae* Gmelin, 1788)

6.1 INTRODUCTION

6.1.1 General Introduction

Falco novaeseelandiae is a comparatively rare member of the Falconidae, endemic to the islands of New Zealand and Auckland Island to the south, and is held in captivity by only a few breeders. There are three recognised forms: the "Bush", the "Eastern" and the "Southern" falcon; the existence of at least two of these forms is under threat (Fox, 1978). A decline in the New Zealand falcon population has been in evidence since the 1950s and 1960s, necessitating the development of a breeding programme for this species. The aim of this programme, run by Dr. N. Fox, is to build up a genetic reservoir, pending reintroduction of the bird to the wild. DNA fingerprinting of Dr. Fox's captive stock of New Zealand falcons was carried out in order to ensure the accuracy of breeding records and heritability studies.

6.1.2 The Islands of New Zealand

New Zealand comprises a number of islands, the two largest being North Island and South Island, which lie 34°- 42° and 40°- 48° south of the equator, respectively (Willett et al., 1986). These islands separated from Gondwanaland about 50 million years ago, isolating much of the island's flora and fauna from the continental species in Asia (Campbell & Lack, 1985). The islands cover an area of approximately 268,676km^2 and have a human population of around 3 million; one of the lowest population densities of any developed country (M^{c}Whirter, 1982).

6.1.3 Taxonomy

KINGDOM	- Animalia
PHYLUM	- Chordata
SUBPHYLUM	- Vertebrata
CLASS	- Aves
ORDER	- Falconiformes
SUBORDER	- Falcones
FAMILY	- Falconidae
SUBFAMILY	- Falconinae
GENUS	- Falco
SPECIES	- novaeseelandiae

(See Chapter 5, Section 5.1.2 for an account of *Falco*'s taxonomic background.)

Since the genus *Falco* is thought to have evolved around 25 million years ago (Grossman & Hamlett, 1965), the species *novaeseelandiae* must have evolved from a colonist that reached (flew to) New Zealand sometime after the Miocene Epoch (Late Tertiary Period), when the islands became isolated (see Section 3.1.2). Fleming (1962) has proposed that this event took place sometime during the Pleistocene Epoch, between 1 million and 15,000 years ago.

The scientific classification of the New Zealand falcon is attributed to Gmelin (1788), but he is thought to have based his description of the bird on that originally presented by Latham in 1781 (Fox, 1988). Until about 30 years ago, the New Zealand falcon was considered to be a primitive raptor displaying several characteristics suggesting an early deviation from the true falcons, and was placed in a separate genus, *Nesierax* (Grzimek, 1972). However, the lack of a comprehensive fossil record has meant that the lineages are difficult to follow (Fleming, 1962). It should be pointed out that there has been a great deal of confusion regarding the exact taxonomic status of this species, and since it was formally described in 1788, at least 23 different names have been used to classify the species now known as *Falco novaeseelandiae* (Fox, 1988).

6.1.4 General Biology and Behaviour

Adult New Zealand falcons are 33cm to 41cm in length with the females being larger than the males (Grossman & Hamlett, 1965). The species exhibits some sexual dimorphism, but this is not pronounced. Males have a blue-black head, neck and back, with barred rufous upper wings and tail coverts; the throat and breast is white or cream, streaked vertically with dark brown stripes; the bill is black, and the legs are deep lemon yellow. The female's head and neck are less blue, and their legs are a paler yellow (Fox, 1988).

Fox (1977) recognises three forms of the falcon: the "Bush", the "Eastern", and the "Southern" falcon, but the taxonomic status of these forms has yet to be decided. The "Bush" falcon, which is found distributed throughout North Island and the northwestern regions of South Island, tends to be smaller and darker than the other forms. The "Eastern" falcon is distributed across the eastern half of South Island and is a larger, paler type. The "Southern" falcon is found only in the southern half of South Island and on Auckland Island (to the south of South Island), and is intermediate in colour and size to the other two (Fox, 1977).

Paired New Zealand falcons occupy their territory all year round and roost in holes or small caves in cliffs where they also occasionally nest, however, they more usually nest in trees (Grzimek, 1972; Fox, 1977). In the wild, the bulk of their diet consists of other birds, e.g. finches, starlings, and songthrushes, with the "Southern" falcon also taking shorebirds. In captivity, their diet of avians is supplemented with hares, mice and rats (Fox, 1979).

The female New Zealand falcon lays 2-4 eggs, each of which is a rich brown colour with dark red blotched markings (Soper, 1965). Once hatched, the young falcons gain full adult plumage in about 16 months (Fox, 1988).

6.1.5 Decline of the New Zealand Falcon

The first review of the numbers of New Zealand falcons present in the wild was produced by Fox in 1978. He estimated that there was a breeding population of at least 2000 pairs, with a possible maximum of 4500 pairs. These pairs were distributed between the three forms of the falcon as shown in Table 6.1.

TABLE 6.1: Estimates of the number of New Zealand falcon pairs in the wild.

Form of falcon	Number of pairs
"Bush"	450-850
"Eastern"	3100-3200
"Southern"	140-280

Dr. Fox's estimates of the sizes of the falcon subpopulations were derived from information which included data gathered from interviewing members of the indigenous population with regard to their recollections of falcon numbers over time. He was frequently told that the falcons appeared to have decreased in numbers, but he suggests that the reliability of some of this information could have been affected by subjective changes in the perception of those interviewed, i.e. the age of the interviewees (Fox, 1978). In fact, the breeding range and numbers of the falcons *have* decreased in the past 3-4 decades, due to deforestation and the changes in land use (conversion to pastureland) that have occurred. In addition, the falcon population in certain places has declined due to the use of organochlorine insecticides (Fox, 1978).

6.1.6 Conservation Measures Taken

All the raptors in New Zealand are protected by law in a similar way to the British Merlin (see Chapter 5, Section 5.1.5), so that the removal of adults, chicks or young from the wild, and the disturbance of nest sites is illegal (Hilton, 1977). In addition, most of the "Southern" falcons live within the Fiordland National Park (southwest, Southern Island), and are thus protected by wardens (Fox, 1978). In 1984, a captive breeding programme was started by Dr. N. Fox with a view to gaining a better understanding of the breeding biology of the falcons in preparation for a reintroduction programme, should such be required (see Section 6.1.8).

6.1.7 Current Status

The "Bush" form of *F. novaeseelandiae* is the most vulnerable to extinction (Fox, 1978). This is due to the continued destruction of New Zealand's forests and their replacement with pastureland for sheep grazing which has deprived the "Bush" falcon of its favoured nesting sites and prey species. In contrast, the "Eastern" falcon appears relatively secure, since it has adapted well to the new

forms of land use and now feeds on some of the introduced species that have colonised this new habitat (Fox, 1978). The "Southern" falcon, whose subpopulation numbers only about 500 individuals, remains at the greatest risk from residual pesticides in the marine environment, most especially around the coasts of Auckland Island (Fox, 1978).

6.1.8 Aims of this Study

As detailed in Section 6.1.6, it is thought that the subpopulations of two of the forms of the New Zealand falcon are vulnerable (the "Bush" and the "Southern"), but it could be some time before a reintroduction policy is needed. This means that the falcons could be in captivity for a number of generations. As with threatened species bred in zoos (see Chapter 1, Section 1.3.3), prolonged periods in captivity can result in changes in the species' genetic composition and alterations in natural behaviour patterns which can be detrimental in the wild (Berry, 1971). Concern has been expressed over the genetic structure of populations composed partly or wholly of birds which have been bred in captivity and reintroduced (Cooper, 1977), and, to reduce the impact of these problems on the captive stock of New Zealand falcons, birds are regularly exchanged with wild individuals (N. Fox, pers. comm., 1990).

The aim of this study was to determine whether sufficient variation existed in the minisatellites of New Zealand falcons to allow them to be individually identified and to allow the level of relatedness between individuals to be deduced from calculations of similarity coefficients (D). The relationships of the wild individuals to the captive birds, if any, is not known and so DNA fingerprinting would hopefully allow wild-caught birds could be paired to the best advantage with birds in the stock.

Also, during this breeding programme, Dr. Fox has conducted extensive heritability studies on the falcons and for these data to be of greatest value, it is important that the captive stock's breeding records are accurate. DNA fingerprinting should allow parental misassignment to be detected, if sufficient minisatellite variation exists, and thus the pairing of related individuals avoided. In addition, like the Merlins (see Chapter 5, Section 5.1.8), DNA fingerprint information could be used as the basis of a registration scheme, should the relevant legislative bodies decide that this measure was necessary to protect the New Zealand falcon, and could assist with ecological and behavioural studies in the wild (for example, see Wetton et al., 1987).

6.2 MATERIALS AND METHODS

6.2.1 General Introduction

The information presented in this section outlines the alterations which were made to the materials and methods presented in Chapter 2 in order to produce a DNA fingerprint for New Zealand falcons. Also presented here are

relevant comments with the numbers of the specific sections in Chapter 2 to which these amendments and comments apply given in parentheses.

6.2.2 Comments on Tissue (2.1.1)

All the blood samples supplied for this study are of the "Eastern" form of the falcon and were kindly donated by Dr. N. Fox to whom I am most grateful. Samples for each individual arrived at the laboratory in two 1.5ml eppendorf tubes. One tube contained blood suspended in 0.5ml EDTA and the other untreated whole blood. The tubes had been sent by arrangement to Dr. Fox prior to sampling with instructions to "place one drop of blood" in each of the EDTA treated tubes and the rest of the blood in the empty tube. Unfortunately, a "drop" is an inexact quantity, and almost all of the samples contained too much blood to use directly in the standard extraction procedure (2.1.1.2 and 2.1.1.3). The samples therefore had to be split into a number of aliquots (from two to four) each of which could then be made up to 0.5ml with the appropriate amount of TE buffer. All these samples were extracted in the normal way with the precipitated pellets of DNA being resuspended together in one tube. The whole blood was stored at -80°C until it was required.

6.2.3 Electrophoresis and Probing (2.1.3 & 2.1.5)

The *HaeIII* restricted DNA samples extracted from the New Zealand falcon blood were electrophoresed for an average of 1500Vh, after which minisatellites of less than 2,000 base pairs (bp) in length had migrated off the end of the gel. Both the probes pSPT19.6 and pSPT18.15 were used to detect the minisatellites present on filters produced by blotting these gels, with the former probe being used more extensively. Probe pSPT18.15 hybridised to more heavy molecular weight minisatellites than did probe pSPT19.6, the latter probe not detecting any minisatellites greater than approximately 20,000bp in length. However, both produced good results, with the minisatellites appearing well distributed along the length of the fingerprint (see Section 6.3).

6.3 RESULTS AND CONCLUSIONS

6.3.1 Initial Analysis

The mean number of pSPT19.6-detected bands scored per individual New Zealand falcon in this study was 13.5 (σ = 2.18) for males, and 14.8 (σ = 2.21), for females; the mean for all individuals, weighted for sex is 14.2 (σ = 2.24). Although these results are not significantly different, it would appear that females have an average of one extra band in their DNA fingerprints compared to males. This could be due to the presence of an allele from a minisatellite locus located on the Z-chromosome, the female being the hemizygous sex in birds. However, this evidence of a possible sex-linked minisatellite is poor as even though large families were available for analysis, it was not possible to find any bands present in an adult female that were exclusively passed to her female offspring (data not

shown).

A total of 53 New Zealand falcons were DNA fingerprinted and the distribution of the similarity coefficients (D) produced by comparisons between them is presented in Figure 6.1.

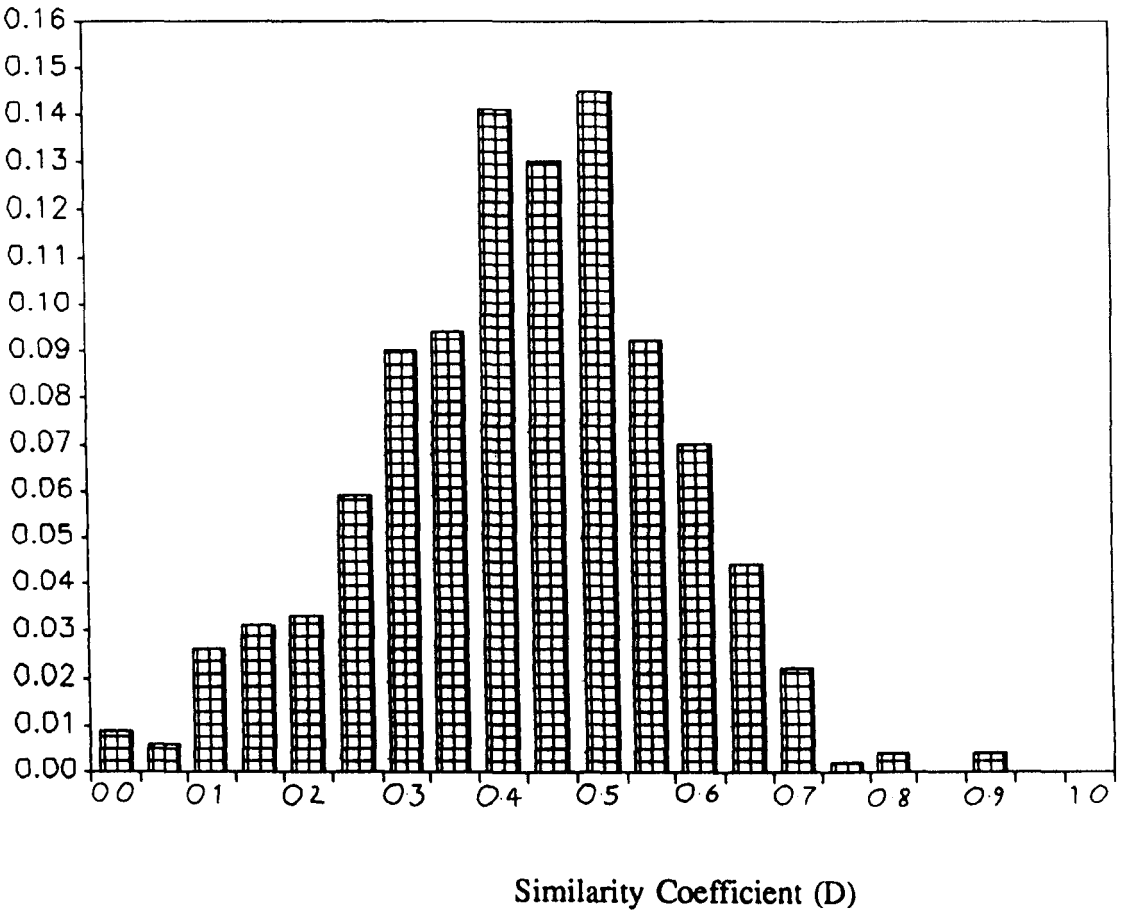


FIGURE 6.1: Graph showing the distribution of all New Zealand falcon similarity coefficients (D) generated in this study. Multilocus probe pSPT19.6 used.

If the graph shown in Figure 6.1 is compared with those generated for the other species analyzed in this volume, namely Rothschild's mynah, the Rodrigues fruit bat and the British Merlin (Figures 3.4, 4.3 and 5.2, respectively), it can be seen that all the distributions are unimodal. This appears to be a common factor for the captive stocks tested here, and this merging of the similarity coefficient distributions that could be expected for defined degrees of relatedness, is probably due to increased numbers of matings between related individuals. The reasoning for this is as follows; the proportion of genes shared between 1st-degree relatives in an ideal population would be approximately 50%; that for 2nd-degree relatives approximately 25%; that for 3rd-degree, 12.5%, and so on. The degree of relationship allocated to a male individual (A) and one of his siblings' offspring

is "2nd-degree relatives", which in an ideal population would mean that they shared approximately 25% of their genes. However, if the mother of this offspring was herself a 2nd-degree relative (specifically a granddaughter) of individual A (not an uncommon occurrence in some captive stocks (see Chapter 3, Figure 3.7)), individual A would be expected to share approximately 37.5% of his genes (25% + 12.5%) with this offspring (all the individuals not specifically referred to in this example e.g. individual A's mate, the offspring's sire, etc., are assumed to be unrelated, a rare event in captive stocks). The effect of a number of generations of this inbreeding would be to produce many individuals with "intermediate" expected proportions of genes shared (see Appendix 1 for a demonstration of this effect in Rothschild's mynahs) and this would merge any previously discreet distributions in the similarity coefficients (D) calculated between individuals of known relationship.

6.3.2 Relationship Allocation

Dr. N. Fox who supplied the New Zealand falcon samples, has maintained extensive records of the relationships of the individuals within the captive stock, and so it has been possible to construct a family tree for this species (see Figure 6.2). DNA fingerprinting was used to assess the accuracy of these breeding records and it has been found that the minisatellite bands detected in every offspring analyzed can be allocated to at least one of that individual's parents (see for example Figure 6.3). It has thus been possible to confirm the accuracy of Dr. Fox's records.

As can be seen from Figure 6.2, there are a number of birds with the letters "UK" before their identity numbers. This indicates that these individuals were caught in the wild, and then imported to this country and registered with the British authorities (N. Fox, pers., comm., 1989). Unfortunately, because of the fact that these birds were previously wild and the fact that such specimens tend to be caught in an opportunistic way, the relationships between these birds are not known. Given the accuracy of Dr. Fox's records and the assumption that the founders of the captive stock (all those individuals with "UK" before their numbers) are unrelated, the expected proportion of genes shared between those individuals for which similarity coefficient (D) data were available was calculated (see Appendix 9 for raw data), and a graph of the two quantities plotted (see Figure 6.4). A regression analysis was performed on these data (*cf.* Rothschild's mynah, Chapter 3, Section 3.3.2), and this produced a highly significant t-value of 14.834 ($p < 0.001$, 542 d.o.f.). This appears to confirm the accuracy of the breeding records for this species and the hypothesis that the founders are indeed unrelated.

