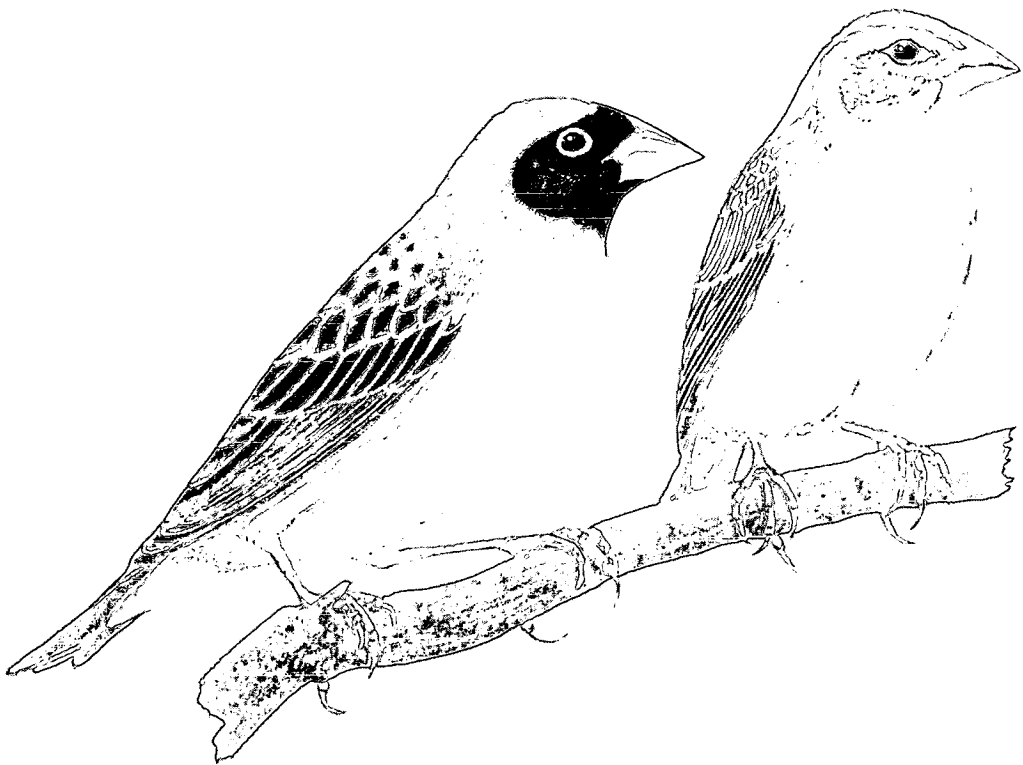


Migration Patterns of the Redbilled Quelea *Quelea quelea* in  
Southern Africa: Genetics, Morphology and Behaviour

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## Abstract

The redbilled quelea *Quelea quelea* is the most abundant bird in the world. Found throughout the drier parts of sub-Saharan Africa, it is a serious pest of grain crops. Better management and control of the redbilled quelea as a pest requires a thorough understanding of its migration patterns. This thesis presents three techniques to analyse the migration patterns of redbilled quelea in southern Africa.

The genetic structure of redbilled quelea from 28 sites across southern Africa was studied using eight polymorphic microsatellite loci. Using indirect molecular evidence no evidence of population division was found. There was no evidence for regular migration pathways or the existence of a migratory divide. However evidence was found for differential dispersal between the sexes. Male quelea dispersed further from their natal flocks than females. In a parentage study, 22.6% of chicks were not related to their social father, while 8.5% of chicks were not related to either of the parents assigned to them by behavioural observations.

The redbilled quelea is sexually dimorphic. Across Africa three subspecies have been described based on the variation in male breeding plumage. Two separate techniques were used to analyse this variation: plumage colour was scored using the human eye and colour variation was assessed using the software package Photoshop. Despite a second subspecies having been described for southern Africa, no evidence was found for geographic variation in male breeding plumage patterns.

Redbilled quelea migration is determined by the regular patterns of seasonal rainfall. In central southern Africa rainfronts approach from two different directions indicating that a migratory divide could exist for redbilled quelea. The direction preferences of redbilled quelea ready to migrate were tested in the wild using Emlen funnels. Two distinct migration directions were preferred by redbilled quelea indicating the possible presence of a migratory divide.

In conclusion, there is no evidence that quelea in southern Africa do not form a panmictic population. However, the behavioural evidence suggests that there is more than one migration pattern that quelea follow in the region. This could have implications for quelea management.

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Lucy Webster, Stuart Blackman and the members of Sporting ICAP FC all added their own special something. Jon Slate, Dave Coltman, Simon Goodman patiently showed me the routines of the lab, and were generally not that annoyed when I broke things. I later realised that this was because they broke things too. Antonis did some sequencing for me, which I've not mentioned in the thesis. Rachel, Dave and Nick Colegrave frequently answered my stats questions. Camilla Blackburn genotyped most of the females for her Zoo 4 project and brought renewed interest into quelea labwork. Finally, my thanks to Becky for, among many things, taking me dancin'.

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# 1 Introduction

The redbilled quelea (*Quelea quelea*) is one of the most abundant birds in the world (Newton 1998). Estimates of population size range as high as 100 billion (Crook & Butterfield 1968). It breeds and feeds in enormous aggregations of individuals that can number many millions. Some describe the sky turning black as flocks of quelea stream overhead. Quelea are also among the most destructive vertebrate pests in the world (Newton 1998), and are responsible for the loss of up to 5% of Africa's grain output (Elliott & Lenton 1989).

Quelea have been an acknowledged threat to crops for centuries (Jackson & Allan 1989). A famine in Ugogo, Tanzania, in 1881 was attributed to a quelea infestation (Haylock 1959). However, it is only since the advent of modern intensive agricultural practices in the 1950s that the threat quelea pose to grain production in Africa has been realised. Government sponsored control programmes, the first in Sudan in 1946 (Disney 1964), involving pesticides and explosives were initiated along with co-ordinated international research programmes into quelea biology and crop damage. Much of this research was limited in its findings by the logistical problems presented by huge population sizes and the large distances that quelea migrate. Understanding the migration patterns of quelea is essential if they are ever to be effectively managed as a pest.

This study uses three different methodologies: genetics, morphology and behaviour, in an attempt to answer some important questions about quelea population structure and migration patterns in southern Africa. The thesis starts by introducing the redbilled quelea in Africa, before examining the specific details of quelea in southern Africa, the region where the study is based. Finally, the aims and structure of the thesis are outlined.

## 1.1 The redbilled quelea *Quelea quelea*

The genus *Quelea* is endemic to sub-Saharan Africa. It consists of three species, the redbilled quelea (*Quelea quelea*) the redheaded quelea (*Q. erythrops*) and the cardinal quelea (*Q. cardinalis*). The genus forms part of the family Ploceidae – the

weaverbirds. *Quelea* are granivorous, with strong conical bills; they build nests woven out of dried grass that are typical of the family. All three species of the genus *Quelea* have sparrow-like plumage and are sexually dimorphic.

The redbilled quelea is the most widespread of the three species. In this thesis, as in common usage, the redbilled quelea will be referred to as ‘the quelea’. Across Africa plumage is highly variable. Males in breeding plumage have a black or white facial mask, with varying degrees of pink or buff on the head. Some of the common plumage patterns are shown in Figure 1.1. Different combinations of plumage colouration are typical of different parts of the continent (Ward 1966). Some of the variation is distinct enough for separate subspecies to have been described for different regions. Even within each region males show polymorphism in their plumage patterning (Ward 1966).

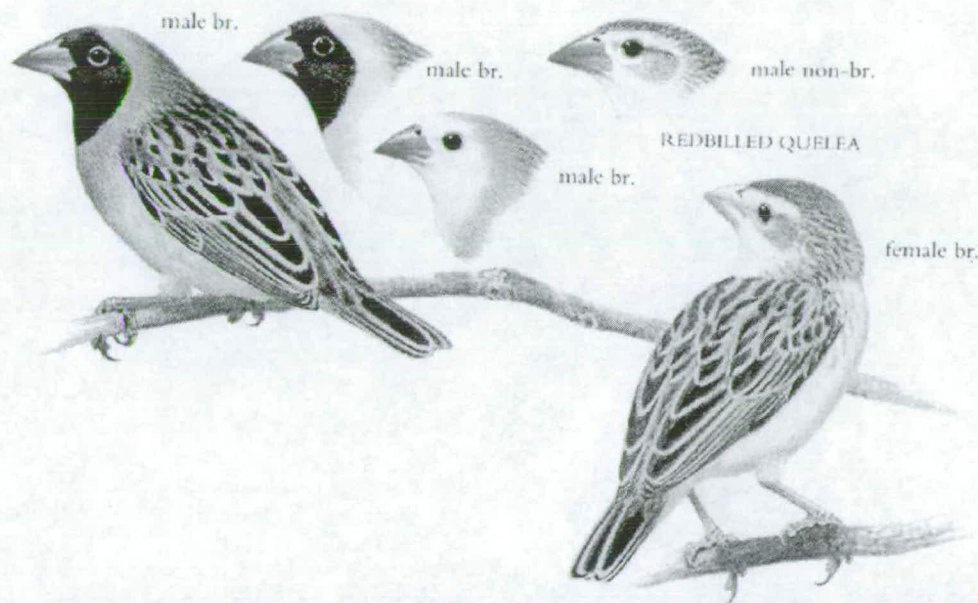


Figure 1.1. The redbilled quelea showing three types of breeding male plumage patterns, a non-breeding male and a breeding female (Sinclair et al. 1993).

### 1.1.1 Distribution

*Quelea* occur throughout the drier parts of sub-Saharan Africa, typically inhabiting arid *Acacia* steppe and shrub savannah. *Quelea* concentrate wherever there is abundant grass seed and plentiful water. They also require dense cover, of thorn trees or reeds, where large aggregations of birds can roost and breed (Allan 1996). Major populations of quelea are found in the inland Niger delta in Mali; the Lake Chad

basin; the Sudan savannahs; the rift valleys of Ethiopia, southern Somalia, Tanzania and Kenya; and in southern Africa, in Botswana, Zimbabwe and South Africa. The distribution of quelea across Africa is shown in Figure 1.2. Map 1 shows Africa with countries mentioned in the text indicated.

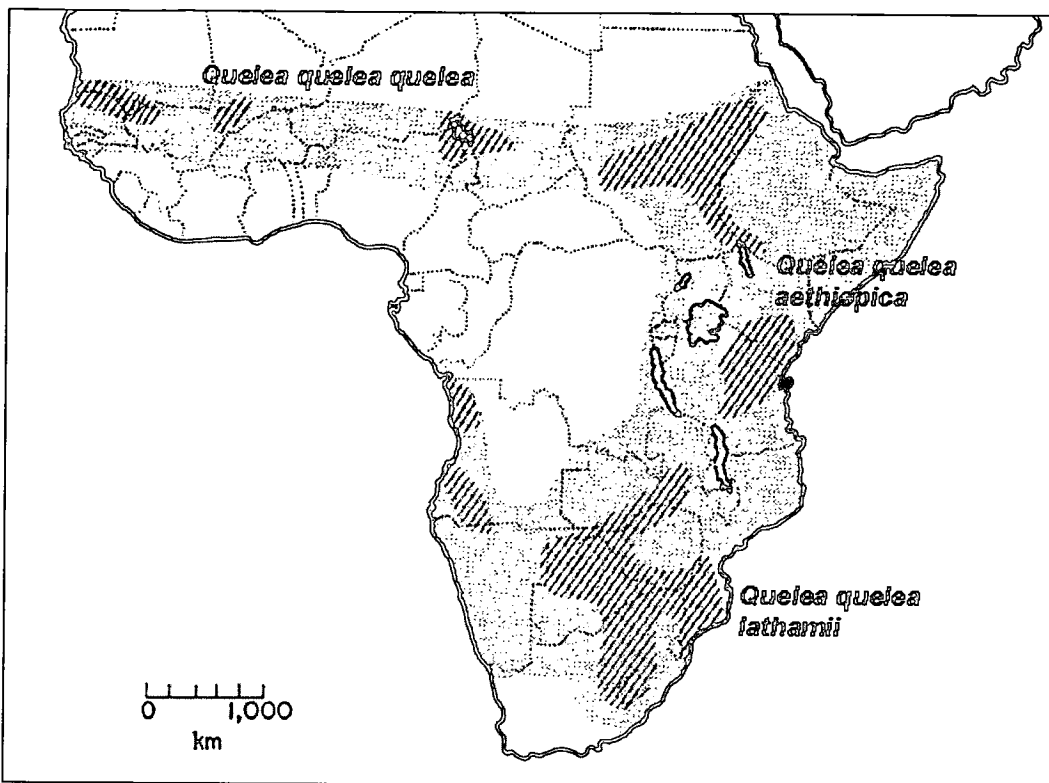


Figure 1.2. The distribution of the redbilled quelea *Quelea quelea* across Africa (shaded). Hatched areas represent the location of the main breeding areas (Ward 1971). The three recognised subspecies are indicated.

### 1.1.2 Diet

Quelea feed on the seeds of annual grasses, principally *Panicum*, *Setaria*, *Urochloa* and *Echinochloa* (Jones & Ward 1976; Jones 1989e). They are therefore pre-adapted to feed on a wide variety of common crops, such as wheat (*Triticum* spp), sorghum (*Sorghum caffrorum*), millet (*Panicum* spp) and rice (*Oryza sativa*). Insects such as termites, caterpillars and beetles are also used as food at the start of the breeding season, as they contain substantially more protein (70% of dry weight compared to 11% for grass seed) (Jones & Ward 1976; Jarvis & Vernon 1989b). At the end of the non-breeding season termites are also an important part of quelea diet (Ward 1965a). Despite feeding on a variety of insects at certain times of the year, quelea remain obligate seed eaters.

### 1.1.3 Subspecies

There are three recognised subspecies of quelea which have been described based on variations in plumage patterns. The nominate *Q.q. quelea* is found in West Africa, *Q.q.aethiopica* in East Africa and *Q.q.lathamii* in southern Africa (Ward 1966) (Figure 1.2).

Male redbilled quelea in breeding plumage show distinct polymorphisms. There are two main areas of plumage that vary. First, male quelea can have either black or white facial mask plumage surrounding the head (Ward 1966). The mask varies further in the extent to which the frontal band of the mask extends above the bill onto the crown. Second, the colour of the plumage on the breast, belly and crown varies from yellow to pink.

Ward (1966) used these variations in plumage patterns to describe the populations of quelea across Africa. He found that the proportion of individuals that were white-faced varied between populations, as did the extent of the frontal band of the face mask and the colouration of the breast and crown. Ward devised a method of scoring the variation in colour patterns, consisting of a 'Mask Index' for the extent of the black frontal band, and an "arbitrary distinction" between pale, medium and dark buff for the crown and breast colour variation.

In West Africa, *Q.q. quelea* was characterised by a facial mask that extends above the bill in a broad band. Similarly, in southern Africa, *lathamii* also had a broad mask above the bill. In eastern Africa, *aethiopica* had the mask restricted to the chin and cheeks, with little, if any spreading to the face above the bill. Quelea from Sudan were palest buff, while in West Africa, birds were darker. For *lathamii* in southern Africa, colouration was so pink ' . . . as to mask the shade of the underlying buff completely.' Lourens (1963) in Whittington-Jones (1999) used similar definition of male colour types, or morphs. In his study, which was restricted to South Africa, males were described as red or buff, and as with or without a dark facial mask. Two other proposed subspecies, *centralis* in eastern Democratic Republic of Congo (DRC) and *intermedia* in southern Somalia, were rejected as the males were too variable and therefore were likely to be 'hybrid swarms'. A third proposed subspecies, *spoliator*, was considered indistinguishable from *lathamii* (Ward 1966).

### 1.1.4 Annual cycle of migration and breeding

Migration is any movement from one temporarily inhabited home area to another (Berthold 1996). This definition is very broad and includes a wide variety of regular and irregular movements. In birds, what distinguishes migration from other forms of dispersal movement is that migrants show a distinct physiological state that includes metabolic adaptations to increase energy supply for the forthcoming journey. These include depositing fat for use as fuel, changes in enzyme composition for storage and then release of energy, hypertrophy of flight muscles and increased haematocrit levels in the blood (Berthold 1996). Berthold (1993) classified quelea movement as 'regular return migration', although there is little evidence that quelea return to the same breeding locations, leading some to argue over their description as true migrants (Tree 1989).

Across Africa, quelea undertake complex long-distance movements throughout the year so that the appearance and disappearance of the birds at a particular location can seem random. Ward (1971) hypothesised that these movements are part of predictable migration patterns based on food availability and rainfall. The Ward hypothesis states that quelea migrate in response to the availability of the annual grass seeds that make up the majority of their food. Such grasses only grow and set seed in the wet season and hence seasonal food availability for the quelea is determined by the patterns of rainfall. There are two elements to the Ward hypothesis. First, quelea move to avoid the rains; second, as the rains are seasonal and predictable, so it should be possible to predict quelea movements.

#### 1.1.4.1 The Ward hypothesis for quelea migration

Rainfall in Africa is highly seasonal; it is concentrated in wet seasons lasting several months and separated by dry seasons when little, if any, rain falls. Rainfall across Africa is controlled by the Inter-Tropical Convergence Zone (ITCZ) which is where northern and southern air masses meet. Rain is produced across a broad latitudinal band. The ITCZ moves north and south across the equator following the sun. North of the equator rain falls between March and November and in the southern tropics between September and May. Wherever the ITCZ goes it brings rain, but the

amounts can be variable (Thompson 1965). The pattern of rainfall in Africa is illustrated in Figure 1.3. The rain patterns north and south of the equator mirror each other but are six months out of phase, although, as discussed in Section 1.2, the rains are more complex in southern Africa. In general the rains further away from the equator start later and end sooner.

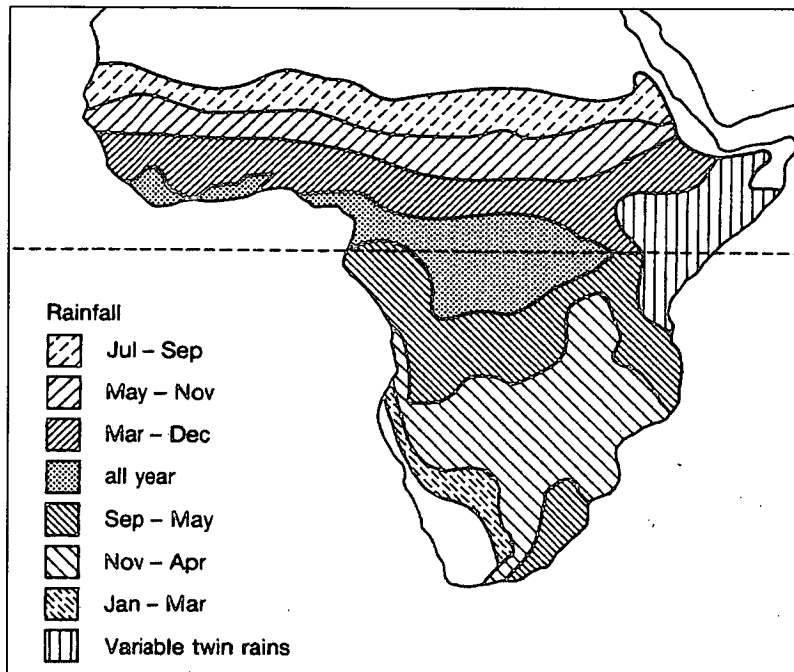


Figure 1.3. Rainfall patterns in sub-Saharan Africa. The dotted line is the equator (Jones 1989e).

During the dry season quelea spend their time searching for food and water. They mainly survive on the seeds of annual grasses produced during the previous wet season (Ward 1965a). As the dry season progresses, the amount of food diminishes. Quelea start to congregate in ever-larger aggregations and roosts near to reliable food and water sources. When the rains brought by the ITCZ arrive the grass seed on which the quelea have been feeding suddenly germinates and begins to grow. Quelea are left in a situation of severe food shortage which requires them to move away from the area in which they have spent the dry season. A schematic representation of seasonal food availability to quelea is shown in Figure 1.4.

A schematic representation of quelea migration in relation to the rains is shown in Figure 1.5. Faced with food shortage, quelea fly ahead of the advancing rainfront to an area where it has not yet rained and there is still adequate ungerminated seed (Figure 1.5 a). However, eventually the rains catch up and quelea are forced to find

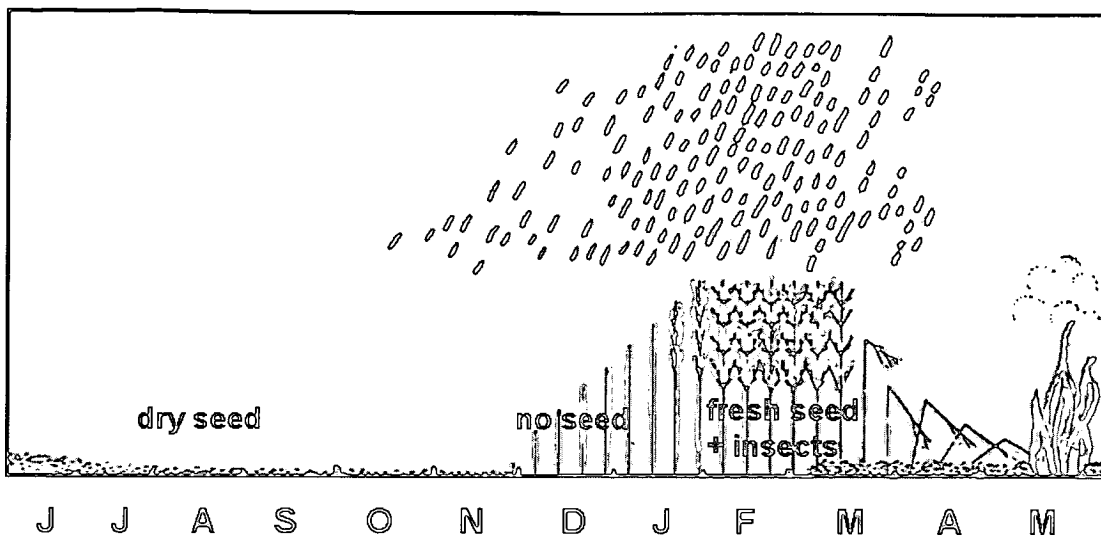


Figure 1.4. Schematic diagram to illustrate seasonal changes in food availability for the quelea. This example follows food availability in Zimbabwe where quelea start to breed in January.

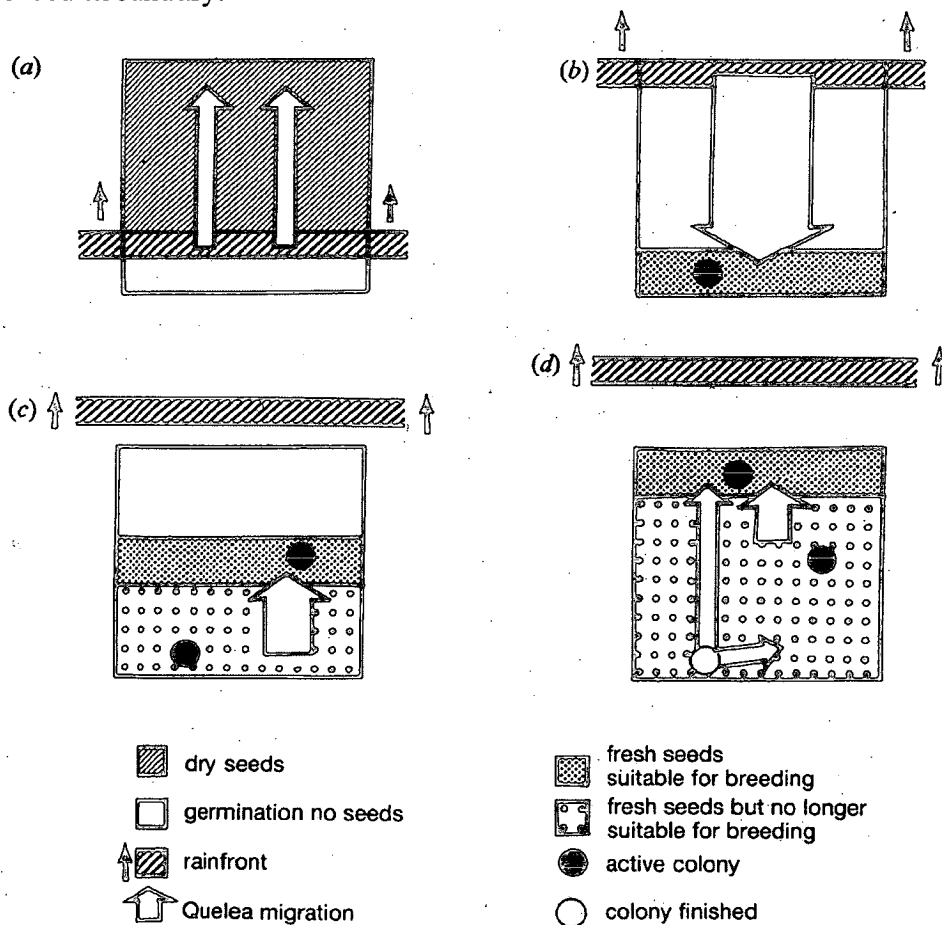


Figure 1.5. Quelea migration patterns (Jones 1989e) (a) quelea in early rains areas are forced ahead of the rains; (b) early rains migration; (c) breeding migration; (d) breeding migration and itinerant breeding.

find areas where fresh seed has already been set in the wake of the rain. For many annual grasses this takes between 6 and 8 weeks. Therefore quelea are eventually forced to fly back over the rainfront to a region where rain fell a sufficiently long period ago for fresh seed to be available (Figure 1.5 b).

Quelea that fly ahead of the rainfront are likely to find food within a relatively short distance. However birds flying back over the rainfront may have to cross a wide region where there is no available food. The width of this region depends on how fast the rainfront moves. If, as in East Africa, the rains move quickly, then quelea have to fly a substantial distance (up to 1200 km) to reach a region where fresh grass seed has set. Even in West Africa, where the rainfronts move the slowest, quelea are likely to have to fly about 300 kms to reach areas of fresh seed set (Jones 1989e). The movement associated with crossing the advancing rainfront to areas of freshly set seed is termed the *early rains migration* (Ward 1971) (Figure 1.5 b).

In common with many other migrant birds, quelea lay down deposits of pre-migratory fat to help fuel the trip (Alerstam 1990). Although small compared to many of the longer distance palaeartic migrants, the amount of fat laid down differs between subspecies and is related to the distance migrated (Ward & Jones 1977). In West Africa the rainfront advances about 300 km in 6-8 weeks. To fly this distance quelea lay down up to 2 g of fat. However, in East Africa, quelea must fly as far as 1200 kms. Up to 4.4 g of fat is laid down by these birds. Quelea are helped in accumulating fat reserves at the onset of the rains by the appearance of an important additional food source. Winged termites emerge at the start of the rains and gather in vast numbers as they try and mate. They are easily caught and provide a rich source of fat (Ward & Jones 1977).

The earliest breeding attempts take place when quelea arrive in the zone where fresh grass seed was set the earliest, the so-called *early rains quarters*. Quelea are sometimes almost ready for breeding before undertaking the early rains migration (Ward 1971). However, most quelea are not ready to breed straight away. Suitable conditions for breeding are often short lived, so by the time quelea are ready, the best environmental conditions for breeding will be some distance away in the wake of the advancing rains. Quelea therefore follow the rains on the so-called *breeding*



*migration* (Figure 1.5 c and d) to locate areas for establishing breeding colonies wherever conditions are suitable. If conditions are good, individuals will often attempt to breed more than once. This breeding migration is not strictly migration in that it does not involve a long distance flight across unsuitable habitat, nor are fat reserves laid down prior to migration.

#### 1.1.4.2 Breeding colonies

Breeding colonies are established from about 6 weeks into the rains until the end. Colonies can be very large; one colony in Zimbabwe (Malilangwe) was estimated to be 20 km long by 1 to 2 km wide. Nest density can be as high as 30 000 per hectare (Allan 1996).

Nest building starts when males arrive at a suitable breeding site. Nest building and display can take three or four days. Females arrive after the males, but can start laying eggs rapidly as yolk development is under way before they choose a mate. As suitable conditions for breeding are so short-lived, breeding is highly synchronous. 90% of eggs are laid and hatched within a few days of each other (Crook 1960a; Ward 1965b). Clutch size varies from 2 to 7 eggs, with an average of around 2.8 (Ward 1965b; Jones & Ward 1976). Nestling development is rapid. The young leave the nest within 11 to 13 days, although they are unable to fly until a few days later (Ward 1965a). The entire breeding cycle can be completed in 5 to 6 weeks, after which adult quelea disperse rapidly, leaving the juveniles alone (Ward 1965a).

After a breeding attempt, quelea can resume the breeding migration following the rainfront. Although quelea have only once been recorded breeding in the same place twice in a row (Thompson 1993), up to 20% of females show signs of developing a new clutch (Ward 1971). Quelea are capable of breeding more than once in a season, an ability termed *itinerant breeding*. Itinerant breeding occurs only if suitable conditions are found again. For example, in Ethiopia, colour marked individuals from breeding colonies established in June were retrieved in September further north in the Awash valley in second breeding colonies (Jaeger et al. 1986). Nearly half the adult birds at the second colony showed an interrupted wing moult that was consistent with having bred earlier in the season.

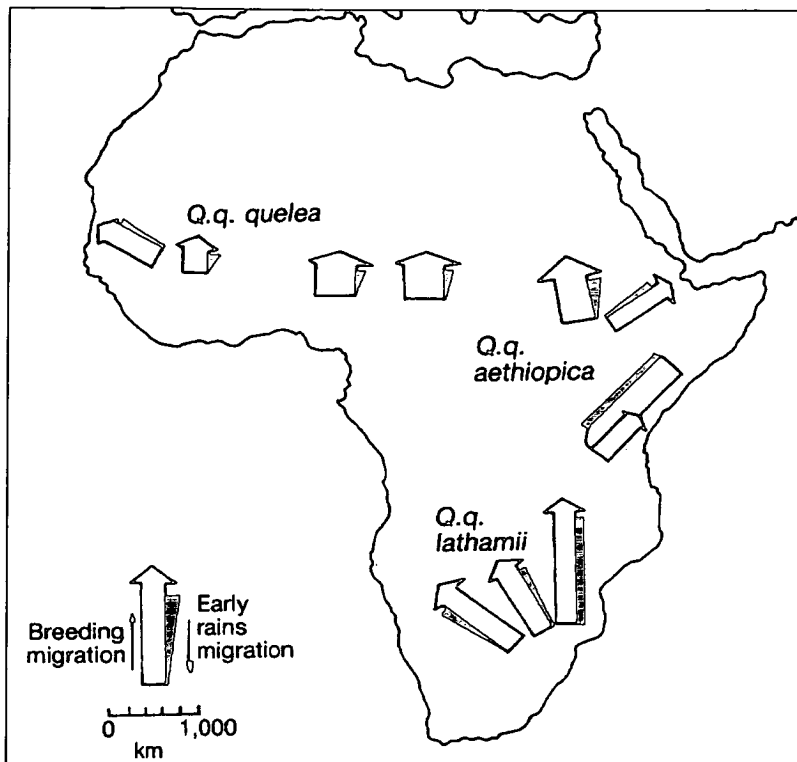


Figure 1.6. Map of Africa showing the suggested migration routes of the three subspecies of quelea. From Jones (1989e).

#### 1.1.4.2.a Migration patterns of quelea subspecies across Africa

The migration patterns for different populations of quelea across Africa are shown in Figure 1.6 (Ward 1971). The illustrated migration patterns are based on the Ward hypothesis and the known passage of rainfronts across the continent. The evidence of quelea movements so far gathered (Bruggers & Elliott 1989; Mundy & Jarvis 1989; Venn et al. 1999; Jones et al. 2000) does provide support for the model, however it remains a hypothesis. The different patterns could play a role in keeping the populations sufficiently isolated that subspecific differences can be maintained. For example, *lathamii* in southern Africa is separated from *aethiopica* by a band of unsuitable habitat. The isolation between the populations is maintained as the patterns of rainfall in the two regions mean that early-rains migrations are in opposite directions. In western Africa, the migration patterns are in parallel. There is no reason for quelea to migrate east-west so population differences can be maintained (Jones 1989e). The situation in southern Africa, where this study is based is discussed in section 1.3.3.

### 1.1.5 *The quelea as a pest*

The redbilled quelea is often cited as the most destructive vertebrate pest in the world (Jackson & Allan 1989; Newton 1998). They are grain eaters which feed off wild grasses, but also off the four most important grain crops in Africa – wheat, rice, sorghum and millet, leading to an immense amount of damage, affecting cereal production in more than 25 countries (Meinzingen 1993). It has been estimated that a relatively modest sized roost of one million birds could destroy as much as 10 tonnes of grain a day (Meinzingen 1993).

Estimates of the percentage crop lost to quelea across Africa range as high as 5% (Elliott & Lenton 1989). However, national and regional estimates of the actual amount of damage solely attributable to quelea are difficult to quantify. On a local scale the impact can be enormous especially to subsistence farmers whose crops can be entirely wiped out by a quelea outbreak (Elliott & Lenton 1989). In many areas, farmers refuse to plant quelea-susceptible crops; several examples come from the 1950s. In 1953 many South African farmers abandoned sorghum after quelea infestations; in 1954, rice production in some areas of Senegal was stopped after 24 acres were destroyed by quelea. In the same year many farmers in Sudan abandoned sorghum after crops were attacked (Disney 1964). More recently, trials of wheat and rice in Swaziland were abandoned due to excessive damage by quelea (Morrison Mbuli pers comm), and the abundance of maize in south east Zimbabwe is partly due to the susceptibility of other crops to quelea (Peter Mundy pers comm).

The impact of quelea on a farming community can therefore be quite severe. In many situations where quelea are threatening crops, the response of the farming community can be dramatic. In Senegal the quelea problem can be traced back hundreds of years (Jackson & Allan 1989) and traditional techniques for dealing with quelea include bird scaring and destroying nests and the trees containing them (Bashir 1989). Chicks are often collected for food, representing an important additional source of protein in rural communities (Elliott & Craig 1999; Mullie 2000). At one colony in southern Zimbabwe (Malilangwe (XX)) in 1998, three and a half tonnes of chicks (roughly a quarter of a million) were collected in three days

In summary, quelea have three impacts on farming communities. First they lead to the loss of crops. Second, time is spent scaring the quelea away from crops or destroying nest sites. Third, the local environment can be degraded through destruction of breeding sites. On the positive side quelea can provide an additional food supply.

The financial and environmental expense of quelea is most acute when government agriculture departments get involved in controlling quelea as a pest. When quelea were first thought of as a problem to agriculture with the advent of large-scale commercial farming in Africa in the 1950s, massive government sponsored programmes of quelea control were initiated (Ward 1979). For example in South Africa between 1954 and 1960 up to 134 million birds were killed by government sponsored control programmes (Ward 1979). The increased mortality of the control operations did not substantially alter natural mortality (Jones 1980) and there was no overall reduction in the number of quelea. Agricultural changes have been cited as the reason for increased numbers of quelea in KwaZulu-Natal (Berruti 1995), the Eastern Cape (Whittington-Jones 1998) and in southern Africa as a whole (Mundy & Herremans 1997).

Large scale control operations involve one of two methods. First, spraying operations use queletox, which contains the avicide organophosphate poison fenthion, which is designed specifically to kill quelea and other small birds. Aerial spraying is commonplace (Geertsema 1999), but backpack and vehicle mounted spraying operations are also carried out (La Grange 1989; Allan 1996). The second control method is even more dramatic. Quelea are blown up with drums of explosive that are raised into the acacia stands where the quelea are breeding. The explosives are detonated after dark when quelea have returned to their nests. Bruggers and Elliott (1989) provide several excellent chapters on quelea control methods in operation.

The ineffectiveness of mass-destruction policies led to a re-think in quelea control (Ward 1979). Present day control actions are only undertaken in cases where quelea are likely to cause imminent damage to crops (Meinzingen 1993; National Department of Agriculture 1994; Jones et al. 2000). Nonetheless there is considerable annual expenditure on quelea control operations. In South Africa alone,

for the 1997/1998 control operations, nearly £400 000 was spent killing an estimated 75 million birds in 200 locations. Over 5000 litres of queletox was sprayed on birds (Geertsema 1998). The South African Directorate of Agricultural Resource Conservation justifies such expense by estimating that £1.3 million of crop damage was prevented. The control programme is therefore seen as enormously beneficial, if expensive. The total number of control actions per year between 1987 and 1999 is shown in Figure 1.7.

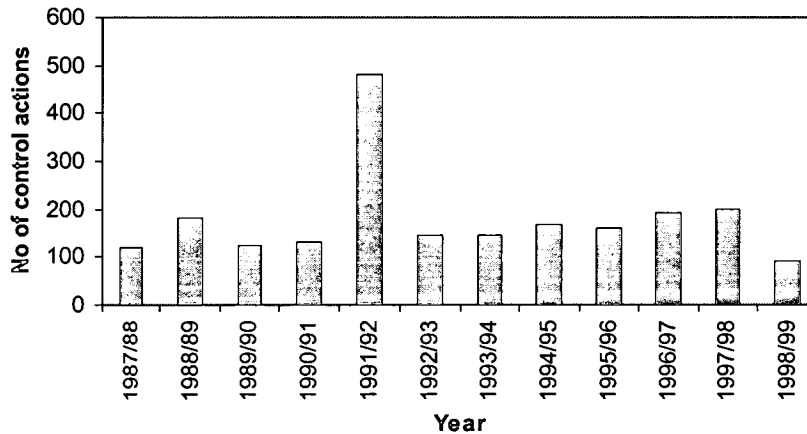


Figure 1.7. The number of control actions against quelea in South Africa from 1987 to 1999. Data from Geertsema (1999).