Of the New Zealand falcons sampled for this study, five had been acquired directly from the wild. Comparisons between the DNA fingerprints of these individuals should allow the approximate background level of band sharing in the natural population to be determined. The mean similarity coefficient (D) calculated between these individuals was 0.284 ($\sigma = 0.149$). The other members

NEW ZEALAND FALCON FAMILY TREE

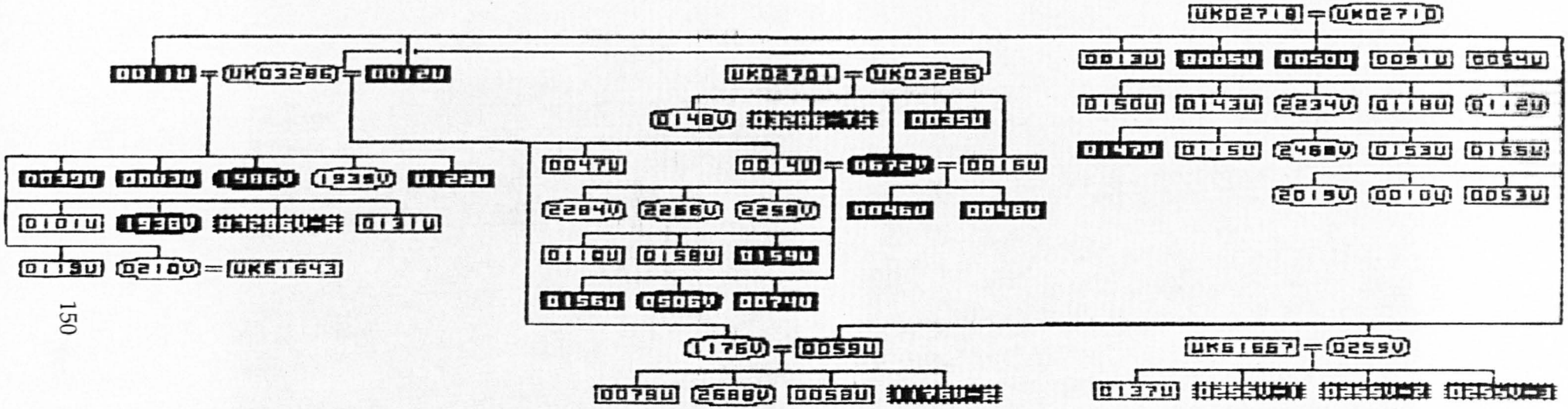


FIGURE 6.2: Family tree for the 1990 captive stock of New Zealand falcons held by Dr. N. Fox.

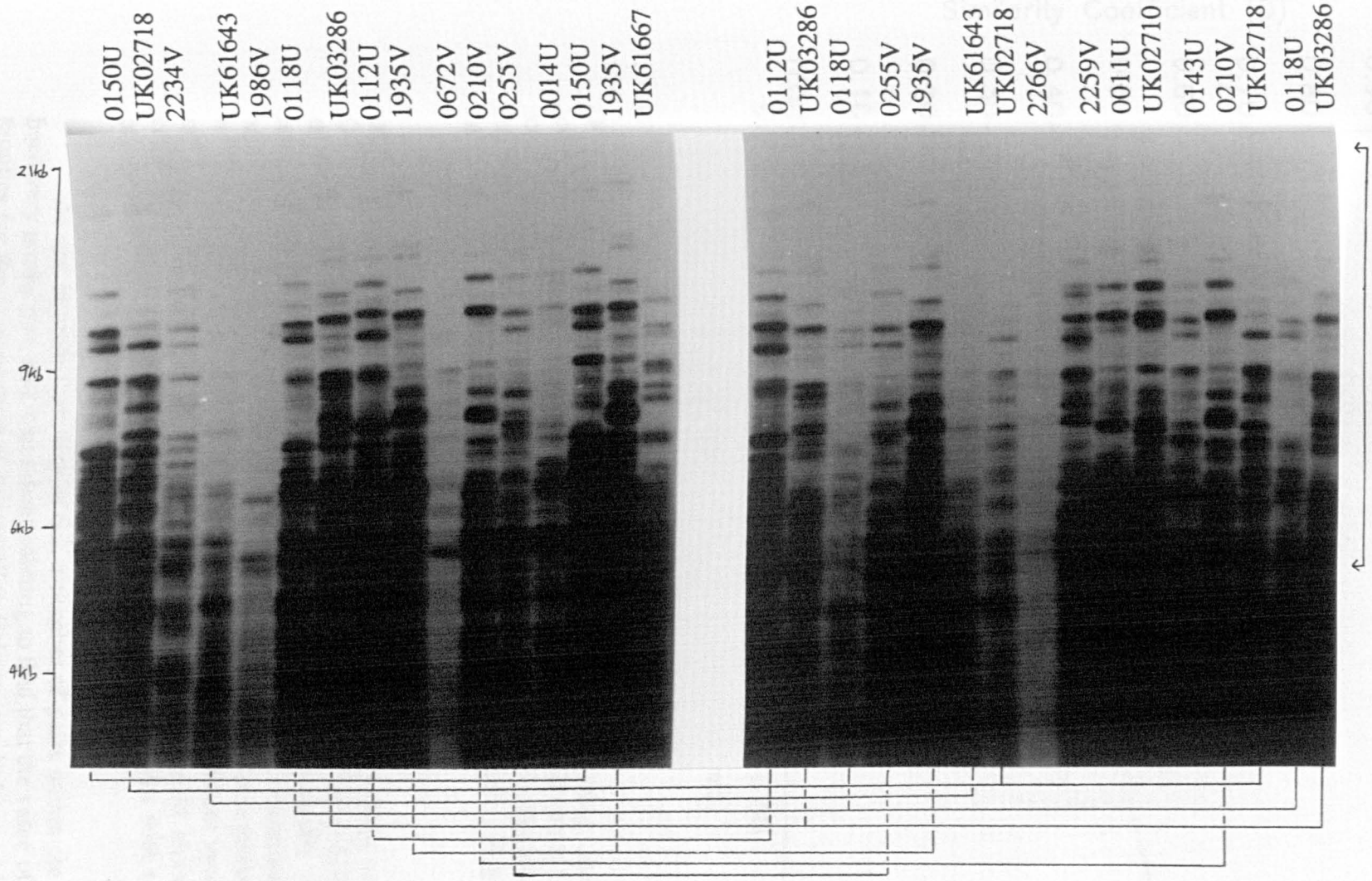


FIGURE 6.3: DNA fingerprints produced using pSPT19.6 to probe samples from 14 individual New Zealand falcons run in different combinations on two gels. The lines at the bottom of the figure connect samples from the same individual present on both gels. The relationships of the individuals tested can be seen from the family tree in Figure 6.2. Arrows to the right of the figure mark the region of the fingerprint across which bands were scored.

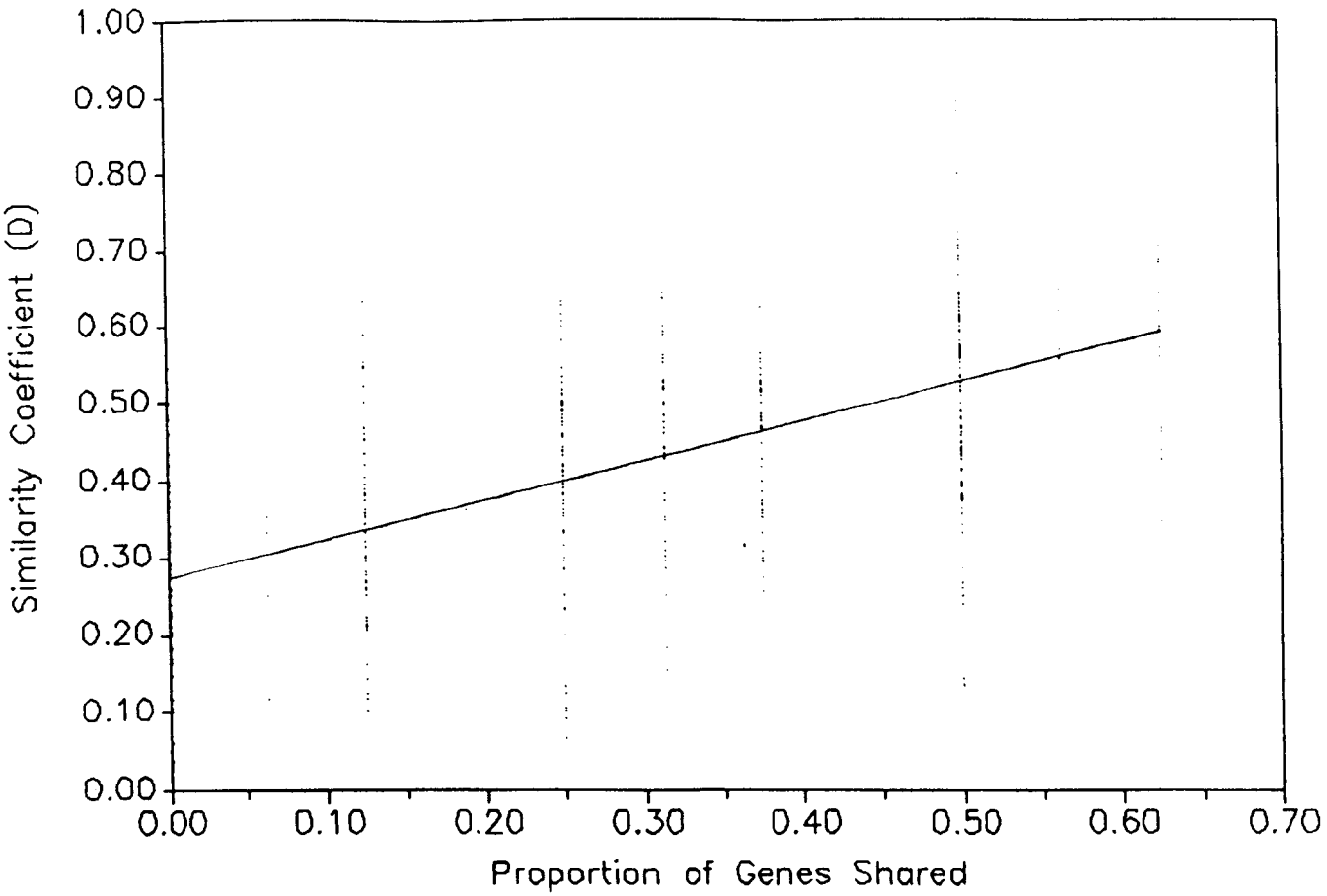


FIGURE 6.4: Graph showing the results of plotting the similarity coefficient data (D) obtained from comparisons between members of Dr. N. Fox's captive stock of New Zealand falcons against the expected proportion of genes shared between them, given that the founders are unrelated. Regression line shown.

of the stock are all descended from one or more of these importees, and the mean D between known 1st-degree relatives is 0.507 ($\sigma = 0.137$). These figures indicate that, like the Merlins (see Chapter 5, Section 5.3), there appears to be ample minisatellite variation in the captive stock for individual characterisation. In addition, if the results from the wild specimens can be taken as a guide (given that *n* for the mean presented is only nine), there should be sufficient variation in the natural population to allow the stock manager to select wild birds that share considerably less than one quarter of their minisatellite alleles with any member of the captive stock.

As can be seen from Figure 6.2, a number of pairs within the stock have been very productive, and it was disappointing to find that the value of these large families for the investigation of minisatellite linkage, sex-linkage and allelism in the species, was rendered worthless by the fact that none of the aforementioned

phenomena could be detected, possibly due to the idiosyncrasies of the species or possibly due to chance.

6.3.3 Comparison of the Results Obtained Using pSPT19.6 and pSPT18.15

Figure 6.5 shows the results of probing the filters shown in Figure 6.3 with the probe pSPT18.15. As can be seen, the latter probe hybridises to considerably more minisatellites than the former, over the region of the DNA fingerprint that was scored, and detects larger alleles. Table 6.2 lists the numbers of bands scored in each individual by hybridisation of the filters shown in Figure 6.3 with either pSPT19.6 or pSPT18.15, and also lists the number of bands which appear to be hybridised by both probes.

TABLE 6.2: Comparison of the number of bands scored using probes pSPT19.6 and pSPT18.15 for the individual New Zealand falcon samples shown in Figures 6.3 and 6.5. Numbers in parentheses in the column headed "pSPT19.6" are the numbers of bands that appear to be hybridised by both probes.

FALCON \ PROBE	pSPT19.6	pSPT18.15
UK02710	16 (12)	25
UK02718	12 (11)	23
UK03286	14 (9)	30
UK61667	9 (9)	20
0011U	13 (7)	26
0014U	10 (10)	21
0112U	13 (12)	25
0118U	10 (9)	35
0143U	11 (8)	20
0150U	15 (10)	27
0210V	16 (14)	31
0255V	10 (10)	25
1935V	16 (13)	32
2234V	10 (8)	24

A comparison between this table and the equivalent produced for the British Merlins (see Chapter 5, Table 5.3), reveals that the results from the two species are similar in that almost all the bands detected by probe pSPT19.6 are also

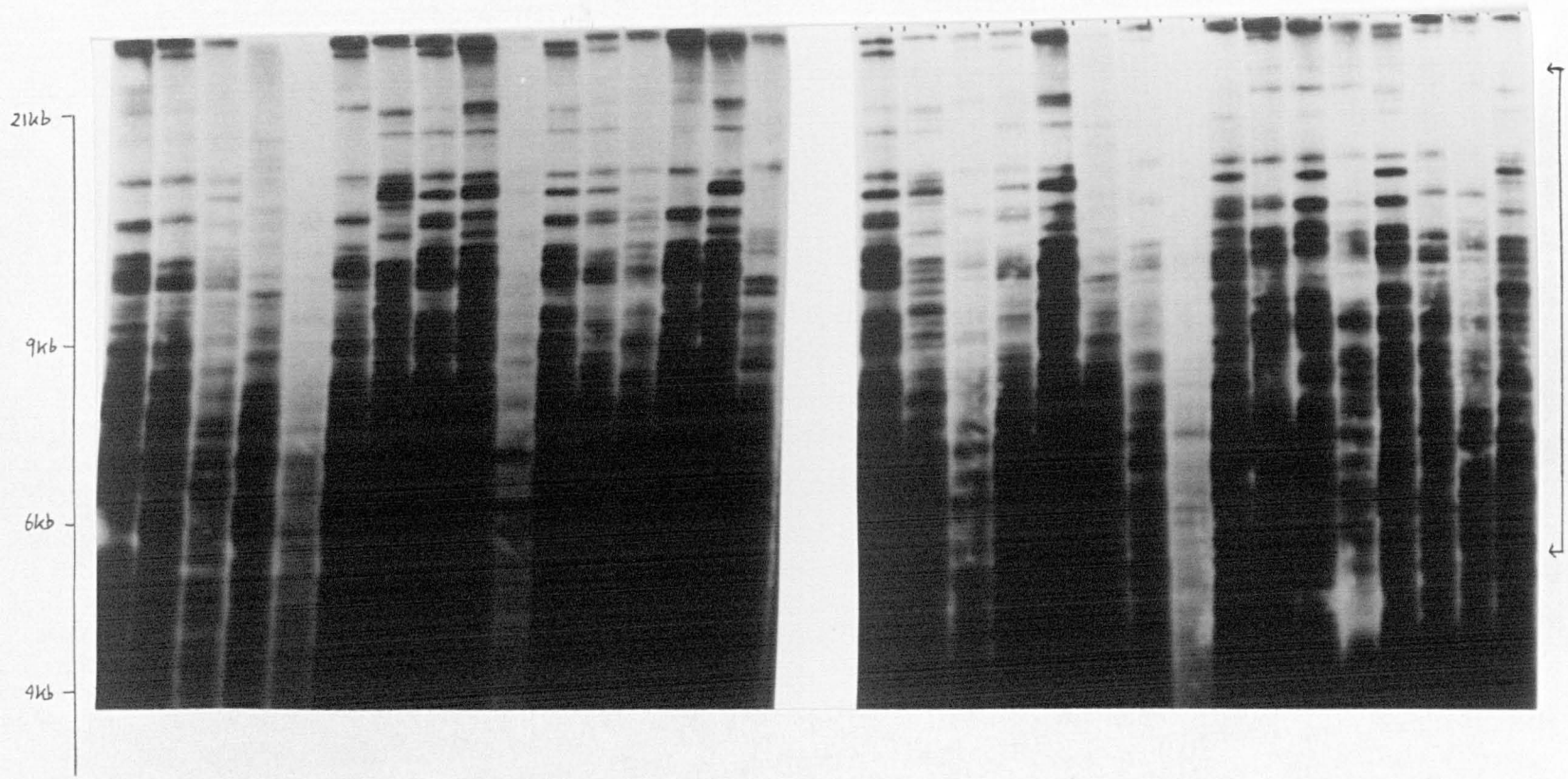


FIGURE 6.5: DNA fingerprints produced using pSPT18.15 to probe the filters shown hybridised with pSPT19.6 in Figure 6.3. See latter figure for details of which samples are from the same individuals. Arrows to the right of the figure mark the region of the fingerprint across which bands were scored.

hybridised by pSPT18.15. Since both these species belong to the genus *Falco*, it is possible that this phenomenon will be found in other members of this taxonomic category. If this is shown to be the case, researchers (if they do not have access to other oligonucleotides) may have to confine themselves to hybridisations using only one of the two human polycore probes used in this study.

However, it is interesting to note that whereas the two probes detect approximately the same numbers of bands in the Merlins, pSPT18.15 detects around twice as many as pSPT19.6 in the New Zealand falcons. Given that the two species probably started to diverge more the 25 million years ago (Grossman & Hamlett, 1965), it appears possible that although the specific sequence of the minisatellites present in both species has remained almost constant over time (as it has in many other species), the number of alleles at the minisatellite loci detected by probe pSPT18.15 in New Zealand falcons has increased with respect to those in the Merlins. It is possible that there is some form of selection pressure being exerted on the number and size of the minisatellite alleles in Merlins which is absent in the New Zealand falcons, but an explanation for the cause and reason for this is not immediately apparent.

6.4 DISCUSSION

It has been possible to confirm during this study that the records maintained for the captive stock of the New Zealand falcon are accurate, unlike those maintained for Rothschild's mynah (see Chapter 3, Section 3.3). Accurate records are the basis of an efficient breeding programme that maintains genetic variation within the stock by keeping the levels of inbreeding low. This is especially important if a captive propagation programme is to be effectively coupled to a reintroduction scheme. Although the New Zealand falcon is not yet in need of serious assistance, should such become necessary, there appears to be a strong base from which to select individuals for return to the wild in this captive stock.

Also in this study, it was possible to show that like the Merlins (see Chapter 5, Section 3.3), sufficient variation is exhibited in the minisatellite alleles of New Zealand falcons to allow individuals to be characterised and parentage confirmed. This means that if the authorities deem it necessary to safeguard the remaining members of the natural population, a registration scheme based upon DNA fingerprint analysis could effectively be established. However, the apparently large overlap between the bands detected by the two human polycore probes, further work should be carried out using only one of them, preferably 33.15 or derivatives thereof. Alternatively, specially developed multilocus and/or single locus probes should provide more useful data.

CHAPTER 7

CONCLUSIONS AND DISCUSSION

7.1 GENERAL SUMMARY

The rate at which species are being driven to extinction is still increasing and the overriding causes of this increase have been the direct and indirect actions of human beings, from hunting for food or "sport" to pollution of the environment and translocation of species. Although conservation of the environment has become a much more prominent issue in recent years, the destruction of the rainforests, continuing dumping of waste in rivers and oceans, and the persistent persecution of particular species, specifically some of the large vertebrates, e.g. whales, rhinos and elephants, demonstrates that the rate of species extinction seems unlikely to drop for many years. Given that these rates of extinction will *increase*, many more species are going to require human assistance if they are not to disappear.

There has been much discussion in the scientific literature (see references in Chapter 1, Section 1.3) regarding the merits of conserving species in reserves rather than in captivity. The former method has its advantages in that a species is thus maintained within the environment to which it is best adapted and so it is unlikely to develop deleterious morphological or behavioral traits which can occur under artificial conditions. In addition, by creating a reserve for a particular species, many other species will also benefit. However, it is sometimes necessary to concentrate efforts upon one particular species and this can only be accomplished by removing specimens from the wild and using modern technological methods (not available in the species' country of origin or not transportable to its habitat) to ensure that the species breeds successfully (Wildt et al., 1992).

When in captivity, a species may lose a greater or lesser proportion of its genetic variability due to factors such as random genetic drift and inbreeding caused by the (probably) small size of the stock. It is important that this be countered using, for example, Wright's "maximum avoidance of inbreeding" (Wright, 1921), especially if the species is to be reintroduced to its native habitat. In order to monitor the levels of variation within captive stocks a number of techniques have been developed, one of the most recent being DNA fingerprinting.

The discovery of minisatellites and the subsequent development of DNA fingerprinting has had a profound impact on a number of areas of biological research. In the field of forensic science it is now possible to identify the perpetrator of a crime (by comparing tissue samples found at the scene with those of suspected individuals) with a degree of accuracy that can be almost absolute. Also, the technique has proved so powerful when applied to the determination of paternity in humans that businesses have been established to exploit its potential.

Since the publication of the papers by Jeffreys, Wilson & Thein (1985a and b), DNA fingerprinting has been applied in a number of ways to problems relating to a great many species (see refs. in Chapter 1, Section 1.7.5). The technique has provided a method by which an individual of almost any species can be characterised, and the parents of that individual identified. These properties have made DNA fingerprinting an indispensable tool for research into breeding biology and its impact on the genetic structure of populations. It has in addition allowed certain evolutionary theories to be tested, for example determining the amount of effort parents put into raising offspring against the genetic benefits accrued in return, i.e. assessing the proportion and number of parent genes that are passed to the next generation. DNA fingerprinting can also be applied to practical problems associated with the conservation of species and this thesis specifically examined the ways in which the data generated by this technique can be used to assist in the management of captive populations. In this respect, it has proved extremely useful, providing data that would not have been available other than by using a battery of different techniques.

During this study, it has been possible to show that at least two of the founders of the British Rothschild's mynah captive stock were probably 1st-degree relatives. In addition, errors in the allocation of offspring to parents have been discovered which call into question the true breeding success of the pair that are shown in the stud book to be the most productive pairing in the country. It has also been possible to establish that the founder who made the greatest single contribution to the genetic variability currently present in the British captive stock, was probably closely related to the birds that were originally exported to establish a breeding programme in Hong Kong in the 1970s. This is important because birds from the Hong Kong breeding programme have been sent to Britain and are currently paired with a number of this particular founder's direct descendants. The inbreeding coefficients for the offspring of these pairings have been calculated (Fisher, unpublished) under the assumption that the birds from Hong Kong were unrelated to the birds with which they have been paired. As this appears not to be the case, it is possible that the inbreeding coefficients for their offspring are higher than those calculated and possibly higher than the maximum allowed by the British stock managers' pairing policy, i.e. 0.125.

Most interestingly, it has been possible during the study on the mynahs to show that there is a very significant positive relationship between similarity coefficients (D) calculated between adult individuals and the inbreeding coefficient (F) calculated for their (potential) offspring. Thus it may be possible to use D to determine which pairings should be avoided. As only half of the British stock were DNA fingerprinted in this study, it would be informative to analyze the other half. This would allow any other mismatches present in the stock to be detected and possibly allow the offspring in such cases to be assigned to the correct parents. It would then be possible to calculate accurate productivity (fertility) rates for the "parent" birds in the stock.

The analysis of the Rodrigues fruit bat captive stock was disappointing as it was shown that it would be difficult if not impossible to determine the parentage

of each individual currently held in captivity. This is due to the high degree to which those bands detected by probe pSPT19.6 were shared between individuals. The results were not unexpected as ten founders (three males and seven females) and their direct offspring are known to have been responsible for all the breeding that has taken place over the past 17 years. This situation is not improved by the fact that there does not seem to be an incest taboo in this species, so that "back-crossing" occurs frequently.

It is possible that further DNA analyses can be used to deduce the breeding structure of the bat stock, but only if either specifically developed single locus probes are used (in the hope that sufficient variation will be found at one or more loci to be of use in this respect), or the recently discovered technique of "internal mapping" is applied (Jeffreys, MacLeod et al., 1991) (see Section 7.2). Using this new technique, it may be possible to trace allelic haplotypes in the population and, given that the ringing system will at least allow the cohorts within the stock to be established, determine the patterns of inheritance. A family tree of some form could then be constructed and future studies should be able to identify both the mother and the father of an offspring.

Studies on the British Merlin revealed that of the four captive species presented in this volume, this stock exhibited the greatest minisatellite allele variation, and it would appear that a coordinated management programme would be able to conserve most of the variation present in those groups currently held in captivity. In addition, from a legal point of view, this relatively high level of minisatellite variation means that individuals can be identified from DNA fingerprints produced using only one multilocus probe, pSPT19.6. This could easily become the basis for a registration scheme that would allow individual Merlins which had been stolen to be identified, and the legal status of captively bred specimens, i.e. whether they are indeed offspring of a pair held by a breeder, confirmed. This would remove the possibilities of unnecessary prosecutions and would greatly facilitate prosecution of private breeders that had illegally taken Merlins from the wild.

The captive stock of New Zealand falcons held by Dr. N. Fox were shown to be correctly accounted for in the breeding records, and no discrepancies could be detected using DNA fingerprinting. Unfortunately, although large families were available, no linkage, sex-linkage or allelism could be detected. This analysis demonstrated that sufficient minisatellite variation remains within the captive stock to characterise individuals and confirm their parent/offspring allocations. In addition, it appears that the wild population of at least the "eastern" form of the New Zealand falcon exhibits ample minisatellite variation, with a number of wild caught individuals showing very little or no band sharing at all. It should thus be possible, assuming logistical difficulties can be overcome, to identify wild individuals that are not related to any members of the current captive stock, and which can thus be beneficially added to the breeding programme.

DNA fingerprinting is an expensive and complex technique whereas maintaining accurate breeding records is relatively cheap. The latter system

ensures that managers of captive stocks are aware of the genetic backgrounds of the individuals within the stock. Accurate records are one of the cornerstones of an effective captive stock management programme and such data allow the loss of variation to be minimised by identifying least related individuals who can then paired.

The technique of DNA fingerprinting can be used to determine which individuals are suitable for establishing a captive breeding programme. After the first breeding, DNA analysis could be used purely as an occasional check on the accuracy of the breeding records. If such a check was performed, for example once a year after the breeding season, problems such as the misallocation of offspring to parents would be readily and rapidly detected, and the chance of deleterious inbreeding could thus be minimised.

7.2 FURTHER RESEARCH

Multilocus DNA fingerprinting has proved to be an extremely powerful technique for determining close relationships, but it does have associated problems. By using a multilocus probe (MLP) which detects many different and dispersed loci, it is not possible to be certain that two bands migrating to the same position in the DNA fingerprints of two individuals are analogous. Neither is it possible to easily determine allelism and linkage which can affect calculations of band sharing. The more recent development of "single locus probes" (SLP) has allowed the allelic variation at a single minisatellite locus to be examined (see for example, Fowler et al., 1988). Wong et al., (1986) purified a single band from a multilocus fingerprint and found that it contained a 6,300 base pair (bp) minisatellite allele consisting of multiple copies of a 37-bp repeat unit, each of which contained the 11-bp core sequence (see Chapter 1, Section 1.7.3). This nucleotide sequence was then used to probe the human genome and they found that, under appropriate conditions, it only hybridized to alleles from a single locus. This locus was found to be highly polymorphic with a sample of 79 individuals exhibiting at least 77 alleles, each composed of 14 to 525 repeats.

SLPs work under similar sets of conditions as MLPs, but the stringency of hybridisation is increased (see Chapter 2, Section 2.1) to ensure that the SLP only binds to alleles from the locus for which it was developed. By using a number of these SLPs, an individual's genotype at a number of highly polymorphic loci can be screened separately. Indeed, it has been determined that the heterozygosity at a number of human hypervariable loci is 90% to 99% (Wong et al., 1986; 1987). If appropriate probes are selected for the analysis, the statistical power of this method can be as great as multilocus fingerprinting because the allelism of the bands is clear. In addition, single locus probes lend themselves to the compilation of allele frequency data bases and thus straightforward statistical analysis.

The single locus banding pattern or "profile" of an individual can contain two, one or no bands. The explanations for these patterns are as follows:

1) *Two bands:* the individual is heterozygous at the minisatellite locus concerned. There is a different allele on each chromosome and these are the two bands seen;

2) *One band:* the individual is either homozygous for the single band seen in the DNA profile or heterozygous for that band and one which is so small that it is lost from the end of the gel during electrophoresis;

3) *No bands:* The individual has two small bands that have been lost from the end of the gel. These bands may or may not represent alleles of the same size.

Single locus probes do have their own limitations. They are not very useful when attempting to determine degrees of relatedness between individuals as there are only two bands per probe in an individual's profile and even two full siblings would be expected to share no bands at all for 25% of probes tested. However, SLPs are extremely useful for establishing parentage because it is easy to determine which alleles are passed to an offspring. Assuming that both parents exhibit two alleles each, all four of which are different, then one of their offspring would also be expected to show two alleles, one from each parent. The likelihood that the father (or mother) is indeed the true biological parent of that offspring is calculated by dividing 0.5 (the probability that the parent being examined has passed the allele that is present in both his/her profile and that of the offspring *to* that offspring), by the frequency of that allele in the general population. This gives a ratio of the likelihood that the father/mother is the true parent against the chance that it is a randomly selected individual from the population. For example, if the allele passed to the offspring by its father has a frequency of 0.05, then the likelihood of paternity based on just this one result is $^{0.5}/_{0.05} = 10$. Thus, the putative father of this offspring is ten times more likely than a randomly selected individual from the population to be the true biological father of that offspring. If a number of SLPs are used, each detecting a locus not linked to any others, the likelihoods generated by each probe can be multiplied together to give an overall probability of parentage. The greater the number of probes used, the greater the probability.

The use of SLPs could probably be effectively applied to the captive stocks of Rothschild's mynah, the British Merlin and the New Zealand falcon discussed in this study, providing that appropriate specific SLPs could be developed. Unfortunately, the results from the multilocus probe analysis of Rodrigues fruit bat suggest that sufficient variation may no longer be present at the minisatellite loci of this species to make SLPs an effective option. However, there has been a recent development in the field of DNA analysis, "internal mapping", that could provide a system powerful enough to determine the breeding structure of even this stock.

Jeffreys, Neumann and Wilson (1990) have shown that variation between alleles at a single locus is sufficient for individual identification, at least in outbred

human populations. This technique is based upon utilizing a minisatellite which has one enzymatic restriction site (targeted by enzyme A) in each of its repeat units, and a second restriction site (targeted by enzyme B) that occurs in only some of the repeats. The minisatellite allele in question is first amplified using the polymerase chain reaction (see references in Jeffreys, MacLeod et al., 1991) and then one aliquot is partially digested using enzyme A, reducing it to a number of fragments of different lengths. A second aliquot is partially digested with enzyme B and the fragments resulting from the two digestions are separated in adjacent lanes by electrophoresis. The two ladders of bands can then be compared allowing the precise locations of the repeat units with the enzyme B cleavage sites to be determined. The repeat units can then be designated in sequence as being either unaffected by enzyme B (U) or cleaved by it (C). This technique allows minisatellite alleles to be "internally mapped", the specific "map" being represented by a string of in this case, Us and Cs. Both alleles in an individual can thus be identified and traced through generations. It is also possible that such alleles can be traced through a population and this would be of great benefit when attempting to deduce the breeding structures of inbred captive stocks such as Rodrigues fruit bat.

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APPENDICES

APPENDIX 1

ROTHSCHILD'S MYNAH: Similarity coefficients and G for all mynah comparisons given relationship models A-E. (mynreg0)

Comparisons	Between	Simil. Coeff	Model A	Model B	Model C	Model D	Model E
127	131	0.800	0.000	0.000	0.500	0.000	0.500
127	133	0.658	0.000	0.000	0.000	0.500	0.500
127	181	0.455	0.000	0.000	0.000	0.250	0.250
127	183	0.243	0.000	0.000	0.000	0.250	0.250
127	254	0.570	0.000	0.000	0.250	0.000	0.250
127	325	0.261	0.000	0.000	0.000	0.000	0.000
127	391	0.174	0.000	0.000	0.250	0.000	0.250
127	434	0.590	0.500	0.500	0.500	0.625	0.625
127	435	0.754	0.500	0.500	0.500	0.625	0.625
127	468	0.585	0.000	0.000	0.250	0.000	0.250
127	475	0.538	0.000	0.000	0.125	0.125	0.500
127	506	0.427	0.000	0.000	0.000	0.000	0.000
127	565	0.781	0.500	0.500	0.563	0.563	0.625
127	628	0.609	0.000	0.000	0.125	0.000	0.125
127	630	0.692	0.000	0.000	0.125	0.000	0.125
127	642	0.385	0.000	0.000	0.000	0.000	0.000
127	643	0.611	0.000	0.000	0.000	0.000	0.000
127	673	0.708	0.500	0.500	0.532	0.594	0.625
127	675	0.506	0.500	0.000	0.125	0.000	0.125
127	693	0.621	0.500	0.500	0.532	0.594	0.625
127	699	0.765	0.500	0.500	0.532	0.594	0.625
127	701	0.593	0.000	0.000	0.063	0.000	0.063
127	702	0.640	0.000	0.000	0.188	0.125	0.313
127	706	0.679	0.500	0.500	0.532	0.594	0.625
127	712	0.483	0.000	0.000	0.063	0.000	0.063
127	722	0.444	0.000	0.000	0.063	0.000	0.063
127	752	0.667	0.000	0.000	0.125	0.125	0.250
131	133	0.640	0.000	0.500	0.000	0.000	0.500
131	181	0.333	0.000	0.250	0.000	0.000	0.250
131	183	0.250	0.000	0.250	0.000	0.000	0.250
131	254	0.533	0.500	0.500	0.500	0.500	0.500
131	325	0.320	0.000	0.000	0.000	0.000	0.000
131	391	0.240	0.500	0.500	0.500	0.500	0.500
131	468	0.385	0.500	0.500	0.500	0.500	0.500
131	475	0.429	0.250	0.375	0.250	0.250	0.375
131	506	0.370	0.000	0.000	0.000	0.000	0.000
131	624	0.686	0.250	0.250	0.250	0.250	0.250
131	625	0.800	0.250	0.250	0.250	0.250	0.250
131	629	0.800	0.250	0.250	0.250	0.250	0.250
131	630	0.811	0.250	0.250	0.250	0.250	0.250
131	631	0.757	0.250	0.250	0.250	0.250	0.250
131	640	0.786	0.000	0.000	0.000	0.000	0.000
131	642	0.564	0.000	0.000	0.000	0.000	0.000
131	692	0.579	0.125	0.125	0.125	0.125	0.125
131	701	0.632	0.125	0.125	0.125	0.125	0.125
131	703	0.757	0.125	0.125	0.125	0.125	0.125
131	710	0.649	0.125	0.125	0.125	0.125	0.125
131	712	0.632	0.125	0.125	0.125	0.125	0.125
131	722	0.684	0.125	0.125	0.125	0.125	0.125