#### 1.1.5.1 Environmental impacts of quelea control

Any control operation that involves a non-specific method such as a general avicide or explosives will inevitably have impacts on non-target species either directly or through secondary poisoning of predators or the environment. Non-target species are often caught in control operations, and when things go wrong, as many as a fifth of the birds killed have not been quelea. In one control operation, carried out inside Spioenkop Nature Reserve, KwaZulu-Natal, South Africa in 1993 at the behest of the local farmers, only 1000 quelea were killed, while 200 of the kill were not quelea (Natal Parks Board 1993). The hazards to non-target species of spraying with queletox are well known. Raptors are commonly poisoned, for example in 1993 an accident while spraying led to the deaths of over 100 steppe buzzards (*Buteo buteo*) (Yeld 1993). Passerines and waterbirds can be killed that use the same habitat as the quelea aggregation being controlled (Meinzingen et al. 1989). For example aerial spraying of quelea roosts near wetlands in Kenya led to the deaths of over eighty

non-target birds. Additionally, the poison killed significant numbers of aquatic insects and detectable residues were still on vegetation and in the water for several days after spraying (Keith et al. 1994).

Effects of using lethal poisons are rarely limited to the target site. Off target drift, even in ideal spraying conditions, can be as much as 3 km (Van der Walt 1998). The dead and dying quelea attract avian and mammal predators to spraying sites which then consume a large number of poisoned birds, leading to secondary poisoning. There is also a poor knockdown rate of sprayed quelea. Birds can escape the immediate vicinity of the spraying site before dying which leads to further secondary poisoning of raptors (Meinzingen et al. 1989). Quelea spraying operations are thought to be a potentially serious cause of mortality for many non-target species (Mundy & Herremans 1997).

#### 1.1.5.2 Summary – Quelea as crop pests

Quelea are a highly visible pest of grain crops in Africa. The actual physical damage done to crops is financially measurable (Geertsema 1998; Geertsema 1999), although debates about the accuracy of such estimates are inevitable (Elliott & Lenton 1989). Some argue that it is simply the spectacle of quelea pillaging a field that has led to their promotion to the major crop pest in Africa rather than the actual damage done (Elliott & Craig 1999). Nonetheless quelea have a serious impact as a crop pest. First, they perform actual, financially measurable, damage to some of Africa's most important crops. Second, the highly visible nature of a quelea outbreak and the potentially serious impact on small-scale subsistence farmers dissuades those farmers from planting certain types of crops. Third, lethal control of quelea aggregations has a serious effect on the environment through non-target kills and secondary poisoning. Finally, quelea control operations are expensive, and involve dedicated highly trained control teams, something that only the richer of Africa's states have been able to afford.

Governments and farmers are always looking for ways to reduce the damage done by quelea and also reduce the amount spent on their control through more efficient management of the pest problem. The only way that any migrating pest can be

reliably managed is if there is a good understanding of its biology and behaviour. Predictions of where and when the next problem will occur could then be made with some degree of accuracy and preventative measures taken. Such a forecasting system already exists for another of Africa's migrant pests, the African armyworm *Spodoptera exempta*. Data on various environmental parameters, such as rainfall and wind direction, are incorporated into a simple model, "WormBase" (Day et al. 1996), to predict where control efforts should be directed.

Southern Africa contains one of the major concentrations of quelea in Africa, based in the Lowveld dry savannahs of Botswana, Zimbabwe and South Africa. Quelea are seen as a major pest of grain crops in the region, especially among the commercial farmers in Zimbabwe and South Africa where intensive agricultural practices, such as year round irrigation and planting dry season wheat, provide ideal conditions for the survival and proliferation of a granivorous bird. Additionally, losses to subsistence agriculture occur mainly to rain-fed sorghum and millet crops shortly before harvest in the wet season (Jones et al. 2000). Damage is done mainly by newly independent juveniles from nearby breeding colonies that were established 5 to 6 weeks previously.

Migration patterns of quelea in southern Africa are potentially complex so that several populations could each follow different migration patterns, leading to possible population differentiation and structure. Both factors could have a major impact on the understanding of how quelea move in the region. The particular aspects of southern Africa and the existing knowledge of quelea in the region will now be discussed.

## 1.2 Southern Africa

Southern Africa is defined as the part of Africa south of the Cunene, Cubango and Zambezi Rivers at about 17° south. It consists of the countries of South Africa, Lesotho, Swaziland, Namibia, Botswana, Zimbabwe and the Mozambique provinces south of the Zambezi, namely: Sul do Save, Manica e Sofala and part of Tete district (Maclean 1993; Newman 1999). To the north of this region there is a band of moist wooded miombo savannah that consists of woodland dominated by *Brachystegia* and

*Julbernardia* tree species. It acts as a dividing line for many species and subspecies of birds, including the division between *Q.q. lathamii* and *Q.q. aethiopica* (Mundy 1989). A map of vegetation types in southern Africa is shown in Figure 1.8. Three quarters of southern Africa is covered by wooded savannah of various sorts.

Rainfall in southern Africa is controlled by the Inter Tropical Convergence Zone. With the exception of the south west tip, rain over most of the subcontinent falls in the summer. The earliest start to the wet season is in the south east and the north west, as shown in Figure 1.9. Rains then spread inland, reaching Zimbabwe in November.

### 1.3 Quelea in southern Africa

#### 1.3.1 Distribution

The most comprehensive information on quelea distribution in southern Africa is contained in the Southern Africa Bird Atlas Project (SABAP) (Harrison et al. 1997). A separate volume covers southern Mozambique (Parker 1999). In southern Africa quelea have been recorded in most vegetation types except Cape fynbos. Quelea prefer woodlands and grassland below 2000m, and prefer to breed in thorny or spiny vegetation below 1000m especially the *Acacia* savannahs of Botswana and the lowveld areas of Zimbabwe and South Africa (Mundy & Herremans 1997). At least 170 million quelea are estimated to live in southern Africa (Yeld 1993).



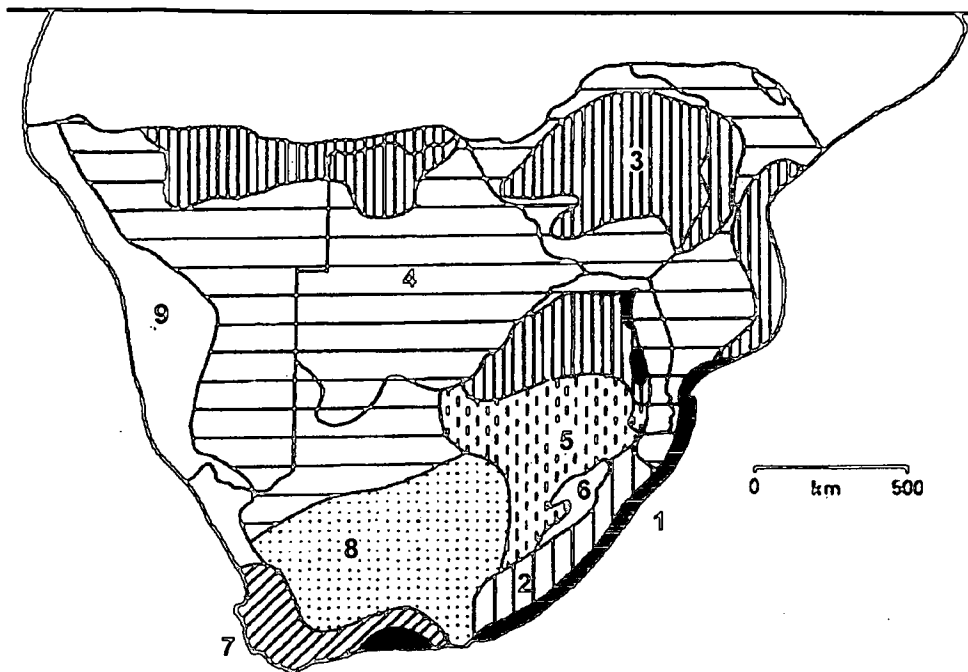


Figure 1.8. A simplified vegetation map of southern Africa. Biomes shown are (1) – coastal and montane forest, evergreen; (2) – scrub forest and tall grassveld; (3) – moist savannah woodlands; (4) – arid savannah woodlands; (5) – Highveld grassland; (6) – montane grassland; (7) – Cape fynbos; (8) – Karoo scrub; (9) – semi-desert and desert (Mundy 1989).

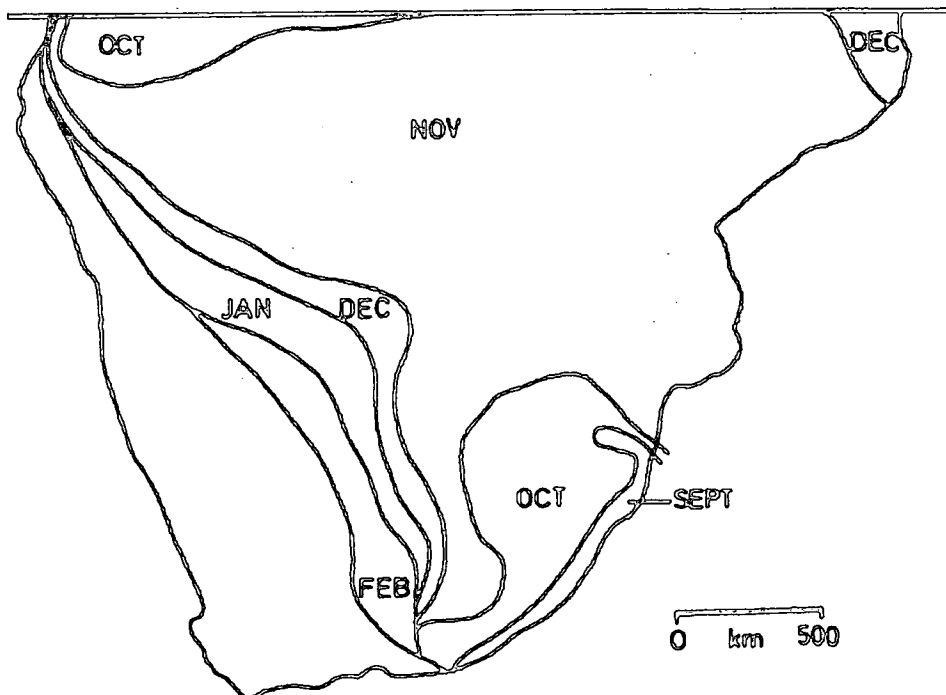


Figure 1.9. Map of southern Africa to show the first month of the wet season when average rainfall exceeds a minimum of 50 mm. Rainfall in the south west falls mainly in the winter (May to August).

### 1.3.2 Subspecies

In southern Africa, the quelea is represented by the subspecies *Q.q. lathamii* Smith 1836 (Ward 1966). However a second subspecies, *Q.q. spoliator*, has been suggested (Clancey 1960). According to Clancey (1960), *spoliator* breeds in the wetter areas of the central Highveld, on north east coast of South Africa and in Swaziland and southern Mozambique. *Q.q. lathamii* breeds in the drier interior of the subcontinent (Figure 1.10). However there is considerable overlap in the distribution of the subspecies (Jones 1989a), with *spoliator* occupying the south eastern part of the range of *lathamii*. Further studies of museum specimens by Clancey (1968; 1973) showed that *spoliator*-like individuals occurred during the non-breeding season (May to November) throughout the interior of southern Africa within the known breeding range of *lathamii*.

The subspecific status of *spoliator* is controversial. It was described from specimens in non-breeding plumage that had grey-brown mantle feathers compared to the warm buff-brown of *lathamii*. Lourens (1961) and Ward (1966) rejected the taxon, instead suggesting that it is part of a highly variable population. Other subspecies of the quelea are described by the type and frequencies of the different plumage morphs of breeding males (Ward 1966). These differences are quantified in large, randomly sampled collections (Ward 1966; Ward 1971; Jaeger et al. 1989b) a study that has never been done for *spoliator*. The morphological evidence for the subspecies therefore remains inconclusive.

There are ecological and behavioural reasons why there is unlikely to be any population subdivision of quelea in southern Africa. In southern Africa, both *lathamii* and the alleged *spoliator* respond in similar ways to the timing and distribution of the rainfall that determines quelea movements and remain sympatric for much of the year, including the breeding season (Jones 1989a). However, if *spoliator*-like individuals reach breeding condition earlier, then they could remain reproductively isolated from *lathamii*. In a study based in Zimbabwe, quelea classified as either *lathamii* or *spoliator* showed no consistent plumage variation. There was no evidence that *spoliator*-like individuals were in a more advanced condition for breeding than *lathamii* (Jones et al. In press).

The proposed *spoliator* subspecies could potentially be restricted to the region of southern Africa that receives the first rain in September and October. This region roughly corresponds with the suggested breeding range of *spoliator* (Clancey 1973). Equally, the potential migratory divide (see Section 1.3.3) is one hypothesis that could account for population subdivision in southern Africa. *Spoliator*-like individuals could fly only to early-rains quarters in the south-east while *lathamii*-like individuals could fly north-west. Mass marking experiments show that quelea flocks can remain as cohesive units (Thompson & Jaeger 1984; Jaeger et al. 1986), so such a migratory divide could function as a barrier to gene flow even though the birds from both populations appear to mix through much of the year.

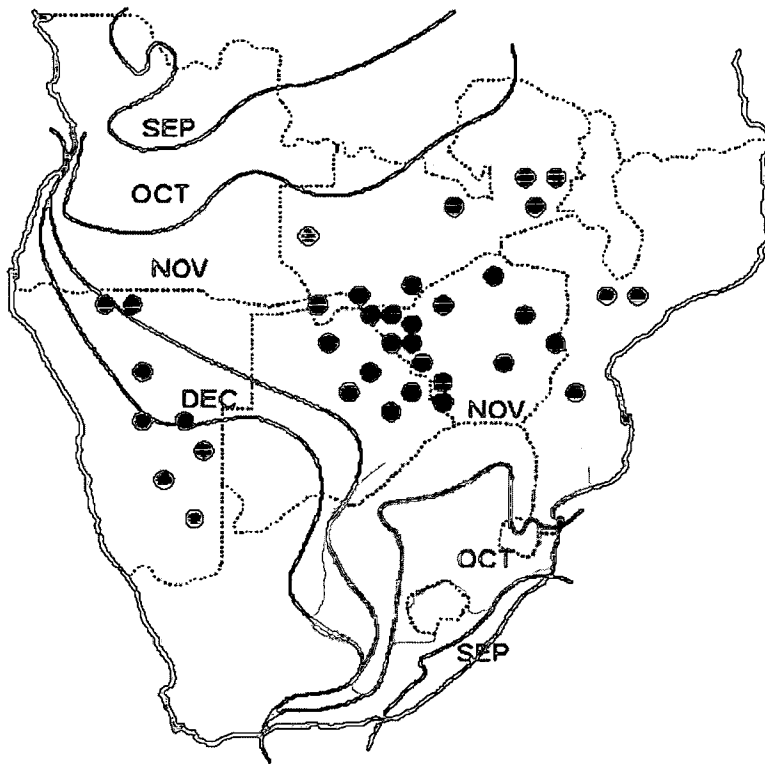


Figure 1.10. Supposed breeding ranges of *Q. q. lathamii* (light grey) and *Q. q. spoliator* (dark grey) in southern Africa (adapted from Magor (1972) and Clancey (1973)). Black dots indicate records of *spoliator* during the non-breeding season (May to November) within the range of *lathamii* (Clancey 1973). Contours indicate the approximate start of the wet season (Thompson 1965) (Jones et al. in press).

### 1.3.3 Migration Routes

In common with quelea throughout Africa, quelea in the south are believed to migrate in response to food availability and rainfall (Ward 1971; Jones 1989a). To recap, the Ward hypothesis suggests that as rains arrive, quelea in these areas have to

move ahead of the rainfront to keep in areas with food. However, eventually no more suitable habitat exists ahead of the rains. Quelea then migrate back across the rainfront to a region where it has rained long enough ago for fresh grass seed to be available. This is the early rains migration. Quelea flocks can then begin to breed, either in these early rains quarters, or later in the season having followed the rainfront back on the breeding migration. The Ward model extended to southern Africa hypothesises a pattern of movement between Zimbabwe/Botswana and South Africa to the south east as well as between Zimbabwe/Zambia and Angola to the north west (Figure 1.11). However, there is little data from Angola at all, so it is not known if quelea use this region, and if they do, the numbers involved (Jones 1989a).

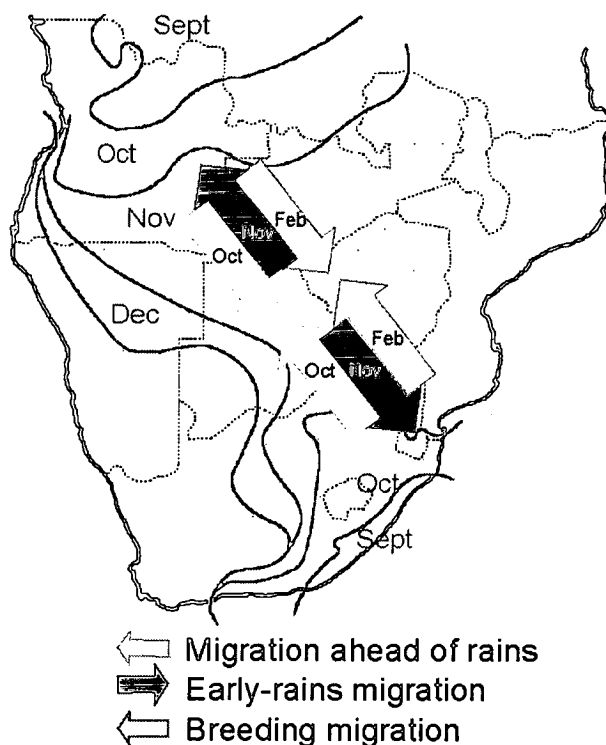


Figure 1.11. The possible timing and direction of quelea migration in southern Africa. Contours mark the start of the wet season (Jones et al. 2000).

There is the potential for two migration routes available to quelea escaping the onset of the rains in central southern Africa. The choice could constitute a migratory divide. A migratory divide develops if migration directions of adjacent migratory populations differ (Berthold 1993). They are often found where migratory birds have to avoid geographic barriers, such as the Alps in Europe, or where separate,

neighbouring, populations try and reach different areas, as is potentially the case with quelea.

Alternatively, if rainfall is both predictable and patchy, in a particular region there may well be food available all year round – especially where agriculture and irrigation are present. Quelea could simply move around nomadically depending on where food and suitable breeding sites are available (Allan 1996). Recent studies on quelea in the Eastern Cape (Whittington-Jones 1999) and KwaZulu-Natal (Berruti 1995) provinces in South Africa have shown that quelea are probably sedentary in those areas. Year round food availability for the birds has led to a loss of migratory behaviour. Even normally migratory quelea populations in Kenya stayed and bred twice in the same location in a year of particularly abundant rainfall (Thompson 1993). Quelea movements, whether migratory or not in the strictest sense, are highly plastic and allow the birds to adapt to whatever resource availability that confronts them.

### 1.3.3.1 Is there any evidence supporting the Ward migration hypothesis in southern Africa?

The Ward migration hypothesis states that quelea movements are determined by food availability and rainfall (Ward 1971). In southern Africa, the hypothesis raises the possibility of a migratory divide. The evidence that could support the hypothesised regular migration patterns comes from three sources, namely: ringing data, bird atlas data and breeding record data. All three offer different approaches to using direct observation techniques to assess migration patterns.

#### 1.3.3.1.a *Ringing*

Bird ringing is a mark-recapture technique. Birds caught in one place are marked with a band of metal indicating where and when they were first caught. If they are then caught elsewhere, the two locations can be used to infer patterns of movement. Between 1948 and 1998 133, 574 quelea were ringed. Of these only 510, or 0.38%, have been recovered (Oschadleus 2000). The low level of recoveries is not surprising considering how far quelea can fly, and how sparsely populated most of southern

Africa is. The longest movement so far recorded is 2545 km, from South Africa to the Democratic Republic of Congo. Most long distance (>500 km) recoveries were between South Africa and Zimbabwe in September, following the start of the rains in KwaZulu-Natal in south-east South Africa. October to December was characterised by short-distance movements within South Africa. Recoveries from January to April were fewer but longer distance, which Oschadleus (2000) ascribed to the breeding migration. Although the pattern revealed is suggestive of movement in the directions and at the times predicted by the Ward hypothesis, the study is severely limited. Most bird enthusiasts in southern Africa live in Zimbabwe and South Africa, it is therefore not surprising that most of the recoveries are in these two countries. The long-distance recovery from the DRC reveals how mobile quelea are, and how inadequate the picture of quelea movements from ringing recoveries is likely to be. The long-distance (>500 km) ringing recoveries for quelea in southern Africa are shown in Figure 1.12.

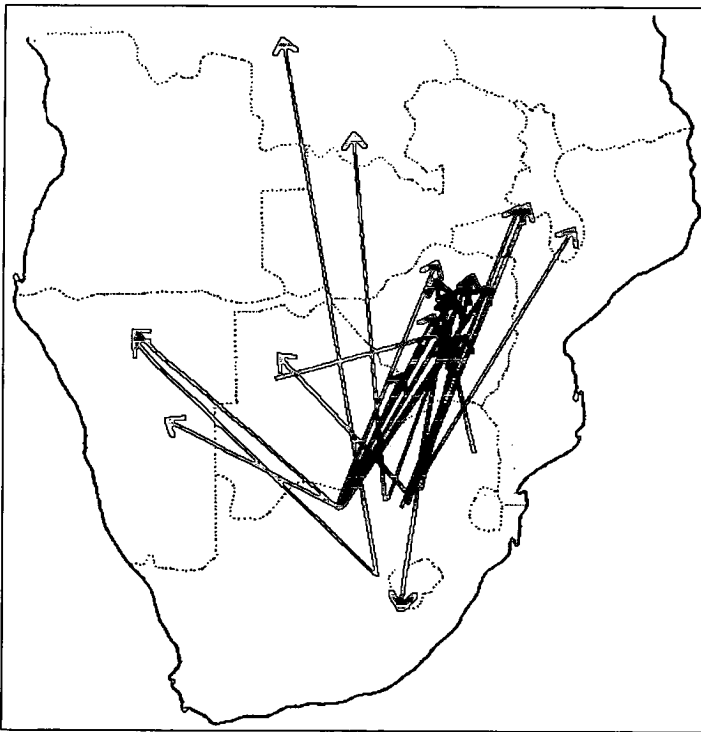


Figure 1.12. Map of long-distance (>500 km) ringing recoveries of quelea in southern Africa (data from Oschadleus 2000).

### 1.3.3.1.b *Bird Atlas Data*

As part of the southern Africa Bird Atlas Project (Harrison et al. 1997; Parker 1999), southern Africa was surveyed for the presence or absence of all the species known to occur in the region. To ensure some degree of accuracy in the survey, levels of effort were standardised. Each quadrat was visited every two months in order to provide seasonal distribution data. The reporting rate was also recorded so that some indication of species abundance was obtained. Hence it is possible to build a picture of the regional and seasonal presence or absence of quelea across southern Africa. The distribution of quelea is shown in Figure 1.13 for southern Africa, excluding Mozambique.

The seasonal patterns of variation in reporting rate shown in the lower half of Figure 1.13 show that there is a seasonal change in the reporting rate of quelea (Mundy & Herremans 1997). The most interesting regions are highlighted in Figure 1.14. In Zimbabwe, there is a reduction in reporting rate at the start of the rains in November. At the same time there is an increase in northern South Africa. This indicates that there may be an exodus of quelea from Zimbabwe with the onset of rains and an influx into areas of earlier rain. However, the data is only presence/absence, and reveals nothing of how many quelea were seen. A single bird is reported in the same way as a million-strong breeding colony. Additionally, each quadrat was surveyed only every two months. Under the Ward hypothesis quelea could be absent from a given area for just six weeks. The survey therefore does not have a high enough resolution for revealing quelea migration patterns. Despite the drawbacks associated with the data type, the Atlas of Breeding Birds does provide evidence of quelea abundance changes consistent with the Ward migration hypothesis.

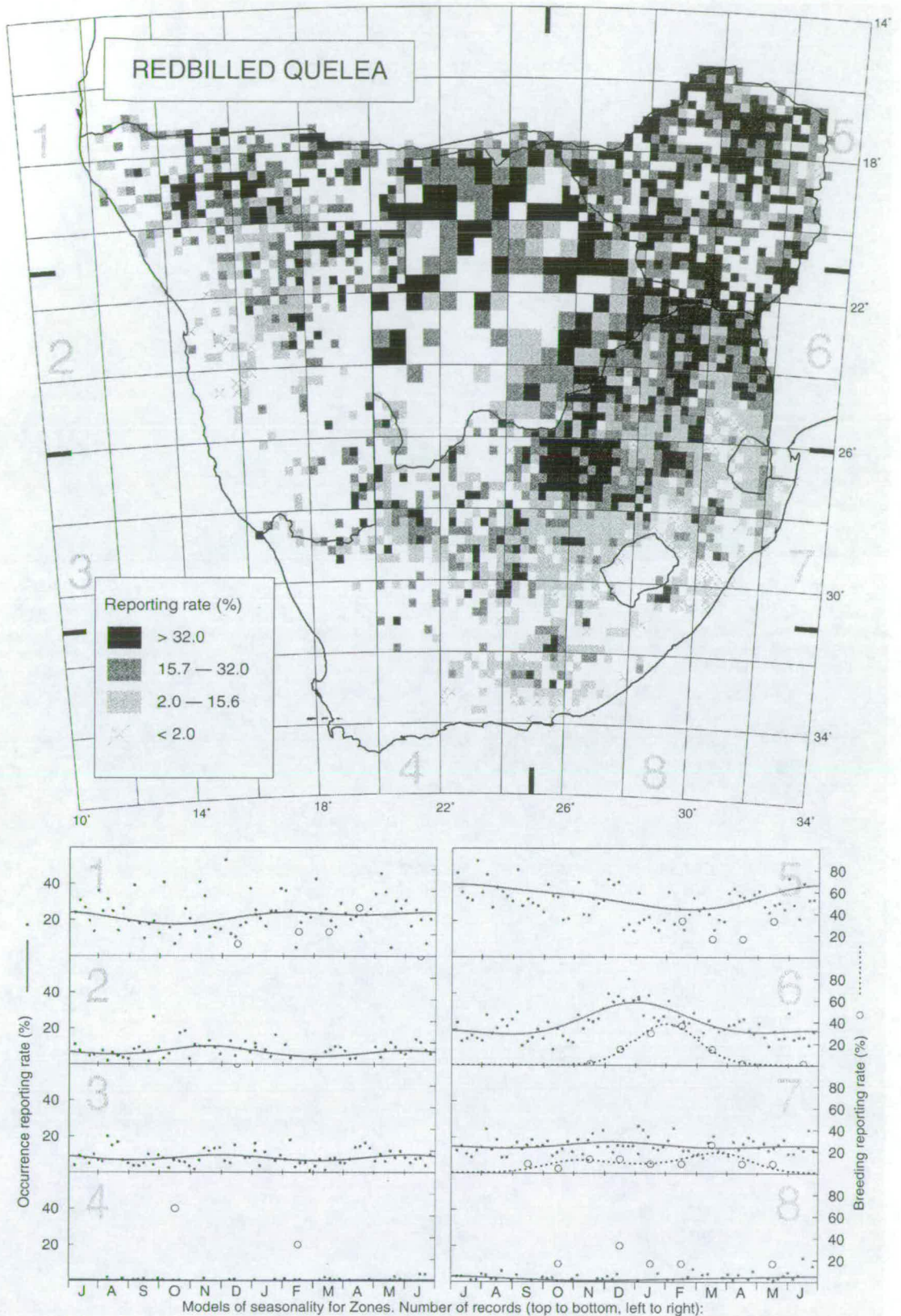


Figure 1.13. Extract from the Southern Africa Bird Atlas Project for quelea (Mundy & Herremans 1997) showing overall distribution and seasonal reporting rate variation in eight regional zones.



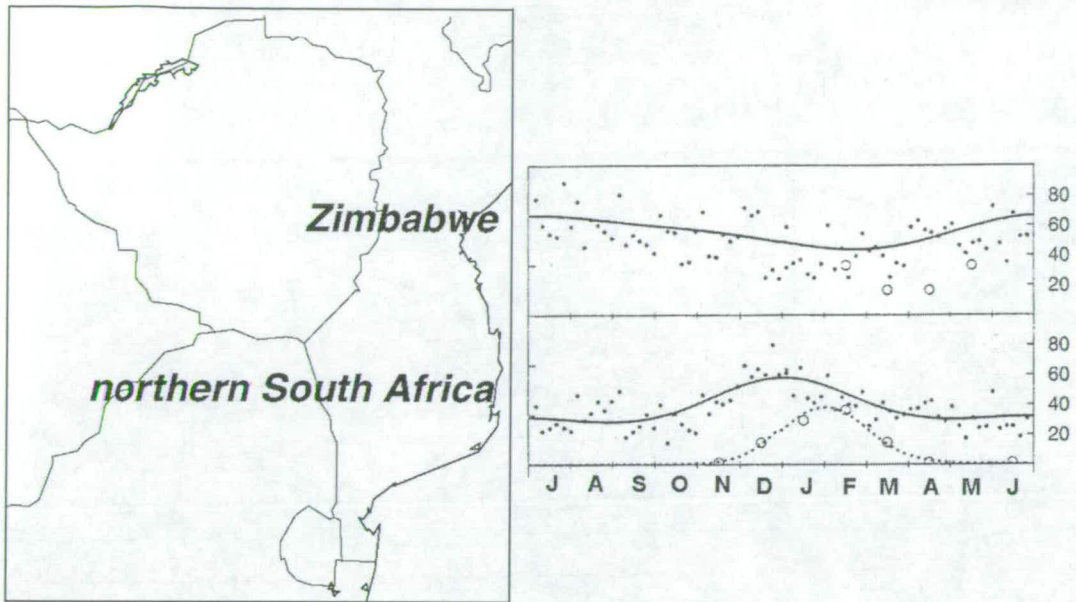


Figure 1.14. Month by month percentage reporting rate for quelea sightings (dots and solid line) and breeding (open circles and dotted line) for Zimbabwe (Zone 5) and northern South Africa (Zone 6). Taken from Mundy and Herremans (1997).

#### 1.3.3.1.c Breeding Records

A database of quelea breeding records extending from 1836 (for South Africa only) to 1974 details the location and egg laying dates, where available, for quelea colonies across southern Africa (Venn et al. 1999). Complete data on the precise timing and location of breeding colonies is only available from the 1950s and data from years after 1974 is still being added. Nonetheless the database represents an important resource and is the backbone for a forecasting model of quelea outbreaks currently under construction (Jones et al. 2000). The distribution of quelea breeding colonies in southern Africa by month from 1911 to 1972 is shown in Figure 1.15. There is a noticeable shift in the location of breeding colonies. In December most colonies are in South Africa, while in January, the location of reported colonies has shifted inland and to the north. By February breeding colonies are reported all over the region. This shift in breeding is precisely what is predicted to happen on the breeding migration as the wet season progresses. Rainfronts move inland, and quelea follow as they search for suitable breeding sites.

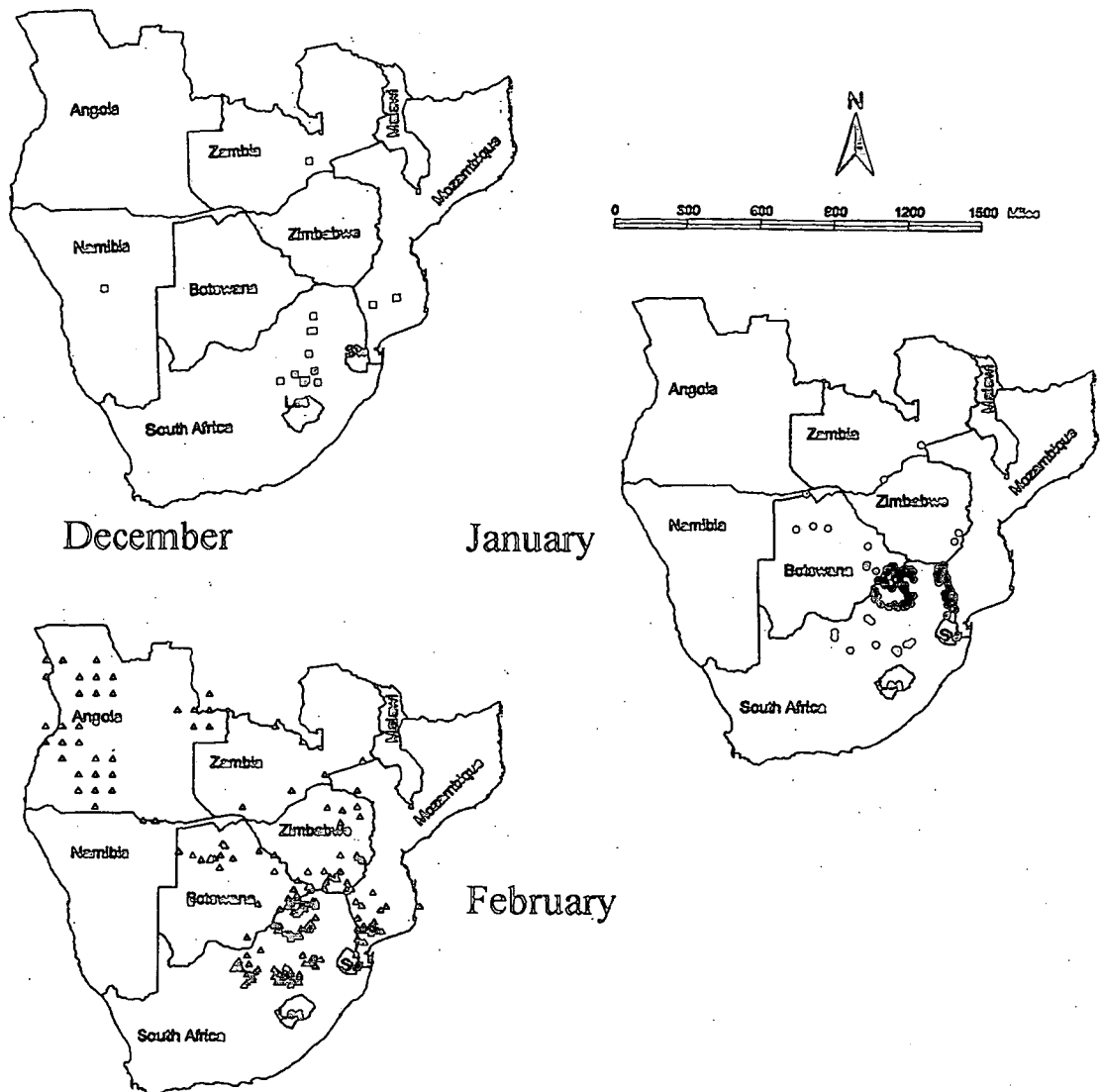


Figure 1.15. *Quelea* breeding colonies reported on a month by month basis in southern Africa from 1911 to 1972. Data from Venn 1999.

#### 1.3.4 Conclusions from previous studies

Previous studies have used different techniques to observe directly the movement of *quelea* in southern Africa as the seasons progress. Ringing studies are capable of mapping the movements of individual birds, but say little about overall abundance and movement. Presence/absence atlas data show broad seasonal patterns of occurrence but nothing of absolute abundance. The breeding records database gives the best indication of where and when *quelea* breed.

Each technique has revealed that there appears to be an underlying pattern to *quelea* movements that is explicable in terms of rainfall and food availability. However, the

three data sources – ringing, atlas data and breeding records – are only consistent with the Ward hypothesis of quelea migration, none of them provides any clear-cut evidence. Each has exceptions and data that do not appear to fit into the recognised pattern and each suffers from the problems associated with using direct observation techniques to infer dispersal patterns, such as an inability to detect long-range and unusual movements (Koenig et al. 1996; Crochet 1996).

#### 1.4 Aims

This thesis attempts to provide definitive evidence on quelea movement patterns in southern Africa. First, can quelea be considered to follow consistent migration patterns based on rainfall and food availability as suggested by Ward (1971)? Alternatively are quelea within southern Africa nomadic opportunists whose movements remain largely without pattern due to complex overlapping rainfronts and widespread intensive agriculture? Second, this thesis will attempt to provide evidence on the occurrence or otherwise of population structure in quelea in southern Africa.

Is it reasonable to expect that different migration patterns can maintain distinct populations? In the rest of Africa quelea subspecies have their own migration pattern based on the rainfall in that part of Africa (Section 1.1.4.2.a). The patterns are sufficiently different to have led to population divisions large enough for the birds in different regions to look quite different and for separate subspecies to be described (Ward 1966; Ward 1971). The migration pathways followed by quelea across Africa are shown in Figure 1.6.

In southern Africa, there are two possible migration pathways that quelea could follow. First, from the central region towards Mozambique/KwaZulu-Natal and the south east, and second, from the central region towards Angola and the north west. There is therefore the potential that quelea could be separated into two populations. Migration patterns could provide a mechanism for the maintenance of population structure and hence the two proposed subspecies in southern Africa.

There is one major difference between southern Africa and the rest of Africa. In southern Africa the two migration pathways share a common dry-season range, while

in the rest of Africa the pathways are completely separate. In such a situation, are the converging rainfronts, the two hypothesised migration patterns and the potential cohesive nature of quelea flocks enough to maintain genetic isolation that could lead to population subdivision?

This thesis uses three different techniques to examine this issue. First, polymorphic microsatellite loci are used to describe the population structure of quelea in southern Africa. Second, geographic variation in plumage patterns is examined, and third, the migration direction preferences of quelea in the wild are tested.

#### *1.4.1 Microsatellite loci*

The direct observation techniques outlined in Sections 1.3 have begun to piece together a pattern of movement for quelea in southern Africa. Microsatellites provide an indirect molecular technique that is capable of revealing relationships and divisions between populations that can then be used to describe population structure and potentially migration patterns. Molecular tools can also be used to examine other issues that direct observations alone can rarely fully describe.

Chapter Two describes the properties of microsatellites and many of the analytical techniques that are used later to attempt to infer population structure in the quelea in southern Africa. The techniques are mainly relevant to Chapter Three, but are also used in Chapters Five and Six.

In Chapter Three a wide-ranging survey of variation in microsatellite loci is carried out. Chapter Six presents evidence for different dispersal behaviours for male and female quelea as revealed by microsatellite loci. In Chapter Seven microsatellite loci are used to accept or reject putative parents that have been assigned to chicks on the basis of behavioural observations.

#### *1.4.2 Plumage Variation*

Across Africa there is immense variation in plumage patterns in breeding male quelea. These variations form the basis for the description of the three subspecies (Ward 1966). Despite two subspecies having been described for southern Africa, no

wide-ranging assessment of plumage variation has previously been carried out in the region. Chapter Four will describe the variation in plumage patterns in male redbilled quelea in southern Africa.

### *1.4.3 Preferred migration directions*

If birds are kept in confined areas at the time of year when they would normally migrate then they orientate themselves in the direction that they would otherwise be flying (Emlen & Emlen 1966; Berthold 1996). Using this technique, the migration orientation behaviour of quelea can be examined. No intra-African migrant has previously been tested in this way. If quelea respond to the technique, then it will be possible to determine whether there are different migration pathways that different birds follow, and hence confirm or refute the presence of a migratory divide in southern Africa. The results of the direct observation of quelea migration orientation behaviour in the wild are given in Chapter Five

## 2 Sampling techniques and molecular methods

This Chapter describes quelea population sampling and methods for collecting and storing tissue samples. The molecular techniques employed and the methods of analysis used in subsequent chapters are discussed.

### 2.1 Sample sites and sampling techniques

#### 2.1.1 Sampling strategy

Quelea are wide ranging migrants. As such they cover enormous distances and have breeding and non-breeding ranges in which they are found, to a varying extent, at different times of the year. Therefore there are few specific locations where quelea are guaranteed to be found year round. Even in the most favoured locations, in some years quelea fail to materialise. Sample collection was therefore unpredictable. Nonetheless, a clear sampling strategy was planned to encompass as much of the range of quelea as possible in the time available.

The first part of the strategy was to cover as wide an area of quelea range in southern Africa (Figure 1.13) as possible, thereby ensuring that as much population variability that may exist was sampled. Three main areas were targeted. First, Zimbabwe in the centre of the region, second the Eastern Cape and KwaZulu-Natal - the supposed range of spoliator and region of early rains, and third as close to the putative Angolan early-rains region as possible – i.e. as far west as possible.

The second aim of the sampling was to cover as wide a time span as possible. In order to assess whether quelea show any philopatry to breeding sites or regions, specimens collected at different times from the different regions were required. Non-breeding sites were also visited with the intention of being able to link breeding and non-breeding regions and therefore show migration routes.

Map 2 shows the location of the sampling sites for quelea in southern Africa. As shown in Table 2.1, the majority of sample sites were from active breeding colonies. Additionally, two non-breeding roosts were sampled, from Bulawayo (BU) and Lake Manyame (LM) in Zimbabwe. There is a good geographic spread of sample sites,

including Namibia in the west and the Eastern Cape, in the supposed spoliator early rains region. However the unpredictability of quelea meant large samples were unavailable from KwaZulu-Natal. There is also a good time spread of samples, especially in south-west Zimbabwe where there are samples of quelea from three different years (HU, JD, XX, XA, and RR).

There is a geographical hierarchy in the location of sample sites. Some are grouped at a local level, and there are also regional groups of sample sites, as defined in Map 2. For example, the sites Nokoneng North (NN) and Nokoneng South (NS) in Botswana represent opposite ends of a single colony, separated by 2.5 km. Samples were also collected from a nearby colony, Gumare (GU), which was only 27.2 km from Nokoneng North (NN). These three colonies give a tight local cluster within the West region, which also included sites in eastern Namibia, Alwyn Farm (AF), Eden Farm (ED) and Wilde Farm (WF), which are also in close proximity to each other, and are about 350 km from the Botswana sites.

### *2.1.2 Sampling methods*

Two types of information were collected from quelea. First, DNA samples were collected for molecular analysis of geographic variation in genotype. Second, plumage patterns were scored and photographed in order to examine geographic variation in phenotype. As geographic variation in plumage is mainly a feature of male quelea, sampling was concentrated on males. Plumage data collection and analysis is discussed in Chapter Four.

Quelea populations were sampled in two main ways. First, quelea were caught in mist-nets. Individuals were bled, killed and their plumage patterns assessed. Second, already dead or dying quelea were collected in the aftermath of control operations. In this case plumage was assessed, before a piece of liver was removed. In some cases quelea were initially frozen until data gathering could be completed. Quelea at Bulawayo (BU) were shot. The samples of nestlings from Tsumcor (TS) and Wilde Farm (WF) were collected as live chicks from nests, or dead chicks were collected from the ground beneath active nests. No more than one chick was collected per nest.

Nestlings from MDA Farm (MD) and Mkhulamini Ranch (MK) were purchased from those collecting chicks for food.

#### 2.1.2.1 Tissue Sample Collection and Storage

Blood samples were taken from the brachial vein on the underside of the wing. Sterile disposable hypodermic needles (12 mm x 0.4 mm, Sherwood Medical Industries Ltd) were used to puncture the vein. Blood was transferred to a sample tube (Sarstedt) using a heparinised capillary tube (1µl microhaematocrit capillary tube). The sample tubes contained a blood preservative consisting of 10mM Tris HCl (pH 8), 100 mM EDTA (pH8) and 2% w/v SDS. Once collected, samples were kept cool until being frozen on return to Edinburgh. Alternatively, a small (5 mm by 5 mm) section of liver was removed and stored in the same preservative. Dissection equipment was cleaned in alcohol each time a liver sample was taken. Samples were imported to the UK under licence from the Scottish Executive.

### 2.2 Molecular methods

The analysis of DNA has become an important tool in studies of evolution, population genetics and systematics (Avice 1994; Hillis & Mable 1996). Compared to morphological techniques or protein markers, there are several advantages of using DNA as a molecular marker to assess population differences and phylogenetic inference. First, the genotype, not phenotype, is assayed directly. Second, a marker system appropriate to the problem can be selected depending on the evolutionary time scale of interest. Finally, DNA can be prepared from small samples, allowing the sampling of endangered or extinct taxa. With careful planning, the appropriate molecular marker can also be near neutral, thereby overcoming the potentially confounding effects of selection when studying relationships within and among populations and species.



Table 2.1. Quelea collection sites in southern Africa, giving full name, date of collection, location, the type of site, how many individuals were sampled at each site and details of the type of sample taken.

Site Code	Full Name	Country	Date	Latitude	Longitude	Type	Sample			Tissue Sample	How Collected	Collected By
							Males	Females	Others			
AF	Alwyn Farm	Namibia	22/04/99	19 19 41	18 51 44	Breeding	54	28	7 Juveniles	Blood	Mist Net	MD
BU	Bulawayo	Zimbabwe	27/11/97	20 8 0	28 35 0	Non-Breeding	76	34	0	Liver	Shot	PJ, RC
ED	Eden Farm	Namibia	24/04/99	19 29 40	18 39 41	Breeding	43	18	21 Juveniles	Blood	Mist Net	MD
GU	Gumare	Botswana	15/03/99	19 17 36	22 10 48	Breeding	63	21	0	Liver	Control Operation	MD, PJ, RC
HU	Humani	Zimbabwe	27/03/97	20 30 0	32 16 0	Breeding	50	0	0	Blood	Mist Net	JD
JD	JDMailangwe97	Zimbabwe	03/97	21 5 0	31 55 0	Breeding	37	22	62 Nestlings	Blood	Mist Net	JD
KR	Kroonstad	South Africa	24/02/99	27 46 21	27 32 10	Breeding	58	21	0	Blood	Mist Net	MD
KW	Klawervallei	South Africa	26/01/00	24 32 40	28 57 40	Breeding	63	25	0	Liver	Control Operation	LG
LM	Lake Manyame	Zimbabwe	15/11/99	17 49 0	30 30 0	Non-Breeding	19	0	32 Adults	Blood	Mist Net	MD, GJ
LT	Lichtenberg	South Africa	16/02/99	26 11 56	25 46 11	Breeding	70	0	0	Liver	Control Operation	LG, JW
MA	Mathangwane	Botswana	09/03/99	20 48 21	26 29 27	Breeding	79	21	0	Blood	Mist Net	MD, PJ, RC
MD	MDA Farm	Zimbabwe	27/03/99	19 53 0	32 22 0	Breeding	0	0	119 Nestlings	Liver	Chick Collection	MD, NC
MK	Mkhulamini Ranch	Swaziland	19/01/00	27 12 0	31 54 0	Breeding	0	0	8 Nestlings	Liver	Chick Collection	MM
NA	Aris	Namibia	26/03/98	22 58 0	17 7 0	Unknown	2	0	0	Blood	Mist Net	PJ, RC
NL	Natal	South Africa	01/02/95	28 42 0	29 12 0	Unknown	2	0	0	Liver	Mist Net	CWJ
NN	Nokoneng North	Botswana	13/03/99	19 32 11	22 8 50	Breeding	80	21	0	Blood	Mist Net	MD, PJ, RC
NS	Nokoneng South	Botswana	14/03/99	19 33 15	22 9 41	Breeding	60	22	14 Juveniles	Blood	Mist Net	MD, PJ, RC
PM	Pietermaritzburg	South Africa	11/12/95	30 22 0	29 35 0	Unknown	2	0	0	Blood	Mist Net	JO
RF	Riverside Farm	South Africa	16/02/99	32 46 0	25 50 0	Breeding	90	29	0	Blood	Mist Net	MD, CWJ
RQ	Riverside Farm	South Africa	12/12/97	32 46 0	25 50 0	Breeding	2	0	0	Liver	Mist Net	CWJ
RR	Reata Ranch	Zimbabwe	24/03/99	21 13 0	29 40 0	Breeding	98	21	0	Liver	Mist Net	MD, NC, DK
SA	Samedupi	Botswana	16/03/99	20 8 13	21 31 66	ex-Breeding	10	14	83 Flying nestlings	Blood	Mist Net	MD, PJ, RC
SH	Shirville Farm	Zimbabwe	16/03/99	19 48 0	28 18 0	Breeding	97	12	64 Non-breeding	Liver	Mist Net	MD, NC, DK
TE	Terminus	South Africa	18/02/99	31 53 0	25 29 0	Breeding	92	17	0	Blood	Mist Net	MD, CWJ
TS	Tsumcor	Namibia	28/04/99	18 42 83	16 54 24	ex-Breeding	6	0	73 Juvs and nestlings	Blood	Mist Net	MD, RS
TTQ	Riverside Farm	South Africa	30/10/98	32 46 0	25 50 0	Breeding	7	0	0	Liver	Mist Net	CWJ
TU	Tuinplaas	South Africa	05/03/99	24 55 11	28 43 34	Breeding	56	0	22	Liver	Control Operation	MD, JW
UP	Upington	South Africa	26/01/99	28 23 40	21 29 15	Breeding	14	0	3 Juveniles	Blood	Mist Net	MD, DS
VK	Volksrust	South Africa	21/01/00	27 21 0	30 0 0	Unknown	1	0	0	Blood	Mist Net	JO
VVQ	Riverside Farm	South Africa	25/11/98	32 46 0	25 25 0	Breeding	5	0	0	Liver	Mist Net	CWJ
WK	White Kopjes Ranch	Zimbabwe	24/03/99	19 41 0	29 10 0	Breeding	33	21	2 Juveniles	Blood	Mist Net	MD, NC
WL	Wilde Farm	Namibia	30/04/99	19 10 24	18 45 99	ex-Breeding	0	0	107 Nestlings	Liver	Chick Collection	MD
WQ	Riverside Farm	South Africa	24/11/97	32 46 0	25 50 0	Breeding	2	0	0	Liver	Mist Net	CWJ
XA	Senuko	Zimbabwe	10/03/98	20 45 0	31 50 0	Breeding	88	23	0	Blood	Mist Net	MD, PJ, PM, NC
XB	Bumi Hills	Zimbabwe	18/03/98	16 54 0	28 12 0	Breeding	98	27	0	Blood	Mist Net	MD, PM, NC
XC	Maitengwe Dam	Zimbabwe	24/03/98	19 55 0	27 5 0	Breeding	97	25	0	Blood	Mist Net	MD, NC, DK
XX	Mailangwe	Zimbabwe	08/03/98	21 5 0	31 55 0	Breeding	96	24	0	Blood	Mist Net	MD, PJ, PM, NC
							Total	1650	446	617		

Latitude and Longitude given in degrees, minutes and seconds

Collectors (for affiliations, see Acknowledgements)

CWJ - Craig Whittington-Jones	JD - Jim Dale	MD - Martin Dallimer	PM - Peter Mundy
DK - Douglas Kabale	JO - Jonas Ornborg	MM - Morrison Mbuli	RC - Bob Cheke
DS - Dirk Steenkamp	JW - Jako Willemsse	NC - Ngoni Cheweshe	RS - Rob Simmons
GJ - Gift Jonasi	LG - Luka Geertsema	PJ - Peter Jones	

## 2.3 Microsatellites

Since their development at the start of the 1990s, microsatellites have become the marker of choice for many molecular studies (Jarne & Lagoda 1996; McDonald & Potts 1997; Goldstein & Schlötterer 1999). They have found applications in all areas of molecular ecology, including parentage studies, conservation biology, population structure and applied fields such as the description of fish stocks. In parentage studies microsatellites have identified the highest frequency of extra-pair paternity yet found in the superb fairy-wren *Malurus cyaneus* (Double et al. 1997), while the social structure of the northern hairy-nosed wombat, *Lasiorhinus kreffi*, (Taylor et al. 1997) pilot whales (Amos et al. 1993), and chimpanzees (Morin et al. 1994) have also been characterised. In conservation biology, microsatellites have been used to identify population units for conservation in the Komodo dragon *Varanus komodoensis* (Ciofi & Bruford 1999) and have tracked the hybridisation of introduced brown hare with native mountain hares in Sweden (Andersson et al. 1999). Phylogenetic studies include the structure of polar bear *Ursus maritimus* populations in the Arctic (Paetkau et al. 1995), and Darwin's finches in the Galapagos (Petren et al. 1999). More applied studies include identifying separate management units for fish stocks, such as the cod *Gadus morhua* off the east coast of Canada (Ruzzante et al. 1998), and analysing the decline in salmon *Salmo salar* stocks in Denmark (Nielsen et al. 1997).

### 2.3.1 Properties of microsatellites

Microsatellites are tandem DNA sequences of between one and five base pairs per repetition unit repeated up to 100 times (Tautz 1993). They are found in the nuclear genome of a wide range of eukaryotes (Valdes et al. 1993) and also in the chloroplast genome in plants. Mutation rates in microsatellites are high. Pedigree analysis in humans suggests a rate of  $10^{-3}$  events per locus per generation (Weber & Wong 1993), and in mice the estimated rate is  $10^{-3}$  to  $10^{-4}$  events per locus per generation (Dallas 1992). The supposed birth of a microsatellite has been documented in a clade of closely related primate species (Messier et al. 1996). Ubiquitous chromosomal distributions have been established in humans (Dib et al. 1996) and mice (Dietrich et

al. 1996), although some clustering of loci in vertebrate genomes has been detected. Dinucleotide repeats, the commonest and most frequently used class, vary widely in the density that they are found in different species. The highest densities recorded are around one locus per 5 kb, although one in every 30-50 kb is more usual (Estoup et al. 1993). In the avian genome, microsatellite density was estimated to be one in every 39 kb (Primmer et al. 1997), which is considerably lower than the estimate for humans of one in every 6kb (Beckmann & Weber 1992). On the complete sequence of human chromosome 22 there are 2666 microsatellite loci, an average of one every 12.5 kb (Dunham et al. 1999).

In terms of repeat structure, there are three main classes of microsatellite, namely: pure, compound and interrupted repeats (Figure 2.1). All combinations of the three basic patterns are found, and all combinations of base composition and repeat length occur. However, most work has concentrated on a few sequence motifs.  $(CA)_n$  repeats are the most common type found in mammals, in insects  $(CT)_n$  is more common, while in plants  $(AT)_n$  is the most common repeat (Primmer et al. 1997). Different alleles at a given locus are identified by their relative electrophoretic migration after specific PCR amplification using a clone sequence or ladder of known size as comparison.

Pure	$(CA)_n, n > 4$
Compound	$(CA)_n(GA)_m, n \text{ and } m > 3$
Interrupted	$(CA)_nN_l(GA)_m$ with $l < 4$ and $n \text{ and } m > 3$

Figure 2.1. Some examples of types of microsatellite repeat structure (Weber 1990).

Microsatellites are co-dominant and alleles are inherited in a Mendelian fashion, which makes interpretation and recognition of genotypes straightforward. They are considered to be near neutral (Jarne & Lagoda 1996; Goldstein & Schlötterer 1999), although some microsatellite trinucleotide repeat arrays are known to be involved in causing some human genetic diseases (Sutherland & Richards 1995; Rubinsztein 1999; Gourdon 2000). Microsatellites tend to be highly polymorphic in natural populations with average expected heterozygosity usually above 50%. Compound and interrupted loci tend to be less polymorphic (Queller et al. 1993; Di Rienzo et al.

1994; Estoup et al. 1995b). Mutation rates tend to be highest in microsatellites with most repeats (Zhu et al. 2000). High mutation rates and high levels of polymorphism in wild populations are positive features in promoting the use of microsatellites as molecular markers, as it means the markers will be sensitive to barriers to gene flow. However, there is considerable disagreement over the way that microsatellites mutate and evolve. An understanding of microsatellite mutation processes will lead to their more appropriate analysis and application.

### *2.3.2 Models of microsatellite evolution*

#### *2.3.2.1 Mutation mechanisms*

The mechanism for the rapid evolution of microsatellites is believed to be due to polymerase slippage whereby repeat units are added or lost at DNA replication (Levinson & Gutman 1987). This is consistent with population studies in which most dinucleotide loci allele sizes differ by even numbers of bases. Large scale studies show that bigger mutation steps do occur, indicating that there must be another mechanism in addition to slippage, probably connected with recombination and DNA repair mechanisms (Strand et al. 1993). Nielsen and Palsbøll (1999) used evidence from nine baleen whale loci to show that multi-step mutations do occur and that there is likely to be a limit, or constraint, on the number of repeats at each locus.

##### *2.3.2.1.a Mutation models*

Two main mutation models have been proposed to allow interpretation of microsatellite data. Each has a different mutation mechanism as its basis, and therefore each can produce radically different interpretations of the same data (Estoup et al. 1995a). However, Angers and Bernatchez (1998) found that combining the two mutation mechanisms allowed them to explain their data better, thus hinting at the complexity of the actual mutation mechanism. The Infinite Allele Model (IAM) assumes that each mutation creates a new allele of random size at rate  $u$ . All alleles differ equally from each other and allele size contains no information. In contrast, the Stepwise Mutation Model (SMM) assumes that mutations add or subtract (with equal probability  $u$ ) a single repeat unit to or from the current allelic

state. Alleles of similar size are therefore more closely related. A development of the SMM is the Two-Phase Model (Di Rienzo et al. 1994), in which mutation modifies the current allele by one unit with a probability  $P$ , and by more than one unit with probability  $1-P$ . Mutation processes other than slippage events are then taken into account. The models make different predictions about variability in populations, and despite testing (Estoup et al. 1995a) there is no agreement which of the models is the most appropriate for microsatellites (Jarne & Lagoda 1996).

As more data on microsatellites are gathered, the variety and complexity of their mutation processes is becoming clearer. An historical study on mutation events in three different avian microsatellite loci showed size expansion in a dinucleotide repeat occurred between different species (Primmer & Ellegren 1998). However there was also a high degree of instability, and the pattern of mutation observed in each of the three loci was variable and depended on the repeat type and structural variation in the primer site. Other studies have shown that the changes in numbers of repeats was broadly in line with the stepwise process even though the mutations they observed were not stepwise in fashion (Zhu et al. 2000).

Homoplasy, the co-occurrence of alleles that are identical in state though not in origin, can further complicate the situation. Where the number of allelic states is limited, homoplasy is probably common due to the rapid mutation of microsatellites. Under the SMM lots of homoplasy is expected and it becomes a problem to ignore it in any analysis as it leads to an underestimate of divergence between populations. Hidden variation in alleles that have identical sizes has been observed (Viard et al. 1998). Incorporating the hidden variation in the size homoplasy into a phylogenetic analysis of three bee species altered the patterns of population structure revealed. Homoplasy is more of a concern where there are constraints on the number of alleles at a given locus.

Within the restrictions of the mutation models, microsatellites are generally considered to evolve in a random, unbiased fashion. However, several studies on the molecular structure of microsatellite evolution have revealed that the mutation processes are, at least in part, non-random, and potentially non-neutral. Some studies have reported a mutation bias so that large alleles tend to get smaller, and small

alleles larger (Garza et al. 1995). Equally there may be a constraint in allele size and mutation directionality (Amos & Rubinsztein 1996). This has been found in loci in coding regions of the genome, suggesting that some microsatellites are under selective pressure (Sutherland & Richards 1995). Biases in mutation direction and a positive relationship between repeat length and mutation rate have also been found (Primmer et al. 1998). Further, large differences between the sizes of pairs of alleles at a given locus may generate recombination instability and lead to the loss of a particular locus, or to a higher mutation rate in heterozygotes (Amos 1999).

The non-amplification of null alleles is an additional potential difficulty with microsatellites (Pemberton et al. 1995). Null alleles are alleles that fail to amplify during PCR. They can occur at frequencies of up to 15% (Paetkau et al. 1995) and can cause mis-identification of heterozygotes as homozygotes leading to apparent heterozygote deficiency (Brookfield 1996), incorrect estimates of gene flow and genetic similarity.

### ***2.3.3 A summary of microsatellites as genetic markers***

The nuclear genome provides a potentially inexhaustible supply of genetic markers. Accessing this variation through microsatellites has proved a powerful technique in many ecological studies. The positive features of microsatellites include their high variability, the ability to score co-dominant alleles and the use of PCR so that extinct and endangered species can be analysed. However, negative aspects include null alleles, a complex mutation process leading to the likely presence of homoplasy and a ceiling in their utility for divergent taxa. Further, despite the routine nature of isolating novel microsatellites for each new study species (Rassman et al. 1991; Estoup 2000), one of the main drawbacks is the need to develop new sets of primers for each species (Newton et al. 1999; Sunnucks 2000). However, there are a substantial number of examples of microsatellite primers that have reasonable cross-species applicability. Therefore a search of the available literature was carried out in order to find suitable avian microsatellites that may be useful for population studies on the redbilled quelea (Dallimer 1999).

## 2.4 Obtaining microsatellite loci for the redbilled quelea

### 2.4.1 Introduction

One major disadvantage of microsatellites is the need to characterise species-specific loci. This is because PCR primers require a high degree of homology in the flanking regions to allow adequate annealing. Mutations in the flanking regions prevent amplification. The probability of mismatch due to mutations is related to the evolutionary distance between the study species. It is therefore expected that primers for microsatellites cloned from one species will amplify homologous products in a closely related species, but not in an evolutionarily distant species.

An economical approach to locating suitable microsatellites may be to survey loci developed for other closely related species in an attempt to find loci that amplify a polymorphic product in the target species. Conservation of primer sites and microsatellite loci has been reported across species, such as cetaceans (Schlötterer et al. 1991), cichlid fish (Zardoya et al. 1996), marine turtles (FitzSimmons et al. 1995) and ruminants (Slate et al. 1998). Avian studies have also provided evidence for the cross-species utility of microsatellites. In a survey of 48 bird species using markers isolated from the swallow *Hirundo rustica* and pied flycatcher *Ficedula hypoleuca*, there was a significant negative relationship between the likelihood that a microsatellite locus amplified a product and the evolutionary distance separating the source and test species (Primmer et al. 1996b).

Other avian studies have tested fewer species, but drawn similar conclusions. In some taxa the markers only work in congeneric species (*Phalacrocorax* spp: Piertney et al. 1998a), while in other studies markers successfully amplified products in species from the same family (Fringillidae: Hanotte et al. 1994; Anatidae: Fields & Scribner 1997, Icterinae: Hughes et al. 1998) or even order (Galliformes: Piertney & Dallas 1997). However the study by Primmer et al. (1996b) is the only one wide ranging enough to have related the success of microsatellite markers in cross-species amplification with a measure of evolutionary distance, taken from the DNA hybridisation work by Sibley and Ahlquist (1990). The authors found that 50% of

loci would successfully amplify a polymorphic product when the test species was separated from the original species by an evolutionary distance of  $\Delta T_{50H}=5$ .

The above examples have all tested novel microsatellites from one species on a range of different species. This thesis had the opposite aim. In order to identify polymorphic microsatellite loci for the quelea, a survey of the available literature on avian microsatellite markers was made and other labs were contacted in order to obtain samples of avian primers. The sources are listed in Table 2.2 and in the Acknowledgements. The avian primer pairs were tested using quelea DNA. Seventy-three primer pairs from sixteen species representing eight families were tested on samples of quelea DNA. The success of the primers in amplifying homologous products on quelea DNA was compared to evolutionary distance (Sibley & Ahlquist 1990).

The evolutionary relationships published by Sibley and Ahlquist (1990) have been controversial and intensely criticised (Harshmann 1994). Some elements of the phylogeny have been shown to be wrong (e.g. Sheldon & Winkler 1993; Sheldon & Gill 1993), but in general the overall shape of the phylogeny has been supported by subsequent studies and reviews (e.g. Moores & Cotgreave 1994; Hedges & Sibley 1994; Gerwin & Zink 1998). Despite the controversy, the standardised measures of evolutionary separation that Sibley and Ahlquist (1990) provide for birds is an ideal framework for attempts to quantify the utility of avian microsatellites in cross-species analyses.

#### *2.4.2 Materials and Methods*

The quelea blood samples used were from the site Bulawayo (BU), Zimbabwe. Complete lab protocols are given in Appendix A. A summary of the lab methods is included below as some of the PCR conditions were different in this pilot survey.

DNA was prepared using the Chelex technique (Walsh et al. 1991). 2  $\mu$ l blood in preservative was added to 200  $\mu$ l 5% Chelex-resin (Chelex®100, Instagene). The mixture was incubated at 65°C for three hours and then boiled for eight minutes. 2  $\mu$ l of the supernatant containing the DNA was used as a PCR template.



A list of the primers and sources is given in Table 2.2. All primers were tested with two different PCR conditions. The initial conditions used the genomic DNA template in a 10 $\mu$ l reaction mix containing 0.1mM dATP, dGTP and dTTP; 0.01mM dCTP; 2pmol each primer; 1X 'Parr' buffer containing 1.5mM MgCl<sub>2</sub> (Cambio); 0.25 unit *Taq* polymerase (Advanced Biotechnologies) and <1 $\mu$ Ci [ $\alpha$ -<sup>32</sup>P] dCTP. The second set of conditions included between 0.5 and 1.5mM MgCl<sub>2</sub> (total MgCl<sub>2</sub> 2.0mM to 3.0mM), and 60mM tetramethylammonium chloride/2.5% formamide (TMACIDE) added to the reaction mix (Gemmell 1997). Reactions were overlaid with one drop of mineral oil and amplified in a Hybaid Omnigene Temperature Cycler. The PCR profile was as follows: 2 min denaturing step at 93°C; 7 cycles of 93°C for 30 s denaturation, 50°C for 1 min annealing and 72°C for 30 s extension; then 25 cycles of 93°C for 30 s denaturation, followed by 52°C for 1 min annealing and 72°C for 30 s extension. Products were separated on 6% polyacrylamide sequencing gels and visualised on X-ray film (Bancroft et al. 1995).

Each marker was tested using between four and ten quelea DNA samples. A sample of DNA from the source species was also included when available. A locus that was detected in quelea DNA was considered homologous when one to two major bands occurred that were similar in size to that expected in the original species. In general positive amplifications would also display characteristic 'stutter' bands that typify microsatellite loci.

### 2.4.3 Results

Of the 73 primer pairs tested, 22 pairs produced homologous amplification products in the quelea (Table 2.2), of which 21 gave a polymorphic product. Loci isolated from species more closely related to the quelea were more likely to amplify successfully (Table 2.3). Six out of eight markers from the most closely related species (Sibley & Ahlquist 1990), *Plocepasser mahali*, gave a product, and 22 of 51 loci (43%) derived from passerines (maximum  $\Delta T_{50H} = 12.8$ ) gave a product, including at least one locus from each species. In contrast, no loci cloned from non-passerines yielded a homologous product. There was a negative relationship between evolutionary distance and the proportion of loci that amplified successfully ( $y = 1.45$

– 0.0991x;  $r^2 = 0.967$ ), as shown in Figure 2.2. If this linear function is appropriate, it suggests that no loci would amplify from species separated from quelea by an evolutionary distance  $\Delta T_{50H}$  14.6 (95% Confidence Interval +/- 1.12) or greater, which is at the upper end of the range  $\Delta T_{50H} = 10 - 15$  (Primmer et al. 1996b). However, the lack of species in the evolutionary distance range between *Malurus* ( $\Delta T_{50H}$  12.8 to quelea) and *Phalacrocorax* ( $\Delta T_{50H}$  21.6 to quelea) in this study means this figure is speculative. Perhaps a more useful measure is the evolutionary distance at which 50% of loci tested may be expected to amplify a product. The value predicted by the linear model is  $\Delta T_{50H} = 9.6$  (95% Confidence Interval +/- 0.55), considerably further in evolutionary distance than the previous estimates of  $\Delta T_{50H} = 6.7$  (Primmer et al. 1996b) and  $\Delta T_{50H} = 3.9$  (Hughes et al. 1998).

Table 2.2. Avian microsatellite loci tested and those giving successful amplification. Families as in Sibley and Ahlquist (1990).

Loci				
Tested	Successful	Species	Family	Source
WBSW1,2,4,7-11	WBSW1	<i>Plocepasser mahali</i>	Passeridae (p)	McRae and Amos 1999
	WBSW2	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW4	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW9	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW10	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW11	<i>Plocepasser mahali</i>	Passeridae (p)	
Pdou3	Pdou3	<i>Passer domesticus</i>	Passeridae (p)	Neumann and Wetton 1996
Esc4	Esc4	<i>Emberiza schoeniclus</i>	Fringillidae (p)	Hannotte et al 1994
Lox1-8	Lox3	<i>Loxia scotia</i>	Fringillidae (p)	Piertney et al 1998b
	Lox6	<i>Loxia scotia</i>	Fringillidae (p)	
	Lox8	<i>Loxia scotia</i>	Fringillidae (p)	
Hru1-10	Hru1	<i>Hirundo rustica</i>	Hirundinidae (p)	Primmer et al 1996b <sup>1</sup>
	Hru5	<i>Hirundo rustica</i>	Hirundinidae (p)	
	Hru6	<i>Hirundo rustica</i>	Hirundinidae (p)	
	Hru7	<i>Hirundo rustica</i>	Hirundinidae (p)	
Phtr1-4	Phtr2	<i>Phylloscopus trochilus</i>	Sylviidae (p)	Fridolfsson et al 1997
	Phtr3	<i>Phylloscopus trochilus</i>	Sylviidae (p)	
Pocc1,2,5,6,8	Pocc6	<i>Phylloscopus occipitalis</i>	Sylviidae (p)	Bensch et al 1997
Fhu1-6	Fhu2	<i>Ficedula hypoleuca</i>	Muscicapidae (p)	Primmer et al 1996b <sup>1</sup>
	Fhu3	<i>Ficedula hypoleuca</i>	Muscicapidae (p)	
	Fhu5	<i>Ficedula hypoleuca</i>	Muscicapidae (p)	
Mcyu1-8	Mcyu4	<i>Malurus cyaneus</i>	Maluridae (p)	Double et al 1997
PcD2,6	None	<i>Phalacrocorax carbo</i>	Phalacrocoracidae	Piertney et al 1998a
PcT3,4	None	<i>Phalacrocorax carbo</i>	Phalacrocoracidae	
LLSD2,7,10	None	<i>Lagopus lagopus</i>	Phasianidae	Piertney et al 1997
LLST1	None	<i>Lagopus lagopus</i>	Phasianidae	
ADL102,158,172,176	None	<i>Gallus gallus</i>	Phasianidae	U.S. Poultry Gene Mapping <sup>2</sup>
Bca5,6,10,11	None	<i>Branta canadensis</i>	Anatidae	Buchholz et al 1998
WFG2,8	None	<i>Anser albifrons</i>	Anatidae	Fields and Scribner 1997
Hhi1,3,5	None	<i>Histrionicus histrionicus</i>	Anatidae	Buchholz et al 1998
44 (Sfi)	None	<i>Somateria fischeri</i>	Anatidae	Fields and Scribner 1997

(p) - passerine families

1 - and refs therein

2 - 'Population Tester Kit'

<http://poultry.mph.msu.edu/index.256.htm>

Table 2.3. Amplification success in the redbilled quelea and evolutionary distance from the source family of successful loci. Family names as Sibley and Ahlquist (1990).

Family	DeltaT50H	Number of Loci			Av. Heterozygosity
		Tested	Successful	Prop'n Success	
Passeridae (p)	6.65	9	7	0.78	0.57
Fringillidae (p)	10	9	4	0.44	0.55
Hirundinidae (p)	11.1	10	4	0.40	0.80
Sylviidae (p)	11.1	9	3	0.33	0.62
Muscicapidae (p)	11.7	6	3	0.50	0.33
Maluridae (p)	12.8	8	1	0.13	0.60
Phalacrocoracidae	21.6	4	0	0.00	-
Phasianidae	28	8	0	0.00	-
Anatidae	28	10	0	0.00	-
	Passerines	51	22	0.43	
	Non-Passerines	22	0	0.00	
	Total	73	22	0.30	

(p) - passerine families

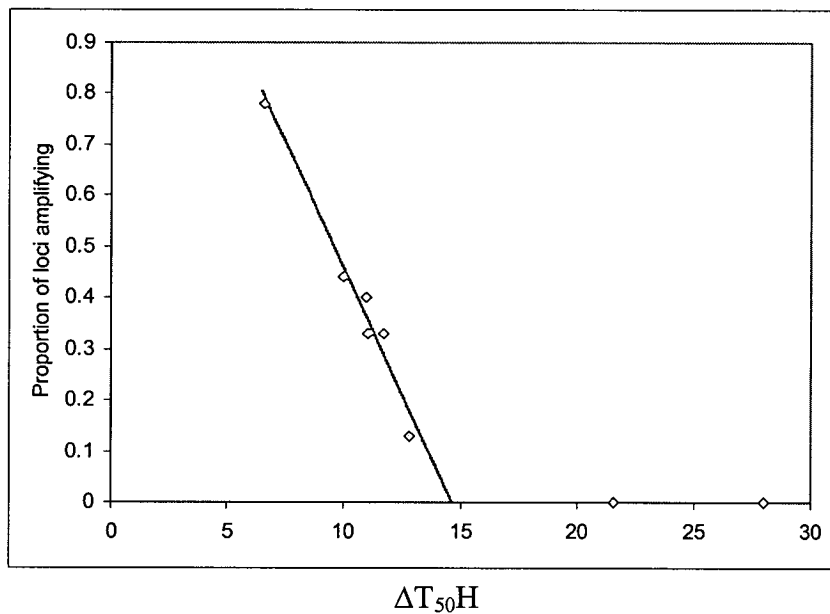


Figure 2.2. Proportion of loci amplifying as a function of evolutionary distance ( $\Delta T_{50H}$ ) between the families of source species and the redbilled quelea. The best fitting model was a linear model:  $y = 1.45 - 0.0991x$ .

#### 2.4.4 Loci chosen for further use

The 12 loci that most efficiently amplified products in redbilled quelea were used in further genetic analysis and are listed in Table 2.4. The initial test conditions outlined above were modified into the protocols presented in Appendix A which were subsequently used to carry out microsatellite work as outlined in later Chapters.

The overall heterozygosity and number of alleles for each locus is given in Table 2.4. Heterozygosity ranged from 0.613 for locus Pdou3 to 0.870 for locus Hru7. The maximum number of alleles detected for any single locus was 45 (Lox8). Only 57 individuals were screened for this locus. At the other end of the distribution, only 16 alleles were detected from over 1000 individuals for locus Phtr2. Most of the loci chosen are dinucleotide repeats, although there are two tetranucleotide (Lox8 and Pdou3) and one mononucleotide repeat locus (Hru5). The high number of alleles detected for both Hru7 and Lox8 made these loci unsuitable for population analyses, as discussed in Section 2.5. Their use was therefore restricted to the parentage study outlined in Chapter Seven for which a large number of alleles is advantageous.