133	181	0.588	0.500	0.500	0.500	0.500	0.500
133	183	0.420	0.500	0.500	0.500	0.500	0.500
133	254	0.487	0.000	0.250	0.000	0.000	0.250
133	325	0.333	0.000	0.000	0.000	0.000	0.000
133	391	0.333	0.000	0.250	0.000	0.000	0.250
133	435	0.564	0.250	0.250	0.250	0.500	0.500
133	468	0.316	0.000	0.250	0.000	0.000	0.250
133	475	0.476	0.250	0.375	0.250	0.250	0.375
133	506	0.100	0.000	0.000	0.000	0.000	0.000
133	565	0.500	0.125	0.188	0.125	0.375	0.438
133	606	0.667	0.125	0.250	0.125	0.250	0.375
133	626	0.800	0.375	0.438	0.375	0.500	0.563
133	627	0.667	0.375	0.438	0.375	0.500	0.563
133	636	0.615	0.250	0.313	0.250	0.250	0.313
133	641	0.538	0.000	0.000	0.000	0.000	0.000
133	642	0.512	0.000	0.000	0.000	0.000	0.000
133	643	0.550	0.000	0.000	0.000	0.000	0.000
133	673	0.684	0.188	0.219	0.188	0.438	0.469
133	675	0.537	0.000	0.125	0.000	0.000	0.125
133	722	0.769	0.000	0.063	0.000	0.000	0.063
133	756	0.615	0.000	0.063	0.000	0.000	0.063
133	760	0.696	0.188	0.219	0.188	0.250	0.281
181	183	0.375	0.500	0.500	0.500	0.500	0.500
181	254	0.636	0.000	0.125	0.000	0.000	0.125
181	325	0.471	0.000	0.000	0.000	0.000	0.000
181	391	0.353	0.000	0.125	0.000	0.000	0.125
181	468	0.444	0.000	0.125	0.000	0.000	0.125
181	475	0.500	0.250	0.313	0.250	0.250	0.313
181	506	0.211	0.000	0.000	0.000	0.000	0.000
183	254	0.314	0.000	0.125	0.000	0.000	0.125
183	296	0.522	0.000	0.000	0.000	0.000	0.000
183	325	0.588	0.000	0.000	0.000	0.000	0.000
183	391	0.274	0.000	0.125	0.000	0.000	0.125
183	435	0.469	0.500	0.500	0.500	0.625	0.625
183	468	0.348	0.000	0.125	0.000	0.000	0.125
183	475	0.300	0.250	0.313	0.250	0.250	0.313
183	506	0.334	0.000	0.000	0.000	0.000	0.000
183	565	0.229	0.125	0.156	0.125	0.250	0.281
183	606	0.503	0.250	0.313	0.250	0.313	0.330
183	624	0.364	0.000	0.063	0.000	0.000	0.063
183	625	0.529	0.000	0.063	0.000	0.000	0.063
183	626	0.382	0.375	0.375	0.375	0.438	0.438
183	627	0.296	0.375	0.375	0.375	0.438	0.438
183	628	0.500	0.000	0.063	0.000	0.000	0.063
183	629	0.432	0.000	0.063	0.000	0.000	0.063
183	630	0.410	0.000	0.063	0.000	0.000	0.063
183	631	0.500	0.000	0.063	0.000	0.000	0.063
183	636	0.588	0.125	0.156	0.125	0.125	0.156
183	640	0.550	0.000	0.000	0.000	0.000	0.000
183	641	0.387	0.000	0.000	0.000	0.000	0.000
183	642	0.524	0.000	0.000	0.000	0.000	0.000
183	643	0.410	0.000	0.000	0.000	0.000	0.000
183	673	0.314	0.313	0.328	0.313	0.438	0.453
183	675	0.447	0.000	0.063	0.000	0.000	0.063

254	296	0.250	0.000	0.000	0.000	0.000	0.000
254	325	0.348	0.000	0.000	0.000	0.000	0.000
254	435	0.575	0.000	0.063	0.125	0.000	0.188
254	468	0.630	0.500	0.500	0.500	0.500	0.500
254	475	0.692	0.500	0.563	0.500	0.500	0.563
254	506	0.380	0.000	0.000	0.000	0.000	0.000
254	565	0.603	0.250	0.281	0.375	0.250	0.406
254	606	0.486	0.250	0.281	0.313	0.250	0.344
254	625	0.514	0.125	0.125	0.125	0.125	0.125
254	626	0.571	0.250	0.344	0.313	0.250	0.407
254	627	0.786	0.250	0.344	0.313	0.250	0.407
254	636	0.526	0.250	0.313	0.250	0.250	0.313
254	641	0.312	0.000	0.000	0.000	0.000	0.000
254	642	0.558	0.000	0.000	0.000	0.000	0.000
254	643	0.650	0.000	0.000	0.000	0.000	0.000
254	673	0.527	0.125	0.172	0.250	0.125	0.297
254	675	0.667	0.500	0.500	0.500	0.500	0.500
254	693	0.462	0.125	0.172	0.250	0.125	0.297
254	699	0.452	0.125	0.172	0.250	0.125	0.297
254	706	0.462	0.125	0.172	0.250	0.125	0.297
296	435	0.276	0.000	0.000	0.000	0.000	0.000
296	606	0.323	0.000	0.000	0.000	0.000	0.000
296	625	0.345	0.000	0.000	0.000	0.000	0.000
296	626	0.273	0.000	0.000	0.000	0.000	0.000
296	627	0.364	0.000	0.000	0.000	0.000	0.000
296	636	0.312	0.000	0.000	0.000	0.000	0.000
296	641	0.385	0.000	0.000	0.000	0.000	0.000
296	673	0.148	0.000	0.000	0.000	0.000	0.000
296	675	0.258	0.000	0.000	0.000	0.000	0.000
325	391	0.444	0.000	0.000	0.000	0.000	0.000
325	468	0.317	0.000	0.000	0.000	0.000	0.000
325	475	0.190	0.000	0.000	0.000	0.000	0.000
325	506	0.300	0.000	0.000	0.000	0.000	0.000
391	468	0.516	0.500	0.500	0.500	0.500	0.500
391	475	0.286	0.250	0.313	0.250	0.250	0.313
391	506	0.262	0.000	0.000	0.000	0.000	0.000
391	606	0.632	0.250	0.281	0.313	0.250	0.344
391	624	0.516	0.125	0.125	0.125	0.125	0.125
391	626	0.714	0.125	0.156	0.187	0.125	0.218
391	628	0.471	0.125	0.125	0.125	0.125	0.125
391	629	0.457	0.125	0.125	0.125	0.125	0.125
391	630	0.595	0.125	0.125	0.125	0.125	0.125
391	631	0.526	0.125	0.125	0.125	0.125	0.125
391	636	0.462	0.125	0.188	0.125	0.125	0.188
391	640	0.474	0.000	0.000	0.000	0.000	0.000
434	435	0.712	0.500	0.500	0.500	0.563	0.563
434	468	0.503	0.000	0.063	0.125	0.000	0.188
434	506	0.467	0.000	0.000	0.000	0.000	0.000
434	565	0.625	0.313	0.329	0.344	0.376	0.422
434	628	0.500	0.000	0.031	0.063	0.000	0.094
434	630	0.609	0.000	0.031	0.063	0.000	0.094
434	642	0.435	0.000	0.000	0.000	0.000	0.000
434	673	0.581	0.406	0.414	0.422	0.469	0.492

434	675	0.526	0.000	0.031	0.063	0.000	0.094
434	693	0.621	0.406	0.414	0.422	0.469	0.492
434	699	0.529	0.406	0.414	0.422	0.469	0.492
434	701	0.583	0.000	0.016	0.031	0.000	0.047
434	702	0.545	0.063	0.109	0.156	0.125	0.266
434	706	0.445	0.406	0.414	0.422	0.469	0.492
434	712	0.462	0.000	0.016	0.031	0.000	0.047
434	722	0.500	0.000	0.016	0.031	0.000	0.047
434	752	0.583	0.063	0.094	0.125	0.125	0.219
435	468	0.780	0.000	0.063	0.125	0.000	0.188
435	506	0.571	0.000	0.000	0.000	0.000	0.000
435	565	0.676	0.313	0.329	0.344	0.376	0.422
435	606	0.762	0.250	0.281	0.313	0.281	0.375
435	625	0.800	0.000	0.031	0.063	0.000	0.094
435	626	0.667	0.375	0.391	0.406	0.469	0.516
435	627	0.606	0.375	0.391	0.406	0.469	0.516
435	628	0.696	0.000	0.031	0.063	0.000	0.094
435	630	0.769	0.000	0.031	0.063	0.000	0.094
435	636	0.698	0.063	0.078	0.094	0.125	0.172
435	641	0.595	0.000	0.000	0.000	0.000	0.000
435	642	0.504	0.000	0.000	0.000	0.000	0.000
435	643	0.634	0.000	0.000	0.000	0.000	0.000
435	673	0.709	0.656	0.664	0.672	0.703	0.727
435	675	0.646	0.000	0.031	0.063	0.000	0.094
435	693	0.647	0.656	0.664	0.672	0.703	0.727
435	699	0.821	0.656	0.664	0.672	0.703	0.727
435	701	0.741	0.000	0.016	0.031	0.000	0.047
435	702	0.640	0.063	0.109	0.156	0.125	0.266
435	706	0.687	0.656	0.664	0.672	0.703	0.727
435	712	0.621	0.000	0.016	0.031	0.000	0.047
435	722	0.593	0.000	0.016	0.031	0.000	0.047
435	752	0.815	0.063	0.094	0.125	0.125	0.219
468	475	0.727	0.250	0.313	0.250	0.250	0.313
468	506	0.443	0.000	0.000	0.000	0.000	0.000
468	565	0.706	0.125	0.156	0.250	0.125	0.281
468	606	0.818	0.250	0.281	0.313	0.250	0.344
468	624	0.649	0.125	0.125	0.125	0.125	0.125
468	626	0.471	0.125	0.156	0.187	0.125	0.218
468	628	0.721	0.125	0.125	0.125	0.125	0.125
468	629	0.634	0.125	0.125	0.125	0.125	0.125
468	630	0.717	0.125	0.125	0.125	0.125	0.125
468	631	0.727	0.125	0.125	0.125	0.125	0.125
468	636	0.533	0.125	0.188	0.125	0.125	0.188
468	640	0.727	0.000	0.000	0.000	0.000	0.000
468	642	0.483	0.000	0.000	0.000	0.000	0.000
468	673	0.606	0.063	0.109	0.188	0.063	0.234
468	675	0.600	0.250	0.250	0.250	0.250	0.250
468	693	0.581	0.063	0.109	0.188	0.063	0.234
468	699	0.722	0.063	0.109	0.188	0.063	0.234
468	701	0.600	0.063	0.063	0.063	0.063	0.063
468	702	0.786	0.250	0.313	0.250	0.250	0.313
468	706	0.693	0.063	0.109	0.188	0.063	0.234
468	712	0.625	0.063	0.063	0.063	0.063	0.063
468	722	0.533	0.063	0.063	0.063	0.063	0.063
468	752	0.800	0.188	0.250	0.188	0.188	0.250

475	506	0.087	0.000	0.000	0.000	0.000	0.000
506	565	0.588	0.000	0.000	0.000	0.000	0.000
506	606	0.585	0.000	0.000	0.000	0.000	0.000
506	624	0.588	0.166	0.166	0.166	0.166	0.166
506	626	0.323	0.000	0.000	0.000	0.000	0.000
506	628	0.541	0.166	0.166	0.166	0.166	0.166
506	629	0.737	0.166	0.166	0.166	0.166	0.166
506	630	0.650	0.166	0.166	0.166	0.166	0.166
506	631	0.732	0.166	0.166	0.166	0.166	0.166
506	636	0.667	0.000	0.000	0.000	0.000	0.000
506	640	0.634	0.000	0.000	0.000	0.000	0.000
506	673	0.424	0.000	0.000	0.000	0.000	0.000
506	675	0.800	0.500	0.500	0.500	0.500	0.500
506	693	0.387	0.000	0.000	0.000	0.000	0.000
506	699	0.500	0.000	0.000	0.000	0.000	0.000
506	706	0.452	0.000	0.000	0.000	0.000	0.000
565	642	0.537	0.000	0.000	0.000	0.000	0.000
565	643	0.632	0.000	0.000	0.000	0.000	0.000
565	673	0.677	0.656	0.664	0.672	0.703	0.727
565	675	0.593	0.125	0.141	0.188	0.125	0.203
565	693	0.667	0.656	0.664	0.672	0.703	0.727
565	699	0.789	0.656	0.664	0.672	0.703	0.727
565	706	0.727	0.656	0.664	0.672	0.703	0.727
606	624	0.564	0.125	0.141	0.156	0.125	0.172
606	625	0.714	0.125	0.141	0.156	0.125	0.172
606	626	0.569	0.219	0.227	0.234	0.266	0.289
606	627	0.557	0.219	0.227	0.234	0.266	0.289
606	628	0.714	0.125	0.141	0.156	0.125	0.172
606	629	0.698	0.125	0.141	0.156	0.125	0.172
606	630	0.756	0.125	0.141	0.156	0.125	0.172
606	631	0.739	0.125	0.141	0.156	0.125	0.172
606	636	0.698	0.094	0.102	0.109	0.125	0.148
606	640	0.739	0.000	0.000	0.000	0.000	0.000
606	641	0.593	0.000	0.000	0.000	0.000	0.000
606	673	0.600	0.234	0.262	0.305	0.258	0.356
606	675	0.636	0.125	0.141	0.156	0.125	0.172
606	722	0.857	0.031	0.039	0.047	0.031	0.055
606	756	0.786	0.031	0.039	0.047	0.031	0.055
606	760	0.640	0.141	0.156	0.156	0.164	0.195
624	625	0.641	0.500	0.500	0.500	0.500	0.500
624	626	0.445	0.031	0.078	0.063	0.031	0.109
624	627	0.560	0.031	0.078	0.063	0.031	0.109
624	628	0.723	0.500	0.500	0.500	0.500	0.500
624	629	0.696	0.500	0.500	0.500	0.500	0.500
624	630	0.622	0.500	0.500	0.500	0.500	0.500
624	631	0.703	0.500	0.500	0.500	0.500	0.500
624	636	0.500	0.031	0.063	0.031	0.031	0.063
624	640	0.540	0.000	0.000	0.000	0.000	0.000
624	641	0.533	0.000	0.000	0.000	0.000	0.000
624	642	0.396	0.000	0.000	0.000	0.000	0.000
624	673	0.560	0.016	0.039	0.078	0.016	0.102
624	675	0.788	0.146	0.146	0.146	0.146	0.146

624	692	0.703	0.500	0.500	0.500	0.500	0.500
624	701	0.757	0.500	0.500	0.500	0.500	0.500
624	703	0.703	0.250	0.250	0.250	0.250	0.250
624	710	0.722	0.500	0.500	0.500	0.500	0.500
624	712	0.649	0.500	0.500	0.500	0.500	0.500
624	722	0.595	0.500	0.500	0.500	0.500	0.500
625	626	0.603	0.031	0.078	0.063	0.031	0.109
625	627	0.504	0.031	0.078	0.063	0.031	0.109
625	628	0.667	0.500	0.500	0.500	0.500	0.500
625	629	0.731	0.500	0.500	0.500	0.500	0.500
625	630	0.927	0.500	0.500	0.500	0.500	0.500
625	631	0.800	0.500	0.500	0.500	0.500	0.500
625	636	0.744	0.031	0.063	0.031	0.031	0.063
625	640	0.621	0.000	0.000	0.000	0.000	0.000
625	641	0.646	0.000	0.000	0.000	0.000	0.000
625	642	0.502	0.000	0.000	0.000	0.000	0.000
625	673	0.490	0.016	0.039	0.078	0.016	0.102
625	675	0.704	0.146	0.146	0.146	0.146	0.146
625	692	0.571	0.250	0.250	0.250	0.250	0.250
625	701	0.667	0.250	0.250	0.250	0.250	0.250
625	703	0.762	0.500	0.500	0.500	0.500	0.500
625	710	0.683	0.250	0.250	0.250	0.250	0.250
625	712	0.667	0.250	0.250	0.250	0.250	0.250
625	722	0.571	0.250	0.250	0.250	0.250	0.250
626	627	0.720	0.531	0.508	0.516	0.531	0.586
626	628	0.511	0.109	0.109	0.109	0.109	0.109
626	629	0.319	0.109	0.109	0.109	0.109	0.109
626	630	0.457	0.109	0.109	0.109	0.109	0.109
626	631	0.464	0.109	0.109	0.109	0.109	0.109
626	636	0.606	0.156	0.180	0.172	0.188	0.227
626	640	0.413	0.000	0.000	0.000	0.000	0.000
626	641	0.461	0.000	0.000	0.000	0.000	0.000
626	642	0.190	0.000	0.000	0.000	0.000	0.000
626	673	0.533	0.203	0.207	0.211	0.227	0.238
626	675	0.545	0.125	0.172	0.156	0.125	0.203
626	722	0.667	0.016	0.039	0.031	0.016	0.055
626	756	0.583	0.016	0.039	0.031	0.016	0.055
626	760	0.667	0.281	0.281	0.289	0.297	0.309
627	628	0.538	0.109	0.109	0.109	0.109	0.109
627	629	0.320	0.109	0.109	0.109	0.109	0.109
627	631	0.552	0.109	0.109	0.109	0.109	0.109
627	636	0.551	0.156	0.180	0.172	0.188	0.227
627	640	0.417	0.000	0.000	0.000	0.000	0.000
627	641	0.480	0.000	0.000	0.000	0.000	0.000
627	642	0.167	0.000	0.000	0.000	0.000	0.000
627	673	0.532	0.203	0.207	0.211	0.227	0.238
627	675	0.615	0.125	0.172	0.156	0.125	0.203
627	722	0.545	0.016	0.039	0.031	0.016	0.055
627	756	0.455	0.016	0.039	0.031	0.016	0.055
627	760	0.632	0.500	0.500	0.500	0.500	0.500
628	629	0.653	0.500	0.500	0.500	0.500	0.500
628	630	0.811	0.500	0.500	0.500	0.500	0.500
628	631	0.775	0.500	0.500	0.500	0.500	0.500