Figure 2.3 shows the allele frequency distributions for all twelve loci. Most loci had a reasonably continuous distribution of alleles. Lox8 and Hru7 do not have an obvious peak in allele frequencies around a most common allele. This may well be due to the loci having such a high number of alleles relative to samples screened meaning that there is not an adequate sample of allele frequencies. Loci WBSW1 and WBSW11 had interrupted repeats with at least two allele frequency peaks. The other 8 loci tended to have one common allele. The frequency of this common allele varied from approaching 50% for allele size 117 in Phtr2 to less than 12% for allele 170 for locus Esc4. Complete tables of overall and population by population allele frequencies for each locus are given in Appendix B.

## 2.5 Analysis of genetic differentiation

### 2.5.1 Introduction

One of the objectives of this thesis is to identify population structure. If a given population has internal structure, then it is likely that there will be genetic differentiation among sampled populations. Genetic differentiation is defined as the acquisition of allele frequencies that differ among populations (Hartl & Clark 1997). A second objective is identifying migration routes. Little is known about specific quelea migration patterns, breeding site philopatry and individual or flock movements. Therefore identifying the appropriate scale at which to study quelea population structure is important. If quelea consistently migrate back and forth

between the same breeding and non-breeding areas, then sample site may be the appropriate division. If quelea populations are more regionally based, and use common migration routes, then a broader sub-structure incorporating several sample sites may be appropriate. If quelea movement is complex and flexible, involving itinerant breeding and dispersal throughout the breeding season, then there may be no informative structure at all.

There are a variety of techniques that can be used to infer information about the differences between populations using microsatellites. Many of these techniques include assumptions and requirements that may or may not be met depending if sampling is either incomplete or has unavoidable biases. The techniques that are later used to analyse microsatellite data generated for the quelea will now be described.

#### 2.5.1.1 Identifying population genetic structure

One of the consequences of population structure is that for each level of structure, there is a reduction in the observed heterozygosity relative to that which would be expected if there were no structure. It is this reduction in heterozygosity as compared to a panmictic population that forms the basis for many of the most popular genetic differentiation analysis tools.

Wright (1951) developed the fixation index to quantify the effect of population substructure on levels of heterozygosity.  $F$  statistics are designed to analyse the distribution of genetic variation within and between populations. A popular  $F$  statistic is  $F_{ST}$ .  $F_{ST}$  measures the genetic differentiation among populations and despite doubts about its absolute accuracy (Whitlock and McCauley 1999), the calculation of  $F_{ST}$  (or some equivalent statistic) is useful in the understanding of genetic structure among populations.

In addition, if a long list of assumptions are true,  $F_{ST}$  can be used to calculate the level of gene flow between populations based on the island model of randomly mating populations with effective size  $N$ , migrating with rate  $m$ .  $Nm$  can then give an indication of the number of effective migrants that could move among populations in each generation. However using estimates of genetic differentiation, such as  $F_{ST}$ , as

Table 2.4. Details of the twelve microsatellite loci selected for study of quelea genetics

Name	Primer Sequence	Annealing Temp	Type of repeat in quelea	Putative repeat motif	N	Range	No. Alleles	Ho
Esc4	TTC CCT CAC AAT TTT CCG AC	F	dinucleotide	(CA) <sub>18</sub>	1042	146-188	22	0.865
	TAT GTG CTG AAG TGA ACC ATC C	R						
Hru5	TCA ACA AGT GTC ATT AGG TTC	F	mononucleotide	(T) <sub>10</sub> (GC) <sub>17</sub>	1042	108-143	27	0.860
	AAC TTA GAT AAG GAA GGT ATA T	R						
Mcyu4	ATA AGA TGA CTA AGG TCT CTG GTG	F	dinucleotide	(GT) <sub>26</sub> AT(GT) <sub>3</sub>	1042	138-192	23	0.824
	TAG CAA TTG TCT ATC ATG GTT TG	R						
Pdou3	CTG TTC ATT AAC TCA CAG GT	F	tetranucleotide	(TCCA) <sub>18</sub>	1042	80-200	27	0.613
	AGT GAA ACT TTA ATC AGT TG	R						
Phtr2	CGC AGG CTC AGA AAT ACT TGA	F	dinucleotide	(CA) <sub>12</sub>	1042	107-183	16	0.754
	GCC CAC AGC TCA ATA GTC TT	R						
Phtr3	ATT TGC ATC CAG TCT TCA GTA ATT	F	dinucleotide	(CA) <sub>23</sub>	1042	126-182	29	0.866
	CTC AAA GAA GTG CAT AGA GAT TTC AT	R						
WBSW1	TAT TTT ATG CTC TGC CCA GTT G	F	dinucleotide	(TG) <sub>15</sub>	1042	141-213	28	0.787
	TAG GCA TTG CCA AGG TTA ATC	R						
WBSW2	AAG GTC ACT GTG CAT CTT GC	F	dinucleotide	(TG) <sub>3</sub> TA(TG) <sub>11</sub>	1042	201-257	28	0.810
	GCA GAC TTG ATA GAT CTT CAC TGT AA	R						
WBSW4	TAC CAC TTG GTC CTC TGG CT	F	dinucleotide	(AC) <sub>21</sub>	1042	131-193	31	0.878
	GGT TAT GCT ACA AAC TGG TCA C	R						
WBSW11	TGA AAA TCC CAG GTC CCT ATT	F	dinucleotide	(AC) <sub>15</sub> (GT) <sub>6</sub>	1042	151-233	37	0.672
	CCA CAT CTT TTT CCA CAG CA	R						
Hru7	GCA TTC ACA GTG TAG ACA ATG	F	dinucleotide	(A) <sub>4</sub> (C) <sub>3</sub> (AAACC) <sub>2</sub> (AAAC) <sub>3</sub>	54	139-259	40	0.870
	GAT CAC TAT GAG TCC CTG GAA	R						
Lox8	TTG TGA AGG TTT GGG ACA TAA G	F	tetranucleotide	(CTTT) <sub>25</sub> (CCTT) <sub>13</sub>	57	194-350	45	0.737
	AGT TGA GGC CAT TAA AAA GAT TC	R						

Ho - Overall observed heterozygosity

N - The number of quelea samples investigated

Phtr 3 is sex-linked (Fridolfsson et al 1997)

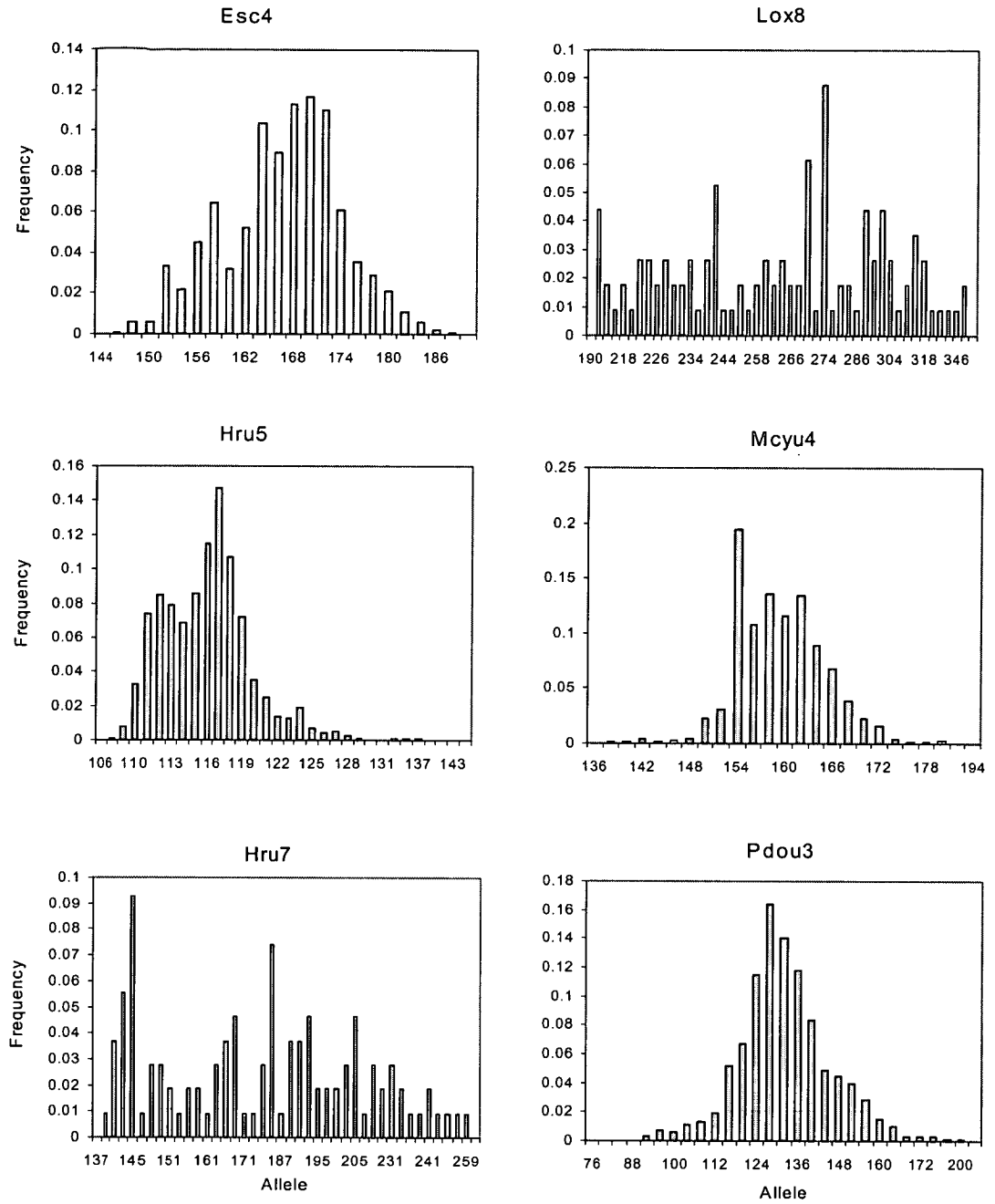


Figure 2.3. (a). Overall allele frequencies for six microsatellite loci. All loci investigated in 1042 samples, except Lox8 ( $n=57$ ) and Hru7 ( $n=54$ ).

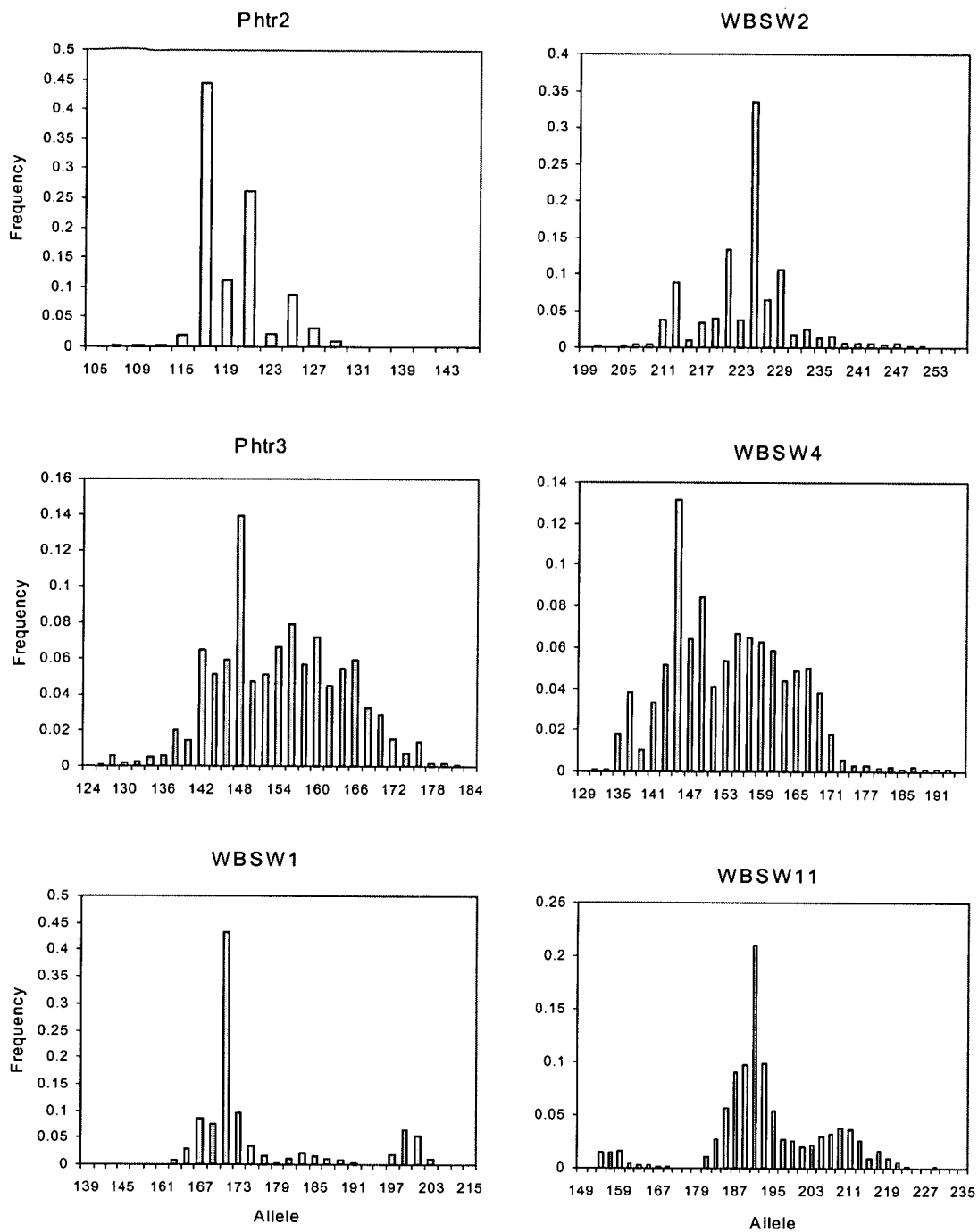


Figure 2.3. (b). Overall allele frequencies for six microsatellite loci. All loci investigated in 1042 samples.



an indirect measure of gene flow and migration between populations has several difficulties not least because the underlying theoretical assumptions of the models are rarely met in ecologically realistic situations and such estimates have been subject to much controversy (Bohonak et al 1998; Whitlock and McCauley 1999).

Under the assumptions of Wright's island model,  $F_{ST}$  has a relatively simple relationship with the number of migrants a population receives per generation,

$$F_{ST} \approx 1/(4Nm+1),$$

however the island model makes a large number of assumptions, including: that there is no selection or mutation in the loci being measured, that all populations contribute equally to the gene pool, there is no spatial structure and migration is completely random, and that an equilibrium has been reached between the forces of migration and genetic drift. In natural populations many of these assumptions are not going to be met. In particular, as ecological conditions are likely to impose some spatial structure on the populations under study. Equally, different populations could easily be acting as sources or sinks for migrating individuals. Finally rapid anthropological change could mean that changing ecological conditions make it unlikely that many species are in mutation-drift equilibrium (Whitlock and McCauley 1999).

F statistics assumes that loci follow the IAM. If, however, microsatellites follow the SMM or some variant, F statistics are no longer valid, and  $F_{ST}$  will generally overestimate the similarity between populations. In such cases the statistic  $R_{ST}$  is more appropriate (Slatkin 1995).  $R_{ST}$  is a genetic differentiation statistic equivalent to  $F_{ST}$ , but it is based instead on the average sum of squares of the difference in allele size. It therefore accounts for the 'memory' that any one allele has of its evolutionary history.

Statistics such as  $F_{ST}$  and  $R_{ST}$  are based on differences in allele frequencies between populations. However as microsatellites become more variable, the high number of alleles at each locus can mean that all individuals are different from all others even with large sample sizes. Allele-frequency based measures also inevitably simplify the information content that is available by summarising in terms of population-wide allele frequencies (Waser & Strobeck 1998). An alternative way to analyse

microsatellite data is to consider individual multilocus genotypes as opposed to population-based allele frequencies. Individuals that come from the same population have more similar genotypes than individuals from different populations. Population structure can be assessed by analysing the way individuals cluster based on their genotypes (Cornuet et al. 1999).

Multilocus genotype data can be used to construct inter population relationships in two different ways. First, relationships can be examined at the individual level (e.g. Bowcock et al. 1994 and Estoup et al. 1995a). At any locus, two individuals share up to two alleles. Across several loci, the proportion of shared alleles becomes an index of genetic similarity (e.g. Nielsen et al. 1997). Second an individual may be assigned to a given population based on the likelihood that its multilocus genotype occurs in the test population (Paetkau et al. 1995; Rannala & Mountain 1997). Both techniques make fewer assumptions about the populations under consideration than R and F statistics, and are potentially much more informative analysis techniques.

### 2.5.2 Multiple tests

Many of the analysis techniques outlined below were performed several times on the same data set. When performing multiple tests it is important to adjust the critical significance value so that the probability of making a type one error – rejecting a true null hypothesis - is no greater than it would be if just one test were performed. This is done by adjusting the original critical significance level,  $\alpha$ , by the number of tests done,  $k$ . The corrected significance value,  $\alpha'$ , is calculated as follows:

$$\alpha' = 1 - (1 - \alpha)^{1/k}$$

This is the Dunn-Šidák method of Bonferroni correction (Sokal & Rohlf 1995), which is a very conservative method. As the main aim of the Bonferroni correction is to be certain that no type one errors have been made at all, the power of each individual test is very low unless the number of tests made,  $k$ , is small. A more appropriate technique in cases where  $k$  is large is the sequential Bonferroni correction (Rice 1989; Sokal & Rohlf 1995). The sequential Bonferroni correction tests the significance of each probability in turn. The lowest probability,  $P_1$ , is compared to  $\alpha_1$  with  $k$  tests, if it is found to be not significant, then all tests are not

significant. If  $P_1$  is significant, then the second smallest probability,  $P_2$  is compared to  $\alpha_1$  with  $k-1$  tests. This procedure is continued until a probability is found that is not significant. All probabilities that are higher are therefore also not significant. In order to maintain the power of the tests, while still being able to cope confidently with type one error, sequential Bonferroni technique was employed on all tests.

### 2.5.3 Genetic variation analysis methods

All the software used to perform the genetic analyses in this thesis is freely available. A list of URLs where the software may be obtained is given in Appendix A.

#### 2.5.3.1 Descriptive statistics

##### 2.5.3.1.a Allele frequencies

The allele frequency is the proportion of all alleles at a locus that are of a specific type. Frequencies can be calculated for each locus across all populations, as shown in Figure 2.3. However, populations that are genetically differentiated are likely to have different allele frequencies, and it is then informative to calculate allele frequencies at each locus separately for each population. Such a procedure allows the identification of private alleles (alleles that are present in only one population) and rare alleles (defined as alleles present at a frequency of less than 0.01) which can give some indication of relationships between populations. Allele frequencies by locus and population were calculated using the software package Genetix (Belkhir 1999).

##### 2.5.3.1.b Heterozygosity

Allele frequencies are more easily interpreted when summarised within a population in terms of heterozygosity. Heterozygosity is calculated as the frequency of heterozygotes at a locus, or averaged across all loci. It is highest when there are many alleles at equal frequency.

Heterozygosity can be calculated in two ways. Observed heterozygosity ( $H_o$ ) gives the proportion of heterozygotes within a sample. This is a simple measure of the

amount of genetic variability on a population by population basis. Expected heterozygosity ( $H_e$ ) is calculated from the observed allele frequencies assuming Hardy-Weinberg equilibrium. The difference between the observed and expected heterozygosities gives an indication of the deviation from the assumptions of the Hardy-Weinberg equilibrium. The most frequently encountered difference is an excess of homozygotes (Hartl and Clarke 1997). This can be attributed to inbreeding, the Wahlund effect (the presence of unsampled population structure), non-random mating, selection or, in the case of microsatellites, the presence of null alleles. Observed and expected heterozygosity were calculated using Genetix (Belkhir 1999).

#### 2.5.3.1.c *Hardy-Weinberg equilibrium*

As F statistics rely on the assumptions of Hardy-Weinberg equilibrium, it is necessary to test that populations do not deviate from equilibrium. When in equilibrium  $H_o$  is approximately equal to  $H_e$ .  $H_o$ ,  $H_e$  and deviations from Hardy-Weinberg expectations were tested using the computer programme Genepop 3.1d (Raymond & Rousset 1995). An exact Hardy-Weinberg test (Guo & Thompson 1992) based on a Markov chain algorithm was used to obtain an unbiased estimate of the exact probability of incorrectly rejecting the null hypothesis of Hardy-Weinberg equilibrium. The Markov chain was set to 10,000 steps with 1000 steps of dememorisation. Markov chain algorithms are randomisation procedures that use the original data as a basis. The dememorisation steps allow the algorithm to 'forget' the original data before proceeding to generate random data sets against which the observed data can be compared for statistical significance. The algorithm was performed in 500 batches of 1000 iterations per batch.

#### 2.5.3.1.d *Genotypic linkage disequilibrium*

Tests for significant pairwise non-random associations between locus genotypes were performed using the 'Linkage Disequilibrium' option in Genepop 3.1d under the null hypothesis that genotypes at one locus are independent from genotypes at the other locus. An exact test was performed together with a Markov chain process to evaluate statistical significance (Guo & Thompson 1992). The Markov chain was set

to 10,000 steps with 1000 steps of dememorisation. The algorithm was performed in 500 batches of 1000 iterations per batch.

### 2.5.3.2 F statistics

F statistics assume that there are three levels of population structure. The first is the individual level, the second is the subpopulation level, and the third is the level of the total population. The differences in the observed and expected heterozygosities at each of these levels give a description of population structure. The most important level in a study of population structure is the fixation index  $F_{ST}$  which measures the extent of population subdivision by quantifying the amount of reduction in heterozygosity at the subpopulation level.

$$F_{ST} = \frac{H_T - \bar{H}_S}{H_T}$$

Where  $H_T$  is the expected heterozygosity in the total population and  $\bar{H}_S$  is the mean heterozygosity across all subpopulations.

Other F statistics measure the reduction in heterozygosity of an individual relative to its sub-population ( $F_{IS}$ ) or of an individual relative to the total population ( $F_{IT}$ ).  $F_{IS}$  is a measure of inbreeding and should generally be close to zero for randomly mating individuals. A positive  $F_{IS}$  indicates inbreeding and therefore a departure from Hardy-Weinberg assumptions (Page & Holmes 1998).  $F_{IS}$  (Weir & Cockerham 1984) was calculated for each locus and for each sub-population using the software package Genetix (Belkhir 1999).

F statistics and their microsatellite equivalents R statistics describe the relative amount of genetic variation within populations. However they cannot give information on the relationships between subpopulations. Weir and Cockerham (1984) extended Wright's original model to allow pairwise comparisons between subpopulations. Weir and Cockerham's  $\theta$  (theta) is based on between-population variance components of allele frequencies. It has been widely used to assess the degree of between population differentiation. A similar pairwise statistic  $\rho$  (rho) has been calculated for R statistics (Slatkin 1995; Michalakis & Excoffier 1996).

### 2.5.3.2.a *The most appropriate statistic*

F and R statistics are based on different mutation models and therefore could generate different patterns of population structure. Intuitively the SMM would seem to be more accurate of the two mutation models as it is based on the observed microsatellite mutation processes. However, as the mutation models make different predictions about population variability, it has been possible to test which is more appropriate to microsatellite data. Estoup et al. (1995a) used a procedure based on the amount of heterozygosity the two models predicted for a given data set and compared this to the observed heterozygosity. Neither model could be rejected, although the probability of rejecting the SMM was higher. DiRienzo et al. (1994) generated a theoretical distribution of alleles to compare with observed data sets using computer simulation. In this case the authors did not reject the SMM, or its close relative the Two-Phase Model, whilst rejecting the IAM. Simulations have also been used to test the performance of  $F_{ST}$  and  $R_{ST}$  based pairwise genetic differentiation measures (Gaggiotti et al. 1999). The authors chose to test the  $F_{ST}$ -based measure  $\theta$  (Weir & Cockerham 1984) and the estimator of  $R_{ST}$ ,  $\rho$  (Michalakis & Excoffier 1996) (see Section 2.5.4.3 for definitions). Using populations with known genetic differentiation, the authors varied several parameters, such as number of loci, number of alleles and sample size. Under ideal conditions with a high number of loci (more than 20) and large sample sizes (greater than 50)  $\rho$  more accurately estimated genetic differentiation for a variety of parameter conditions than  $\theta$ . When the number of loci and sample sizes fell much below this  $\theta$  was always better.  $F_{ST}$  was therefore concluded to be the better choice in studies that did not have the high numbers of loci and samples they recommended. However, as more data become available it seems that no single representation of the microsatellite mutation process is appropriate.

## 2.5.4 *Testing for genetic differentiation using F statistics*

### 2.5.4.1 Overall $F_{ST}$

Locus by locus estimates of  $F_{ST}$  according to Weir and Cockerham (1984) were carried out using the software package Fstat 2.9.1 (Goudet 2000). Fstat also evaluates

the variance components from which  $F_{ST}$  statistics are calculated. The variance components give an indication of the way in which overall  $F_{ST}$  is compartmentalised in the three different levels: sig a (the between sample variance component), sig b (the between individual within sample component) and sig w (the within individual component).

Global  $F_{ST}$  across all populations and loci was calculated in Genetix. An indication of the significance of  $F_{ST}$  in the entire population was obtained using a global permutation test at the level of the individual. The procedure allows the significance of the observed value of  $F_{ST}$  to be assessed. The global permutation test works as follows. Under the null hypothesis  $F_{ST} = 0$  (i.e. there is no differentiation between populations) all the population samples can be considered as one. If  $F_{ST}$  is calculated many times without any structure to the population, a distribution of  $F_{ST}$  under the null hypothesis is produced. By comparing the actual value of  $F_{ST}$  with the null distribution, an estimate of the probability of a value greater than that observed by chance is obtained. If the probability is less than 5%, it can be concluded that there is significant genetic structure at the population sample level. 10,000 permutations were performed.

#### 2.5.4.2 Overall $R_{ST}$

Locus by locus estimates of  $R_{ST}$  were calculated according to Rousset (1996) in Fstat 2.9.1. The different components of variance outlined above were also calculated. Overall  $R_{ST}$  was estimated with each locus weighted by the amount of allelic variance (Rousset 1996). It was not possible to perform permutation testing on overall  $R_{ST}$  values due to the limitations in the software packages available.

#### 2.5.4.3 Pairwise statistics

There are a number of estimators of pairwise  $F_{ST}$  based on allele size frequencies. This study uses  $\theta$  (Weir & Cockerham 1984), the least biased and most widely used statistic (Gaggiotti et al. 1999). An estimator of pairwise  $R_{ST}$ ,  $\rho$  (Michalakis & Excoffier 1996) is also used.  $\rho$  is based on the sum of the size differences between

alleles over all loci for pairs of microsatellite haplotypes.  $\theta$  and  $\rho$  were estimated using the software package Arlequin 2.000 (Schneider et al. 2000).

Pairwise  $\theta$  and  $\rho$  values were calculated between all population pairs. To test the significance of the estimates, a null distribution under the hypothesis of no difference between the populations was calculated by permutation testing with 10,000 permutations. The p-values represent the proportion of the permuted values that had pairwise statistics larger than the observed value.

Arlequin calculates pairwise F statistics by counting the number of different alleles between two haplotypes. This corresponds to a weighted  $F_{ST}$  over all loci and is equivalent to  $\theta$  (Weir & Cockerham 1984; Michalakis & Excoffier 1996). Pairwise  $R_{ST}$  is estimated by counting the sum of the squared number of repeat differences between two haplotypes. This then corresponds to Slatkin's pairwise  $R_{ST}$ ,  $\rho$  (Slatkin 1995; Michalakis & Excoffier 1996).

#### 2.5.4.4 AMOVA

The AMOVA (Analysis of MOlecular VAriance) technique examines the genetic structure that may be present in a population by grouping populations in meaningful ways. AMOVA was developed to provide an analysis that does not contain any underlying assumptions about the evolution of genetic systems but quantifies the extent of genetic differentiation within and among populations and groups (Excoffier et al. 1992).

The technique builds upon analysis of variance. Observations are grouped into levels of a hierarchy; for example, individuals are grouped into populations, and populations into regions. The percentage of the overall variance that each level explains is then used to determine the significance of the groupings. AMOVA was performed using Arlequin 2.000 (Schneider et al. 2000). Arlequin allows the user to define a group structure to reflect the pattern of genetic differentiation found, or to reflect the pattern that may be expected under a specific hypothesis. A grouping is supported if a significant component of the total genetic differentiation can be attributed to the between groups element. Equally, the within groups component should contain a low, non-significant, amount of the total variance. A non-parametric



permutation approach with 10,000 permutations to ensure accuracy is used (Guo & Thompson 1992; Excoffier et al. 1992). A significant result at the group level implies that a significant amount of the total genetic variation sampled is accounted for by the user-defined population structure.

### 2.5.5 *Multilocus genotype techniques for measuring population differentiation*

Any method that relies on pre-defined populations and allele frequencies could miss the impact that rare events have on genetic differentiation (Davies et al. 1999). This is especially true of techniques that rely on population-wide allele frequencies. Rare migrants are not picked up because they represent a small proportion of the overall population. An alternative approach therefore is to consider the individual as the unit of study rather than the population sample. In the assignment test, the likelihood that an individual belongs to the population that it was sampled in is measured (Paetkau et al. 1995; Rannala & Mountain 1997). However assignment tests still rely on *a priori* defined populations. A second approach, the shared allele distance, computes a genetic distance between pairs of individuals based on the number of alleles that they share (Bowcock et al. 1994; Estoup et al. 1995a). Finally, multilocus genotypes can be considered like any other multivariate data and analysed using multiple correspondence analysis (Guinand et al. 1996).

#### 2.5.5.1 The assignment test

The principle of the assignment test is to assign an individual to the population in which the individual's genotype is most likely to occur. There are several steps to the calculation. First the individual's genotype is removed from the population in which it was sampled. The allele frequencies at each locus are estimated. Then the expected frequency of the individual's genotype at each locus is determined, before multiplying across all loci and log transforming the result to give the assignment index for how likely that individual is to have come from that population. The same procedure is carried out for the same individual for all the other test populations. The individual is assigned to the population in which it has the highest probability of

occurring (Waser & Strobeck 1998). This is the frequency method employed by Paetkau et al. (1995).

One problem with the frequency method is rare and unique alleles. If the test individual has a unique allele, when it is removed from the source population, the unique allele is also removed, therefore making it less likely that the individual will be assigned to that population. Two solutions have been proposed to deal with this problem. The first involves adding the individual's genotype to all the sampled populations. The second adds all alleles to all populations at a low frequency – this can either be a constant frequency, or the inverse of the number of gene copies sampled in each population (Cornuet et al. 1999). Both methods introduce their own bias when there are many rare alleles.

Perhaps a more appropriate way of dealing with rare alleles is to take a different approach to calculating assignment indices altogether. Rannala and Mountain (1997) used a Bayesian approach to detect immigrants to a population. This procedure was modified to produce a variant of the assignment test that is not affected by the presence or otherwise of rare alleles (Cornuet et al. 1999). The Bayesian method has been shown to be a more powerful technique than the frequency method. A completely correct assignment rate can be achieved by scoring 10 microsatellite loci for about 30 to 50 individuals from 10 populations with an  $F_{ST}$  of about 0.1 (Cornuet et al. 1999).

Bayesian methods use the observed data and incomplete or subjective knowledge about the prior distribution of the input parameters to provide a probability distribution for the parameter of interest (Luikart & England 1999; Cornuet 2000). In this case, Rannala and Mountain assumed an equal prior probability density for all the allele frequencies of each locus in each population. They calculated a probability of observing an individual in each population. This probability can then be used in the same way as in the frequency method above, with individuals assigned to the population in which their genotype is most likely to occur. An assignment index is calculated, which represents the negative log likelihood of the individual being assigned to a given population. The Bayesian method was used to calculate

assignment indices in the software package GeneClass version 1.0.02 (Cornuet et al. 1999).

In all assignment tests, the test individual can have an effect on its probability of assignment. An individual is more likely to be assigned to its source population simply because its genotype is in that population. Whilst this effect will diminish with increasing sample size, it will still have an influence (Cornuet et al. 1999). The test individual was therefore removed from its original population - the 'Leave One Out' option in GeneClass. Only individuals with a maximum of one missing genotype from the microsatellite loci were included in the analysis.

Assignment tests are always able to assign an individual to a population regardless of how likely that individual is to have come from the population. In other words there is always a closest population for each individual. In GeneClass, the probability of belonging compares the assignment index of the test individual relative to the indices of the members of that population. If the test individual's index is similar to that of the members of the population, then it is sensible to assign that individual to that population. However, when the test individual's index is very different from the rest of the population, it is sensible not to assign that individual to any population (Cornuet et al. 1999). GeneClass allows the user to determine the threshold probability level below which an individual remains unassigned. This process is carried out by simulating multilocus genotypes by randomly choosing alleles according to their frequencies in the population. For the assignments in this study, 10,000 simulations were performed, with a threshold probability of 0.05.

It is also possible to assign 'unknown' individuals to reference populations. This is particularly useful where only a small sample is available from a particular location. By assigning these individuals to reference populations with adequate sample sizes, a picture of the likely relationship between reference populations and 'unknown' individuals can be established. The test is also performed in GeneClass, under the same principles as outlined above, with individuals from unknown sources assigned to the population to which they have the highest probability of belonging.

Assignment tests allow an assessment of which populations are most closely related to which others through the phenomenon of mis-assigned individuals. Populations that are more closely related will have individuals with multilocus genotypes that are similar. When test individuals are assigned, there is a greater chance that the assignment will be incorrect if populations are closely related. Hence the higher the number of individuals that have been mis-assigned between a pair of populations, the more closely related those populations are (Luikart & England 1999).

#### 2.5.5.2 Shared allele distance

The shared allele distance between a pair of individuals is defined as one minus half the average number of shared alleles per locus. The shared allele distance between a maximum of ten individuals (listed in Table 3.11) with complete datasets from each population was calculated using the 'Individual to Individual Genetic Distance Calculator' (Brzustowski 1999).

Distances were used to draw a neighbour-joining tree (Saitou & Nei 1987) so that relationships between individuals can be visualised. The neighbour-joining method uses a cluster analysis tree-building algorithm. In cluster analysis, tree building starts by selecting the two most similar populations. Populations are added one at a time in order of decreasing similarity. In neighbour-joining, the minimum evolution principle is used at each step in the algorithm so that the resulting tree topology has the minimum sum of branch lengths (Nei & Kumar 2000).

#### 2.5.5.3 Multiple correspondence analysis

Multiple correspondence analysis (MCA) is similar to many types of multivariate techniques in that it allows the variation of complex data sets to be visualised and understood in fewer, still meaningful, dimensions (Greenacre 1984). The main difference between MCA and other multivariate techniques is that MCA is specifically designed to allow the analysis of categorical rather than continuous variables. It is also assumption free at the population genetic level. Hence the data set is examined and visualised based solely on the information in the data, and without

many of the pre-conditions that come attached to many of the more traditional methods of analysing patterns in genetic data.

MCA allows the exploration of variables included in two, or multi-way tables. Although initially developed for categorical variables, it has been adapted for genetic data sets consisting of allele frequencies (Guinand et al. 1996). Individuals can be represented as points in multi-dimensional hyperspace, which has as many dimensions as there are alleles. MCA searches for independent ordination axes in the hyperspace that represent as much of the original variation as possible. Each of these axes is defined in terms of the 'inertia' of the original data set, i.e. the proportion of the variance in the original data that the new axis explains. Further axes are defined successively containing less and less of the variance in the original data. The data can then be visualised by plotting up to three axes.

Application of MCA to population genetics has so far been limited (Guinand et al. 1996; Guinand & Easteal 1996; Lugon-Moulin et al. 1999) as MCA does not quantify the significance of observed patterns. However as a method for exploring data free from assumptions, it is potentially invaluable. Not only will the technique reveal patterns in the data set, but the locus that has most influence on each of the ordination axes can be identified as the locus with the highest correlation ratio on a given axis. Patterns of genetic differentiation can then be partitioned among loci depending on whether genetic differentiation has been estimated from the first, second or third ordination axis (Guinand 1996).

MCA was performed using Genetix (Belkhir 1999). Genetix allows the user to specify the number of dimensions that are calculated. It also gives the proportion of the total variance that each axis expresses. The data were plotted in two dimensions. Outlying points were identified and removed from the analysis.

### **2.5.6 Genetic distances**

A common way to analyse genetic information is to calculate genetic distances. The main aim of calculating distances is to examine the relationships between the test populations in an evolutionary and geographic context. Genetic distances use the raw genotypic data to generate a matrix of pairwise distances between each population.

Each genetic distance has a set of assumptions and biases that may not be appropriate for a given situation. Hence a pattern revealed by one distance might be more to do with the assumptions underlying that distance than any actual properties of the data. Three distance measures were used, each with a different theoretical background. Any pattern that is universally revealed is therefore likely to be robust. The distances chosen were Nei's (1978) unbiased genetic distance; a distance measure based on the stepwise mutation model,  $D_{SW}$  (Shriver et al. 1995); and  $D_{LR}$  (Paetkau et al. 1997) based on assignment indices.

#### 2.5.6.1 Nei's unbiased genetic distance

Nei's unbiased genetic distance (Nei 1978) is based on the infinite allele model of mutation. All loci are assumed to have the same level of neutral mutation, with mutation producing fresh unique alleles. The genetic variability initially in the population is at equilibrium between mutation and genetic drift. Effective population size of each population remains constant. The distance increases linearly with time, so is suitable for reconstructing relationships between populations. However as Nei's unbiased distance is based on the infinite allele model, it may not be suitable for microsatellite data. Nei's unbiased distance was calculated using Genetix (Belkhir 1999).

#### 2.5.6.2 $D_{SW}$

$D_{SW}$  (Shriver et al. 1995) is a genetic distance measure that was developed specifically for loci following the stepwise mutation model with high mutation rates and high levels of heterozygosity.  $D_{SW}$  (stepwise weighted genetic distance) weights the probability that two alleles are different by the absolute value of the difference in the number of repeats between the two loci. It therefore takes into account the fact that under the SMM, alleles that are of similar size are more closely related. It is potentially a more appropriate distance measure for microsatellites, which are believed to evolve under the SMM.  $D_{SW}$  diverges linearly with time (Shriver et al. 1995), an important property when investigating phylogenetic relationships. Empirical (Paetkau et al. 1997) and simulation (Shriver et al. 1995) studies show that

$D_{SW}$  accurately reflects known relationships between populations.  $D_{SW}$  was calculated using the programme GeneDist (Brzustowski 1999).

### 2.5.6.3 $D_{LR}$

Nei's unbiased distance and  $D_{SW}$  both take the type of mutation model as the starting point for how they attempt to represent evolutionary relationships between populations.  $D_{LR}$  takes a different approach. It was developed to compare the likelihood of complete multilocus genotypes in two populations (Paetkau et al. 1997) and is based on the frequency method of assignment index calculation (Paetkau et al. 1995). A  $D_{LR}$  of two means that the genotypes of individuals from the two populations under consideration are, on average, two orders of magnitude more likely to occur in the individuals' own population than in the other population.  $D_{LR}$  performs well in distinguishing fine-scaled population structure (Paetkau et al. 1997).  $D_{LR}$  was calculated using the Assignment Calculator (Brzustowski 1999).

### 2.5.6.4 Visualising and representing genetic distances

One reason for calculating genetic distances is to represent the pattern of geographic variation between populations in an evolutionarily meaningful way. As the resulting pairwise matrices of data are multidimensional, it is necessary to summarise and reduce the data in some way so that the information in the data is maintained yet is more easily visualised.

The standard approach to visualising a matrix of pairwise genetic distances is to use phylogenetic tree drawing algorithms to produce a bifurcating, hierarchical representation of the multi-dimensional relationships between populations. A tree-like diagram therefore comes to represent the genetic relationships between populations. However, there is no *a priori* reason to assume, before any analysis, that the relationships between the populations will be tree-like at all. There are many cases in which the relationships would be better visualised as a network, or as a cline (Lessa 1990). Indeed Smouse (1998) maintains that as the main aim of tree drawing algorithms is to produce a graphical representation of the genetic data, there are many more appropriate techniques available to achieve this that do not impose any

predetermined structure. Trees are also increasingly difficult to construct with large numbers of populations, and a more informative visualisation can be obtained using multivariate statistical techniques (Cavalli-Sforza 1998).

As tree drawing has limitations, it is worth considering other ways of representing genetic data. One increasingly popular method is the multivariate technique multidimensional scaling (MDS) (McConnel et al. 1997; Ruzzante et al. 1998; Shaw et al. 1999). The rationale behind using MDS is as follows. Each population is a single point in an array of observations in multidimensional space, with each allele or gene frequency represented by a dimension. Pairwise genetic distances summarise the multidimensional relationships between the populations. Multivariate techniques, such as MDS, reduce the multidimensional relationships to a few dimensions without substantial loss of information.

Multidimensional scaling can be defined as the search for a low dimensional space in which the points in the space represent the original objects. The distances between the calculated points match the original distances as closely as possible (Cox & Cox 1994). The data used in MDS does not have to be linear, and so the most appropriate genetic distances can be used. As with all multivariate techniques, the relationships that are in the data can be visualised in a two or three dimension scatter plot. The main advantage of MDS is that non-hierarchical relationships, such as networks and clines, are more easily detected than they would be with a hierarchy imposed on them from a tree drawing algorithm. Indeed, the main drawback in using MDS and genetic distances appears to be the assumptions inherent in the genetic distances themselves as opposed to MDS (Lessa 1990). Relationships between populations are therefore represented as a series of MDS plots carried out using the statistical package SPSS. Tree-drawing algorithms have not been used.



### 3 Population genetics of the redbilled quelea in southern Africa

#### 3.1 Introduction

Quelea are highly destructive grain crop pests. Despite an organised control programme that has been established in many countries for several decades, there has been no decline in quelea numbers, and no decline in the amount of damage that the birds are responsible for. Indeed as agriculture and land degradation spread, so do the quelea.

In many ways the control programmes lack basic information that is essential for the efficient control of a pest species. Little is known about quelea population structure and movements beyond the basic overall patterns. There is therefore a need to define migration pathways and recognise distinct population units of quelea, if they exist, so that control measures can be targeted at those units that are responsible for the majority of the crop damage.

Attempts have been made to follow quelea movements using direct methods such as ringing studies (Oschadleus 2000), mass-marking studies (in East Africa, Jaeger et al. 1986; Johns et al. 1989) (in southern Africa, Luka Geertsema pers comm) and radio-telemetry (Bruggers 1989). The results have been at best in agreement with the hypothesised migration routes of quelea, and at worst completely uninformative. This is not surprising considering the large distances that quelea are capable of migrating, and the low chance of recovering marked individuals. The use of direct methods in studying bird populations has several biases, such as the difficulty in detecting long-range migrants (Crochet 1996), and the problems with using direct observations to infer dispersal patterns in vertebrates has long been recognised (Koenig et al. 1996). The advent of molecular markers gives another, indirect, way to assess movements of quelea and define population units for management.

### 3.1.1 *Evolutionarily Significant Units and Management Units*

Effective conservation programmes need unambiguous population units to be defined that adequately encompass the evolutionary lineage and genetic diversity of a species (Avice 1994). For quelea, separate units also need to be identified for management. The management at issue, however, is not conservation, but control. And just as in conservation there are questions relating to what level of division should be recognised before separate management policies are implemented (Burke 1999).

A conservative level of division is the Evolutionarily Significant Unit (ESU) (Moritz 1994; Newton et al. 1999) which is defined as a population or aggregation of populations that is reproductively isolated from other conspecifics, and represents an important component in the evolution of a species. Describing a population as an ESU requires detailed genetic evidence in support and hence may not always be the most appropriate definition. Moritz (1994) defined a more practical ‘management unit’ as demographically independent breeding units identified as populations having distinct allele frequencies regardless of phylogenetic structure and the level of genetic divergence. This is a very similar definition to that of the stock concept, which is used extensively in fisheries management. A stock is defined as a group of organisms whose demographic genetic trajectory is largely independent from other such groups (Waples 1998). The debate over the most appropriate level for conservation is a contentious one and open to many interpretations (Bowen 1999). The role of policy and priorities is also important and should be taken into consideration early in any scientific investigation that intends to lead to management recommendations (Taylor & Dizon 1999).

The above concepts were developed with conservation, not control, in mind, but the principles are the same. If quelea are divided into separate stocks in southern Africa, then they need to be managed in different ways. The aim is not to preserve the evolutionary history that is present, or to maintain a viable population for harvesting, but to give structure to the management decisions of a pest control operation.

The definition that is more useful for the purposes of this thesis is that of a management unit. There is no interest in preserving unique lineages, only in defining independent demographic units. This is a task for which molecular methods are

appropriate but are certainly not the only source of information. Genetic differences are only a part of what is important in defining the population units of control for quelea in southern Africa. Some examples will now be given of how molecular methods have been used to determine the population structure and management units for a range of species that share common features with quelea.

### *3.1.2 Population structure of the redbilled quelea in southern Africa*

Two theories suggest that there may be a division in the population of quelea in southern Africa. The first is based on the hypothesised existence of a migratory divide, with quelea from different sides of this divide regularly and faithfully sticking to one migration route. Such a pattern could provide the reproductive isolation necessary to maintain genetic differentiation. The second connected piece of evidence is the description of the *spoliator* subspecies of quelea in south east South Africa. The detailed background to both ideas was explained in Chapter One.

### *3.1.3 Identifying migration routes using molecular markers*

There are demographic reasons why it may be appropriate to describe two separate management units for quelea in southern Africa. However the simple process of surveying the variation in allele frequencies across populations from different parts of the subcontinent is complicated by the fact that quelea are long distant migrants. Indeed it is quite conceivable that the non-breeding population is a mixture of two separate breeding ones. Defining management units and population structure could therefore be a complex task. Nonetheless, molecular methods have been informative in defining migration routes in many circumstances.

The small migratory noctule bat *Nyctalus noctula* can fly up to 1600 km between summer and winter roosts. Females are known to be philopatric to their natal roosts. Petit and Mayer (Petit & Mayer 2000) used mitochondrial DNA control region sequences to show that wintering roosts were more genetically diverse than summer roosts, indicating that winter roosts are used by bats from a variety of summer roosts. The summer and winter roosts between which individuals migrated should also not be genetically differentiated. Therefore by linking genetically similar winter and

summer roosts the authors were able to show possible migration routes that were consistent with the ringing and marking data available. A mtDNA population genetic approach was successful in defining migration routes of a highly mobile bat species.

RAPD markers were used to distinguish between populations of migratory shore birds such as Hudsonian godwit *Limosa haemastica* and the red-necked phalarope *Phalaropus lobatus* (Haig et al. 1997). Individuals were assigned to putative breeding locations using the frequency method of Paetkau et al. (1995). Assignments were more certain when the populations were more genetically differentiated. However, the genetic differentiation was only sufficient to be able to assign non-breeding individuals to either eastern or western breeding populations. Nevertheless, the authors recommended using molecular markers to track migratory birds. Even the coarse detail of migration routes described was a notable increase in the knowledge about these particular birds.

The dunlin *Calidris alpina* is a widespread shore bird that shows a high degree of morphological variation. MtDNA sequences were used to show that non-breeding dunlin could be assigned to either eastern or western lineages according to which haplotypes they possessed (Wenink & Baker 1996). The authors further concluded that non-breeding populations of dunlin consisted of a mixture of breeding populations that join on the southward migration. The breeding origins of dunlin could further be distinguished by including some morphological measures along with the mtDNA haplotypes (Wennerberg et al. 1999). However the estimates of dunlin origin remained crude, with large confidence intervals. Nonetheless, molecular techniques combined with morphology were used to give a likelihood that a specific individual came from a particular dunlin breeding region.

Variation in the control region of mtDNA of the beluga whale *Delphinapterus leucas* revealed that individuals at five summer grounds off Alaska that were sampled were demographically distinct (O'Corry-Crowe et al. 1997). The authors were also able to show evidence for summer-ground philopatry even though beluga whales from several summer grounds share winter regions, and are capable of migrating thousands of kilometres.

The above examples have one thing in common – the authors were able to make new discoveries about wide-ranging, long distance migrants based on the properties of a set of molecular markers. These inferences were possible despite the fact that the study animals invariably mixed during the non-breeding season before exhibiting some level of philopatry so as to separate out into distinguishable units for breeding. This chapter aims to quantify the amount of genetic differentiation between quelea populations. If the observed genetic differentiation warrants it, management units will be described. Further, the genetic relationships between populations of quelea will be used to define migration routes and decide whether quelea form mixed non-breeding aggregations regardless of the migration pattern they follow.

## 3.2 Methods

### 3.2.1 Sampling

Of the samples collected and listed in Table 2.1, 1042 samples were used for further analysis. Up to 48 males were used from 28 sites. Sites were selected that comprised at least 45 male samples. Other sites with fewer males were also used if they represented geographically distinct regions for which no other sample was available. The sites used in this part of the thesis are shown in Figure 3.1, and on Map 2. Details of sites are given in Table 3.1. Some of the sites in Table 3.1 have only a few samples associated with them. Nonetheless these sites were important as they represented geographic extremes. Tsumcor (TS) and Aris (NA) in Namibia were the most westerly sites, while Volksrust (VK), Natal (NL) and Pietermaritzburg (PM) in South Africa were the most easterly. The samples WQ, RQ, TTQ, and VVQ represent a time series from Riverside Farm (RF). The sample from Lake Manyame (LM), Zimbabwe, consisted of non-breeding adults, 19 of which were identified as male.

Variation in the sample size of genotyped individuals from the populations could lead to difficulties in interpreting results. The main problem is that the standard errors for the population genetic parameters will vary among populations, meaning that the accuracy of the estimates from small populations could be doubtful. Hence

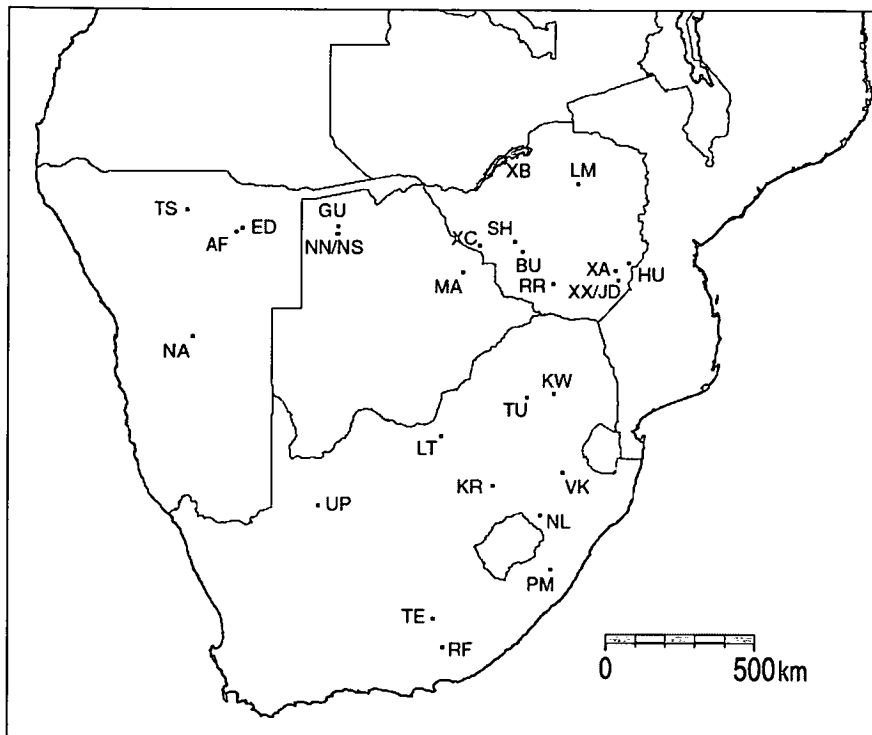


Figure 3.1. Sampling sites in southern Africa used in population genetics analysis. Each site is represented by a two letter code. See Map 2 or Table 3.1 for full site names.

care is needed in interpreting some of the results. However, for other analysis techniques, such as the shared allele tree building, and assignment of unknown individuals, population sample size is unimportant as the analysis technique uses the individual, not the population. Therefore small populations were only included in certain of the analyses as appropriate.

The population structure of male quelea is the focus for this chapter for three reasons. First, male quelea show plumage polymorphism that is described in Chapter Four. Second, quelea subspecies are defined on the basis of variation in male plumage patterns. Finally, as shown in Chapter Six, there are detectable differences in the dispersal of males and females. It was therefore sensible to keep the sexes separate for population genetic analysis.

In addition to the quelea samples, blood samples from 25 male red bishops *Euplectes orix* (collected by Jonas Örnberg at Volksrust, South Africa) were obtained. Red bishops are from a closely related genus to quelea, and are in the same family, Ploceidae (Maclean 1993).

Table 3.1. The number of samples genotyped at each of the 32 sites. For site locations see Map 2 or Figure 3.1.

Site	Full Name	Country	Date	Type	No of Genotypes		
					Males	Females	Other
AF	Alwyn Farm	Namibia	22/04/99	Breeding	45	28	
BU	Bulawayo	Zimbabwe	27/11/97	Non-Breeding	45	34	
ED	Eden Farm	Namibia	24/04/99	Breeding	44		
GU	Gumare	Botswana	15/03/99	Breeding	45		
HU	Humani	Zimbabwe	27/03/97	Breeding	45		
JD	JDMalilangwe97	Zimbabwe	03/97	Breeding	37	22	62#
KR	Kroonstad	South Africa	24/02/99	Breeding	45	21	
KW	Klawervallei	South Africa	26/01/00	Breeding	45		
LM	Lake Manyame	Zimbabwe	15/11/99	Non-Breeding	19		32*
LT	Lichtenberg	South Africa	16/02/99	Breeding	45		
MA	Mathangwane	Botswana	09/03/99	Breeding	46		
NA	Aris	Namibia	26/03/98	Unknown	2		
NL	Natal	South Africa	01/02/95	Unknown	2		
NN	Nokoneng North	Botswana	13/03/99	Breeding	46		
NS	Nokoneng South	Botswana	14/03/99	Breeding	45	22	
PM	Pietermaritzburg	South Africa	11/12/95	Unknown	2		
RF	Riverside Farm	South Africa	16/02/99	Breeding	45	29	
RQ	Riverside Farm	South Africa	12/12/97	Breeding	2		
RR	Reata Ranch	Zimbabwe	24/03/99	Breeding	45		
SH	Shirville Farm	Zimbabwe	16/03/99	Breeding	45		
TE	Terminus	South Africa	18/02/99	Breeding	45		
TS	Tsumcor	Namibia	28/04/99	ex-Breeding	6		
TTQ	Riverside Farm	South Africa	30/10/98	Breeding	7		
TU	Tuinplaas	South Africa	05/03/99	Breeding	44		
UP	Upington	South Africa	26/01/99	Breeding	14		
VK	Volkstrust	South Africa	21/01/00	Unknown	1		
VVQ	Riverside Farm	South Africa	25/11/98	Breeding	5		
WQ	Riverside Farm	South Africa	24/11/97	Breeding	2		
XA	Senuko	Zimbabwe	10/03/98	Breeding	48		
XB	Bumi Hills	Zimbabwe	18/03/98	Breeding	47	27	
XC	Maitengwe Dam	Zimbabwe	24/03/98	Breeding	48		
XX	Malilangwe	Zimbabwe	08/03/98	Breeding	48	24	
					1010	207	94

\* - Non-breeding adults

# - Nestlings not included in population genetic analysis.

### 3.2.2 Molecular methods and data analysis

The protocols for DNA extraction and PCR amplification of microsatellite loci are outlined in Appendix A. The 10 loci used for this study are listed in Table 2.4. Lox 8 and Hru7 were not used for population genetics (see section 2.4.4). After screening, two further loci (Pdou3 and WBSW11) were not used in population genetic analysis as they consistently deviated from Hardy-Weinberg equilibrium, as described in

Section 3.3.2. Eight loci were therefore used in the population genetics analysis. Data analyses were performed as described in Chapter 2.

### 3.3 Results

A total of 9844 genotypes were obtained from the 1042 quelea screened for 10 microsatellite loci. In addition, a further 185 genotypes were obtained from 25 red bishops screened for the same 10 loci. The number of genotypes for each locus and population is listed in Table 3.3.

#### 3.3.1 Allele Frequencies

The allele frequencies for each population, and over all quelea populations are presented in Appendix C. There was tremendous variability in allele frequencies between populations, but it was difficult to notice any trends in a data set of 268 different alleles across 28 populations. The only clear trend was that red bishops (JRB) had consistently different allele frequencies across all loci compared to quelea. In contrast among quelea populations the most common allele varied between populations. For example, for locus Esc4, allele 168 was the most common in Mathengwane (MA) with a frequency of 0.20, while for Terminus (TE) the most common allele was 164, with a lower frequency of 0.14. In most populations (19 out of 26) locus Mcyu4 allele 154 was the most common, indicating little among population variation for this locus, with frequencies ranging from 0.15 to 0.26. Private alleles were common, with 29 being detected in total. Nine loci had at least one private allele in one population; locus Hru5 had seven private alleles; 16 of the 26 populations had at least one private allele. Populations Maitengwe Dam (XC), Humani (HU) and Shirville Farm (SH) all had three private alleles. Only six populations that had at least 40 samples did not have private alleles, while none of the smaller ( $n < 40$ ) populations had any. Private alleles occurred at frequencies up to 0.033 for Shirville Farm (SH) locus Hru5, allele 108.

The high number of private alleles detected raises the question of whether sample sizes were adequate to have completely sampled all the alleles in a population. In other words, if sample sizes were increased, would new alleles keep on being



discovered, and would alleles that are ‘private’ simply be rare. One way to address this question is to examine the way in which the average number of alleles detected per locus and population varies with sample size. Figure 3.2 shows that as sample size increased, the number of alleles detected starts to level off at around a sample size of 45 to 50 individuals. Therefore with a standard sample size of 45, most of the alleles have been detected that are present in a population. Increasing sample size does not substantially increase the number of alleles found.

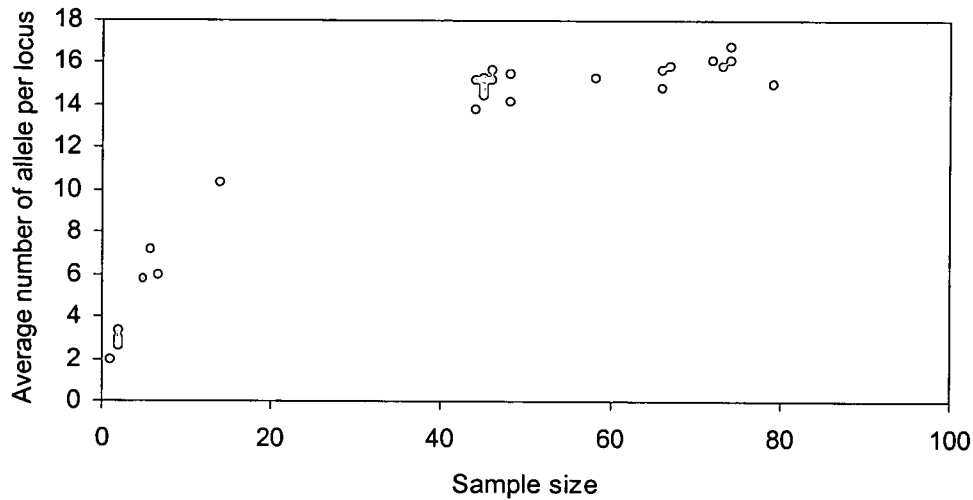


Figure 3.2. Average number of alleles detected per locus according to sample size. Based on six loci used in Chapter Six (*Esc4*, *Hru5*, *Mcyu4*, *Phtr2*, *WBSW2*, *WBSW4*). Populations with  $N > 48$  include female genotypes described in Chapter Six.

### 3.3.2 Hardy-Weinberg Equilibrium

Significant deviations from Hardy-Weinberg equilibrium at the 0.05 level were detected in 81 out of 258 population by locus comparisons, as shown in Table 3.2. All loci, except *Phtr2* had at least one population that showed significant deviation from equilibrium conditions. However, because such a large number of tests were performed, it is likely that a number of these significant results are type one errors.

After correcting for multiple tests 29 population-by-locus comparisons remained significantly different from Hardy-Weinberg expectations at the 0.05 level. 15 of the significant tests were for locus *Pdou3* and 11 for locus *WBSW11*. In addition, for locus *Mcyu4*, one population (LT) showed significant deviation from equilibrium,

Table 3.2. Locus-Population combinations and significance of deviation from Hardy-Weinberg expectations. P-values in italics indicate significance at the 0.05 level before correction for multiple tests, P-values in bold italics indicate significance after sequential Bonferroni correction (Rice 1989).

Popn	Esc4		Hru5		Mcyu4		Pdou3		Phtr2		Phtr3		WBSW1		WBSW2		WBSW4		WBSW11	
	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.
AF	0.6284	0.014	0.0592	0.007	<i>0.0016</i>	0.001	<b><i>0.0000</i></b>	0.000	0.3205	0.009	0.8402	0.011	0.7511	0.013	<i>0.0186</i>	0.004	<i>0.0174</i>	0.004	<b><i>0.0000</i></b>	0.000
BU	<i>0.0130</i>	0.003	<i>0.0345</i>	0.004	0.2297	0.010	0.3761	0.012	0.9896	0.001	<i>0.0022</i>	0.001	0.3442	0.015	0.9518	0.006	0.4645	0.017	<i>0.0138</i>	0.004
ED	0.5901	0.012	0.1621	0.011	0.7498	0.006	<b><i>0.0000</i></b>	0.000	0.2120	0.009	0.5985	0.017	0.7394	0.014	<i>0.0472</i>	0.005	0.9332	0.007	<i>0.0008</i>	0.001
GU	0.8636	0.009	0.2734	0.014	0.3363	0.011	<i>0.0024</i>	0.001	0.3596	0.008	<i>0.0302</i>	0.005	0.8072	0.015	0.4884	0.019	0.2096	0.013	<i>0.0129</i>	0.004
HU	0.3762	0.013	<i>0.0075</i>	0.002	0.1238	0.007	<b><i>0.0000</i></b>	0.000	0.2161	0.007	<i>0.0080</i>	0.003	0.8769	0.011	0.4664	0.016	0.4147	0.016	<i>0.1448</i>	0.013
JD	0.0746	0.008	<i>0.0010</i>	0.001	<i>0.0085</i>	0.003	<b><i>0.0000</i></b>	0.000	0.1343	0.006	<i>0.0020</i>	0.001	0.1125	0.009	0.4677	0.014	<i>0.0080</i>	0.002	<b><i>0.0000</i></b>	0.000
KR	<i>0.0466</i>	0.006	0.2891	0.014	0.5432	0.011	<b><i>0.0000</i></b>	0.000	0.6791	0.008	0.5750	0.018	0.8095	0.012	0.7115	0.009	0.0907	0.009	<i>0.0009</i>	0.001
KW	0.6960	0.014	0.2933	0.014	0.4913	0.011	0.3192	0.016	0.1830	0.010	0.7445	0.013	0.7873	0.013	0.0588	0.006	0.6305	0.014	<b><i>0.0000</i></b>	0.000
LM	0.0812	0.007	0.1543	0.009	<i>0.0279</i>	0.003	<b><i>0.0000</i></b>	0.000	0.9566	0.003	<b><i>0.0000</i></b>	0.000	0.2059	0.014	<i>0.0370</i>	0.005	<i>0.0271</i>	0.005	<b><i>0.0000</i></b>	0.000
LT	0.2761	0.010	<i>0.0154</i>	0.003	<b><i>0.0000</i></b>	0.000	<i>0.0013</i>	0.001	0.7460	0.010	0.2034	0.013	0.9277	0.008	<i>0.0381</i>	0.006	0.1517	0.011	<i>0.0093</i>	0.002
MA	0.7033	0.014	<i>0.0323</i>	0.005	0.4861	0.011	<b><i>0.0000</i></b>	0.000	0.3208	0.012	<b><i>0.0000</i></b>	0.000	0.4236	0.017	0.0838	0.009	0.2838	0.015	<b><i>0.0002</i></b>	0.000
NN	<i>0.0431</i>	0.005	0.2154	0.013	<i>0.0047</i>	0.001	<b><i>0.0000</i></b>	0.000	0.1021	0.006	<i>0.0013</i>	0.001	0.1511	0.011	0.1508	0.013	0.0852	0.008	<b><i>0.0000</i></b>	0.000
NS	0.5038	0.015	0.1757	0.009	0.0771	0.007	<b><i>0.0000</i></b>	0.000	0.6392	0.008	0.1043	0.010	0.3532	0.018	0.4895	0.016	0.5457	0.017	<b><i>0.0000</i></b>	0.000
RF	0.3931	0.013	0.5113	0.014	0.2564	0.013	<b><i>0.0000</i></b>	0.000	0.1028	0.006	<i>0.0225</i>	0.004	0.5146	0.017	0.0852	0.008	0.0509	0.007	<i>0.0003</i>	0.000
RR	0.8552	0.010	0.2019	0.011	0.8951	0.005	<i>0.0101</i>	0.002	0.4054	0.010	0.2920	0.014	0.6615	0.015	0.3399	0.012	0.0703	0.009	<i>0.0126</i>	0.003
SH	0.1525	0.010	0.2262	0.013	0.2942	0.011	<i>0.0120</i>	0.003	0.8382	0.007	0.1837	0.013	0.6562	0.015	0.4406	0.016	<i>0.0010</i>	0.001	<i>0.0010</i>	0.001
TE	0.2126	0.013	0.7277	0.012	0.1673	0.009	<i>0.0012</i>	0.001	0.2394	0.010	0.8214	0.010	0.2625	0.017	0.7523	0.015	<i>0.0169</i>	0.003	<i>0.0299</i>	0.007
TS	1.0000	0.000	1.0000	0.000	0.4272	0.006	0.2150	0.006	0.9544	0.002	0.4847	0.010	0.6043	0.008	0.1739	0.006	0.3897	0.013	<i>0.0009</i>	0.000
TTQ	1.0000	0.000	0.8101	0.007	0.1751	0.006	1.0000	0.000	0.7460	0.000	1.0000	0.000	1.0000	0.000	0.3388	0.004	0.4238	0.012	1.0000	0.000
TU	0.7285	0.013	0.2739	0.012	0.1285	0.009	<b><i>0.0000</i></b>	0.000	0.8077	0.006	<i>0.0191</i>	0.005	0.4972	0.015	0.2100	0.013	0.5348	0.017	<b><i>0.0000</i></b>	0.000
UP	0.9797	0.003	0.0619	0.006	0.0611	0.007	<i>0.0142</i>	0.003	0.3287	0.004	0.2516	0.015	0.5530	0.014	0.5397	0.013	0.7008	0.011	<i>0.0181</i>	0.003
VVQ	-	-	1.0000	0.000	0.4743	0.006	0.4850	0.006	1.0000	0.000	1.0000	0.000	1.0000	0.000	0.5004	0.005	0.7901	0.005	0.6927	0.004
XA	0.0877	0.008	<i>0.0274</i>	0.005	<i>0.0033</i>	0.001	<b><i>0.0000</i></b>	0.000	0.1374	0.006	<i>0.0239</i>	0.005	0.3974	0.017	0.2445	0.013	0.2066	0.014	<b><i>0.0000</i></b>	0.000
XB	0.0641	0.007	0.3697	0.013	0.2567	0.011	<b><i>0.0000</i></b>	0.000	0.6126	0.007	0.5285	0.015	0.9521	0.006	<i>0.0308</i>	0.006	0.3836	0.016	<b><i>0.0000</i></b>	0.000
XC	0.0834	0.008	0.3715	0.011	0.1377	0.008	<b><i>0.0000</i></b>	0.000	0.1739	0.006	0.0666	0.009	0.3886	0.017	0.3535	0.016	0.4525	0.017	<i>0.0009</i>	0.001
XX	0.0842	0.008	<i>0.0423</i>	0.005	0.5560	0.011	<b><i>0.0000</i></b>	0.000	0.8120	0.007	0.1619	0.011	<i>0.0131</i>	0.004	0.5391	0.015	0.1433	0.012	<b><i>0.0000</i></b>	0.000

and two populations (LM and MA) were not in equilibrium for locus Phtr3. All the deviations were due to heterozygote deficiency. The most extreme examples were for locus Pdou3, where for seven comparisons, less than half the number of expected heterozygotes was observed. These were for populations XA, XB, XC, XX, NN, MA and JD.

There are several explanations for deviations from Hardy-Weinberg equilibrium. Errors could be due to selection on the microsatellite loci, non-random mating, and sampling across unknown population substructure (the Wahlund effect). However, as the incidences of deviation were concentrated in just two loci, the most likely explanation in the case of loci Pdou3 and WBSW11 is the presence of null alleles. Due to non-amplification of an allele, null alleles cause a heterozygote to be scored homozygous. As the extent of deviation from Hardy-Weinberg in these two loci was so extreme and widespread, and as many of the techniques for investigating genetic structure assume Hardy-Weinberg equilibrium, loci Pdou3 and WBSW11 were excluded from all further analyses. The other three population-locus comparisons that deviated from equilibrium were not consistent by locus or population, and hence were included in further analysis.

The sample of red bishops (JRB) showed consistently high values of  $F_{IS}$  across loci. There is also a consistent deficit in heterozygotes with  $H_o$  consistently lower than  $H_e$ . Both indicate deviation from Hardy-Weinberg expectations, most probably due to null alleles. Nonetheless, the JRB samples are included in some further analyses with the caveat that, whilst the trends revealed for their dissimilarity, or otherwise, from quelea are probably robust, any estimates of phylogenetic relatedness are unlikely to be valid.

### ***3.3.3 Heterozygosity and inbreeding***

Observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity and values of  $F_{IS}$  (Weir & Cockerham 1984) – the inbreeding coefficient – are given in Table 3.3. For normal, outbreeding populations,  $F_{IS}$  should be close to zero. For all population-locus comparisons that were in Hardy-Weinberg equilibrium this was generally true.  $F_{IS}$

ranged from  $-0.061$  for KR/Hru5 to  $0.188$  for NN/Mcyu4 among populations for which sample size was greater than 40.

Observed heterozygosity was generally high, ranging from  $0.544$  for MA/Phtr2 to  $0.97$  for TU/Esc4. Locus WBSW4 was the most heterozygous, with  $H_o$  ranging between  $0.80$  and  $0.96$ . Phtr2 was the least heterozygous, with  $H_o$  as low as  $0.544$ . Except for populations already noted as being out of Hardy-Weinberg equilibrium, there was no trend for  $H_o$  to be lower than  $H_e$ .

### *3.3.4 Genotypic linkage disequilibrium*

Exact tests for genotypic linkage disequilibrium showed five cases of significant non-random associations between loci, as shown in Table 3.4. Three of these involve loci Pdou3 and WBSW11, leaving only two: Phtr2 and WBSW4 and Phtr3 and Hru5, between pairs of loci that were used in subsequent analysis.

### *3.3.5 Genetic differentiation and population substructure*

The amount of genetic differentiation and sub-structure was calculated using two different parameters,  $F$  and  $R$  statistics. First the amount of sub-structuring across all populations was estimated; second, pairwise population comparisons were made using pairwise  $F_{ST}$  and  $R_{ST}$ . Finally AMOVA was used to test for structure between defined groups of populations that might better reflect the actual relationships than the population samples alone.

Table 3.3 Observed (Ho) and expected (He) heterozygosity, and Fis for each locus and population.  
 N gives number of genotypes per locus. Mean and standard errors (S.E.) calculated for each locus for quelea samples only.