628	636	0.651	0.031	0.063	0.031	0.031	0.063
628	640	0.584	0.000	0.000	0.000	0.000	0.000
628	641	0.516	0.000	0.000	0.000	0.000	0.000
628	642	0.361	0.000	0.000	0.000	0.000	0.000
628	643	0.552	0.000	0.000	0.000	0.000	0.000
628	673	0.462	0.016	0.039	0.078	0.016	0.102
628	675	0.647	0.146	0.146	0.146	0.146	0.146
628	701	0.583	0.250	0.250	0.250	0.250	0.250
628	702	0.678	0.281	0.313	0.281	0.281	0.313
628	706	0.476	0.016	0.039	0.078	0.016	0.102
628	712	0.538	0.250	0.250	0.250	0.250	0.250
628	713	0.714	0.281	0.313	0.281	0.281	0.313
628	714	0.759	0.625	0.625	0.625	0.625	0.625
628	719	0.769	0.281	0.313	0.281	0.281	0.313
628	722	0.417	0.250	0.250	0.250	0.250	0.250
628	752	0.769	0.250	0.266	0.281	0.250	0.297
628	754	0.583	0.031	0.078	0.063	0.031	0.109
628	756	0.667	0.250	0.250	0.250	0.250	0.250
628	760	0.560	0.031	0.078	0.063	0.031	0.109
629	630	0.794	0.500	0.500	0.500	0.500	0.500
629	631	0.739	0.500	0.500	0.500	0.500	0.500
629	636	0.636	0.031	0.063	0.031	0.031	0.063
629	640	0.578	0.000	0.000	0.000	0.000	0.000
629	641	0.533	0.000	0.000	0.000	0.000	0.000
629	642	0.411	0.000	0.000	0.000	0.000	0.000
629	673	0.480	0.016	0.039	0.078	0.016	0.102
629	675	0.667	0.146	0.146	0.146	0.146	0.146
629	692	0.649	0.250	0.250	0.250	0.250	0.250
629	701	0.703	0.250	0.250	0.250	0.250	0.250
629	703	0.649	0.250	0.250	0.250	0.250	0.250
629	710	0.667	0.250	0.250	0.250	0.250	0.250
629	712	0.703	0.250	0.250	0.250	0.250	0.250
629	722	0.649	0.250	0.250	0.250	0.250	0.250
630	631	0.870	0.500	0.500	0.500	0.500	0.500
630	636	0.609	0.031	0.063	0.031	0.031	0.063
630	640	0.651	0.000	0.000	0.000	0.000	0.000
630	642	0.531	0.000	0.000	0.000	0.000	0.000
630	643	0.506	0.000	0.000	0.000	0.000	0.000
630	692	0.564	0.250	0.250	0.250	0.250	0.250
630	701	0.641	0.250	0.250	0.250	0.250	0.250
630	702	0.682	0.281	0.313	0.281	0.281	0.313
630	703	0.718	0.250	0.250	0.250	0.250	0.250
630	706	0.508	0.016	0.039	0.078	0.016	0.102
630	710	0.684	0.250	0.250	0.250	0.250	0.250
630	712	0.644	0.250	0.250	0.250	0.250	0.250
630	713	0.839	0.281	0.313	0.281	0.281	0.313
630	714	0.875	0.375	0.375	0.375	0.375	0.375
630	719	0.897	0.281	0.313	0.281	0.281	0.313
630	722	0.564	0.250	0.250	0.250	0.250	0.250
630	752	0.676	0.250	0.266	0.281	0.250	0.297
630	754	0.519	0.031	0.078	0.063	0.031	0.109
630	756	0.600	0.250	0.250	0.250	0.250	0.250
630	760	0.500	0.031	0.078	0.063	0.031	0.109
631	636	0.723	0.031	0.063	0.031	0.031	0.063

631	640	0.637	0.000	0.000	0.000	0.000	0.000
631	641	0.647	0.000	0.000	0.000	0.000	0.000
631	642	0.493	0.000	0.000	0.000	0.000	0.000
631	673	0.483	0.016	0.039	0.078	0.016	0.102
631	675	0.865	0.146	0.146	0.146	0.146	0.146
631	692	0.513	0.250	0.250	0.250	0.250	0.250
631	701	0.564	0.250	0.250	0.250	0.250	0.250
631	703	0.718	0.250	0.250	0.250	0.250	0.250
631	710	0.632	0.250	0.250	0.250	0.250	0.250
631	712	0.564	0.250	0.250	0.250	0.250	0.250
631	722	0.462	0.250	0.250	0.250	0.250	0.250
636	640	0.553	0.000	0.000	0.000	0.000	0.000
636	641	0.575	0.000	0.000	0.000	0.000	0.000
636	673	0.505	0.078	0.098	0.109	0.141	0.191
636	675	0.711	0.125	0.156	0.125	0.125	0.156
636	722	0.800	0.016	0.031	0.016	0.016	0.031
636	756	0.867	0.016	0.031	0.016	0.016	0.031
636	760	0.667	0.047	0.059	0.055	0.063	0.082
640	641	0.759	0.000	0.000	0.000	0.000	0.000
640	642	0.750	0.000	0.000	0.000	0.000	0.000
640	673	0.333	0.000	0.000	0.000	0.000	0.000
640	675	0.437	0.000	0.000	0.000	0.000	0.000
640	692	0.762	0.500	0.500	0.500	0.500	0.500
640	701	0.714	0.500	0.500	0.500	0.500	0.500
640	703	0.667	0.000	0.000	0.000	0.000	0.000
640	710	0.732	0.500	0.500	0.500	0.500	0.500
640	712	0.762	0.500	0.500	0.500	0.500	0.500
640	722	0.810	0.500	0.500	0.500	0.500	0.500
641	642	0.483	0.000	0.000	0.000	0.000	0.000
641	673	0.442	0.000	0.000	0.000	0.000	0.000
641	675	0.514	0.000	0.000	0.000	0.000	0.000
641	722	0.600	0.000	0.000	0.000	0.000	0.000
641	756	0.667	0.500	0.500	0.500	0.500	0.500
641	760	0.593	0.000	0.000	0.000	0.000	0.000
642	643	0.844	0.000	0.000	0.000	0.000	0.000
642	673	0.428	0.000	0.000	0.000	0.000	0.000
642	675	0.408	0.000	0.000	0.000	0.000	0.000
642	692	0.634	0.000	0.000	0.000	0.000	0.000
642	701	0.614	0.000	0.000	0.000	0.000	0.000
642	702	0.400	0.000	0.000	0.000	0.000	0.000
642	703	0.683	0.500	0.500	0.500	0.500	0.500
642	706	0.333	0.000	0.000	0.000	0.000	0.000
642	710	0.650	0.000	0.000	0.000	0.000	0.000
642	712	0.652	0.000	0.000	0.000	0.000	0.000
642	722	0.687	0.000	0.000	0.000	0.000	0.000
642	752	0.444	0.000	0.000	0.000	0.000	0.000
643	673	0.650	0.000	0.000	0.000	0.000	0.000
643	675	0.651	0.000	0.000	0.000	0.000	0.000
643	702	0.533	0.000	0.000	0.000	0.000	0.000
643	713	0.545	0.000	0.000	0.000	0.000	0.000
643	714	0.529	0.000	0.000	0.000	0.000	0.000
643	719	0.452	0.000	0.000	0.000	0.000	0.000

643	752	0.500	0.000	0.000	0.000	0.000	0.000
643	754	0.621	0.000	0.000	0.000	0.000	0.000
643	756	0.625	0.000	0.000	0.000	0.000	0.000
643	760	0.600	0.000	0.000	0.000	0.000	0.000
673	675	0.523	0.063	0.086	0.125	0.063	0.148
673	693	0.812	0.578	0.582	0.586	0.594	0.605
673	699	0.703	0.578	0.582	0.586	0.594	0.605
673	706	0.625	0.578	0.582	0.586	0.594	0.605
675	693	0.462	0.063	0.086	0.125	0.063	0.148
675	699	0.500	0.063	0.086	0.125	0.063	0.148
675	706	0.462	0.063	0.086	0.125	0.063	0.148
692	701	0.850	0.500	0.500	0.500	0.500	0.500
692	703	0.550	0.125	0.125	0.125	0.125	0.125
692	710	0.821	0.500	0.500	0.500	0.500	0.500
692	712	0.850	0.500	0.500	0.500	0.500	0.500
692	722	0.800	0.500	0.500	0.500	0.500	0.500
693	699	0.686	0.578	0.582	0.586	0.594	0.605
693	706	0.667	0.578	0.582	0.586	0.594	0.605
699	706	0.800	0.578	0.582	0.586	0.594	0.605
701	702	0.615	0.125	0.125	0.125	0.125	0.125
701	703	0.650	0.125	0.125	0.125	0.125	0.125
701	706	0.560	0.008	0.016	0.039	0.008	0.047
701	710	0.872	0.500	0.500	0.500	0.500	0.500
701	712	0.850	0.500	0.500	0.500	0.500	0.500
701	722	0.793	0.500	0.500	0.500	0.500	0.500
701	752	0.714	0.141	0.156	0.141	0.141	0.156
702	706	0.696	0.094	0.125	0.156	0.156	0.250
702	712	0.571	0.125	0.125	0.125	0.125	0.125
702	713	0.759	0.531	0.563	0.531	0.531	0.563
702	714	0.600	0.297	0.344	0.297	0.297	0.344
702	719	0.741	0.531	0.563	0.531	0.531	0.563
702	722	0.506	0.125	0.125	0.125	0.125	0.125
702	752	0.760	0.281	0.344	0.281	0.281	0.344
702	754	0.480	0.094	0.129	0.117	0.109	0.168
702	756	0.643	0.141	0.156	0.141	0.141	0.156
702	760	0.538	0.094	0.129	0.117	0.109	0.168
703	710	0.667	0.125	0.125	0.125	0.125	0.125
703	712	0.600	0.125	0.125	0.125	0.125	0.125
706	712	0.519	0.008	0.016	0.039	0.008	0.047
706	722	0.400	0.008	0.016	0.039	0.008	0.047
706	752	0.640	0.086	0.117	0.117	0.048	0.210
710	712	0.769	0.500	0.500	0.500	0.500	0.500
710	722	0.718	0.500	0.500	0.500	0.500	0.500
712	722	0.825	0.500	0.500	0.500	0.500	0.500
712	752	0.600	0.141	0.156	0.141	0.141	0.156

713	714	0.727	0.297	0.344	0.297	0.297	0.344
713	719	0.867	0.531	0.563	0.531	0.531	0.563
713	752	0.667	0.281	0.344	0.281	0.281	0.344
713	754	0.571	0.094	0.129	0.117	0.109	0.168
713	756	0.645	0.141	0.156	0.141	0.141	0.156
713	760	0.483	0.094	0.129	0.117	0.109	0.168
714	719	0.774	0.297	0.344	0.297	0.297	0.344
714	752	0.643	0.188	0.211	0.234	0.188	0.258
714	754	0.483	0.023	0.059	0.047	0.023	0.082
714	756	0.687	0.188	0.188	0.188	0.188	0.188
714	760	0.533	0.023	0.059	0.047	0.023	0.082
719	752	0.800	0.281	0.344	0.281	0.281	0.344
719	754	0.462	0.094	0.129	0.117	0.109	0.168
719	756	0.621	0.141	0.156	0.141	0.141	0.156
719	760	0.370	0.094	0.129	0.117	0.109	0.168
722	752	0.643	0.141	0.156	0.141	0.141	0.156
722	756	0.800	0.250	0.250	0.250	0.250	0.250
722	760	0.667	0.008	0.020	0.016	0.008	0.027
752	754	0.522	0.133	0.145	0.148	0.148	0.176
752	756	0.615	0.125	0.133	0.141	0.125	0.148
752	760	0.417	0.133	0.145	0.148	0.148	0.176
754	756	0.593	0.008	0.020	0.016	0.008	0.027
754	760	0.880	0.500	0.500	0.500	0.500	0.500
756	760	0.619	0.008	0.020	0.016	0.008	0.027

MULTIPLE LINEAR REGRESSION

Dependent Variable: D

Variable		Mean	Parameter Estimate	Standard Error	T for H0: parameter=0
Intercept		a =	0.518	0.009	56.750
Model A	X =	0.162	-0.088	0.260	-0.339
Model B		0.179	0.020	0.171	0.117
Model C		0.178	0.654	0.194	3.376
Model D		0.173	0.003	0.175	0.016
Model E		0.208	-0.198	0.164	-1.209

Source	DF	Sum of Squares	Mean Square	F-Value
Model	5.000	2.693	0.539	30.532
Error	485.000	8.554	0.018	
Total	490.000	11.247		

Dependent Mean	Y =	0.583
Root Mean Square Error		0.133
Coefficient of Variation		22.791
R-Square		0.239
Adjusted R-Square		0.232

APPENDIX 2

ROTHSCHILD'S MYNAHS:Comparison of D for a pair of adults against F for their offspring (myndvsf1)

Female	Male	D	F	$(D-\bar{D})$	$(F-\bar{F})$	$(D-\bar{D})(F-\bar{F})$
183	127	0.243	0.000	-0.372	-0.104	0.039
254	133	0.487	0.000	-0.128	-0.104	0.013
468	434	0.503	0.000	-0.112	-0.104	0.012
325	506	0.300	0.000	-0.315	-0.104	0.033
475	127	0.781	0.000	0.166	-0.104	-0.017
565	434	0.625	0.156	0.010	0.052	0.001
565	435	0.676	0.156	0.061	0.052	0.003
565	506	0.588	0.000	-0.027	-0.104	0.003
565	673	0.677	0.328	0.062	0.224	0.014
565	675	0.593	0.063	-0.022	-0.041	0.001
565	693	0.667	0.328	0.052	0.224	0.012
565	699	0.789	0.328	0.174	0.224	0.039
624	506	0.588	0.148	-0.027	0.044	-0.001
624	606	0.564	0.031	-0.051	-0.073	0.004
624	625	0.641	0.250	0.026	0.146	0.004
624	626	0.445	0.016	-0.170	-0.088	0.015
624	627	0.560	0.016	-0.055	-0.088	0.005
624	629	0.696	0.250	0.081	0.146	0.012
624	640	0.540	0.000	-0.075	-0.104	0.008
624	641	0.533	0.000	-0.082	-0.104	0.009
624	673	0.560	0.008	-0.055	-0.096	0.005
624	675	0.788	0.105	0.173	0.001	0.001
624	692	0.703	0.250	0.088	0.146	0.013
624	710	0.722	0.250	0.107	0.146	0.016
624	712	0.649	0.250	0.034	0.146	0.005
630	435	0.769	0.000	0.154	-0.104	-0.016
630	625	0.927	0.250	0.312	0.146	0.046
630	626	0.457	0.016	-0.158	-0.088	0.014
630	629	0.794	0.250	0.179	0.146	0.026
630	640	0.651	0.000	0.036	-0.104	-0.004
630	692	0.564	0.125	-0.051	0.021	-0.001
630	702	0.682	0.141	0.067	0.037	0.002
630	710	0.684	0.125	0.069	0.021	0.001
630	712	0.644	0.125	0.029	0.021	0.001
630	713	0.839	0.141	0.224	0.037	0.008
630	719	0.897	0.141	0.282	0.037	0.010
631	506	0.732	0.148	0.117	0.044	0.005
631	606	0.739	0.031	0.124	-0.073	-0.009
631	625	0.800	0.250	0.185	0.146	0.027
631	626	0.464	0.016	-0.151	-0.088	0.013
631	627	0.552	0.016	-0.063	-0.088	0.006
631	629	0.739	0.250	0.124	0.146	0.018
631	640	0.637	0.000	0.022	-0.104	-0.002
631	641	0.647	0.000	0.032	-0.104	-0.003

631	673	0.483	0.008	-0.132	-0.096	0.013
631	675	0.865	0.105	0.250	0.001	0.001
631	692	0.513	0.125	-0.102	0.021	-0.002
631	710	0.632	0.125	0.017	0.021	0.001
631	712	0.564	0.125	-0.051	0.021	-0.001
642	435	0.504	0.000	-0.111	-0.104	0.012
642	625	0.502	0.000	-0.113	-0.104	0.012
642	626	0.190	0.000	-0.425	-0.104	0.044
642	627	0.167	0.000	-0.448	-0.104	0.047
642	629	0.411	0.000	-0.204	-0.104	0.021
642	640	0.750	0.000	0.135	-0.104	-0.014
642	641	0.483	0.000	-0.132	-0.104	0.014
642	673	0.428	0.000	-0.182	-0.104	0.019
642	692	0.634	0.000	0.019	-0.104	-0.002
642	702	0.400	0.000	-0.215	-0.104	0.022
642	710	0.650	0.000	0.035	-0.104	-0.004
642	712	0.652	0.000	0.037	-0.104	-0.004
706	435	0.687	0.328	0.072	0.224	0.016
706	673	0.625	0.328	0.010	0.224	0.002
706	693	0.667	0.328	0.052	0.224	0.012
706	699	0.800	0.328	0.185	0.224	0.041
706	702	0.696	0.047	0.081	-0.057	-0.005
706	712	0.519	0.004	-0.096	-0.100	0.010
714	702	0.600	0.164	-0.015	0.060	-0.001
714	713	0.727	0.164	0.112	0.060	0.007
714	719	0.774	0.164	0.159	0.060	0.001
				$\sum^{1}_{k}$ 1.628	$\sum^{1}_{=}$ 0.912	Σ 0.653
			Var =	0.024	0.013	0.010

MULTIPLE LINEAR REGRESSION

Dependent Variable: D

Variable		Mean	Parameter Estimate	Standard Error	T for H0: parameter=0
Intercept		a =	0.540	0.021	25.563
F	X =	0.104	0.719	0.137	5.261

Source	DF	Sum of Squares	Mean Square	F-Value
Model	1.000	0.472	0.472	27.683
Error	68.000	1.158	0.017	
Total	69.000	1.630		

Dependent Mean	Y =	0.615
Root Mean Square Error		0.131
Coefficient of Variation		21.218
R-Square		0.289
Adjusted R-Square		0.279

APPENDIX 3

ROTHSCHILD'S MYNAH: Similarity coefficients for all known mynah 1st-degree relatives