																									Total/		JRB		
	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX		Mean	S.E.
Esc4 N	45	26	43	39	44	35	45	33	45	18	44	42	45	45	45	29	45	6	3	33	14	0	44	47	48	48	911	21	
He	0.912	0.894	0.905	0.913	0.919	0.889	0.910	0.883	0.913	0.869	0.879	0.914	0.906	0.922	0.922	0.907	0.890	0.861	0.611	0.907	0.875	0.000	0.884	0.899	0.926	0.912	0.855	0.036	0.857
Ho	0.911	0.962	0.907	0.949	0.932	0.829	0.911	0.909	0.867	0.833	0.864	0.810	0.867	0.889	0.978	0.897	0.889	1.000	1.000	0.970	1.000	0.000	0.841	0.830	0.792	0.854	0.865	0.037	1.000
Fis	0.013	-0.057	0.010	-0.026	-0.003	0.083	0.010	-0.014	0.062	0.069	0.029	0.126	0.055	0.047	-0.050	0.029	0.013	-0.071	-0.500	-0.054	-0.106	-	0.061	0.087	0.155	-0.073	0.002	0.024	-0.143
Hru5 N	45	30	41	44	43	36	45	41	44	44	46	46	45	44	43	45	45	5	7	43	14	5	48	41	48	48	986	21	
He	0.882	0.908	0.916	0.899	0.916	0.895	0.906	0.912	0.904	0.904	0.898	0.909	0.887	0.903	0.902	0.909	0.904	0.820	0.827	0.906	0.816	0.880	0.908	0.908	0.908	0.907	0.894	0.006	0.821
Ho	0.800	0.767	0.829	0.841	0.884	0.750	0.911	0.902	0.886	0.773	0.913	0.826	0.800	0.909	0.930	0.844	0.911	1.000	1.000	0.814	0.643	1.000	0.896	0.854	0.854	0.833	0.860	0.016	0.476
Fis	0.104	0.172	0.107	0.076	0.047	0.175	0.006	0.023	0.031	0.156	-0.006	0.102	0.110	0.005	-0.020	0.082	0.004	-0.111	-0.135	0.114	0.248	-0.026	0.024	0.073	0.069	0.092	0.059	0.017	0.440
Mcyu4 N	45	43	39	41	43	35	36	42	45	44	39	37	36	43	35	32	42	6	6	43	14	5	43	43	46	46	929	22	
He	0.853	0.874	0.815	0.864	0.888	0.871	0.853	0.892	0.888	0.875	0.871	0.884	0.835	0.884	0.880	0.888	0.869	0.778	0.833	0.877	0.901	0.820	0.876	0.873	0.863	0.859	0.864	0.005	0.819
Ho	0.711	0.884	0.821	0.829	0.814	0.771	0.917	0.952	0.822	0.705	0.846	0.730	0.750	0.791	0.914	0.844	0.738	0.667	0.833	0.837	0.714	1.000	0.837	0.907	0.826	0.957	0.824	0.017	0.727
Fis	0.177	0.001	0.006	0.052	0.095	0.129	-0.061	-0.055	0.085	0.206	0.041	0.188	0.115	0.117	-0.024	0.065	0.162	0.231	0.091	0.057	0.242	-0.111	0.056	-0.027	0.054	-0.103	0.069	0.019	0.135
Pdou3 N	43	44	42	45	45	37	42	43	44	45	45	43	45	45	43	44	45	6	6	43	14	5	46	46	46	47	999	0	
He	0.883	0.893	0.914	0.898	0.900	0.899	0.891	0.904	0.885	0.872	0.896	0.888	0.903	0.890	0.911	0.897	0.887	0.819	0.875	0.903	0.862	0.820	0.888	0.892	0.887	0.895	0.887	0.004	0.000
Ho	0.605	0.841	0.643	0.644	0.622	0.432	0.524	0.837	0.591	0.644	0.422	0.442	0.556	0.600	0.884	0.705	0.733	0.667	1.000	0.512	0.643	0.800	0.370	0.413	0.391	0.426	0.613	0.033	0.000
Fis	0.326	0.069	0.308	0.293	0.318	0.529	0.422	0.086	0.342	0.271	0.537	0.511	0.394	0.336	0.041	0.226	0.184	0.273	-0.053	0.443	0.289	0.135	0.591	0.544	0.566	0.532	0.327	0.035	-
Phtr2 N	45	41	44	44	45	35	45	43	45	44	46	46	45	45	43	43	45	6	5	44	14	3	47	47	48	48	1006	14	
He	0.678	0.693	0.731	0.769	0.721	0.711	0.705	0.655	0.656	0.635	0.641	0.735	0.667	0.683	0.637	0.752	0.721	0.750	0.660	0.718	0.696	0.611	0.706	0.747	0.653	0.664	0.692	0.008	0.857
Ho	0.600	0.732	0.705	0.841	0.844	0.686	0.778	0.605	0.711	0.705	0.544	0.652	0.733	0.689	0.744	0.814	0.844	1.000	0.800	0.773	0.786	1.000	0.723	0.872	0.771	0.646	0.754	0.021	0.714
Fis	0.126	-0.043	0.048	-0.082	-0.161	0.050	-0.092	0.088	-0.073	-0.098	0.163	0.123	-0.088	0.003	-0.158	-0.070	-0.164	-0.250	-0.103	-0.065	-0.092	-0.500	-0.015	-0.157	-0.171	0.038	-0.067	0.027	0.202
Phtr3 N	45	40	44	45	45	36	45	43	45	45	44	44	45	45	41	39	45	6	4	44	14	3	47	45	44	47	990	24	
He	0.929	0.931	0.918	0.930	0.925	0.896	0.920	0.931	0.926	0.899	0.904	0.927	0.924	0.917	0.913	0.923	0.925	0.847	0.781	0.919	0.918	0.833	0.916	0.925	0.917	0.908	0.908	0.007	0.837
Ho	0.933	0.800	0.977	0.867	0.800	0.694	0.911	0.954	0.644	0.822	0.727	0.773	0.911	1.000	0.902	0.897	1.000	0.833	1.000	0.818	0.857	1.000	0.851	0.867	0.864	0.809	0.866	0.019	0.833
Fis	0.007	0.153	-0.053	0.079	0.146	0.238	0.021	-0.012	0.314	0.097	0.206	0.177	0.025	-0.079	0.024	0.041	-0.070	0.107	-0.143	0.121	0.103	0.000	0.081	0.074	0.069	0.120	0.071	0.020	0.025
WBSW1 N	45	43	44	45	45	37	45	44	45	45	46	46	45	45	45	45	45	6	5	44	14	5	48	47	48	45	1017	19	
He	0.758	0.795	0.676	0.684	0.824	0.751	0.808	0.748	0.798	0.738	0.792	0.672	0.797	0.778	0.776	0.716	0.787	0.833	0.580	0.762	0.773	0.800	0.783	0.789	0.752	0.791	0.760	0.011	0.658
Ho	0.778	0.861	0.705	0.733	0.933	0.622	0.800	0.864	0.756	0.800	0.696	0.544	0.778	0.778	0.822	0.778	0.844	0.833	0.800	0.750	0.857	1.000	0.771	0.851	0.750	0.756	0.787	0.018	0.474
Fis	-0.014	-0.071	-0.031	-0.060	-0.121	0.186	0.021	-0.143	0.064	-0.072	0.133	0.202	0.035	0.012	-0.048	-0.075	-0.061	0.091	-0.280	0.027	-0.072	-0.143	0.025	-0.068	0.014	0.056	-0.015	0.021	0.305
WBSW2 N	45	40	44	45	45	36	45	42	45	45	46	46	43	45	41	44	45	6	5	44	14	5	48	47	48	48	1007	18	
He	0.838	0.838	0.851	0.806	0.830	0.819	0.852	0.796	0.844	0.864	0.845	0.865	0.827	0.860	0.797	0.824	0.834	0.736	0.740	0.827	0.842	0.680	0.863	0.851	0.800	0.862	0.823	0.009	0.849
Ho	0.756	0.900	0.773	0.756	0.756	0.806	0.800	0.786	0.778	0.822	0.826	0.826	0.907	0.778	0.829	0.818	0.778	0.667	1.000	0.818	0.857	0.800	0.854	0.851	0.729	0.792	0.810	0.013	0.611
Fis	0.110	-0.062	0.103	0.074	0.101	0.031	0.072	0.025	0.090	0.059	0.033	0.056	-0.085	0.107	-0.029	0.018	0.078	0.184	-0.250	0.022	0.019	-0.067	0.021	0.010	0.099	0.092	0.035	0.017	0.306
WBSW4 N	45	41	44	45	45	35	45	44	44	45	44	42	44	45	43	44	45	6	7	44	14	5	46	47	44	46	999	25	
He	0.925	0.920	0.929	0.929	0.923	0.933	0.926	0.930	0.936	0.923	0.923	0.930	0.922	0.941	0.932	0.929	0.928	0.861	0.878	0.932	0.888	0.760	0.930	0.933	0.926	0.928	0.916	0.007	0.000
Ho	0.778	0.927	0.955	0.933	0.889	0.800	0.822	0.955	0.841	0.889	0.886	0.833	0.886	0.911	0.814	0.909	0.800	0.833	1.000	0.886	0.929	0.800	0.891	0.872	0.909	0.870	0.878	0.011	0.000
Fis	0.170	0.005	-0.016	0.007	0.048	0.157	0.123	-0.015	0.113	0.048	0.051	0.116	0.050	0.043	0.138	0.032	0.149	0.123	-0.063	0.061	-0.009	0.059	0.052	0.075	0.030	0.074	0.062	0.012	-
WBSW11 N	45	43	44	44	45	26	45	43	45	45	46	46	45	45	40	45	44	6	6	43	14	5	48	47	47	48	1000	21	
He	0.927	0.903	0.884	0.873	0.880	0.890	0.914	0.912	0.900	0.892	0.891	0.908	0.895	0.898	0.919	0.916	0.895	0.833	0.847	0.908	0.872	0.780	0.894	0.876	0.898	0.877	0.888	0.006	0.872
Ho	0.711	0.767	0.659	0.727	0.800	0.654	0.733	0.721	0.556	0.689	0.630	0.522	0.689	0.622	0.775	0.733	0.773	0.333	1.000	0.605	0.643	0.800	0.583	0.575	0.660	0.500	0.672	0.025	0.762
Fis	0.244	0.161	0.265	0.178	0.102	0.283	0.208	0.221	0.392	0.238	0.303	0.434	0.241	0.317	0.169	0.210	0.148	0.655	-0.091	0.344	0.297	0.086	0.357	0.353	0.275	0.439	0.263	0.027	0.150

Table 3.4. Genotypic linkage disequilibrium for all locus pairs. P-values for each locus pair across all populations (Fisher's method).

Locus pair		Chi <sup>2</sup>	df	P-value
HrU5	Esc4	22.95	46	1.00
Mcyu4	Esc4	21.55	44	1.00
Mcyu4	HrU5	9.79	44	1.00
Pdou3	Esc4	10.81	46	1.00
Pdou3	HrU5	18.38	46	1.00
Pdou3	Mcyu4	19.64	44	1.00
Phtr2	Esc4	33.11	46	0.92
Phtr2	HrU5	42.51	46	0.62
Phtr2	Mcyu4	24.79	46	1.00
Phtr2	Pdou3	Infinity	46	highly significant
Phtr3	Esc4	12.74	44	1.00
Phtr3	HrU5	Infinity	44	highly significant
Phtr3	Mcyu4	6.24	44	1.00
Phtr3	Pdou3	20.74	44	1.00
Phtr3	Phtr2	18.84	44	1.00
WBSW1	Esc4	25.36	48	1.00
WBSW1	HrU5	13.05	46	1.00
WBSW1	Mcyu4	28.59	46	0.98
WBSW1	Pdou3	28.36	46	0.98
WBSW1	Phtr2	47.81	48	0.48
WBSW1	Phtr3	13.45	44	1.00
WBSW2	Esc4	23.65	46	1.00
WBSW2	HrU5	10.36	46	1.00
WBSW2	Mcyu4	30.02	48	0.98
WBSW2	Pdou3	45.16	46	0.51
WBSW2	Phtr2	27.70	50	1.00
WBSW2	Phtr3	17.03	44	1.00
WBSW2	WBSW1	30.28	48	0.98
WBSW4	Esc4	8.92	46	1.00
WBSW4	HrU5	3.76	48	1.00
WBSW4	Mcyu4	14.68	46	1.00
WBSW4	Pdou3	4.60	46	1.00
WBSW4	Phtr2	Infinity	46	highly significant
WBSW4	Phtr3	6.41	44	1.00
WBSW4	WBSW1	29.38	46	0.97
WBSW4	WBSW2	23.73	46	1.00
WBSW11	Esc4	Infinity	46	highly significant
WBSW11	HrU5	7.07	46	1.00
WBSW11	Mcyu4	11.40	44	1.00
WBSW11	Pdou3	15.83	46	1.00
WBSW11	Phtr2	Infinity	46	highly significant
WBSW11	Phtr3	8.52	44	1.00
WBSW11	WBSW1	33.33	46	0.92
WBSW11	WBSW2	20.04	46	1.00
WBSW11	WBSW4	7.26	46	1.00

Overall F and R statistics for quelea populations only are given in Table 3.5. Permutation tests showed that  $F_{ST}$  and  $F_{IS}$  were both significantly different from zero. As  $F_{ST}$  represents the amount of the total genetic variance ( $F_{IT}$ ) that is due to differences between populations, this result indicates that there was some sub-structure present. The components sig a, sig b and sig w represent the components of variance among populations, individuals and within individuals respectively. Sig a was 0.013, which was over 500 times less than the value for sig w (6.597). This indicated that the majority of the variance was accounted for at the within-individual level. Overall  $R_{ST}$  was 0.002, and was divided up into its component variance parts in a similar way to  $F_{ST}$ . The level of variance among populations (sig a = 0.3) is nearly 400 times less than the within individual level (sig w = 119.1). Whilst there was some indication of significant population structure, the overall pattern was that the majority of the variance was partitioned at the within individual level.

Table 3.5. Overall F statistics ( $F_{IT}$ ,  $F_{ST}$ ,  $F_{IS}$ ) and  $R_{ST}$  across all populations. P-value gives the significance after permutation testing with 10,000 permutations. Sig a represents the component of total variance among populations. Sig b is the component of variance among individuals within populations. Sig w is the component of variance within individuals.

	Observed Value	p-value	sig a	sig b	sig w
Fit	0.0439	0.000	0.013	0.292	6.597
Fst	0.0019	0.010	-	-	-
Fis	0.0421	0.000	-	-	-
Rst	0.0020	-	0.300	8.800	119.100

Pairwise population comparisons for  $\theta$  are shown in Table 3.6. The values between quelea populations were uniformly low, ranging from  $-0.067$  for the TTQ-LM comparison to  $0.006$  for the JD-AF comparison (Figure 3.3). Most values were at or around zero with a long tail of negative values. Negative values of  $\theta$  indicate that there was more variance within the samples than there was between them and essentially indicates that there was no differentiation among populations.  $\theta$  between JRB and quelea populations ranged from  $0.126$  for the JRB-XB comparison to  $0.170$  for the JRB-TTQ comparison. The significance of the pairwise population differentiation was tested using a permutation approach. Forty-two of the 650 comparisons were significant. After correcting for multiple tests, only 26

comparisons remained significant. The only significant differences between population pairs were between red bishops (JRB) and quelea populations.

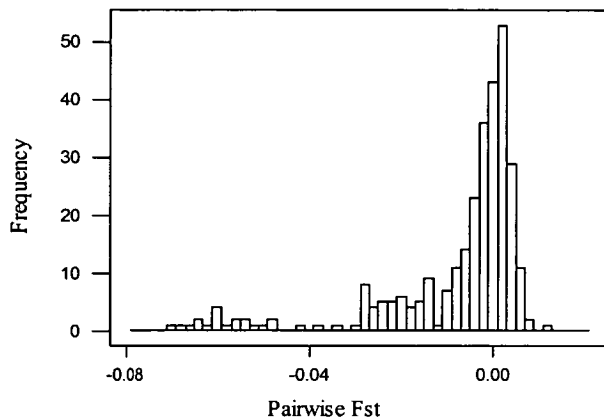


Figure 3.3. The distribution of pairwise  $F_{ST}$ ,  $\theta$ , between all quelea population pairs

Pairwise population comparisons for  $\rho$  are presented in Table 3.7. Values were uniformly low, ranging from  $-0.109$  for the TTQ-HU comparison to  $0.061$  for the TS-AF comparison (Figure 3.4). Most values were at or around zero with a long tail of negative values. Values for  $\rho$  for the comparison between JRB and quelea populations were higher and ranged from  $0.416$  to  $0.480$ . The significance of the pairwise population differentiation was estimated using a permutation approach. Forty of the 650 pairwise tests were significant at the 0.05 level. Only 26 remained significant after correcting for multiple tests using a sequential Bonferroni correction. Once again, the only significant comparisons were between red bishops (JRB) and quelea.

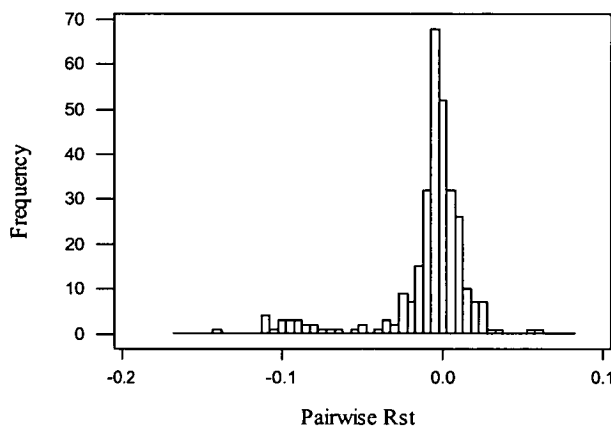


Figure 3.4. The distribution of pairwise  $R_{ST}$ ,  $\rho$ , between all quelea population pairs.



Table 3.6. Estimates of  $\theta$  for relationships between populations (lower matrix) and significance values (upper). Estimates significant at the 0.05 level are in italics. Those significant after sequential Bonferroni correction are in bold italics.

	AF	BU	ED	GU	HU	JD	JRB	KR	KW	LM	LT	MA	NN
AF	-	1.000	0.109	0.434	0.242	<i>0.043</i>	<b>0.000</b>	0.601	0.968	0.384	1.000	0.125	0.674
BU	-0.026	-	1.000	0.999	1.000	0.999	<b>0.000</b>	1.000	0.951	1.000	0.996	1.000	1.000
ED	0.003	-0.021	-	0.435	0.079	<i>0.028</i>	<b>0.000</b>	0.159	0.716	0.138	1.000	<i>0.028</i>	0.280
GU	0.001	-0.015	0.000	-	0.091	0.053	<b>0.000</b>	<i>0.028</i>	0.335	0.300	0.999	<i>0.023</i>	0.378
HU	0.002	-0.022	0.003	0.003	-	0.052	<b>0.000</b>	0.566	0.982	0.655	1.000	0.328	0.788
JD	0.006	-0.015	0.006	0.005	0.005	-	<b>0.000</b>	0.350	0.722	0.267	1.000	0.095	0.331
JRB	0.139	0.154	0.138	0.140	0.127	0.144	-	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>
KR	0.000	-0.028	0.002	0.004	0.000	0.001	0.129	-	0.988	0.947	1.000	0.114	0.207
KW	-0.004	-0.007	-0.002	0.000	-0.005	-0.002	0.154	-0.005	-	1.000	0.975	0.996	0.918
LM	0.001	-0.025	0.003	0.001	0.000	0.002	0.132	-0.003	-0.007	-	1.000	0.637	0.955
LT	-0.022	-0.010	-0.024	-0.010	-0.018	-0.016	0.136	-0.028	-0.005	-0.025	-	1.000	1.000
MA	0.003	-0.020	0.005	0.005	0.001	0.004	0.141	0.003	-0.006	0.000	-0.019	-	0.004
NN	0.000	-0.020	0.002	0.001	-0.001	0.002	0.139	0.002	-0.003	-0.002	-0.019	0.009	-
NS	0.001	-0.028	0.002	0.002	0.001	0.005	0.142	0.002	-0.005	-0.003	-0.025	0.003	0.005
RF	0.001	-0.027	0.001	0.002	-0.001	0.002	0.127	-0.001	-0.009	-0.001	-0.020	0.002	0.000
RR	-0.004	-0.025	0.003	0.000	-0.003	-0.002	0.145	-0.001	-0.009	-0.006	-0.031	0.004	0.000
SH	-0.014	-0.014	-0.007	-0.007	-0.014	-0.007	0.146	-0.007	-0.003	-0.016	-0.004	-0.003	-0.006
TE	0.002	-0.027	0.000	0.000	0.001	0.002	0.128	-0.001	-0.007	-0.003	-0.021	0.000	0.002
TS	0.002	-0.017	-0.001	-0.002	-0.005	0.008	0.182	-0.007	0.003	-0.001	-0.019	0.002	0.004
TTQ	-0.061	-0.033	-0.051	-0.055	-0.059	-0.056	0.170	-0.065	-0.038	-0.067	-0.027	-0.051	-0.054
TU	-0.004	-0.015	-0.004	-0.001	-0.002	-0.003	0.135	-0.004	-0.001	-0.005	-0.005	-0.002	-0.002
UP	0.002	-0.021	0.006	0.005	0.006	0.005	0.147	-0.001	-0.003	-0.001	-0.019	0.006	-0.001
XA	0.004	-0.019	0.002	0.004	0.000	0.003	0.133	-0.001	0.000	-0.002	-0.015	0.004	0.003
XB	0.003	-0.017	0.005	0.002	0.004	0.003	0.126	0.000	-0.001	-0.002	-0.024	0.007	-0.001
XC	0.004	-0.023	0.001	0.002	0.002	0.003	0.135	0.000	-0.007	0.000	-0.029	0.004	0.001
XX	0.004	-0.023	0.003	0.006	0.002	0.005	0.136	-0.003	-0.004	-0.001	-0.026	0.005	0.002

	NS	RF	RR	SH	TE	TS	TTQ	TU	UP	XA	XB	XC	XX
AF	0.456	0.325	0.976	1.000	0.295	0.557	1.000	0.989	0.422	0.061	0.115	0.058	0.051
BU	1.000	1.000	1.000	0.998	1.000	0.805	0.936	1.000	0.984	1.000	1.000	1.000	1.000
ED	0.191	0.262	<i>0.062</i>	0.993	0.418	0.520	0.999	0.983	0.117	0.206	<i>0.016</i>	0.363	0.136
GU	0.157	0.141	0.442	0.993	0.535	0.540	0.999	0.661	0.109	<i>0.032</i>	0.094	0.138	<i>0.010</i>
HU	0.269	0.664	0.940	1.000	0.317	0.751	1.000	0.893	0.107	0.500	<i>0.030</i>	0.145	0.230
JD	0.063	0.328	0.740	0.990	0.289	0.377	0.997	0.903	0.249	0.133	0.130	0.187	0.054
JRB	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>
KR	0.203	0.611	0.642	0.991	0.703	0.797	1.000	0.990	0.573	0.717	0.358	0.489	0.979
KW	0.978	1.000	0.998	0.792	0.999	0.274	0.974	0.536	0.613	0.352	0.611	0.999	0.975
LM	0.976	0.713	1.000	1.000	0.976	0.706	1.000	0.999	0.766	0.906	0.923	0.564	0.821
LT	1.000	1.000	1.000	0.931	1.000	0.964	0.981	0.974	0.999	1.000	1.000	1.000	1.000
MA	0.090	0.185	<i>0.043</i>	0.896	0.518	0.473	0.998	0.794	0.152	<i>0.040</i>	<i>0.005</i>	<i>0.043</i>	<i>0.033</i>
NN	<i>0.035</i>	0.601	0.542	0.989	0.259	0.480	0.999	0.827	0.702	0.093	0.726	0.468	0.242
NS	-	0.745	0.358	0.997	0.995	0.865	1.000	0.998	0.423	0.242	0.014	0.137	0.184
RF	-0.001	-	0.887	1.000	0.586	0.640	1.000	1.000	0.335	0.530	0.145	0.088	0.363
RR	0.000	-0.003	-	0.991	0.930	0.475	0.997	1.000	0.680	0.196	0.497	0.948	0.776
SH	-0.008	-0.016	-0.008	-	1.000	0.770	0.941	0.993	0.970	0.971	1.000	1.000	0.999
TE	-0.005	0.000	-0.003	-0.014	-	0.668	1.000	1.000	0.741	0.862	0.454	0.251	0.307
TS	-0.010	-0.003	-0.003	-0.012	-0.004	-	0.889	0.827	0.173	0.528	0.493	0.566	0.682
TTQ	-0.059	-0.060	-0.062	-0.029	-0.064	-0.048	-	0.997	0.998	0.998	1.000	1.000	0.999
TU	-0.006	-0.006	-0.008	-0.008	-0.006	-0.008	-0.042	-	0.342	0.669	0.983	0.937	0.725
UP	0.001	0.002	-0.003	-0.013	-0.002	0.012	-0.071	0.002	-	0.459	0.237	0.168	0.381
XA	0.001	0.000	0.001	-0.005	-0.002	-0.001	-0.048	-0.001	0.000	-	0.188	<i>0.042</i>	0.786
XB	0.005	0.002	-0.001	-0.011	0.000	-0.001	-0.063	-0.004	0.003	0.002	-	0.463	0.061
XC	0.002	0.003	-0.003	-0.011	0.001	-0.001	-0.058	-0.003	0.005	0.004	0.000	-	0.177
XX	0.002	0.001	-0.002	-0.010	0.001	-0.003	-0.055	-0.001	0.002	-0.001	0.003	0.002	-

Table 3.7. Estimates of  $\rho$  for relationships between populations (lower matrix) and significance values (upper). Estimates significant at the 0.05 level are in italics, those significant after sequential Bonferroni correction are in bold italics.

	AF	BU	ED	GU	HU	JD	JRB	KR	KW	LM	LT	MA	NN
AF	-	1.000	<i>0.006</i>	<i>0.023</i>	0.119	0.238	<b><i>0.000</i></b>	0.130	0.846	0.089	1.000	0.334	0.948
BU	-0.020	-	0.327	0.590	0.862	0.657	<b><i>0.000</i></b>	1.000	0.913	0.999	0.864	0.998	0.872
ED	0.024	0.003	-	0.782	0.400	0.223	<b><i>0.000</i></b>	0.568	0.261	0.387	1.000	0.326	0.115
GU	0.015	-0.003	-0.005	-	0.722	0.442	<b><i>0.000</i></b>	0.328	0.382	0.476	0.999	0.704	0.355
HU	0.009	-0.007	0.001	-0.004	-	0.354	<b><i>0.000</i></b>	0.141	0.539	0.301	1.000	0.808	0.967
JD	0.004	-0.005	0.005	-0.001	0.002	-	<b><i>0.000</i></b>	0.341	0.149	0.314	1.000	0.992	0.290
JRB	0.452	0.452	0.466	0.446	0.460	0.466	-	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>
KR	0.009	-0.023	-0.001	0.002	0.009	0.003	0.448	-	0.998	0.596	1.000	0.361	0.409
KW	-0.007	-0.010	0.003	0.000	-0.002	0.007	0.468	-0.014	-	0.976	0.981	0.610	0.922
LM	0.010	-0.019	0.001	-0.001	0.003	0.003	0.452	-0.002	-0.010	-	1.000	0.753	0.823
LT	-0.025	-0.008	-0.022	-0.015	-0.026	-0.027	0.461	-0.039	-0.011	-0.020	-	1.000	1.000
MA	0.002	-0.017	0.002	-0.004	-0.005	-0.013	0.463	0.002	-0.003	-0.005	-0.035	-	0.457
NN	-0.009	-0.008	0.010	0.002	-0.009	0.004	0.480	0.002	-0.009	-0.006	-0.022	0.000	-
NS	0.019	-0.004	0.015	0.012	0.014	0.016	0.471	0.009	-0.001	-0.008	-0.016	0.011	0.012
RF	0.011	-0.023	-0.006	-0.008	0.005	-0.002	0.451	-0.010	-0.014	-0.004	-0.035	-0.006	-0.004
RR	0.001	-0.012	0.002	-0.003	-0.007	-0.007	0.465	-0.002	-0.006	-0.003	-0.052	-0.006	-0.001
SH	-0.004	-0.006	0.023	0.015	0.004	-0.004	0.454	0.001	0.007	-0.002	-0.011	-0.005	0.008
TE	0.011	-0.004	-0.007	-0.007	-0.003	-0.002	0.469	-0.003	-0.003	-0.001	-0.026	-0.003	0.003
TS	0.061	0.024	0.011	0.010	0.022	0.031	0.493	0.024	0.026	0.003	-0.011	0.021	0.054
TTQ	-0.110	-0.068	-0.074	-0.095	-0.109	-0.063	0.416	-0.109	-0.091	-0.105	-0.051	-0.087	-0.111
TU	0.009	-0.006	-0.008	-0.009	-0.007	-0.004	0.457	-0.007	-0.002	-0.009	-0.013	-0.011	-0.002
UP	-0.005	-0.031	-0.002	-0.007	0.004	0.003	0.476	-0.017	-0.026	-0.014	-0.027	-0.004	-0.009
XA	0.012	0.003	-0.006	-0.005	0.000	0.003	0.450	-0.003	0.001	0.005	-0.012	0.002	0.004
XB	0.023	-0.004	-0.003	-0.006	-0.001	0.004	0.463	0.006	-0.001	-0.006	-0.018	-0.002	0.006
XC	0.001	-0.026	0.018	0.013	0.015	-0.003	0.478	0.003	-0.005	0.000	-0.035	-0.001	0.009
XX	0.008	-0.016	-0.001	-0.003	0.007	-0.010	0.468	-0.001	-0.002	-0.002	-0.031	-0.009	0.005

	NS	RF	RR	SH	TE	TS	TTQ	TU	UP	XA	XB	XC	XX
AF	<i>0.020</i>	0.073	0.345	0.625	0.070	<i>0.030</i>	1.000	0.091	0.593	0.062	<i>0.007</i>	0.389	0.115
BU	0.656	1.000	0.965	0.748	0.656	0.254	0.972	0.780	0.985	0.334	0.686	1.000	0.995
ED	<i>0.042</i>	0.836	0.312	<i>0.010</i>	0.915	0.317	0.998	0.923	0.518	0.824	0.639	<i>0.023</i>	0.476
GU	0.056	0.917	0.618	<i>0.032</i>	0.856	0.276	0.998	0.950	0.609	0.791	0.839	<i>0.048</i>	0.641
HU	0.060	0.213	0.895	0.275	0.644	0.232	1.000	0.883	0.382	0.465	0.537	<i>0.045</i>	0.177
JD	<i>0.045</i>	0.556	0.813	0.621	0.514	0.154	0.989	0.640	0.382	0.312	0.264	0.571	0.940
JRB	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>	<b><i>0.000</i></b>
KR	0.133	0.992	0.607	0.393	0.641	0.247	1.000	0.875	0.951	0.685	0.204	0.315	0.560
KW	0.446	0.999	0.764	0.147	0.572	0.166	0.995	0.531	0.987	0.371	0.454	0.749	0.530
LM	0.932	0.711	0.600	0.535	0.513	0.425	1.000	0.951	0.892	0.227	0.845	0.453	0.545
LT	0.999	1.000	1.000	0.976	1.000	0.633	0.987	0.997	0.999	0.998	1.000	1.000	1.000
MA	0.079	0.828	0.832	0.709	0.645	0.236	0.998	0.990	0.556	0.346	0.559	0.493	0.951
NN	0.086	0.719	0.506	0.157	0.296	0.096	1.000	0.581	0.740	0.282	0.211	0.133	0.233
NS	-	0.516	0.231	0.182	0.179	0.634	1.000	0.680	0.769	<i>0.045</i>	0.417	0.134	0.072
RF	-0.001	-	0.876	0.749	0.942	0.335	1.000	0.999	0.960	0.955	0.644	0.364	0.810
RR	0.004	-0.007	-	0.923	0.868	0.302	0.997	1.000	0.576	0.628	0.344	0.231	0.724
SH	0.006	-0.006	-0.009	-	0.080	0.301	0.967	0.273	0.543	<i>0.012</i>	0.068	0.994	0.422
TE	0.006	-0.008	-0.007	0.011	-	0.316	1.000	0.985	0.680	0.878	0.752	0.087	0.839
TS	-0.013	0.007	0.011	0.010	0.008	-	0.880	0.629	0.317	0.229	0.551	0.139	0.214
TTQ	-0.099	-0.100	-0.089	-0.055	-0.098	-0.086	-	0.998	0.998	0.999	1.000	0.998	0.998
TU	-0.004	-0.013	-0.015	0.003	-0.010	-0.015	-0.082	-	0.777	0.534	0.999	0.588	0.926
UP	-0.011	-0.018	-0.005	-0.005	-0.008	0.019	-0.141	-0.012	-	0.644	0.601	0.605	0.561
XA	0.016	-0.008	-0.003	0.022	-0.007	0.023	-0.095	-0.002	-0.006	-	0.454	<i>0.043</i>	0.829
XB	0.000	-0.003	0.001	0.012	-0.005	-0.009	-0.094	-0.012	-0.005	0.000	-	0.059	0.463
XC	0.008	0.001	0.005	-0.015	0.010	0.035	-0.088	-0.003	-0.005	0.015	0.013	-	0.575
XX	0.012	-0.006	-0.005	0.000	-0.006	0.022	-0.079	-0.008	-0.004	-0.006	-0.001	-0.002	-

A potential explanation for there being significant overall  $R_{ST}$  and  $F_{ST}$  in quelea, but no significant pairwise differences, is that the loci were not all equally informative; some revealed structure, while others did not. Heterozygote instability leading to an increased mutation rate (Amos 1999) could mean that more heterozygous loci are highly variable within populations. This variation could be greater than the variability between populations and hence no inter-population structure would be revealed. This idea can be tested by examining locus by locus estimates of inter-population variation. Locus by locus overall  $F_{ST}$  and  $R_{ST}$ , (Table 3.8) showed that all eight loci were equally uninformative, with low levels of between population differentiation compared to the within individual level. Values of  $R_{ST}$  by locus ranged from  $-0.003$  (WBSW2) to  $0.014$  (Hru5). Locus Hru5 exhibited the most structure according to  $R_{ST}$  ( $0.014$ ), but revealed no structure at all according to  $F_{ST}$  ( $0.000$ ). Values of  $F_{ST}$  ranged from  $-0.001$  (WBSW4) to  $0.006$  (Esc4). There was no relationship between observed heterozygosity ( $H_o$ ) and  $F_{ST}$  by locus ( $y = 0.0091 - 0.0089x$ ,  $r^2 = 0.034$ ,  $p = 0.661$ ) or  $R_{ST}$  by locus ( $y = -0.0301 + 0.0401x$ ,  $r^2 = 0.118$ ,  $p = 0.404$ ). With this set of markers, it was not possible to decrease the amount of noise in the genetic signal by excluding one or more particularly uninformative loci. Nor was there any relationship between the information content of a locus and its variability.

### 3.3.5.1 AMOVA

Both  $\rho$  and  $\theta$  revealed a similar pattern of lack of genetic differentiation at the population sample level. However, there could still have been meaningful genetic structuring at a higher level. To test for structuring at a regional scale, an Analysis of Molecular Variance (AMOVA) was performed. As both  $\theta$  and  $\rho$  showed equal levels of population pairwise differentiation,  $\theta$  was used in the AMOVA as it is believed to be the most informative for the sample size and number of loci available (Gaggiotti et al. 1999). It is also recommended for estimating  $F_{ST}$  in high gene flow species (Cockerham & Weir 1993).

Table 3.8. Overall  $F_{ST}$  and  $R_{ST}$  by locus across all populations.

(a) Overall Fst				
	Fst	sig a	sig b	sig w
Esc4	0.006	0.005	0.029	0.886
Hru5	0.000	0.000	0.062	0.850
Mcyu4	0.002	0.001	0.057	0.825
Phtr2	0.002	0.002	-0.029	0.728
Phtr3	0.001	0.001	0.074	0.857
WBSW1	0.003	0.002	-0.002	0.770
WBSW2	0.001	0.001	0.040	0.805
WBSW4	-0.001	-0.001	0.063	0.876
Total	0.002	0.013	0.292	6.597

(b) Overall Rst				
	Rst	sig a	sig b	sig w
Esc4	0.004	0.100	0.700	13.000
Hru5	0.014	0.200	1.800	11.400
Mcyu4	0.002	0.000	1.300	6.700
Phtr2	0.005	0.000	0.100	2.500
Phtr3	0.003	0.100	1.700	20.700
WBSW1	-0.002	-0.100	0.900	28.500
WBSW2	-0.003	0.000	0.700	12.600
WBSW4	0.003	0.100	1.500	23.700
Total	0.002	0.300	8.800	119.100

Two different group structures were tested, 'Regional' and 'Merged Populations'. The Regional groups were called West, Central and South (see Map 2). The Merged Populations consist of 6 groups of geographically close populations. Populations were only included in a group if they were all collected in the same season, and were all breeding colonies. Each of the six groups contained two or three populations, listed in Table 3.9.

The results of the AMOVA for the two defined groupings are given in Table 3.9. For both groups, 100% of the variation was accounted for at the within population level. The percentage of variation accounted for by the defined groups was negative. This indicated that there was no genetic differentiation between the defined groups. Choosing group structure for analysis is an *ad hoc* way of approaching the question of supra-population level genetic structure. However, the presence of inter-group genetic differences has now been tested at three different levels – at the level of the sample site, at the level of neighbouring sample sites being grouped together, and at a regional level. If further group structures were tested, then greater levels of inter group genetic differentiation could be detected. However, such a result is unlikely to

be any more than a statistical artefact given the overall lack of genetic differentiation in quelea.

Table 3.9. AMOVA analysis of two group structures.

(a) 'Merged' group structure. Six groups defined as (TS,AF,ED), (GU,NN,NS), (SH,MA,RR), (XA,XX), (TU, LT,KR) and (TE,RF).						
Source of variation	d.f.	sum of squares	components of variance	p-value	Percentage variation	
Among groups	5	9.731	-0.003 Va	0.777	-0.08	
Among populations	10	25.961	-0.007 Vb	1.000	-0.23	
Within populations	1358	4350.863	3.204 Vc	1.000	100.31	
Total	1373	4386.555	3.194			

(b) 'Regions' group structure. Three groups defined as illustrated on Map 2: West, Central and South						
Source of variation	d.f.	sum of squares	components of variance	p-value	Percentage variation	
Among groups	2	3.124	-0.00037 Va	0.729	-0.01	
Among populations	29	63.249	-0.0159 Vb	1.000	-0.50	
Within populations	2040	6476.766	3.17489 Vc	1.000	100.52	
Total	2071	6543.139	3.15861			

### 3.3.6 Multilocus Genotype Techniques

#### 3.3.6.1 MCA

The results of the multiple correspondence analysis are presented in Figure 3.5 and Figure 3.6. Figure 3.5 includes red bishop samples. The red bishop samples (open circles) were distributed in a wide scatter, while the quelea samples (closed circles) were together in a tight cluster. MCA was therefore able to distinguish between these two species. However, the two displayed axes represent only 2.81% of the total variation in the data set. The inclusion of the red bishop samples also obscured any pattern that might have been visible in the cluster of quelea samples. Table 3.10a lists the outlying samples removed from the analysis and the proportion of the total variation represented in the first four calculated dimensions. As more dimensions were calculated, there was only a small drop in the proportion of the total variation expressed by a single dimension indicating that calculating further dimensions would not have not significantly added to the information content of the analysis.

A scatter plot of the MCA of quelea samples is shown in Figure 3.6. Each individual is represented by its two-letter population code. There was no discernible pattern in the cluster. The two displayed axes only represent 1.72% of the variation in the data set. MCA was therefore unable to find any differences between quelea samples.

Table 3.10b gives the outlying samples removed from the analysis and the proportion of the total variation represented in the first four dimensions. All four dimensions represent a similarly small amount of the total variation.

Table 3.10. Details of multiple correspondence analysis. Variation gives the amount of total variation represented by an axis. Percent and cumulative represent the percentage and cumulative percentage of the total variation this represented by the number of axes. Outliers left out of the analysis are also shown.

(a) Quelea and red bishop samples					
axis	variation	percent	cumulative	outliers	
1	0.2796	1.76	1.76	JRB21M	JRB8
2	0.1675	1.05	2.81	JRB10M	JRB7
3	0.1512	0.95	3.77	JRB14	
4	0.1436	0.90	4.67	JRB13	
Total	15.8906				

(b) Only quelea samples					
axis	variation	percent	cumulative	outliers	
1	0.1215	0.86	0.86	LM26M	LT15M
2	0.1209	0.86	1.72	XC35M	HU25M
3	0.1192	0.84	2.56	SH35M	XA68M
4	0.1188	0.84	3.40	JD409M	SH8M
Total	14.1332			NN64M	TU41M
				WQ3M	SH18M



## 3.3.6.2 Shared allele tree

The neighbour-joining tree of shared allele distances is shown Figure 3.7. Individuals included in the tree are given in Table 3.11. There is little definition of clusters in the shared allele tree. There are five main clades, none of which is well defined, each includes individuals from all regions and populations. There is no grouping of individuals by region, country or year within the shared allele tree. The only recognisable cluster is the group of ten red bishop samples (JRB). The shared allele tree therefore is capable of grouping together more similar individuals. However, as the quelea samples are all equally similar, there is no pattern in the arrangement of clades that the shared allele tree produces.