Comparison	Between	Relation	D	Year Hatched	Other Parent or Parents
127	434	p/o	0.590	1980	183
127	435	p/o	0.817	1980	183
127	565	p/o	0.875	1984	475
131	254	p/o	0.533	1976	132
131	391	p/o	0.240	1979	132
131	468	p/o	0.385	1981	132
133	181	p/o	0.588	1973	134
133	183	p/o	0.353	1973	134
181	183	sibs	0.375	1973	133.134
183	435	p/o	0.412	1980	127
254	391	sibs	0.435	1976	131.132
254	468	sibs	0.630	1976	131.132
254	475	p/o	0.692	1981	174
254	675	p/o	0.649	1988	506
391	468	sibs	0.516	1976	131.132
434	435	sibs	0.712	1980	127.183
506	675	p/o	0.800	1988	254
624	625	sibs	0.615	1987	603.591
624	628	sibs	0.704	1987	603.591
624	629	sibs	0.696	1987	603.591
624	630	sibs	0.622	1987	603.591
624	631	sibs	0.661	1987	603.591
624	692	p/o	0.703	1989	640
624	701	p/o	0.757	1989	640
624	710	p/o	0.722	1989	640
624	712	p/o	0.649	1989	640
624	722	p/o	0.595	1989	640
625	628	sibs	0.667	1987	603.591
625	629	sibs	0.731	1987	603.591
625	630	sibs	0.927	1987	603.591
625	631	sibs	0.815	1987	603.591
625	703	p/o	0.683	1989	642
626	627	sibs	0.720	1987	399.527
627	760	p/o	0.632	1990	642
628	629	sibs	0.653	1987	603.591
628	630	sibs	0.830	1987	603.591
628	631	sibs	0.775	1987	603.591

628	714	p/o	0.759	1989	595
629	630	sibs	0.794	1987	603.591
629	631	sibs	0.739	1987	603.591
630	631	sibs	0.870	1987	603.591
640	692	p/o	0.762	1989	624
640	701	p/o	0.714	1989	624
640	710	p/o	0.732	1989	624
640	712	p/o	0.762	1989	624
640	722	p/o	0.810	1989	624
641	756	p/o	0.667	1990	624
642	703	p/o	0.683	1989	625
692	701	sibs	0.850	1989	624.640
692	710	sibs	0.821	1989	624.640
692	712	sibs	0.850	1989	624.640
692	722	sibs	0.800	1989	624.640
701	710	sibs	0.872	1989	624.640
701	712	sibs	0.850	1989	624.640
701	722	sibs	0.793	1989	624.640
702	713	sibs	0.759	1989	584.591
702	719	sibs	0.741	1989	584.591
710	712	sibs	0.769	1989	624.640
710	722	sibs	0.718	1989	624.640
712	722	sibs	0.825	1989	624.640
713	719	sibs	0.867	1989	584.591
754	760	sibs	0.880	1990	627.640
Np =	27	$\tilde{X}p$ =	0.651	SDp =	0.153
Ns =	35	$\tilde{X}s$ =	0.740	SDs =	0.123
Np+s =	62	$\tilde{X}p+s$ =	0.701	SDp+s =	0.143

MULTIPLE LINEAR REGRESSION

Dependent Variable: D

Variable		Mean	Parameter Estimate	Standard Error	T for H0: parameter=0
Intercept		a =	-38.985	5.484	-7.109
Year	X =	1985.806	0.020	0.003	7.237

Source	DF	Sum of Squares	Mean Square	F-Value
Model	1.000	0.582	0.582	52.375
Error	60.000	0.667	0.011	
Total	61.000	1.249		

Dependent Mean	Y =	0.701
Root Mean Square Error		0.105
Coefficient of Variation		15.046
R-Square		0.466
Adjusted R-Square		0.457

APPENDIX 4

ROTHSCHILD'S MYNAH: Comparison of the similarity coefficients (D) obtained using probes pSPT19.6 and pSPT18.15.

Bird	Against	D(.6)	D(.15)
624	625	0.615	0.353
624	626	0.545	0.455
624	627	0.560	0.455
624	628	0.759	0.545
624	629	0.714	0.720
624	631	0.812	0.560
624	640	0.593	0.333
624	641	0.533	0.636
624	642	0.370	0.600
625	626	0.600	0.353
625	627	0.522	0.353
625	628	0.667	0.118
625	629	0.692	0.600
625	631	0.800	0.500
625	640	0.560	0.001
625	641	0.643	0.353
625	642	0.400	0.133
626	627	0.842	0.727
626	628	0.522	0.182
626	629	0.273	0.560
626	631	0.538	0.400
626	640	0.381	0.333
626	641	0.417	0.364
626	642	0.190	0.400
627	628	0.538	0.091
627	629	0.320	0.480
627	631	0.552	0.320
627	640	0.417	0.333
627	641	0.519	0.364
627	642	0.167	0.500
628	629	0.690	0.480
628	631	0.788	0.520
628	640	0.500	0.333
628	641	0.516	0.364
628	642	0.286	0.300
629	631	0.750	0.786
629	640	0.444	0.381
629	641	0.533	0.720
629	642	0.296	0.522
631	640	0.580	0.286
631	641	0.647	0.560
631	642	0.387	0.261
640	641	0.759	0.556
640	642	0.615	0.750
641	642	0.483	0.700

MULTIPLE LINEAR REGRESSION

Dependent Variable: D(.6)

Variable		Mean	Parameter Estimate	Standard Error	T for H0: parameter=0
Intercept		a =	0.434	0.063	6.882
pSPT19.15	X =	0.436	0.244	0.134	1.826

Source	DF	Sum of Squares	Mean Square	F-Value
Model	1.000	0.088	0.088	3.333
Error	43.000	1.130	0.026	
Total	44.000	1.217		

Dependent Mean	Y =	0.541
Root Mean Square Error		0.162
Coefficient of Variation		29.973
R-Square		0.072
Adjusted R-Square		0.050

APPENDIX 5

RODRIGUES FRUIT BAT:Similarity coefficients (D) calculated between members of the captive stock at JWPT.

Comparison	Between	D
1258	1260	0.606
1258	1261	0.714
1258	1274	0.769
1258	1275	0.667
1258	1277	0.615
1258	1278	0.846
1258	1279	0.800
1258	1280	0.545
1258	1310	0.667
1258	1341	0.643
1258	1347	0.741
1258	1372	0.815
1260	1261	0.811
1260	1263	0.895
1260	1274	0.727
1260	1275	0.581
1260	1277	0.788
1260	1278	0.667
1260	1279	0.687
1260	1280	0.552
1260	1307	0.857
1260	1309	0.923
1260	1310	0.581
1260	1314	0.842
1260	1316	0.895
1260	1319	0.900
1260	1341	0.686
1260	1347	0.824
1260	1348	0.872
1260	1366	0.889
1260	1372	0.706
1260	1420	0.842
1261	1274	0.643
1261	1275	0.692
1261	1277	0.500
1261	1278	0.643
1261	1279	0.667
1261	1280	0.500
1261	1307	0.824
1261	1309	0.842
1261	1310	0.538
1261	1314	0.865
1261	1316	0.811
1261	1319	0.872
1261	1341	0.533
1261	1347	0.690
1261	1348	0.842
1261	1366	0.800
1261	1372	0.690

1261	1420	0.811
1263	1307	0.914
1263	1309	0.872
1263	1314	0.947
1263	1316	0.947
1263	1319	0.850
1263	1348	0.923
1263	1366	0.833
1263	1420	0.947
1274	1275	0.667
1274	1277	0.769
1274	1278	0.769
1274	1279	0.800
1274	1280	0.545
1274	1310	0.583
1274	1341	0.643
1274	1347	0.889
1274	1372	0.815
1275	1277	0.667
1275	1278	0.750
1275	1279	0.870
1275	1280	0.800
1275	1310	0.818
1275	1341	0.538
1275	1347	0.720
1275	1372	0.800
1277	1278	0.692
1277	1279	0.720
1277	1280	0.636
1277	1310	0.667
1277	1341	0.714
1277	1347	0.815
1277	1372	0.741
1278	1279	0.800
1278	1280	0.636
1278	1310	0.750
1278	1341	0.500
1278	1347	0.741
1278	1372	0.963
1279	1280	0.762
1279	1310	0.783
1279	1341	0.667
1279	1347	0.846
1279	1372	0.846
1280	1310	0.600
1280	1341	0.583
1280	1347	0.609
1280	1372	0.696
1307	1309	0.778
1307	1314	0.914
1307	1316	0.914
1307	1319	0.811
1307	1348	0.889
1307	1366	0.848
1307	1420	0.857
1309	1314	0.872
1309	1316	0.872
1309	1319	0.927
1309	1348	0.900
1309	1366	0.919
1309	1420	0.923
1310	1341	0.462
1310	1347	0.640
1310	1372	0.720
1314	1316	0.947
1314	1319	0.900
1314	1348	0.974
1314	1366	0.889
1314	1420	0.947
1316	1319	0.900
1316	1348	0.974
1316	1366	0.889
1316	1420	0.895
1319	1348	0.927
1319	1366	0.895
1319	1420	0.850
1341	1347	0.690
1341	1372	0.552
1347	1372	0.786
1348	1366	0.919
1348	1420	0.923
1366	1420	0.889
	$\bar{X}$ =	0.772
	SD =	0.127
	N =	131

APPENDIX 6

RODRIGUES FRUIT BAT : Taxon Report ID numbers for individuals contributing samples for allozyme analysis.

	PGD		Est.		Dia.		PGI		MDH
1)	1258	1)	234	1)	1258	1)	1258	1)	234
2)	1260	2)	1264	2)	1260	2)	1260	2)	1264
3)	1261	3)	1265	3)	1261	3)	1261	3)	1265
4)	1263	4)	1285	4)	1263	4)	1263	4)	1285
5)	1271	5)	1287	5)	1271	5)	1271	5)	1287
6)	1274	6)	1289	6)	1274	6)	1274	6)	1289
7)	1275	7)	1290	7)	1275	7)	1275	7)	1290
8)	1277	8)	1292	8)	1277	8)	1277	8)	1292
9)	1278	9)	1293	9)	1278	9)	1278	9)	1293
10)	1279	10)	1313	10)	1279	10)	1279	10)	1313
11)	1280	11)	1318	11)	1280	11)	1280	11)	1318
12)	1281	12)	1220	12)	1281	12)	1281	12)	1220
13)	1307	13)	1221	13)	1307	13)	1307	13)	1221
14)	1309	14)	1254	14)	1309	14)	1309	14)	1254
15)	1310	15)	1256	15)	1310	15)	1310	15)	1256
16)	1314	16)	1261	16)	1314	16)	1314	16)	1261
17)	1316	17)	1282	17)	1316	17)	1316	17)	1282
18)	1317			18)	1317	18)	1317		
19)	1319			19)	1319	19)	1319		
20)	1341			20)	1341	20)	1341		
21)	1347			21)	1347	21)	1347		
22)	1348			22)	1348	22)	1348		
23)	1366			23)	1366	23)	1366		
24)	1372			24)	1372	24)	1372		
25)	1420			25)	1420	25)	1420		

APPENDIX 7

BRITISH MERLINS: Similarity coefficients (D) for all comparisons between tested Merlins.

Comparison	Between	D
UK00963	UK01093	0.286
UK00963	UK1475	0.250
UK00963	UK10223	0.111
UK00963	UK10228	0.211
UK00963	UK60659	0.375
UK00963	UK70745	0.154
UK00963	UK70758	0.190
UK00963	UK71311	0.125
UK00963	UK76128	0.000
UK00963	UK77701	0.222
UK00963	0406P	0.250
UK00963	0721P	0.125
UK00963	0721R	0.235
88-00963-1	UK10228	0.200
88-00963-1	UK60659	0.111
88-00963-1	UK7116	0.133
88-00963-1	UK71302	0.000
88-00963-1	UK77701	0.211
88-00963-1	0116R	0.118
88-00963-1	0197R	0.200
88-00963-1	0406P	0.235
88-00963-1	0721P	0.222
88-00963-1	1277P	0.333
88-00963-1	1887P	0.444
88-00963-1	2015R	0.375
88-00963-1	2187R	0.200
88-00963-1	3283P	0.105
88-00963-1	4134R	0.429
UK01093	UK1475	0.348
UK01093	UK10223	0.320
UK01093	UK10228	0.385
UK01093	UK60659	0.348
UK01093	UK70745	0.300
UK01093	UK70758	0.429
UK01093	UK71311	0.348
UK01093	UK76128	0.400
UK01093	UK77701	0.240
UK01093	0406P	0.348
UK01093	0721P	0.522
UK01093	0721R	0.417
UK1475	UK10223	0.200
UK1475	UK10228	0.286
UK1475	UK60659	0.222
UK1475	UK70745	0.400
UK1475	UK70758	0.087
UK1475	UK71311	0.111
UK1475	UK76128	0.300

UK1475	UK77701	0.200
UK1475	0406P	0.333
UK1475	0721P	0.111
UK1475	0721R	0.316
UK10223	UK10228	0.783
UK10223	UK60659	0.300
UK10223	UK70745	0.353
UK10223	UK70758	0.480
UK10223	UK71311	0.400
UK10223	UK76128	0.545
UK10223	UK77701	0.000
UK10223	0197R	0.348
UK10223	0406P	0.500
UK10223	0596R	0.375
UK10223	0721P	0.300
UK10223	0721R	0.286
UK10223	1277P	0.190
UK10223	1736R	0.111
UK10223	1887P	0.286
UK10223	2187R	0.348
UK10223	2386P	0.000
UK10223	3283P	0.182
UK10223	4268R	0.261
UK10223	E0060	0.211
UK10228	UK60659	0.282
UK10228	UK7116	0.421
UK10228	UK70745	0.333
UK10228	UK70758	0.385
UK10228	UK71311	0.286
UK10228	UK76128	0.435
UK10228	UK77701	0.174
UK10228	0116R	0.381
UK10228	0406P	0.571
UK10228	0721P	0.386
UK10228	0721R	0.455
UK10228	3283P	0.261
UK10228	E0060	0.200
UK10228	19-80S	0.000
UK10228	8EM8	0.750
UK10228	88-3	0.000
UK60659	UK7116	0.118
UK60659	UK70745	0.400
UK60659	UK70758	0.261
UK60659	UK71311	0.000
UK60659	UK76128	0.100
UK60659	UK77701	0.098
UK60659	0116R	0.211
UK60659	0406P	0.380
UK60659	0721P	0.222
UK60659	0721R	0.261
UK60659	3283P	0.095
UK60659	E0060	0.111
UK60659	19-80S	0.235
UK60659	8EM8	0.273
UK60659	88-3	0.190
UK7116	UK71302	0.143
UK7116	UK77701	0.222
UK7116	0116R	0.125
UK7116	0197R	0.526
UK7116	0406P	0.500
UK7116	0721R	0.471
UK7116	1277P	0.353
UK7116	1887P	0.471
UK7116	2015R	0.133
UK7116	2187R	0.316
UK7116	3283P	0.444
UK7116	4134R	0.000
UK7116	E0060	0.400
UK7116	19-80S	0.000
UK7116	8EM8	0.211
UK7116	88-3	0.000
UK7116	YOUNG	0.125
UK70745	UK70758	0.400
UK70745	UK71311	0.267
UK70745	UK76128	0.353
UK70745	UK77701	0.248
UK70745	0197R	0.222
UK70745	0406P	0.133
UK70745	0596R	0.364
UK70745	0721P	0.267
UK70745	0721R	0.500
UK70745	1277P	0.250
UK70745	1736R	0.154
UK70745	1887P	0.375
UK70745	2187R	0.222
UK70745	2386P	0.154
UK70745	3283P	0.235
UK70745	4268R	0.444
UK70745	E0060	0.286
UK70745	YOUNG	0.550
UK70758	UK71311	0.609
UK70758	UK76128	0.400
UK70758	UK77701	0.344
UK70758	0116R	0.174
UK70758	0406P	0.261
UK70758	0721P	0.522
UK70758	0721R	0.417
UK70758	1736R	0.381
UK70758	2386P	0.190
UK70758	2515R	0.308
UK70758	3283P	0.240
UK70758	4268R	0.462
UK70758	19-80S	0.286
UK70758	8EM8	0.308
UK70758	88-3	0.160
UK70758	YOUNG	0.390
UK71302	0197R	0.105