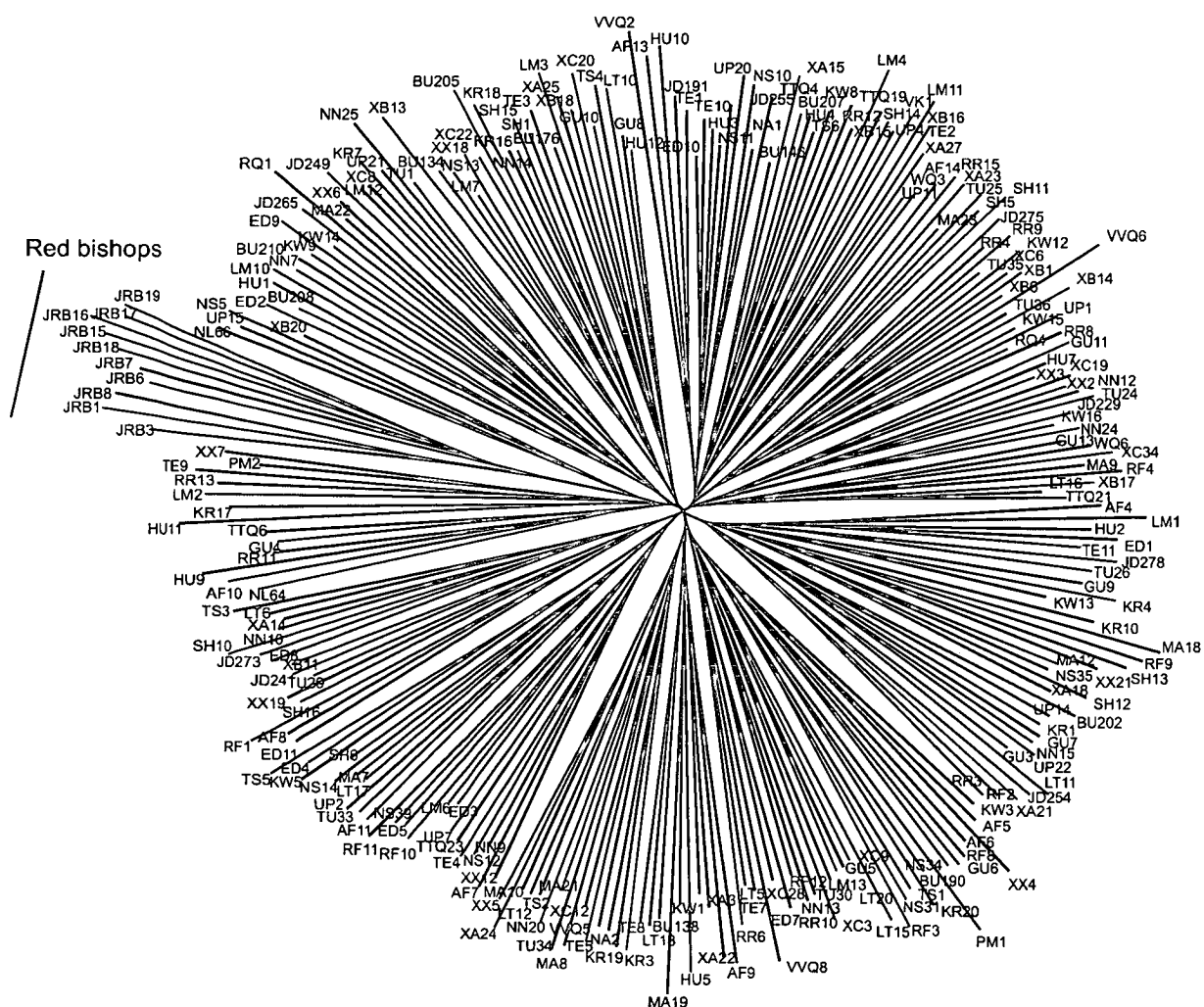


Figure 3.7. Neighbour-joining tree based on shared allele distances between 266 individuals from 33 populations. Individuals included are listed in Table 3.11. All individuals are rebilled quelea except for the clade marked Red bishops.



Table 3.11. List of samples included in the shared allele distance analysis. Individuals included represent up to the first 10 from each site.

Sample Site	Individual	Sample Site	Individual	Sample Site	Individual	Sample Site	Individual		
Alwyn Farm	AF4M	Kroonstad	KR1M	Nokoneng South	NS5M	Tuinplaas	TU1M		
	AF5M		KR3M		NS10M		TU24M		
	AF6M		KR4M		NS11M		TU25M		
	AF7M		KR7M		NS12M		TU26M		
	AF8M		KR10M		NS13M		TU29M		
	AF9M		KR16M		NS14M		TU30M		
	AF10M		KR17M		NS31M		TU33M		
	AF11M		KR18M		NS34M		TU34M		
	AF13M		KR19M		NS35M		TU35M		
	AF14M		KR20M		NS39M		TU36M		
Bulawayo	BU134M	Klawervallei	KW1M	Pietermaritzburg	PM1U	Upington	UP1M		
	BU138M		KW3M		PM2U		UP2M		
	BU146M		KW5M		Riverside Farm		RF1M	UP4M	
	BU176M		KW8M				RF2M	UP7M	
	BU190M		KW9M				RF3M	UP11M	
	BU202M		KW12M				RF4M	UP14M	
	BU205M		KW13M				RF8M	UP15M	
	BU207M		KW14M				RF9M	UP20M	
	BU208M		KW15M				RF10M	UP21M	
	BU210M		KW16M				RF11M	UP22M	
Eden Farm	ED1M	Lake Manyame	LM1M	Riverside Farm		RF12M	Volksrust	VK1U	
	ED2M		LM2U			RQ1M		Riverside Farm	VVQ2M
	ED3M		LM3U		RQ4M			VVQ5M	
	ED4M		LM4M		Reata Ranch	RR3M		VVQ6M	
	ED5M		LM6U			RR4M		VVQ8M	
	ED7M		LM7M			RR6M		Riverside Farm	WQ3M
	ED8M		LM10U			RR8M			WQ6M
	ED9M		LM11U			RR9M		Senuko	XA14M
	ED10M		LM12U			RR10M			XA15M
	ED11M		LM13M			RR11M			XA18M
Gumare	GU3M	Lichtenberg	LT5M	RR12M		XA21M			
	GU4M		LT6M	RR13M		XA22M			
	GU5M		LT10M	RR15M		XA23M			
	GU6M		LT11M	Shirville Farm	SH1M	XA24M			
	GU7M		LT12M		SH5M	XA25M			
	GU8M		LT15M		SH6M	XA27M			
	GU9M		LT16M		SH10M	XA31M			
	GU10M		LT17M		SH11M	Bumi Hills	XB1M		
	GU11M		LT18M		SH12M		XB6M		
	GU13M		LT20M		SH13M		XB11M		
Humani	HU1M	Maitengwane	MA7M		SH14M		XB13M		
	HU2M		MA8M		SH15M		XB14M		
	HU3M		MA9M		SH16M		XB15M		
	HU4M		MA10M	Terminus	TE1M		XB16M		
	HU5M		MA12M		TE2M		XB17M		
	HU7M		MA18M		TE3M		XB18M		
	HU9M		MA19M		TE4M		XB20M		
	HU10M		MA21M		TE5M	Maitengwe Dam	XC3M		
	HU11M		MA22M		TE7M		XC6M		
	HU12M		MA23M		TE8M		XC8M		
JDMalilangwe97	JD191M	Aris	NA1M		TE9M		XC9M		
	JD229M		NA2M		TE10M		XC12M		
	JD241M	Natal	NL64M		TE11M		XC19M		
	JD249M		NL66M	Tsumcor	TS1M		XC20M		
	JD254M	Nokoneng North	NN7M		TS2M		XC22M		
	JD255M		NN9M		TS3M		XC28M		
	JD265M		NN10M		TS4M		XC34M		
	JD273M		NN12M		TS5M	Malilangwe	XX2M		
	JD275M		NN13M		TS6M		XX3M		
	JD278M		NN14M		TS6M		XX3M		
Red Bishops	JRB1M		Riverside Farm		TTQ4M		TTQ4M	XX4M	
	JRB3M				TTQ6M		TTQ6M	XX5M	
	JRB6M				TTQ19M		TTQ19M	XX6M	
	JRB7M			TTQ21M	TTQ21M		XX7M		
	JRB8M	TTQ23M		TTQ23M	XX12M				
	JRB15M				XX18M				
	JRB16M				XX19M				
	JRB17M				XX21M				
	JRB18M								
	JRB19M								

### 3.3.6.3 Assignment test

The mean likelihood and probability of belonging for each population pair are given in Appendix D. However, as the main advantage of multilocus genotype techniques is that the individual is considered, not the population, these tables should only be considered a summary of the results. The more informative results are the cross-assignments, as given in Table 3.12 and Table 3.13. Cross assignment matrices give the number of individuals from the source population (the rows) that are assigned to the other, test, population (the columns). The same data are presented in both tables, organised in different ways to highlight two possible types of among population relationships. In Table 3.12, the populations are grouped by region, as defined in Map 2, while in Table 3.13, the populations are grouped by collection year.

Within quelea populations, there was only one population (Lichtenberg (LT)) for which more individuals were assigned to it than to another population. Furthermore, four populations had none of their own individuals assigned to them. For nine populations, more individuals were left unassigned than could be assigned to any population. Indeed, more individuals (99) from across all populations were left unassigned than were assigned to any single population. There was no clear pattern for populations from one region, or from one year to have more of their individuals assigned to other populations from the same region or year. The only consistent result was that most (13 out of 24) red bishops (JRB) were assigned to their own population. Of the 11 others, nine were unassigned. The assignment test was therefore unable to distinguish between quelea populations, while successfully managing to separate red bishop genotypes. There were no concentrations of cross-assignments to populations that shared the same region, or year of sampling as the source population.

Table 3.12. The number of individuals assigned from the source population (rows) to test populations (columns). The bold figures indicate the number of individuals assigned to their own population. The boxes show populations grouped by region. C - Central, S - South, W - West

Region	Popn	C	C	C	C	C	C	C	C	C	C	C	S	S	S	S	S	S	S	W	W	W	W	W	*		
		BU	HU	JD	LM	MA	RR	SH	XA	XB	XC	XX	KR	KW	LT	RF	TE	TU	UP	AF	ED	GU	NN	NS	JRB	Un	N
C	BU	<b>0</b>	3	1	3	1	0	0	1	0	1	1	0	1	0	4	3	1	0	2	1	0	4	1	0	4	32
C	HU	0	<b>6</b>	0	1	2	0	0	1	2	0	2	2	2	1	4	0	2	0	2	0	5	5	0	0	7	44
C	JD	2	0	<b>0</b>	3	1	0	2	3	3	1	2	2	0	1	3	5	1	0	0	1	1	1	1	0	2	35
C	LM	1	3	0	<b>3</b>	1	0	0	2	2	0	1	1	1	1	5	3	5	0	2	0	5	2	1	0	6	45
C	MA	2	6	0	2	<b>3</b>	0	1	4	3	0	1	4	2	0	1	1	2	0	0	0	6	2	1	0	3	44
C	RR	0	5	0	2	0	<b>1</b>	0	1	4	2	2	4	1	0	2	3	1	0	1	0	0	4	0	0	4	37
C	SH	0	5	0	4	1	1	<b>1</b>	2	2	1	0	0	2	0	1	0	4	0	2	1	4	3	0	0	0	34
C	XA	1	5	0	5	0	0	1	<b>2</b>	4	1	3	6	0	1	3	5	0	0	1	1	3	2	0	0	4	48
C	XB	0	4	0	4	0	1	3	2	<b>5</b>	1	2	4	0	1	1	1	1	0	0	1	4	3	0	0	8	46
C	XC	0	3	0	4	2	0	3	2	2	<b>4</b>	1	2	1	0	4	1	1	0	0	0	7	1	1	0	8	47
C	XX	1	2	0	1	4	3	3	4	4	2	<b>4</b>	2	0	0	3	3	3	0	0	0	2	2	2	0	2	47
S	KR	1	3	1	2	0	3	0	3	2	1	2	<b>2</b>	0	1	6	2	1	0	1	0	1	5	2	0	6	45
S	KW	0	6	2	1	0	3	1	5	1	1	1	2	1	0	6	1	1	0	1	0	3	1	0	0	2	39
S	LT	1	3	0	2	0	1	2	2	6	1	1	3	0	<b>8</b>	1	0	0	0	0	2	3	1	3	0	4	44
S	RF	3	7	1	2	0	0	2	2	4	1	4	4	1	0	<b>3</b>	0	1	0	2	3	1	3	0	0	0	44
S	TE	1	6	0	4	1	2	2	3	6	1	2	3	1	0	1	<b>0</b>	3	0	1	0	2	3	1	0	2	45
S	TU	1	1	1	3	0	0	2	0	4	1	6	2	0	1	7	3	1	0	2	1	2	2	0	0	4	44
S	UP	0	0	0	2	0	1	0	1	0	0	1	1	0	0	1	1	1	0	0	0	1	2	1	0	1	14
W	AF	2	1	1	0	0	1	3	3	3	1	2	1	3	1	3	2	1	0	<b>3</b>	0	4	6	1	0	3	45
W	ED	2	6	1	2	1	0	1	2	5	1	1	5	0	0	3	3	2	0	0	1	1	4	0	0	3	44
W	GU	2	5	1	2	1	0	1	2	3	2	2	1	0	1	3	3	1	0	2	0	4	2	1	0	5	44
W	NN	0	7	0	3	0	2	1	1	5	0	1	4	0	1	1	1	1	1	3	0	1	4	1	0	7	45
W	NS	5	3	1	4	0	2	1	1	2	1	2	3	0	0	4	2	1	0	0	1	1	4	1	0	5	44
-*	JRB	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	<b>13</b>	9	24
Total		25	91	10	59	18	21	31	49	72	25	44	61	16	18	71	43	36	1	25	13	61	66	18	13	99	980

\* - No region defined for JRB (red bishop) samples

Un - Number of unassigned individuals with a probability of belonging to any population < 0.05

N - Sample size

Total - the total number of individuals assigned to the population

Table 3.13. The number of individuals assigned from the source population (rows) to test populations (columns). The bold figures indicate the number of individuals assigned to their own population. The boxes show populations sampled in the same year.

Year	Popn	1997 BU	1997 HU	1997 JD	1998 LM	1998 XA	1998 XB	1998 XC	1998 XX	1999 AF	1999 ED	1999 GU	1999 KR	1999 LT	1999 MA	1999 NN	1999 NS	1999 RF	1999 RR	1999 SH	1999 TE	1999 TU	1999 UP	2000 KW	* JRB	Un	N	
1997	BU	<b>0</b>	3	1	3	1	0	1	1	2	1	0	0	0	1	4	1	4	0	0	3	1	0	1	0	4	32	
1997	HU	0	<b>6</b>	0	1	1	2	0	2	2	0	5	2	1	2	5	0	4	0	0	0	2	0	2	0	7	44	
1997	JD	2	0	<b>0</b>	3	3	3	1	2	0	1	1	2	1	1	1	1	3	0	2	5	1	0	0	0	2	35	
1998	LM	1	3	0	<b>3</b>	2	2	0	1	2	0	5	1	1	1	2	1	5	0	0	3	5	0	1	0	6	45	
1998	XA	1	5	0	5	<b>2</b>	4	1	3	1	1	3	6	1	0	2	0	3	0	1	5	0	0	0	0	4	48	
1998	XB	0	4	0	4	2	<b>5</b>	1	2	0	1	4	4	1	0	3	0	1	1	3	1	1	0	0	0	8	46	
1998	XC	0	3	0	4	2	2	<b>4</b>	1	0	0	7	2	0	2	1	1	4	0	3	1	1	0	1	0	8	47	
1998	XX	1	2	0	1	4	4	2	<b>4</b>	0	0	2	2	0	4	2	2	3	3	3	3	3	0	0	0	2	47	
1999	AF	2	1	1	0	3	3	1	2	<b>3</b>	0	4	1	1	0	6	1	3	1	3	2	1	0	3	0	3	45	
1999	ED	2	6	1	2	2	5	1	1	0	1	1	5	0	1	4	0	3	0	1	3	2	0	0	0	3	44	
1999	GU	2	5	1	2	2	3	2	2	2	0	4	1	1	1	2	1	3	0	1	3	1	0	0	0	5	44	
1999	KR	1	3	1	2	3	2	1	2	1	0	1	2	1	0	5	2	6	3	0	2	1	0	0	0	6	45	
1999	LT	1	3	0	2	2	6	1	1	0	2	3	3	<b>8</b>	0	1	3	1	1	2	0	0	0	0	0	0	4	44
1999	MA	2	6	0	2	4	3	0	1	0	0	6	4	0	<b>3</b>	2	1	1	0	1	1	2	0	2	0	3	44	
1999	NN	0	7	0	3	1	5	0	1	3	0	1	4	1	0	4	1	1	2	1	1	1	1	0	0	7	45	
1999	NS	5	3	1	4	1	2	1	2	0	1	1	3	0	0	4	1	4	2	1	2	1	0	0	0	5	44	
1999	RF	3	7	1	2	2	4	1	4	2	3	1	4	0	0	3	0	<b>3</b>	0	2	0	1	0	1	0	0	44	
1999	RR	0	5	0	2	1	4	2	2	1	0	0	4	0	0	4	0	2	1	0	3	1	0	1	0	4	37	
1999	SH	0	5	0	4	2	2	1	0	2	1	4	0	0	1	3	0	1	1	1	0	4	0	2	0	0	34	
1999	TE	1	6	0	4	3	6	1	2	1	0	2	3	0	1	3	1	1	2	2	<b>0</b>	3	0	1	0	2	45	
1999	TU	1	1	1	3	0	4	1	6	2	1	2	2	1	0	2	0	7	0	2	3	1	0	0	0	4	44	
1999	UP	0	0	0	2	1	0	0	1	0	0	1	1	0	0	2	1	1	1	0	1	1	0	0	0	1	14	
2000	KW	0	6	2	1	5	1	1	1	1	0	3	2	0	0	1	0	6	3	1	1	1	0	<b>1</b>	0	2	39	
-*	JRB	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	<b>13</b>	9	24
Total		25	91	10	59	49	72	25	44	25	13	61	61	18	18	66	18	71	21	31	43	36	1	16	13	99	980	

\* - No year defined for JRB (red bishop) samples

Un - Number of unassigned individuals with a probability of belonging to any population < 0.05

N - Sample size

Total - the total number of individuals assigned to the population

## 3.3.6.4 Assignment of unknown individuals

Individuals from populations that had only a small number of samples (NL, PM, TS, RQ, TTQ, VVQ, WQ) were considered as ‘unknown’ individuals, i.e., the source population was considered to be unknown. Each of these unknown individuals was therefore assigned to a test population, giving an indication of the relatedness of the unknown individual to larger known populations. Test populations were defined as those that had a large enough sample of individuals to include in the assignment testing in Section 3.3.6.3. The results of assigning the unknown individuals are given in Table 3.14. There was no clear pattern of assignments, with individuals assigned apparently at random to the test populations. Only one individual from the South region (NL64M) was assigned to a population in the South region. Similarly TS6M was the only individual from the West region assigned to its own region. Individuals RQ1M to WQ6M were all from the same site as Riverside Farm (RF) but sampled in different years. None of them was assigned to Riverside Farm (RF).

Table 3.14. ‘Unknown’ individuals from the source populations and regions were assigned to the population and region with the highest probability of belonging (Highest P).

Individual	Source		Highest P	Assignment	
	Population	Region		Population	Region
NL64M	NL	S	0.784	TU	S
PM1U	PM	S	0.020	Unassigned	-
PM2U	PM	S	0.678	NN	W
RQ1M	RQ	S	1.000	RR	C
RQ4M	RQ	S	0.904	GU	W
TTQ4M	TTQ	S	0.221	NN	W
TTQ19M	TTQ	S	0.551	BU	C
TTQ21M	TTQ	S	0.845	LM	C
TTQ23M	TTQ	S	1.000	XB	C
VVQ5M	VVQ	S	0.539	NN	W
VVQ6M	VVQ	S	0.392	BU	C
WQ3M	WQ	S	0.502	GU	W
WQ6M	WQ	S	0.416	NN	W
VK1U	VK	S	0.267	XX	C
NA1M	NA	W	0.935	TU	S
NA2M	NA	W	0.592	KR	S
TS1M	TS	W	0.670	SH	C
TS2M	TS	W	0.778	HU	C
TS3M	TS	W	0.274	TU	S
TS4M	TS	W	0.992	KR	S
TS5M	TS	W	0.111	KR	S
TS6M	TS	W	0.739	NS	W

### 3.3.7 Genetic Distances

Nei's unbiased genetic distance, Shriver's stepwise mutation model based distance ( $D_{SW}$ ) and Paetkau's assignment index based distance ( $D_{LR}$ ) were calculated for all population relationships (Tables 3.14, 3.15 and 3.16 respectively). Nei's distance varied between -0.064 for the TS-NS comparison to 0.140 for TTQ-ED comparison. Most values were in the range 0 to 0.04. Nei's distances to red bishop samples were about ten times greater, varying between 1.210 for the UP comparison to 1.426 for the AF comparison. For  $D_{SW}$ , the majority of distances fell in the range 0.02 to 0.05, with the smallest distance at 0.012 between TU and TE. The largest within quelea distance was 0.180 for the TS-AF comparison. Again distances involving red bishops (JRB) were larger – from 0.981 for the ED comparison to 1.237 for TS. Finally,  $D_{LR}$  had its lowest value, -0.947, for the UP-TE pair. Most values were in the range 0.1 to 0.8. Only four values were above 1.0, with the highest being 1.419 for the RR-MA pair, indicating that for only four population comparisons was an individual more likely to have come from its own population than from the other population, based on its multilocus genotype. Values for comparisons involving red bishops (JRB) typically ranged between 12.6 (UP) to 15.47 (AF).

Between-population relationships were visualised with multiple dimensional scaling (MDS) plots. The patterns revealed by each of the three distance measures were similar in two respects. First, as expected, JRB was well separated from the quelea populations. Second, the smaller samples (TS, TTQ, UP) were also separated from the remaining populations. Hence for establishing the relationships between quelea populations alone these outlying points were removed. This allowed the within quelea relationships to be more easily visualised. An example of an MDS plot including the red bishop sample and the small quelea samples is shown for  $D_{SW}$  in Figure 3.8.

Table 3.15. Nei's unbiased genetic distance (1978) between all population pairs.

	AF	BU	ED	GU	HU	JD	JRB	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE	TS	TTQ	TU	UP	XA	XB	XC
AF	-																								
BU	-0.005	-																							
ED	0.026	0.005	-																						
GU	0.012	0.011	0.005	-																					
HU	0.008	0.005	0.018	0.020	-																				
JD	0.041	0.021	0.046	0.033	0.031	-																			
JRB	1.426	1.263	1.246	1.288	1.268	1.263	-																		
KR	0.012	-0.001	0.010	0.034	0.009	0.026	1.294	-																	
KW	0.018	0.008	0.024	0.015	0.004	0.010	1.313	0.020	-																
LM	0.008	-0.010	0.024	0.017	-0.003	0.019	1.353	0.002	-0.002	-															
LT	0.029	0.012	0.017	0.046	0.037	0.031	1.303	0.010	0.025	0.015	-														
MA	0.030	0.011	0.034	0.033	0.009	0.029	1.300	0.018	-0.007	0.010	0.034	-													
NN	0.016	0.002	0.017	0.012	0.005	0.022	1.299	0.018	0.014	0.004	0.023	0.051	-												
NS	0.020	-0.013	0.013	0.022	0.018	0.045	1.355	0.007	0.022	0.001	0.036	0.023	0.037	-											
RF	0.007	-0.012	0.012	0.020	-0.007	0.013	1.266	0.007	-0.016	-0.004	0.043	0.020	0.015	0.004	-										
RR	0.006	-0.004	0.032	0.021	-0.002	0.004	1.264	0.002	-0.002	-0.010	0.022	0.028	0.009	0.007	0.006	-									
SH	0.006	0.000	0.025	-0.004	-0.003	0.025	1.303	0.025	0.016	0.001	0.048	0.028	0.002	0.015	-0.004	0.015	-								
TE	0.010	-0.015	0.002	0.003	0.005	0.016	1.243	-0.002	-0.001	-0.017	0.029	0.003	0.021	-0.020	-0.003	-0.002	-0.002	-							
TS	0.014	-0.056	-0.024	-0.018	-0.033	0.043	1.411	-0.038	0.020	-0.006	0.041	0.003	0.026	-0.064	-0.016	-0.026	-0.018	-0.021	-						
TTQ	0.094	0.053	0.140	0.035	0.099	0.052	1.293	0.069	0.068	0.027	0.128	0.100	0.065	0.082	0.103	0.019	0.057	0.060	0.118	-					
TU	0.003	-0.009	0.000	-0.001	0.008	0.001	1.244	0.012	-0.001	-0.007	0.032	0.015	0.016	0.009	-0.014	0.005	-0.005	-0.013	-0.031	0.091	-				
UP	0.012	0.006	0.039	0.039	0.039	0.032	1.210	0.015	0.014	-0.011	0.027	0.042	0.008	0.025	0.014	-0.001	0.009	-0.014	0.108	0.041	0.037	-			
XA	0.032	0.005	0.012	0.024	0.005	0.022	1.268	-0.001	0.026	-0.005	0.026	0.026	0.023	0.016	0.006	0.025	0.018	-0.010	-0.005	0.100	0.009	0.008	-		
XB	0.024	0.022	0.028	0.025	0.031	0.027	1.189	0.017	0.031	-0.008	0.022	0.050	0.008	0.044	0.018	0.013	0.023	0.005	-0.026	0.060	0.005	0.024	0.020	-	
XC	0.029	0.008	0.013	0.027	0.015	0.018	1.219	0.013	0.004	0.005	0.006	0.031	0.013	0.030	0.022	-0.004	0.017	0.012	-0.016	0.076	0.016	0.028	0.028	0.006	-
XX	0.028	0.006	0.018	0.040	0.013	0.033	1.358	-0.011	0.016	-0.004	0.010	0.035	0.021	0.026	0.007	0.010	0.020	0.009	-0.016	0.104	0.021	0.012	-0.005	0.028	0.012

Table 3.16.  $D_{sw}$  (Shriver et al 1995) pairwise genetic distance between all population pairs.

	AF	BU	ED	GU	HU	JD	JRB	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE	TS	TTQ	TU	UP	XA	XB	XC
AF	-																								
BU	0.029	-																							
ED	0.058	0.042	-																						
GU	0.051	0.035	0.022	-																					
HU	0.035	0.046	0.035	0.028	-																				
JD	0.036	0.028	0.036	0.025	0.039	-																			
JRB	1.051	1.022	0.981	1.033	1.048	1.067	-																		
KR	0.034	0.027	0.022	0.039	0.037	0.035	1.004	-																	
KW	0.024	0.017	0.028	0.026	0.028	0.026	1.022	0.018	-																
LM	0.033	0.023	0.037	0.028	0.028	0.029	1.090	0.027	0.015	-															
LT	0.044	0.035	0.034	0.038	0.050	0.022	1.037	0.027	0.030	0.037	-														
MA	0.032	0.023	0.033	0.021	0.027	0.012	1.063	0.032	0.019	0.019	0.026	-													
NN	0.021	0.037	0.043	0.039	0.017	0.040	1.048	0.029	0.023	0.028	0.044	0.032	-												
NS	0.048	0.026	0.048	0.044	0.050	0.044	1.128	0.039	0.025	0.021	0.051	0.034	0.052	-											
RF	0.040	0.022	0.028	0.021	0.036	0.024	1.044	0.030	0.015	0.018	0.037	0.019	0.043	0.026	-										
RR	0.027	0.035	0.035	0.030	0.024	0.021	1.087	0.026	0.023	0.025	0.029	0.020	0.026	0.033	0.029	-									
SH	0.030	0.024	0.061	0.044	0.045	0.027	1.070	0.047	0.034	0.036	0.044	0.025	0.046	0.035	0.032	0.025	-								
TE	0.042	0.025	0.019	0.017	0.029	0.019	1.036	0.027	0.017	0.019	0.026	0.015	0.037	0.025	0.016	0.021	0.037	-							
TS	0.180	0.122	0.127	0.108	0.144	0.131	1.237	0.153	0.134	0.123	0.149	0.115	0.180	0.088	0.101	0.116	0.107	0.104	-						
TTQ	0.102	0.105	0.140	0.130	0.115	0.136	1.149	0.112	0.096	0.109	0.145	0.128	0.093	0.111	0.130	0.115	0.135	0.110	0.242	-					
TU	0.040	0.025	0.019	0.012	0.028	0.016	1.039	0.028	0.019	0.018	0.028	0.013	0.037	0.028	0.012	0.019	0.028	0.012	0.091	0.127	-				
UP	0.039	0.024	0.048	0.037	0.051	0.040	1.046	0.032	0.020	0.027	0.040	0.035	0.038	0.035	0.030	0.040	0.046	0.030	0.152	0.101	0.035	-			
XA	0.048	0.027	0.026	0.019	0.037	0.026	1.005	0.029	0.023	0.031	0.029	0.026	0.044	0.038	0.020	0.035	0.046	0.012	0.116	0.114	0.018	0.031	-		
XB	0.055	0.033	0.031	0.019	0.039	0.026	1.078	0.042	0.026	0.020	0.036	0.019	0.046	0.030	0.018	0.032	0.043	0.016	0.084	0.128	0.012	0.037	0.021	-	
XC	0.028	0.026	0.048	0.044	0.051	0.021	1.100	0.034	0.026	0.030	0.030	0.022	0.045	0.038	0.031	0.026	0.022	0.036	0.128	0.142	0.026	0.041	0.044	0.035	-
XX	0.036	0.026	0.028	0.027	0.038	0.017	1.060	0.027	0.023	0.027	0.020	0.017	0.041	0.038	0.021	0.023	0.031	0.019	0.118	0.134	0.017	0.040	0.020	0.024	0.018



Table 3.17.  $D_{LR}$  (Paetkau et al 1997) pairwise genetic distances between all population pairs.

	AF	BU	ED	GU	HU	JD	JRB	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE	TU	UP	XA	XB	XC	
AF	-																							
BU	0.410	-																						
ED	0.551	0.161	-																					
GU	0.194	0.306	0.063	-																				
HU	0.354	0.528	0.440	0.240	-																			
JD	0.569	0.464	0.395	0.294	0.842	-																		
JRB	15.470	13.859	13.779	14.049	14.907	14.455	-																	
KR	0.290	0.179	0.292	0.400	0.372	0.301	14.234	-																
KW	0.167	0.453	0.528	0.038	0.239	0.267	14.657	0.269	-															
LM	-0.033	0.264	0.254	-0.035	0.298	0.145	14.754	0.301	0.287	-														
LT	1.008	0.811	0.778	0.638	1.188	0.380	14.070	0.972	0.948	0.560	-													
MA	0.578	0.520	0.502	0.110	0.159	0.487	14.614	0.716	0.338	0.398	1.080	-												
NN	-0.117	0.080	-0.032	-0.195	-0.156	0.534	13.780	-0.023	0.016	-0.201	0.605	0.299	-											
NS	0.415	0.161	0.146	0.123	0.279	0.332	15.016	0.320	0.505	-0.164	0.604	0.298	0.287	-										
RF	0.208	0.237	0.186	0.299	-0.124	0.173	13.491	0.047	-0.388	0.057	0.960	0.588	0.001	0.024	-									
RR	0.079	0.596	0.852	0.379	0.834	0.301	14.975	0.318	0.144	0.680	1.348	1.419	0.149	0.432	0.286	-								
SH	-0.097	0.637	0.478	0.029	0.254	-0.012	14.490	0.605	0.206	0.119	0.780	0.379	-0.056	0.114	0.173	0.574	-							
TE	0.283	-0.151	-0.159	-0.317	0.344	0.044	14.206	0.321	-0.051	-0.059	0.722	0.257	0.134	-0.274	-0.013	-0.018	0.052	-						
TU	-0.224	0.294	0.109	-0.233	0.108	0.010	13.833	0.082	-0.050	-0.391	0.635	0.236	-0.309	-0.001	-0.428	-0.041	-0.368	-0.301	-					
UP	-0.211	0.032	-0.350	-0.108	0.510	-0.205	12.600	-0.041	-0.112	-0.565	0.405	0.213	-0.486	0.021	-0.159	-0.093	-0.351	-0.947	-0.058	-				
XA	0.420	0.566	0.462	0.285	0.032	0.158	14.294	0.108	0.603	0.169	0.947	0.244	0.150	0.354	-0.014	0.799	0.164	-0.057	-0.012	-0.450	-			
XB	0.093	0.879	0.298	0.176	0.893	0.516	13.226	0.396	0.974	0.016	0.611	0.851	-0.037	0.749	0.403	0.865	0.260	0.124	-0.004	-0.097	0.233	-		
XC	0.463	0.643	0.570	0.242	0.910	0.174	14.315	0.489	0.378	0.476	0.866	0.775	0.430	0.770	0.315	0.675	0.114	0.370	0.297	0.088	0.712	0.371	-	
XX	0.763	0.483	0.590	0.142	0.452	0.526	15.230	0.132	0.465	0.247	0.617	0.718	0.160	0.398	0.260	0.544	0.372	0.417	0.050	-0.021	0.123	0.554	0.724	

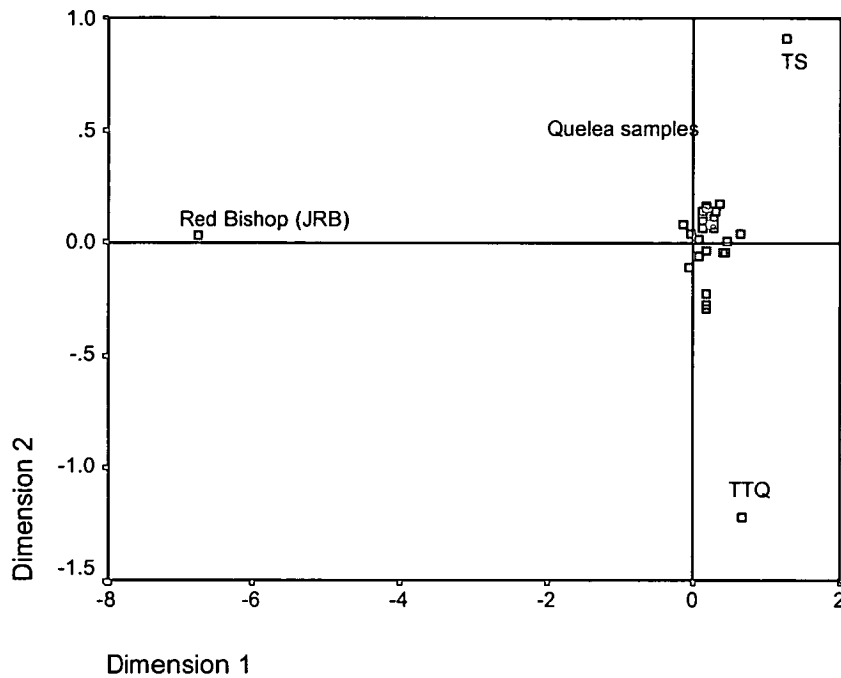


Figure 3.8. MDS plot of  $D_{SW}$  for all populations, including red bishops (JRB). Stress=0.056,  $R^2=0.996$ .

The effectiveness of MDS in representing the original matrix of pairwise distances is given by two numbers, 'stress' and  $R^2$ . The lower the stress, the better the original scaled data are represented by the new distance relationships in the plot, with zero meaning the fit is perfect. Typically stress should be below 0.05 to indicate a good fit to the data, and below 0.1 for a reasonable fit. Stress values above 0.2 indicate that the original data are represented poorly by the MDS plot (Everitt & Dunn 1991). Stress is reduced by increasing the number of dimensions used to represent the data. However, as the purpose of MDS is to visualise relationships easily, there is little point increasing the number of dimensions required beyond two.  $R^2$  gives the proportion of the variance in the original scaled data accounted for in the MDS distances. Therefore stress gives an indication of how well the MDS distances represent the original scaled distances, while  $R^2$  shows how much of the original variance is now represented in the MDS distances. Table 3.18 gives the stress and  $R^2$  for each of the plots illustrated. Among the large quelea samples, for two dimensions, stress is generally high, and  $R^2$  ranges from 0.665 to 0.742. Both stress and  $R^2$  do improve for a 3 dimensional representation of the data. However the improvement is not substantial enough to warrant the consequent loss in ease of interpretation.

Table 3.18. Goodness of fit (stress) and proportion of total variance represented ( $R^2$ ) for MDS plots.

Dimensions	Stress			$R^2$		
	1	2	3	1	2	3
<u>All Samples</u>						
Nei 1978	0.088	0.073	0.066	0.992	0.993	0.994
Dsw	0.071	0.056	0.048	0.994	0.996	0.997
Dlr	0.089	0.075	0.066	0.991	0.993	0.994
<u>Quelea Samples Only</u>						
Nei 1978	0.421	0.245	0.153	0.440	0.665	0.826
Dsw	0.440	0.227	0.116	0.429	0.742	0.898
Dlr	0.418	0.246	0.170	0.527	0.724	0.814

The MDS plot for Nei's genetic distance (Figure 3.9) for quelea populations only, revealed no clear clustering. There were some instances where geographically close populations were close together (for example Terminus (TE) and Riverside Farm (RF) in South Africa, and Alwyn Farm (AF) and Gumare (GU) in Namibia/Botswana). However there were many others where populations are geographically close, but were dissimilar in the MDS plot. One example was NN and NS, which are samples from different parts of the same colony (Nokoneng) in Botswana, but were on opposite sites of the MDS plot indicating that a relatively large genetic distance separates them.