UK71302	1277P	0.353
UK71302	1887P	0.235
UK71302	2015R	0.133
UK71302	2187R	0.105
UK71302	4134R	0.154
UK71302	E0060	0.133
UK71302	19-80S	0.143
UK71302	88-3	0.222
UK71302	YOUNG	0.125
UK71311	UK76128	0.400
UK71311	UK77701	0.400
UK71311	0197R	0.095
UK71311	0406P	0.222
UK71311	0596R	0.143
UK71311	0721P	0.333
UK71311	0721R	0.316
UK71311	1277P	0.316
UK71311	1736R	0.250
UK71311	1887P	0.211
UK71311	2187R	0.190
UK71311	2386P	0.000
UK71311	3283P	0.500
UK71311	4268R	0.381
UK71311	E0060	0.353
UK76128	UK77701	0.000
UK76128	0116R	0.200
UK76128	0406P	0.300
UK76128	0721P	0.300
UK76128	0721R	0.380
UK76128	1736R	0.111
UK76128	2386P	0.222
UK76128	2515R	0.435
UK76128	3285R	0.273
UK76128	4268R	0.261
UK76128	19-80S	0.222
UK76128	8EM8	0.261
UK76128	88-3	0.364
UK77701	0116R	0.100
UK77701	0406P	0.200
UK77701	0721P	0.200
UK77701	0721R	0.286
UK77701	1736R	0.222
UK77701	2386P	0.111
UK77701	2515R	0.174
UK77701	3283P	0.182
UK77701	4268R	0.348
UK77701	E0060	0.211
UK77701	19-80S	0.222
UK77701	8EM8	0.087
UK77701	88-3	0.091
UK77701	YOUNG	0.390
0116R	0406P	0.222
0116R	0721R	0.211
0116R	1736R	0.000
0116R	2386P	0.125
0116R	2515R	0.190
0116R	3283P	0.200
0116R	4268R	0.286
0116R	E0060	0.000
0116R	19-80S	0.250
0116R	8EM8	0.476
0116R	88-3	0.200
0197R	0596R	0.471
0197R	0721P	0.190
0197R	1277P	0.273
0197R	1736R	0.105
0197R	1887P	0.364
0197R	2015R	0.300
0197R	2187R	0.333
0197R	2386P	0.211
0197R	3283P	0.174
0197R	4134R	0.111
0197R	4268R	0.250
0197R	E0060	0.300
0197R	19-80S	0.211
0197R	88-3	0.261
0197R	YOUNG	0.000
0406P	0721P	0.333
0406P	0721R	0.316
0406P	3283P	0.400
0406P	E0060	0.000
0406P	19-80S	0.250
0406P	8EM8	0.381
0406P	88-3	0.200
0596R	0721P	0.286
0596R	1277P	0.267
0596R	1736R	0.167
0596R	1887P	0.400
0596R	2187R	0.471
0596R	2386P	0.000
0596R	3283P	0.375
0596R	4268R	0.353
0596R	E0060	0.308
0721P	0721R	0.316
0721P	1277P	0.632
0721P	1736R	0.500
0721P	1887P	0.737
0721P	2187R	0.381
0721P	2386P	0.000
0721P	3283P	0.300
0721P	4268R	0.286
0721P	E0060	0.353
0721R	3283P	0.286
0721R	E0060	0.333
0721R	19-80S	0.118

0721R	8EM8	0.273
0721R	88-3	0.095
1277P	1736R	0.235
1277P	1887P	0.700
1277P	2015R	0.222
1277P	2187R	0.273
1277P	2386P	0.235
1277P	3283P	0.286
1277P	4134R	0.375
1277P	4268R	0.364
1277P	E0060	0.556
1277P	19-80S	0.118
1277P	88-3	0.095
1277P	YOUNG	0.211
1736R	1887P	0.471
1736R	2187R	0.211
1736R	2386P	0.000
1736R	2515R	0.105
1736R	3283P	0.222
1736R	4268R	0.316
1736R	E0060	0.533
1736R	19-80S	0.000
1736R	8EM8	0.211
1736R	88-3	0.000
1887P	2015R	0.300
1887P	2187R	0.303
1887P	2386P	0.235
1887P	3283P	0.381
1887P	4134R	0.111
1887P	4268R	0.364
1887P	E0060	0.484
1887P	19-80S	0.211
1887P	88-3	0.261
1887P	YOUNG	0.000
2015R	2187R	0.500
2015R	4134R	0.143
2015R	E0060	0.250
2015R	19-80S	0.133
2015R	88-3	0.211
2015R	YOUNG	0.235
2187R	2386P	0.211
2187R	3283P	0.174
2187R	4134R	0.111
2187R	4268R	0.417
2187R	E0060	0.300
2187R	19-80S	0.105
2187R	88-3	0.174
2187R	YOUNG	0.095
2386P	2515R	0.105
2386P	3283P	0.000
2386P	4268R	0.105
2386P	E0060	0.267
2386P	19-80S	0.143
2386P	8EM8	0.105
2386P	88-3	0.111
2515R	3285R	0.522
2515R	4268R	0.667
2515R	19-80S	0.000
2515R	8EM8	0.250
2515R	88-3	0.087
3283P	4268R	0.348
3283P	E0060	0.316
3283P	19-80S	0.111
3283P	8EM8	0.087
3283P	88-3	0.182
4134R	E0060	0.429
4134R	19-80S	0.154
4134R	88-3	0.118
4134R	YOUNG	0.400
4268R	E0060	0.400
4268R	19-80S	0.000
4268R	8EM8	0.417
4268R	88-3	0.000
E0060	19-80S	0.000
E0060	8EM8	0.200
E0060	88-3	0.000
E0060	YOUNG	0.353
19-80S	8EM8	0.105
19-80S	88-3	0.630
19-80S	YOUNG	0.125
8EM8	88-3	0.087
88-3	YOUNG	0.100
	$\bar{X}$ =	0.270
	SD =	0.167
	N =	343

APPENDIX 8

BRITISH MERLINS: Similarity coefficients (D) calculated between individuals using bands hybridised by pPST19.6 and pSPT18.15

Comparison	Between	D(.6)	D(.15)
UK00963	88-00963-1	0.645	0.500
UK00963	UK01093	0.286	0.310
UK00963	UK1475	0.250	0.450
UK00963	UK10228	0.211	0.500
UK00963	UK60659	0.375	0.125
UK00963	UK70745	0.154	0.100
UK00963	UK70758	0.190	0.200
UK00963	UK71311	0.125	0.130
UK00963	UK76128	0.000	0.200
UK00963	0116R	0.343	0.450
UK00963	19-80S	0.176	0.213
UK00963	8EM8	0.429	0.321
UK00963	88-3	0.294	0.307
88-00963-1	UK01093	0.121	0.132
88-00963-1	UK1475	0.483	0.520
88-00963-1	UK10228	0.200	0.210
88-00963-1	UK60659	0.111	0.176
88-00963-1	UK70745	0.276	0.251
88-00963-1	UK70758	0.229	0.299
88-00963-1	UK71311	0.059	0.104
88-00963-1	UK76128	0.258	0.500
88-00963-1	0116R	0.118	0.184
88-00963-1	19-80S	0.160	0.092
88-00963-1	8EM8	0.424	0.325
88-00963-1	88-3	0.194	0.248
UK01093	UK1475	0.348	0.385
UK01093	UK10228	0.385	0.385
UK01093	UK60659	0.348	0.310
UK01093	UK70745	0.300	0.175
UK01093	UK70758	0.429	0.612
UK01093	UK71311	0.348	0.350
UK01093	UK76128	0.400	0.250
UK01093	0116R	0.500	0.214
UK01093	19-80S	0.133	0.210
UK01093	8EM8	0.211	0.300
UK01093	88-3	0.111	0.167
UK1475	UK10228	0.286	0.235
UK1475	UK60659	0.222	0.247
UK1475	UK70745	0.400	0.368
UK1475	UK70758	0.087	0.063
UK1475	UK71311	0.111	0.132
UK1475	UK76128	0.300	0.400
UK1475	0116R	0.267	0.260
UK1475	19-80S	0.231	0.468
UK1475	8EM8	0.167	0.157
UK1475	88-3	0.063	0.063
UK10228	UK60659	0.282	0.265
UK10228	UK70745	0.333	0.354
UK10228	UK70758	0.385	0.426
UK10228	UK71311	0.286	0.324
UK10228	UK76128	0.435	0.378
UK10228	0116R	0.381	0.400
UK10228	19-80S	0.000	0.063
UK10228	8EM8	0.750	0.685
UK10228	88-3	0.000	0.120
UK60659	UK70745	0.400	0.320
UK60659	UK70758	0.261	0.420
UK60659	UK71311	0.000	0.000
UK60659	UK76128	0.100	0.130
UK60659	0116R	0.211	0.250
UK60659	19-80S	0.235	0.320
UK60659	8EM8	0.273	0.300
UK60659	88-3	0.190	0.240
UK70745	UK70758	0.400	0.400
UK70745	UK71311	0.267	0.250
UK70745	UK76128	0.353	0.310
UK70745	0116R	0.467	0.512
UK70745	19-80S	0.308	0.421
UK70745	8EM8	0.294	0.263
UK70745	88-3	0.063	0.000
UK70758	UK71311	0.609	0.503
UK70758	UK76128	0.400	0.290
UK70758	0116R	0.174	0.200
UK70758	19-80S	0.286	0.290
UK70758	8EM8	0.308	0.346
UK70758	88-3	0.160	0.175
UK71311	UK76128	0.400	0.345
UK71311	0116R	0.171	0.235
UK71311	19-80S	0.129	0.130
UK71311	8EM8	0.205	0.260
UK71311	88-3	0.000	0.000
UK76128	0116R	0.200	0.260
UK76128	19-80S	0.222	0.190
UK76128	8EM8	0.261	0.245
UK76128	88-3	0.364	0.374
0116R	19-80S	0.250	0.260
0116R	8EM8	0.476	0.284
0116R	88-3	0.200	0.300
19-80S	8EM8	0.105	0.125
19-80S	88-3	0.630	0.750
8EM8	88-3	0.087	0.104

MULTIPLE LINEAR REGRESSION

Dependent Variable:

Variable		Mean	Parameter Estimate	Standard Error	T for H0: parameter=0
Intercept		a =	0.034	0.021	1.648
D(.15)	X =	0.279	0.825	0.066	12.517

Source	DF	Sum of Squares	Mean Square	F-Value
Model	1.000	1.293	1.293	156.665
Error	89.000	0.735	0.008	
Total	90.000	2.028		

Dependent Mean	Y =	0.264
Root Mean Square Error		0.091
Coefficient of Variation		34.353
R-Square		0.638
Adjusted R-Square		0.634

APPENDIX 9

NEW ZEALAND FALCONS: Similarity coefficients (D) and genes shared.

Comparison	Between	Simil. Coeff.	Genes shared
UK02710	UK02718	0.198	0.000
UK02710	UK61667	0.037	0.000
UK02710	1935V	0.458	0.250
UK02710	0150U	0.552	0.500
UK02710	0014U	0.445	0.250
UK02710	0255V	0.560	0.500
UK02710	0210V	0.607	0.250
UK02710	0143U	0.572	0.500
UK02710	0011U	0.569	0.500
UK02710	UK03286	0.396	0.000
UK02710	0112U	0.600	0.500
UK02710	2688V	0.467	0.375
UK02710	2266V	0.395	0.125
UK02710	1986V	0.414	0.250
UK02710	2284V	0.320	0.125
UK02710	03286-5	0.370	0.250
UK02710	0255V-6	0.381	0.125
UK02710	0051U	0.396	0.500
UK02710	0118U	0.366	0.500
UK02710	2259V	0.314	0.125
UK02710	2234V	0.515	0.500
UK02710	UK61643	0.308	0.000
UK02710	0005U	0.556	0.500
UK02710	0054U	0.545	0.500
UK02710	0147U	0.500	0.500
UK02718	0143U	0.526	0.500
UK02718	0255V	0.451	0.500
UK02718	0150U	0.593	0.500
UK02718	0011U	0.441	0.500
UK02718	0112U	0.575	0.500
UK02718	UK03286	0.393	0.000
UK02718	0210V	0.511	0.250
UK02718	1935V	0.438	0.250
UK02718	0014U	0.391	0.250
UK02718	UK61667	0.341	0.000
UK02718	0051U	0.707	0.500
UK02718	0118U	0.479	0.500
UK02718	UK61643	0.500	0.000
UK02718	1986V	0.444	0.250
UK02718	03286-5	0.091	0.250
UK02718	2284V	0.500	0.125
UK02718	2266V	0.302	0.125
UK02718	2259V	0.547	0.125
UK02718	2688V	0.400	0.375
UK02718	0255V-6	0.250	0.125
UK02718	0005U	0.629	0.500
UK02718	0054U	0.562	0.500
UK02718	2234V	0.468	0.500
UK02718	0147U	0.581	0.500
UK03286	UK61667	0.279	0.000

UK03286	1935V	0.643	0.500
UK03286	0150U	0.279	0.000
UK03286	0014U	0.378	0.500
UK03286	0255V	0.309	0.000
UK03286	0210V	0.434	0.500
UK03286	0143U	0.375	0.000
UK03286	0011U	0.181	0.000
UK03286	0112U	0.386	0.000
UK03286	2234V	0.400	0.000
UK03286	0118U	0.291	0.000
UK03286	1176V-2	0.385	0.500
UK03286	0255V-1	0.500	0.000
UK03286	0255V-4	0.545	0.000
UK03286	0255V-6	0.421	0.000
UK03286	03286-5	0.643	0.500
UK03286	03286-8	0.687	0.500
UK03286	0059U	0.519	0.000
UK03286	0122U	0.400	0.500
UK03286	0074U	0.381	0.500
UK03286	2259V	0.560	0.500
UK03286-5	1935V	0.382	0.500
UK03286-5	2688V	0.519	0.313
UK03286-5	0014U	0.300	0.375
UK03286-5	2266V	0.435	0.313
UK03286-5	2284V	0.182	0.313
UK03286-5	0011U	0.381	0.500
UK03286-5	0255V-6	0.161	0.125
UK03286-5	0051U	0.000	0.250
UK03286-5	0118U	0.105	0.250
UK03286-5	2259V	0.348	0.313
UK03286-5	0143U	0.381	0.250
UK03286-5	1176V-2	0.308	0.313
UK03286-5	0255V-1	0.333	0.125
UK03286-5	0255V-4	0.455	0.125
UK03286-5	03286-8	0.687	0.500
UK03286-5	0074U	0.286	0.313
UK03286-5	0059U	0.370	0.250
UK03286-5	0122U	0.480	0.500
UK03286-8	1176V-2	0.467	0.313
UK03286-8	0255V-1	0.357	0.125
UK03286-8	0255V-4	0.462	0.125
UK03286-8	0255V-6	0.385	0.125
UK03286-8	0137U	0.522	0.125
UK03286-8	0059U	0.516	0.250
UK03286-8	0122U	0.483	0.500
UK03286-8	0074U	0.320	0.313
UK03286-8	1935V	0.610	0.500
UK03286-8	2688V	0.526	0.313
UK03286-8	0053U	0.526	0.250
UK03286-8	0153U	0.421	0.250
UK03286-8	0115U	0.316	0.250
UK03286-8	0079U	0.500	0.313
UK03286-8	0010U	0.632	0.250
UK03286-8	0150U	0.444	0.250
UK03286-8	0058U	0.353	0.313

UK61643	UK61667	0.100	0.000
UK61643	0210V	0.182	0.000
UK61643	0143U	0.480	0.000
UK61643	0011U	0.462	0.000
UK61643	2259V	0.333	0.000
UK61643	1935V	0.286	0.000
UK61643	0255V	0.320	0.000
UK61643	0014U	0.211	0.000
UK61643	2266V	0.320	0.000
UK61643	1986V	0.261	0.000
UK61667	1935V	0.169	0.000
UK61667	0150U	0.292	0.000
UK61667	0014U	0.224	0.000
UK61667	0255V	0.200	0.000
UK61667	0210V	0.163	0.000
UK61667	0143U	0.205	0.000
UK61667	0011U	0.199	0.000
UK61667	0112U	0.291	0.000
UK61667	2234V	0.105	0.000
UK61667	0118U	0.316	0.000
UK61667	2468V	0.061	0.000
UK61667	2688V	0.229	0.000
UK61667	0058U	0.250	0.000
UK61667	0153U	0.368	0.000
UK61667	0115U	0.250	0.000
UK61667	0079U	0.258	0.000
UK61667	0101U	0.320	0.000
UK61667	0047U	0.148	0.000
UK61667	0131U	0.222	0.000
UK61667	0010U	0.118	0.000
UK61667	2259V	0.250	0.000
UK61667	2266V	0.240	0.000
UK61667	1986V	0.000	0.000
0005U	0143U	0.606	0.500
0005U	0015U	0.703	0.500
0005U	0054U	0.703	0.500
0005U	2234V	0.706	0.500
0005U	0118U	0.429	0.500
0005U	0112U	0.629	0.500
0005U	0147U	0.500	0.500
0010U	2468V	0.894	0.500
0010U	2688V	0.535	0.375
0010U	0210V	0.512	0.250
0010U	0058U	0.429	0.375
0010U	0153U	0.450	0.500
0010U	0115U	0.565	0.500
0010U	0079U	0.471	0.375
0010U	0101U	0.359	0.250
0010U	0047U	0.390	0.250
0010U	0131U	0.390	0.250
0010U	0137U	0.500	0.250
0010U	0255-6	0.250	0.250
0010U	0053U	0.500	0.500

0010U	1935V	0.444	0.250
0010U	0150U	0.400	0.500
0011U	1935V	0.394	0.500
0011U	0150U	0.492	0.500
0011U	0014U	0.492	0.250
0011U	0255V	0.440	0.500
0011U	0210V	0.595	0.500
0011U	0143U	0.518	0.500
0011U	0112U	0.612	0.500
0011U	2688V	0.500	0.375
0011U	2266V	0.354	0.125
0011U	1986V	0.414	0.500
0011U	2284V	0.269	0.125
0011U	0255V-6	0.133	0.250
0011U	0051U	0.286	0.500
0011U	0118U	0.398	0.500
0011U	2259V	0.280	0.125
0011U	2234V	0.385	0.500
0011U	0039U	0.615	0.500
0011U	0122U	0.133	0.500
0011U	0101U	0.267	0.500
0011U	0131U	0.471	0.500
0011U	0046U	0.000	0.000
0011U	0116U	0.435	0.000
0011U	0050U	0.429	0.500
0011U	0255V-1	0.235	0.250
0011U	0059U	0.625	0.500
0011U	0047U	0.231	0.250
0011U	0672V	0.148	0.000
0011U	0110U	0.296	0.125
0011U	0158U	0.345	0.125
0011U	0159U	0.296	0.125
0011U	0156U	0.222	0.125
0011U	0074U	0.364	0.125
0014U	1935V	0.527	0.375
0014U	0150U	0.474	0.250
0014U	0255V	0.396	0.250
0014U	0210V	0.477	0.375
0014U	0143U	0.440	0.250
0014U	0112U	0.499	0.250
0014U	2688V	0.256	0.375
0014U	2266V	0.464	0.625
0014U	1986V	0.545	0.375
0014U	2284V	0.278	0.625
0014U	0255V-6	0.143	0.125
0014U	0051U	0.400	0.250
0014U	0118U	0.334	0.250
0014U	2259V	0.441	0.625
0014U	0047U	0.621	0.500
0014U	0672V	0.067	0.250
0014U	0110U	0.600	0.625
0014U	0158U	0.687	0.625
0014U	0159U	0.467	0.625
0014U	0156U	0.333	0.625
0014U	0074U	0.400	0.625

0014U	0116U	0.529	0.375
0014U	2468V	0.412	0.250
0014U	0115U	0.516	0.250
0014U	0153U	0.400	0.250
0014U	0155U	0.581	0.250
0014U	0053U	0.296	0.250
0014U	0079U	0.516	0.375
0014U	0058U	0.483	0.375
0014U	2234V	0.300	0.250
0039U	1935V	0.615	0.500
0039U	0122U	0.286	0.500
0039U	0101U	0.143	0.500
0039U	0131U	0.625	0.500
0039U	0210V	0.375	0.500
0039U	0046U	0.125	0.250
0039U	0116U	0.500	0.500
0039U	0050U	0.462	0.250
0039U	0255V-1	0.250	0.125
0039U	0059U	0.400	0.250
0046U	1935V	0.235	0.250
0046U	0122U	0.333	0.250
0046U	0101U	0.444	0.250
0046U	0131U	0.200	0.250
0046U	0210V	0.100	0.250
0046U	0116U	0.400	0.250
0046U	0050U	0.118	0.000
0046U	0255V-1	0.100	0.000
0046U	0059U	0.000	0.000
0047U	0672V	0.296	0.250
0047U	2284V	0.519	0.375
0047U	2266V	0.370	0.375
0047U	2259V	0.429	0.375
0047U	0110U	0.519	0.375
0047U	0158U	0.552	0.375
0047U	0159U	0.370	0.375
0047U	0156U	0.296	0.375
0047U	0074U	0.364	0.375
0047U	1935V	0.480	0.000
0047U	0116U	0.516	0.250
0047U	2468V	0.400	0.250
0047U	2688V	0.524	0.375
0047U	0210V	0.556	0.375
0047U	0058U	0.387	0.375
0047U	0153U	0.364	0.250
0047U	0115U	0.513	0.250
0047U	0079U	0.579	0.375
0047U	0101U	0.375	0.375
0047U	0131U	0.529	0.375
0050U	1935V	0.286	0.250
0050U	0122U	0.400	0.250
0050U	0101U	0.133	0.250
0050U	0131U	0.353	0.250
0050U	0210V	0.353	0.250

0050U	0116U	0.353	0.250
0050U	0255V-1	0.471	0.250
0050U	0059U	0.500	0.500
0051U	1935V	0.364	0.250
0051U	2688V	0.370	0.375
0051U	2266V	0.261	0.125
0051U	2284V	0.455	0.125
0051U	0255V-6	0.333	0.250
0051U	0118U	0.463	0.500
0051U	2259V	0.435	0.125
0051U	0054U	0.359	0.500
0051U	2234V	0.882	0.500
0051U	0112U	0.516	0.500
0051U	0147U	0.500	0.500
0053U	2468V	0.343	0.500
0053U	0115U	0.625	0.500
0053U	0153U	0.500	0.500
0053U	0155U	0.437	0.500
0053U	0059U	0.375	0.500
0053U	0079U	0.353	0.375
0053U	2688V	0.375	0.375
0053U	0058U	0.419	0.375
0053U	2259V	0.214	0.125
0053U	1935V	0.370	0.250
0053U	0137U	0.600	0.250
0053U	0255V-6	0.250	0.250
0053U	0150U	0.400	0.500
0054U	0143U	0.400	0.500
0054U	2234V	0.581	0.500
0054U	0118U	0.400	0.500
0054U	0112U	0.500	0.500
0054U	0147U	0.545	0.500
0058U	2468V	0.514	0.375
0058U	0115U	0.486	0.375
0058U	0153U	0.509	0.375
0058U	0155U	0.529	0.375
0058U	0079U	0.649	0.563
0058U	2688V	0.568	0.563
0058U	2259V	0.333	0.188
0058U	1935V	0.375	0.313
0058U	0059U	0.706	0.625
0058U	0210V	0.303	0.313
0058U	0101U	0.552	0.313
0058U	0131U	0.516	0.313
0058U	0137U	0.667	0.188
0058U	0255V-6	0.143	0.188
0058U	0150U	0.308	0.375
0059U	1935V	0.408	0.250
0059U	0122U	0.284	0.250
0059U	0101U	0.235	0.250
0059U	0131U	0.421	0.250

0059U	0210V	0.421	0.250
0059U	0116U	0.421	0.250
0059U	0255V-1	0.385	0.250
0059U	2468V	0.564	0.500
0059U	0115U	0.667	0.500
0059U	0153U	0.571	0.500
0059U	0155U	0.611	0.500
0059U	2688V	0.595	0.625
0059U	2259V	0.357	0.125
0059U	0079U	0.684	0.625
0059U	1176V-2	0.560	0.625
0059U	0255V-4	0.286	0.250
0059U	0255V-6	0.333	0.250
0059U	0074U	0.100	0.125
0074U	0672V	0.348	0.625
0074U	2284V	0.435	0.500
0074U	2266V	0.435	0.500
0074U	2259V	0.333	0.500
0074U	0110U	0.261	0.500
0074U	0158U	0.240	0.500
0074U	0159U	0.522	0.500
0074U	0156U	0.435	0.500
0074U	1935V	0.444	0.313
0074U	0116U	0.296	0.313
0074U	1176V-2	0.316	0.219
0074U	0255V-1	0.353	0.063
0074U	0255V-4	0.400	0.063
0074U	0255V-6	0.333	0.063
0074U	0122U	0.444	0.313
0079U	2468V	0.517	0.375
0079U	0115U	0.615	0.375
0079U	0153U	0.487	0.375
0079U	0155U	0.579	0.375
0079U	2688V	0.729	0.563
0079U	2259V	0.471	0.188
0079U	1935V	0.485	0.313
0079U	0210V	0.500	0.313
0079U	0101U	0.556	0.313
0079U	0131U	0.474	0.313
0079U	0137U	0.667	0.188
0079U	0255V-6	0.471	0.188
0079U	0150U	0.625	0.375
0101U	1935V	0.133	0.500
0101U	0122U	0.000	0.500
0101U	0131U	0.417	0.500
0101U	0210V	0.428	0.500
0101U	0116U	0.444	0.500
0101U	0255V-1	0.222	0.125
0101U	2468V	0.316	0.250
0101U	2688V	0.600	0.313
0101U	0153U	0.581	0.250
0101U	0115U	0.432	0.250
0110U	0672V	0.429	0.625

0110U	2284V	0.429	0.500
0110U	2266V	0.500	0.500
0110U	2259V	0.345	0.500
0110U	0158U	0.800	0.500
0110U	0159U	0.714	0.500
0110U	0156U	0.500	0.500
0110U	1935V	0.154	0.313
0110U	0116U	0.562	0.313
0112U	1935V	0.484	0.250
0112U	0150U	0.607	0.500
0112U	0255V	0.608	0.500
0112U	0210V	0.627	0.250
0112U	0143U	0.481	0.500
0112U	2259V	0.552	0.125
0112U	2234V	0.489	0.500
0112U	2266V	0.333	0.125
0112U	0118U	0.379	0.500
0112U	0147U	0.516	0.500
0115U	2468V	0.574	0.500
0115U	0153U	0.631	0.500
0115U	0155U	0.722	0.500
0115U	2688V	0.450	0.375
0115U	2259V	0.250	0.125
0115U	1935V	0.444	0.250
0115U	0120V	0.488	0.250
0115U	0131U	0.462	0.250
0115U	0137U	0.400	0.250
0115U	0255V-6	0.500	0.250
0115U	0150U	0.533	0.500
0116U	1935V	0.343	0.500
0116U	0122U	0.556	0.500
0116U	0131U	0.800	0.500
0116U	0210V	0.500	0.500
0116U	0255V-1	0.100	0.125
0116U	0672V	0.250	0.250
0116U	2284V	0.375	0.313
0116U	2266V	0.375	0.313
0116U	2259V	0.303	0.313
0116U	0158U	0.588	0.313
0116U	0159U	0.562	0.313
0116U	0156U	0.250	0.313
0118U	1935V	0.294	0.250
0118U	2688V	0.273	0.375
0118U	2266V	0.333	0.125
0118U	2284V	0.588	0.125
0118U	0255V-6	0.615	0.250
0118U	2259V	0.405	0.125
0118U	0143U	0.459	0.500
0118U	2234V	0.573	0.500
0118U	0147U	0.250	0.500
0118U	0210V	0.420	0.250
0118U	0255V	0.438	0.500
0118U	0150U	0.640	0.500

0122U	1935V	0.137	0.500
0122U	0131U	0.444	0.500
0122U	0210V	0.333	0.500
0122U	0255V-1	0.206	0.125
0122U	1176V-2	0.435	0.313
0122U	0255V-4	0.421	0.125
0122U	0255V-6	0.125	0.125
0131U	1935V	0.471	0.500
0131U	0210V	0.500	0.500
0131U	0255V-1	0.100	0.125
0131U	2468V	0.250	0.250
0131U	2688V	0.429	0.313
0131U	0153U	0.545	0.250
0137U	0255V-6	0.500	0.500
0137U	2688V	0.500	0.188
0137U	0153U	0.400	0.250
0137U	0150U	0.316	0.250
0137U	1935V	0.545	0.125
0143U	1935V	0.370	0.250
0143U	0150U	0.692	0.500
0143U	0255V	0.557	0.500
0143U	0210V	0.503	0.250
0143U	2688V	0.333	0.375
0143U	2266V	0.267	0.125
0143U	1986V	0.357	0.250
0143U	2284V	0.211	0.125
0143U	0255V-6	0.400	0.250
0143U	2259V	0.217	0.125
0143U	2234V	0.667	0.500
0143U	0147U	0.414	0.500
0147U	2234V	0.267	0.500
0150U	1935V	0.381	0.250
0150U	0255V	0.567	0.500
0150U	0210V	0.495	0.250
0150U	0255V-6	0.533	0.250
0150U	2688V	0.400	0.375
0150U	0153U	0.535	0.500
0150U	2234V	0.480	0.500
0153U	2468V	0.442	0.500
0153U	0155U	0.514	0.500
0153U	2688V	0.359	0.375
0153U	2259V	0.258	0.125
0153U	1935V	0.444	0.250
0153U	0210V	0.457	0.250
0153U	0255V-6	0.375	0.250
0155U	2468V	0.615	0.500
0155U	2688V	0.486	0.375
0155U	2259V	0.250	0.125
0155U	1935V	0.516	0.250

0156U	0672V	0.571	0.625
0156U	2284V	0.500	0.500
0156U	2266V	0.500	0.500
0156U	2259V	0.621	0.500
0156U	0158U	0.467	0.500
0156U	0159U	0.571	0.500
0156U	1935V	0.462	0.313
0158U	0672V	0.400	0.625
0158U	2284V	0.467	0.500
0158U	2266V	0.400	0.500
0158U	2259V	0.581	0.500
0158U	0159U	0.600	0.500
0158U	1935V	0.286	0.313
0159U	0672V	0.500	0.625
0159U	2284V	0.429	0.500
0159U	2266V	0.500	0.500
0159U	2259V	0.483	0.500
0159U	1935V	0.385	0.313
0210V	1935V	0.607	0.500
0210V	0255V	0.489	0.250
0210V	1986V	0.560	0.500
0210V	0255V-1	0.300	0.125
0210V	2468V	0.381	0.250
0210V	2688V	0.636	0.313
0210V	2259V	0.443	0.313
0210V	2234V	0.444	0.250
0210V	2266V	0.296	0.313
0255V	1935V	0.358	0.250
0255V	2259V	0.389	0.125
0255V	2234V	0.636	0.500
0255V	2266V	0.467	0.125
0255V	1986V	0.357	0.250
0255V-1	1935V	0.118	0.125
0255V-1	1176V-2	0.364	0.188
0255V-1	0255V-4	0.444	0.500
0255V-1	0255V-6	0.535	0.500
0255V-4	1176V-2	0.500	0.188
0255V-4	0255V-6	0.615	0.500
0255V-4	1935V	0.632	0.125
0255V-6	1935V	0.375	0.125
0255V-6	2688V	0.190	0.188
0255V-6	2266V	0.118	0.063
0255V-6	03286V-5	0.250	0.063
0255V-6	2284V	0.161	0.125
0255V-6	0143U	0.118	0.063
0255V-6	1176V-2	0.471	0.188
0672V	2284V	0.643	0.625
0672V	2266V	0.500	0.625

0672V	2259V	0.621	0.625
0672V	1935V	0.385	0.250
1176V-2	1935V	0.522	0.313
1935V	2688V	0.530	0.313
1935V	2266V	0.498	0.313
1935V	1986V	0.667	0.500
1935V	2284V	0.431	0.313
1935V	2259V	0.474	0.313
1935V	2468V	0.294	0.250
1935V	2234V	0.370	0.250
1986V	2266V	0.643	0.313
1986V	0143U	0.593	0.313
2234V	2259V	0.276	0.125
2234V	2266V	0.296	0.125
2259V	2688V	0.413	0.250
2259V	2266V	0.557	0.563
2259V	2284V	0.527	0.563
2259V	2468V	0.343	0.125
2266V	2688V	0.615	0.250
2266V	2284V	0.619	0.563
2284V	2688V	0.480	0.250
2268V	2688V	0.563	0.375

MULTIPLE LINEAR REGRESSION

Dependent Variable: D

Variable		Mean	Parameter Estimate	Standard Error	T for HO: parameter=0
Intercept		a =	0.275	0.011	24.654
Genes shared	X =	0.317	0.456	0.031	14.834

Source	DF	Sum of Squares	Mean Square	F-Value
Model	1.000	3.586	3.586	220.061
Error	541.000	8.815	0.016	
Total	542.000	12.400		

Dependent Mean	Y =	0.420
Root Mean Square Error		0.128
Coefficient of Variation		30.404
R-Square		0.289
Adjusted R-Square		0.288

APPENDIX 10

NEW ZEALAND FALCONS: Similarity coefficients (D) calculated using bands hybridised by pSPT19.6 and pSPT18.15.

Comparison	Between	D(.6)	D(.15)
UK02710	UK02718	0.198	0.370
UK02710	UK03286	0.396	0.303
UK02710	0011U	0.569	0.571
UK02710	0112U	0.600	0.714
UK02710	0118U	0.366	0.571
UK02710	0143U	0.572	0.500
UK02710	0210V	0.607	0.687
UK02710	0255V	0.560	0.538
UK02710	1935V	0.458	0.588
UK02718	UK03286	0.393	0.500
UK02718	UK61667	0.341	0.348
UK02718	0011U	0.441	0.741
UK02718	0014U	0.391	0.471
UK02718	0112U	0.575	0.593
UK02718	0118U	0.479	0.741
UK02718	0143U	0.526	0.609
UK02718	0150U	0.593	0.759
UK02718	0210V	0.511	0.635
UK02718	0255V	0.451	0.670
UK02718	1935V	0.438	0.667
UK02718	2234V	0.468	0.640
UK03286	UK61667	0.279	0.276
UK03286	0011U	0.181	0.364
UK03286	0014U	0.378	0.333
UK03286	0112U	0.386	0.364
UK03286	0118U	0.291	0.323
UK03286	0143U	0.375	0.414
UK03286	0150U	0.279	0.457
UK03286	0210V	0.434	0.575
UK03286	0255V	0.309	0.516
UK03286	1935V	0.643	0.667
UK03286	2234V	0.400	0.581
UK61667	0014U	0.224	0.095
UK61667	0112U	0.291	0.167
UK61667	0118U	0.316	0.320
UK61667	0150U	0.292	0.308
UK61667	0210V	0.163	0.276
UK61667	0255V	0.200	0.261
UK61667	1935V	0.169	0.333
UK61667	2234V	0.105	0.364
0011U	0112U	0.612	0.643
0011U	0118U	0.398	0.714
0011U	0143U	0.518	0.667
0011U	0210V	0.595	0.687
0011U	0255V	0.440	0.692
0011U	1935V	0.394	0.647

0014U	0112U	0.499	0.480
0014U	0118U	0.334	0.462
0014U	0150U	0.474	0.444
0014U	0210V	0.477	0.467
0014U	0255V	0.396	0.583
0014U	1935V	0.527	0.581
0014U	2234V	0.300	0.261
0112U	0118U	0.379	0.714
0112U	0143U	0.481	0.500
0112U	0150U	0.607	0.533
0112U	0210V	0.627	0.667
0112U	0255V	0.608	0.741
0112U	1935V	0.484	0.558
0112U	2234V	0.489	0.462
0118U	0143U	0.459	0.500
0118U	0150U	0.640	0.581
0118U	0210V	0.420	0.562
0118U	0255V	0.438	0.643
0118U	1935V	0.294	0.588
0118U	2234V	0.573	0.519
0143U	0210V	0.503	0.714
0143U	0255V	0.557	0.455
0143U	1935V	0.370	0.600
0150U	0210V	0.495	0.571
0150U	0255V	0.567	0.483
0150U	1935V	0.381	0.611
0150U	2234V	0.480	0.714
0210V	0255V	0.489	0.687
0210V	1935V	0.607	0.840
0210V	2234V	0.444	0.516
0255V	1935V	0.358	0.667
0255V	2234V	0.636	0.480
1935V	2234V	0.370	0.500

MULTIPLE LINEAR REGRESSION

Dependent Variable: D(.6)

Variable		Mean	Parameter Estimate	Standard Error	T for H0: parameter=0
Intercept		a =	0.148	0.040	3.660
D(.15)	X =	0.531	0.541	0.073	7.410

Source	DF	Sum of Squares	Mean Square	F-Value
Model	1.000	0.538	0.538	54.911
Error	77.000	0.755	0.010	
Total	78.000	1.293		

Dependent Mean	Y =	0.435
Root Mean Square Error		0.099
Coefficient of Variation		22.759
R-Square		0.416
Adjusted R-Square		0.409