MDS scatter plot for Shriver's genetic distance  $D_{SW}$  again shows no clear relationships or clustering (Figure 3.10). RF and TE were again close together, but AF and GU were not. The pair of samples from the same location in different years (JD and XX) were positioned close to each other. However, again there is no consistent pattern, and few similarities with Nei's genetic distance.

The MDS plot for  $D_{LR}$  is shown in Figure 3.11. JD and XX remained close to each other, but RF and TE were now split. The samples from the same part of Botswana (GU, NN and NS) were all close together. However there was once again no consistent clear pattern of relationships represented by this plot. Indeed, there are no common elements that were revealed by all three genetic distances. This indicates, in common with the other analysis techniques presented, that there was no meaningful genetic structure within the tested populations of quelea. In contrast the techniques were able to resolve the deeper relationship between red bishops and quelea.

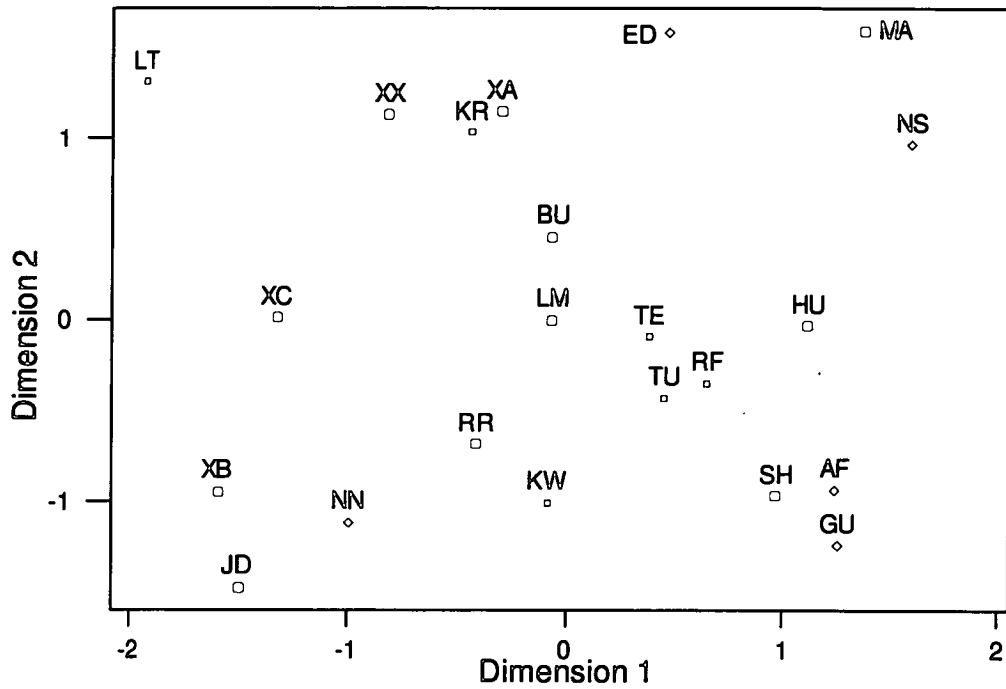


Figure 3.9. MDS two-dimensional plot of Nei (1978) unbiased genetic distance for all quelea populations,  $N > 40$ . Colours represent region of origin of the populations (see Map 2) as follows: Red – Central, Blue – South, Green – West. Stress = 0.245,  $R^2 = 0.665$ .

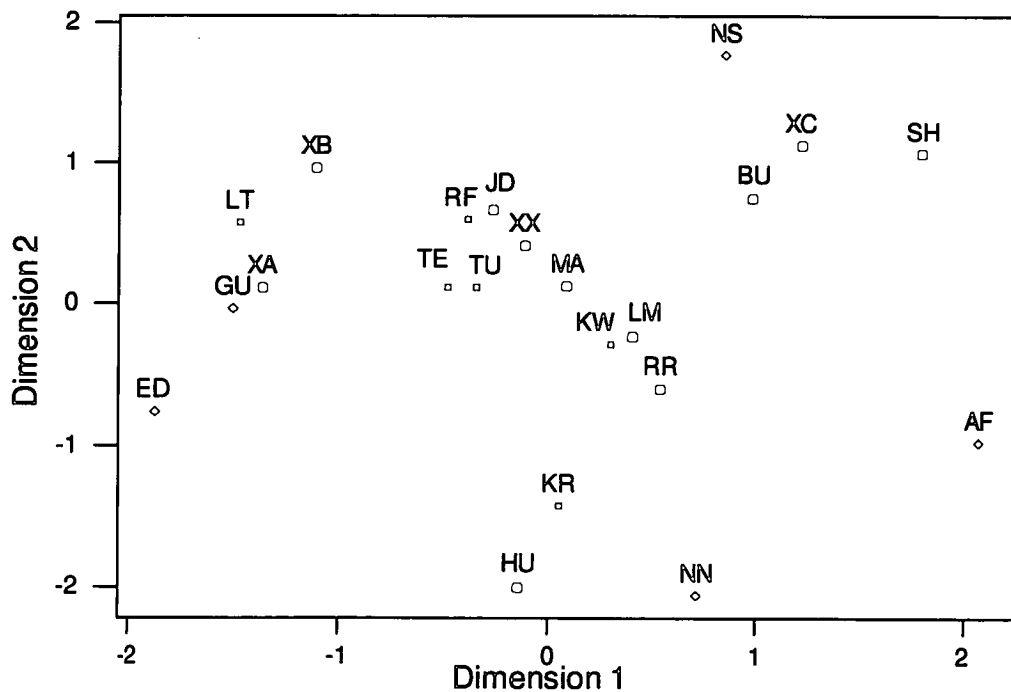


Figure 3.10. MDS two-dimensional plot of  $D_{sw}$  genetic distance for all quelea populations,  $N > 40$ . Colours represent region of origin of the populations (see Map 2) as follows: Red – Central, Blue – South, Green – West. Stress = 0.227,  $R^2 = 0.0742$ .

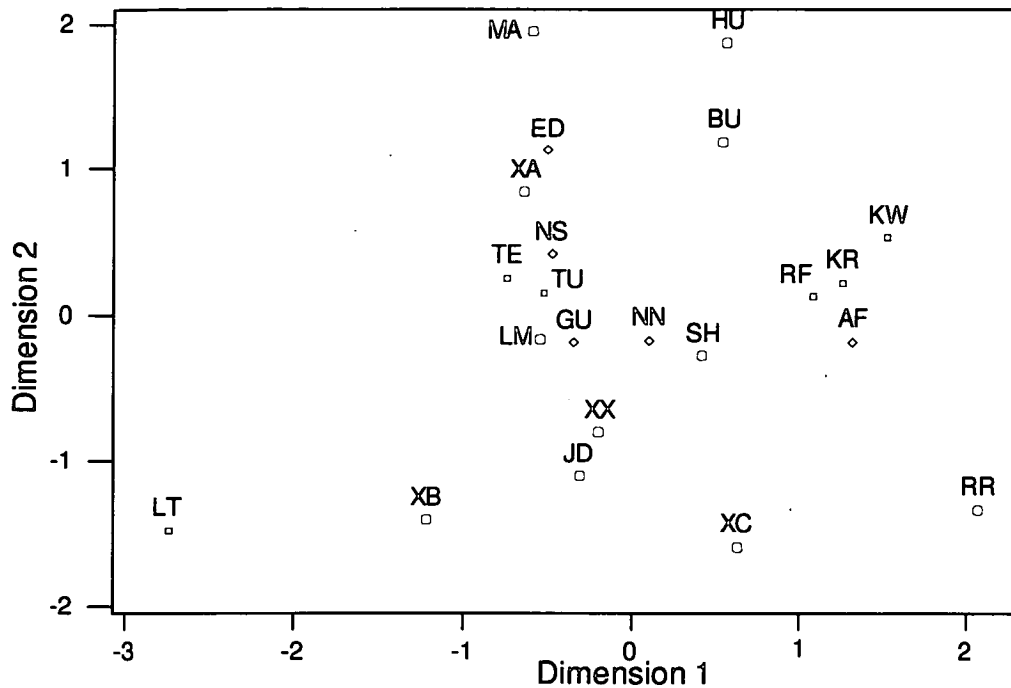


Figure 3.11. MDS two-dimensional plot of  $D_{LR}$  genetic distance for all quelea populations,  $N > 40$ . Colours represent region of origin of the populations (see Map 2) as follows: Red – Central, Blue – South, Green – West. Stress = 0.246,  $R^2 = 0.724$ .

### 3.4 Discussion

No evidence indicating that there is significant genetic structure among populations of redbilled quelea in southern Africa was found. This was in spite of using a full range of analytical techniques had there been a distinction to find. Neither was it possible to define migration patterns by linking geographically distant populations that were genetically similar.

There are three possible outcomes for a study into gene flow between populations, and each outcome has its own associated ambiguities that could bias the way results are interpreted (Bossart & Pashley Prowell 1998a), namely:

1. no genetic structure or differentiation among populations
2. significant genetic structure, no geographic pattern to the structure
3. significant genetic differentiation and geographically structured populations.

Quelea in southern Africa are an example of the first outcome – no genetic differentiation. There are at least two explanations for this result. First, there is no differentiation because of huge population sizes and a high level of gene flow between quelea populations. Second, quelea demographic characteristics mean that the genetic information gathered is inappropriate to detect the differentiation that may be present. Some of the limitations of using molecular markers for high gene flow species will now be discussed before the results are put in to the context of quelea behaviour and ecology.

### 3.4.1 Analysis techniques

Any study attempting to measure gene flow among interbreeding populations may be unable to detect differentiation. The molecular markers may not have adequate power to resolve a difference that is actually there. Equally, there are many limitations and assumptions placed on data by conventional population genetics models such as  $F_{ST}$  which seek to establish levels of gene flow based on variances in allele frequencies among populations.

As a range of analytical techniques have shown the same pattern of lack of genetic differentiation, this chapter cannot contribute to the growing debate about the suitability of measures such as  $F_{ST}$  and  $R_{ST}$  to highly variable markers like microsatellites. Many authors say that caution is essential in interpreting such measures.  $F_{ST}$  is strongly influenced by within-population diversity and hence for loci with high levels of diversity, such as microsatellites,  $F_{ST}$  is a poor measure of between population diversity (Charlesworth 1998). Whitlock and McCauley (1999) advise care to be taken in interpreting  $F_{ST}$ , claiming that while  $F_{ST}$  provides an estimate of gene flow ‘within a few orders of magnitude’ it is unlikely to be any more accurate.

Techniques based on multi-locus genotypes can extract more of the information from the genetic data (Sunnucks 2000), and assignment tests are specifically designed to identify migrants, so have fewer of the assumptions of migration-mutation equilibrium that can invalidate many of the conventional F statistic-based parameters. Nonetheless, both conventional F statistics and more recently developed

multi-locus genotype techniques failed to reveal structure, thus indicating that it is not the assumptions made in the analysis techniques themselves that are responsible for the lack of pattern, but that there is no pattern to detect.

### 3.4.2 Genetic differentiation and life history parameters

There are many examples of the relationship between  $F_{ST}$  and life history parameters. In general, the greater the ability of the study organism to disperse, the lower the value of  $F_{ST}$  reported (Bohonak et al. 1998). The level of genetic differentiation detected in shore birds was directly related to life history patterns (Haig et al. 1997). The lowest value of  $F_{ST}$  found was for the red-necked phalarope, which has a wide-ranging Holarctic breeding distribution and shows little philopatry, while the highest was for the Hudsonian godwit that has distinct breeding grounds and is highly philopatric. Indeed the  $F_{ST}$  of 0.685 for the godwit is the highest yet reported for birds. The value for the phalarope (0.095) is much more typical. Genetic differentiation also depended on life history pattern for estuarine and pelagic fish species (Gold & Richardson 1998). Pelagic, wide ranging fish showed low levels of structure, while those that had a restricted distribution, such as estuarine species, showed higher levels of differentiation. Similar patterns have been shown for water mites (Bohonak 1999a), forest herbs (Williams & Guries 1994), migrating versus non-migrating bats (Petit & Mayer 1999), birds (Crochet 1996) and in a correlation study of dispersal ability and genetic differentiation measured by  $F_{ST}$  across a wide range of taxa (Bohonak 1999b).

#### 3.4.2.1 Genetic differentiation in abundant highly mobile, high gene flow species

Despite some groups of animals having high dispersal capabilities, significant amounts of genetic structure can still be found. In cosmopolitan marine species, Graves (1998) showed that there was considerable genetic differentiation in fish that have global, or near global distributions, thus underlining the need for adequate knowledge of each species to be managed before stock definitions and decisions are made. Cosmopolitan species such as the blue marlin *Makaira nigricans* and the yellowfin tuna *Thunnus albacares* both showed genetic structuring between oceans,

but none within ocean basins. Global population structure was also found for the highly mobile swordfish *Xiphias gladius* (Alvarado Bremer et al. 1996).

Cetaceans are wide-ranging species that have complex patterns of genetic differentiation (Hoelzel 1998). Whales often have seasonal patterns of movements and long-range migration abilities, both of which combine to produce patterns of genetic variation that are sometimes not obviously related to geography. One problem facing the management and conservation of whale stocks is that many whale species (e.g. minke *Balaenoptera acutorostrata* (Bakke et al. 1996), humpback *Megaptera noveangliae* (Baker et al. 1994), beluga *Delphinapterus leucas* (O'Corry-Crowe et al. 1997)) have distinct breeding populations that mix on common feeding grounds. Humpback whales show significant genetic structure within and between populations (Baker et al. 1994) despite humpbacks being capable of migrating 10,000 km between winter and summer grounds and having mixed feeding grounds. Both these examples illustrate the need for any management policy to take into account that, even though there is no obvious genetic structure in a particular region, this may just be an artefact of where and when samples were taken. Knowledge of the distinctiveness of the populations of whale is important in designing conservation and management initiatives.

#### 3.4.2.2 Overcoming noise and errors

Marine species are often characterised by high levels of gene flow and many are capable of long range dispersal, either as adults, such as many cetaceans, or during a pelagic larval phase. In a literature survey of 57 marine species, the median  $F_{ST}$  value was 0.02 (Ward et al. 1994). In such circumstances, the normal errors associated with estimating gene flow become relatively more important. Thus genetic techniques alone cannot and should not be used as the sole basis for management decisions such as defining the number of separate fish stocks and describing the relationships between them (Waples 1998; Taylor & Dizon 1999).

The existence of population structure should not be ruled out simply because the species in question is wide-ranging and can disperse or migrate over long distances. Instead, it is important to be aware of the limitations associated with using genetic



techniques with species that could exhibit high levels of gene flow. In high gene flow species, the signal relating to any population structure is going to be weak. In addition, sources of noise and error become more important and could more systematically bias the estimates of gene flow (Waples 1998). Hence, because a statistical test gives a significant result, this is not enough to provide much biologically meaningful information. A second option is to evaluate the power of the statistical tests (Taylor & Dizon 1996; Waples 1998). If the tests are too powerful, then divisions will be picked up whether they are biologically meaningful or not (type one error). However if the tests are too weak, or the signal too weak, then actual, meaningful population divisions will be missed – a type two error. The best way to ensure that the measured estimates are a reliable indication of the biological situation is to replicate sampling over time. If in a different time cycle (year, breeding season), the same picture of differentiation is discovered, then this is a good indication that a meaningful pattern has been detected. In other words, it is important to have a good understanding of the ecology of the target species before drawing any conclusions. There is therefore immense value in observation and mark-recapture techniques that can measure demographic parameters directly (Bossart & Pashley Prowell 1998a).

#### 3.4.2.3 Microsatellites as genetic markers in high gene flow species

Microsatellites have been used to study mobile, abundant species with varied success, providing evidence of population structure in cod *Gadus morhua* (Ruzzante et al. 1998), and salmon *Salmo salar* (McConnel et al. 1997). In contrast, studies on the dunlin were able to distinguish between breeding populations based on mtDNA haplotypes (Wenink & Baker 1996; Wennerberg et al. 1999). Microsatellites were used to try to give further discrimination, but they were discovered to be too variable to throw any light on the origins of migrating dunlin (Wennerberg et al. 1998). Equally, microsatellites did not provide evidence of population structure in the noctule bat (Petit & Mayer 1999) when mtDNA sequences could. In other words the very properties of variability that make microsatellites the favoured marker system for many studies – especially with closely related individuals and groups of

organisms – is the same thing that could make them unsuitable for studies of wide-ranging species.

Although many aspects of microsatellite mutation and evolution remain open to debate, there appears to be agreement that there are constraints on the number of states that an individual microsatellite locus can have – there is a finite number of alleles per locus (Paetkau et al. 1997; Gaggiotti et al. 1999; Estoup & Cornuet 1999; Jarne 2000). Under such constraints, mutation therefore acts as a homogenising force (Nauta & Weissing 1996). Each mutation could potentially result in a state that had previously been lost, or a state that is typical of another population, leading to homoplasy confounding any genetic signal present. Microsatellites are unlikely to be informative when population sizes are large if there are range constraints (Gaggiotti et al. 1999). In such situations  $Nm$  (the number of migrants between populations) is overestimated, and populations appear more similar than they actually are.

Microsatellites are potentially not good genetic markers to study population subdivision when population sizes are very large and heterozygosity is high. Under these circumstances, homoplasy is very high and will lead to little genetic differentiation between populations even when there is some degree of isolation. In quelea, however, no relationship was found between heterozygosity and  $F_{ST}$  among loci. There is therefore no evidence that the use of less polymorphic markers, with lower mutation rates (Amos 1999) would have been more informative. Present agricultural conditions mean that quelea population numbers could be rapidly expanding, therefore quelea are unlikely to be in mutation-drift equilibrium. Whatever results had been gleaned from whatever set of molecular markers, this observation would mean that a conservative approach would be needed in interpreting any evidence regarding the levels of gene flow between populations (Slatkin 1993; Whitlock & McCauley 1999).

### ***3.4.3 Quelea population structure in southern Africa***

The intention of this chapter was to identify and define management units and migration pathways that could be used to inform strategic decisions on managing quelea as a pest species. A further intention was to examine whether there is any

phylogenetic information to confirm or refute the existence of two subspecies of quelea in southern Africa. If the observed lack of structure is assumed to be real, and not just an artefact of the chosen techniques, then both aims can be answered. First, there is no genetic basis for defining management units or migration patterns, and, second there is no basis for defining two subspecies of quelea. Many authors maintain that genetic information alone should not be used to define management units. A lack of genetic discrimination should not be the sole justification for saying categorically that quelea can safely be managed as a single entity. However, quelea populations were sampled from three different years. There was a lack of genetic differentiation regardless of year, indicating that the absence of pattern is probably a general phenomenon, not a temporal one.

Direct studies of movement complement indirect genetic studies, despite the biases and logistic problems that can arise (Crochet 1996; Bossart & Pashley Prowell 1998a). The most significant bias is that direct studies tell nothing about historical processes and little about rare processes (Bohonak et al. 1998; Whitlock & McCauley 1999). Direct studies do however provide information on dispersal range and the influence of habitat type and patchiness. They also provide an ecological context for gene flow studies, something which indirect techniques cannot do (Bossart & Pashley Prowell 1998b).

In the case of quelea, there are many more pieces of evidence from direct studies that point in the same direction. Ringing studies (Oschadleus 2000), breeding records (Venn et al. 1999) and presence/absence data (Mundy & Herremans 1997) all suggest that quelea follow some seasonal movement patterns. However, none suggests population division, or provide definitive evidence of defined migration pathways. It is therefore highly unlikely that such divisions and migration pathways exist in present day quelea populations in southern Africa.

If divisions had been present in the past, the recent well documented range expansion of quelea in the Eastern Cape (Whittington-Jones 1998; 1999), if repeated elsewhere in the subcontinent, would have allowed current levels of gene flow to obscure the genetic signal of past structure in a marker system that evolves as rapidly as microsatellites. The genetic structure of any species is greatly influenced by

historical demographics. The mtDNA lineages of even currently abundant species, such as the red-winged blackbird *Agelaius phoeniceus* can show signs of much reduced population sizes (Awise et al. 1988). Therefore if questions of quelea population structure are more from scientific interest than driven by a practical need to investigate present day processes, a more slowly evolving marker system, such as mitochondrial DNA, could recover evidence of past divisions that have subsequently been wiped out by anthropogenically assisted range expansion.

#### 3.4.4 Summary

There is no evidence of population genetic subdivision in quelea in southern Africa or evidence for regular migration pathways. Therefore there is no reason to define separate management units for controlling quelea as a pest. There is also no evidence that there is more than a single subspecies of quelea in the region. Nonetheless, the lack of genetic differentiation does not necessarily preclude the possibility that quelea use more than one migratory system in response to the annual variation in their food supply.

Microsatellites may not be the most appropriate molecular marker to study such an abundant, wide-ranging species as the redbilled quelea. Constraints on allele size and rapid mutation rates mean that in large populations, homoplasy can be a problem, and that microsatellites can lose information content. Individuals with apparently similar multilocus genotypes occur throughout the range and it then becomes near impossible to identify the signal of whatever genetic differentiation may be present. Despite the results of the population genetics survey, geographic variation in plumage patterns (Chapter Four) and observations of quelea migration behaviour (Chapter Five) could still provide evidence for the presence of more than one migratory pattern for quelea in southern Africa.

## 4 Geographic variation in plumage patterns in the redbilled quelea in southern Africa

### 4.1 Introduction

Colour patterns in animals have a wide variety of functions, from thermoregulation to courtship. For some purposes, such as intraspecific communication, colour patterns should be as conspicuous as possible, while for others, such as the avoidance of predators, the colours used should be at least inconspicuous against the background, if not actually cryptic (Endler 1990; Savalli 1995).

The redbilled quelea is a sexually dimorphic weaverbird. During the breeding season males moult into brightly coloured plumage that shows a high level of variability. The variation in the male breeding plumage contrasts with the drab brown of both sexes in the non-breeding season, colouration that females retain through breeding.

The maintenance and variation of secondary sexual characteristics in animals has received a great deal of interest in recent years. In many species, conspicuous sexually dimorphic colouration acts as an indicator of individual quality and allows conspecifics to make informed choices about the quality of a mate (e.g. house finches *Carpodacus mexicanus* (Hill et al. 1999), and the blue grosbeak *Guiraca caerulea* (Keyser & Hill 2000)), or the likely success of an aggressive encounter within a sex (e.g. the ring-necked pheasant *Phasianus colchicus* (Mateos & Carranza 1997), the great tit *Parus major* (Wilson 1992)). In other species, geographic variation in sexually dimorphic colouration provides researchers with information on phylogenetic relationships and historical processes that affect populations (e.g. the West Canary Island lizard *Gallotia galloti* (Thorpe et al. 1996), the house sparrow *Passer domesticus* in North America (Johnston & Selander 1964)). Colour variation, whether sexually dimorphic or not, is important in defining endangered taxa for conservation purposes (e.g. the least tern *Sterna antillarum* (Johnson et al. 1998)), or investigating the spread of introduced alien species and hybrids through populations of a species of conservation interest, (e.g. the great crested newt *Triturus cristatus* in southern England (Brede et al. 2000)).

In the redbilled quelea, variation in the frequency of male breeding plumage patterns has been used to identify subspecies (Ward 1966) and infer relationships between breeding colonies (Jaeger et al. 1989b; Manikowski et al. 1989). The role of the plumage variation in intraspecific communication has also been addressed (Dale 2000). This Chapter aims to assess the variation in colour patterns of male quelea across southern Africa.

#### 4.1.1 Plumage variation in the redbilled quelea

The redbilled quelea has streaked greyish buff upperparts and a mottled grey belly (Maclean 1993). However during the breeding season males moult into secondary sexual plumage colours that are conspicuous and extremely variable from individual to individual. The facial mask around the beak can be black, or brown or white. The colour of the breast, belly and crown can vary from light straw to deep pink. Male quelea in breeding plumage show distinct polymorphisms (Figure 1.1). Ward (1966) used these variations in plumage patterns to describe the populations of quelea across Africa leading to the definition of three subspecies.

Many subsequent studies of quelea distribution have been based on the description of quelea plumage polymorphisms outlined by Ward. The '*Manual of Techniques used in Research on Quelea Birds*' (Ward 1973) underlined the importance of plumage polymorphism in quelea research. It included, in a section on Colour Pattern Determination (p23), instructions for recording both Mask Index for assessing the width of the frontal facial band, and a scoring system for the yellow to pink variation of the crown and breast. Recording Mask Index has remained an important part of quelea monitoring techniques (Elliott & Lenton 1989; Meinzingen 1993; Allan 1996).

Quelea male plumage patterns have been used as a signal of identity for populations from different regions. Mask index scores from over 26,000 birds were used to describe local population divisions in eastern Africa (Jaeger et al. 1989b). The authors went on to suggest that the subspecies division of *aethiopica* was not appropriate for the ' . . . complex and dynamic situation existing within the region . . .' (p123). Instead they believed that quelea plumage polymorphisms were best

represented as a series of clines in geographic character variation. Mask index and patterns of moult were used by the same authors to suggest origins for quelea sampled in Ethiopia and Somalia. Different frequencies of mask index indicated that the samples had different geographic origins. In Kenya, similarities in mask index composition led the authors to suggest that quelea from the central Kenyan Rift Valley were more similar to quelea from Ethiopia and northern Kenya than those from Tanzania.

In West Africa, greater variation was found in the proportions of the different mask indices in Senegal and Niger which suggested that quelea plumage patterns were more complex than proposed by Ward (Manikowski et al. 1989). At the extremes of the supposed ranges of *quelea* and *aethiopica*, as many as 31% of males did not have the typical plumage patterning. The authors believed this observation made dividing quelea into subspecies based on plumage patterns ‘misleading’.

The wide range of variation in plumage patterns inevitably leads to disagreement in describing subspecies, races and populations. However, even authors who disagree with established subspecies labels do agree that plumage patterns vary geographically (Ward 1966; Jaeger et al. 1989b; Manikowski et al. 1989). In addition, the available evidence indicates that the proportions of males with certain mask types and plumage colours at a given site remains constant over time. Over a 26-year period, there were no significant differences in the number of white and black morphs of males at four sites in Senegal and Mali (Manikowski et al. 1989).

Plumage variation is therefore clearly present among breeding male quelea. However, there is no obvious evidence that quelea plumage patterns signal the breeding quality of a given male (Dale 2000). Plumage colour (buff or pink/white or black) does not correlate with physical condition or age nor with reproductive success as measured by clutch size. One possibility is that plumage is a signal of individual identity (Dale 2000). An ability to identify and be identified by neighbours could be selectively advantageous in reducing the number of antagonistic interactions in a species that nests in dense, synchronous colonies.

Quelea plumage polymorphisms represent a pool of information that could be used to identify local populations. Identifying which colonies are most similar to each other in terms of plumage polymorphisms offers a way to indicate possible inter-colony relationships. Plumage patterns are stable through time at a population level. They are also genetically determined. Both these facts mean that if, as described above, plumage patterns at given locations represent distinct places on a geographic cline of variation, then a sufficiently broad scale survey of plumage patterns could reveal much about patterns of relationships between populations. Migration pathways could be identified by linking geographically distant populations with similar plumage characteristics.

#### 4.1.1.1 Quelea in southern Africa

Two subspecies of quelea have been proposed for southern Africa: *lathamii* and *spoliator*. *Spoliator* (Clancey 1960) has been rejected by several authors (Lourens 1961; Ward 1966; Jones et al. In press). Nonetheless it is included in many standard ornithological texts for the region (Irwin 1981; Clancey 1998). Quelea subspecies have been defined on the basis of the frequencies of breeding male plumage patterns. *Spoliator*, on the other hand, was described from non-breeding birds on the basis of variation in mantle feather colour. *Spoliator* are supposedly colder and greyer than *lathamii*, which are meant to be warm and buff. Ward (1966) dismissed *spoliator*, as it was not dissimilar enough from *lathamii*. Lourens (1961) claimed that the two forms bred together and that offspring were of either colour. Jones et al. (Jones et al. in press) presented evidence that suggested that not only are the morphological distinctions unclear, but that there is no behavioural or ecological evidence in support of separate subspecies.

This Chapter presents the first large scale sampling of male quelea from across southern Africa and therefore across the supposed divide between the ranges of *lathamii* and *spoliator*. The results from a previous study (Clancey 1973) only used 23 *spoliator* individuals and are probably unreliable. This is the first opportunity to examine the variation in plumage with regard to the subspecific status of *spoliator*, and answer subsequent questions. Is it possible to distinguish quelea reliably on the basis of mantle feather colour? If so, does mantle feather colour either (a), act as a



reliable indicator of where the birds were sampled, or (b) vary in concert with a suite of other plumage characters in such a way that mantle feather colour could act as a single summary character.

#### 4.1.2 Techniques for assessing colour variation

There are three main methods that have been used to assess colour: subjective colour scoring using human vision, electronic equipment such as spectrophotometers, and the relatively recent use of digital images and computer software.

Human observers scored differences in plumage patterns to show altitudinal variation in *Nesospiza* finches in the Tristan da Cunha archipelago (Ryan et al. 1994).

Subjective scores were also used as an indicator of condition in relation to parasite load in passerines (Harper 1999; Figuerola et al. 1999). Spectrophotometers have been used to quantify condition dependent sexual selection based on variation in plumage reflectance of UV in the blue tit *Parus caeruleus* (Andersson et al. 1998) and the role of mate choice in bluethroats *Luscinia s. svecica* (Johnsen et al. 1998). In recent years the use of digital techniques has become more common. Examples include the objective measurement of badge size as an indicator of male quality in the great tit *Parus major* (Figuerola & Senar 2000) and assessing the colour inside nestlings' mouths as a begging signal in reed buntings *Emberiza schoeniclus* (Kilner & Davies 1998).

Using human vision alone is context dependent, subjective, unreliable, and often non-repeatable (Endler 1990). Assessment of differences is also restricted to visible wavelengths (400-700 nm) and can therefore miss variation in the UV range that is important for birds such as the starling *Sturnus vulgaris* (Bennett et al. 1997) and the blue tit (Andersson et al. 1998).

Spectrophotometers are less susceptible to observer bias as they offer a graphical representation of the physical reflectance properties of a given area. They also contain a large amount of information on the colour characteristics of a sample that is independent of the properties of the human eye or any other signal receiver.

Spectrophotometers offer superior resolution and range compared to human vision, which is needed when infrared or ultraviolet wavelengths are important (Cuthill et al.

1999; Grill & Rush 2000). However, spectrophotometers have their own disadvantages including expense. Often complicated methodological restrictions limit their application to environments where the illumination can be controlled, and to flat, uniformly coloured body regions (Villafuerte & Negro 1998).

Photography and computer software can also quantify colour. As camera equipment records the same range of wavelengths as the human eye, the technique is only useful where UV wavelengths are not of interest. In such circumstances digital images can provide increased sensitivity and objectivity to the measurement of colour, and can reveal novel phenomena. For instance, in the red-legged partridge *Alectoris rufa*, digital photography revealed a previously unknown sexual dimorphism and provided a finer discrimination between two subspecies than human observers were able to achieve based on scoring methods (Villafuerte & Negro 1998).

Techniques for subjective scoring plumage variation and using digital images have both been used to assess quelea plumage variation. This chapter continues to use both techniques to assess the plumage pattern variation in male redbilled quelea in southern Africa. A comparison of the two techniques will therefore be possible.

## 4.2 Methods

### 4.2.1 Sampling

Male quelea were collected for plumage analysis from the sites shown in Figure 4.1. The number of males collected and analysed from each site is given in Table 4.1. Males were collected from breeding colonies as described in Chapter Two. Only sites with more than 30 males were included in colour pattern analysis. All the males were used in the analysis based on plumage scores. A subsample of five sites (AF, NN, RR, LT and TE) representing the largest sample sizes from geographically distant regions was used for the digital image analysis.

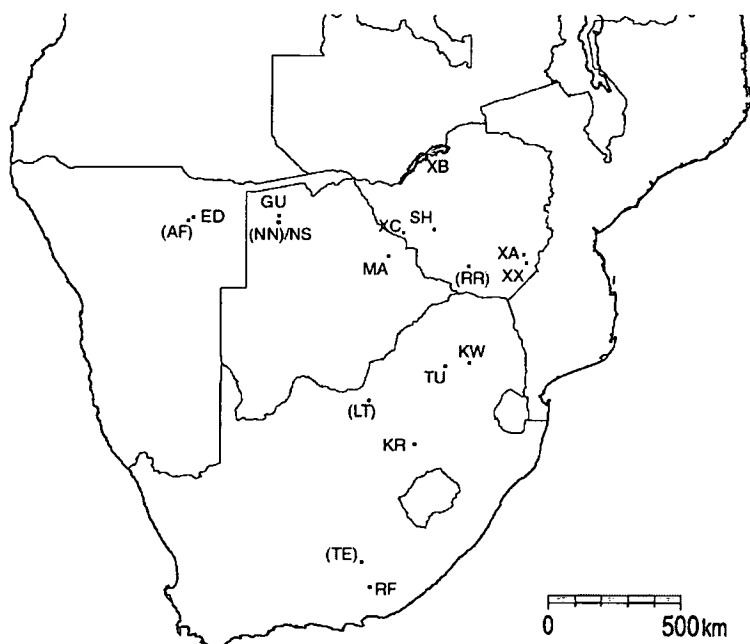


Figure 4.1. A map showing the location of sampling sites in southern Africa used in plumage analysis. Males from the five sites in brackets were analysed for quantitative colour variation using Photoshop 5.02.

Table 4.1. List of sites and the number of males (N) sampled at each. 1397 individuals were sampled in total.

Full Name	Site Code	Country	Region	N
Alwyn Farm*	AF	Namibia	West	53
Eden Farm	ED	Namibia	West	43
Gumare	GU	Botswana	West	63
Kroonstad	KR	South Africa	South	57
Klawervallei	KW	South Africa	South	62
Lichtenberg*	LT	South Africa	South	70
Mathangwane	MA	Botswana	Central	79
Nokoneng North*	NN	Botswana	West	80
Nokoneng South	NS	Botswana	West	59
Riverside Farm	RF	South Africa	South	90
Reata Ranch*	RR	Zimbabwe	Central	98
Shirville Farm	SH	Zimbabwe	Central	96
Terminus*	TE	South Africa	South	92
Tuinplaas	TU	South Africa	South	55
White Kopjes Ranch	WK	Zimbabwe	Central	33
Senuko	XA	Zimbabwe	Central	88
Bumi Hills	XB	Zimbabwe	Central	97
Maitengwe Dam	XC	Zimbabwe	Central	90
Malilangwe	XX	Zimbabwe	Central	92

\* - site included in analysis of colour measures using digital images

### 4.2.2 Morphological variation

The variation of plumage patterns in male redbilled quelea was assessed in two different ways. First a range of plumage characters was chosen to describe the observed variation. Each character was then scored and measured on quelea in the field. Second, photographs of the dorsal and ventral sides of each bird were taken under standardised conditions. Variation in colour patterns were then later assessed using the software package Photoshop 5.02 (Kilner & Davies 1998; Dale 2000). Each method will now be described in turn.

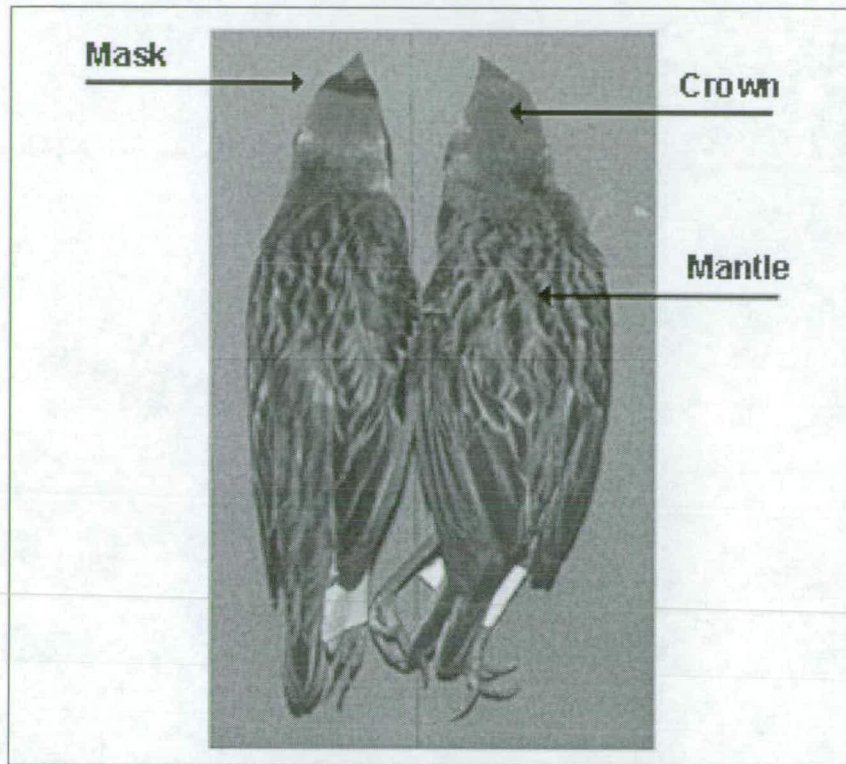
### 4.2.3 Recording plumage variation

#### 4.2.3.1 Scores

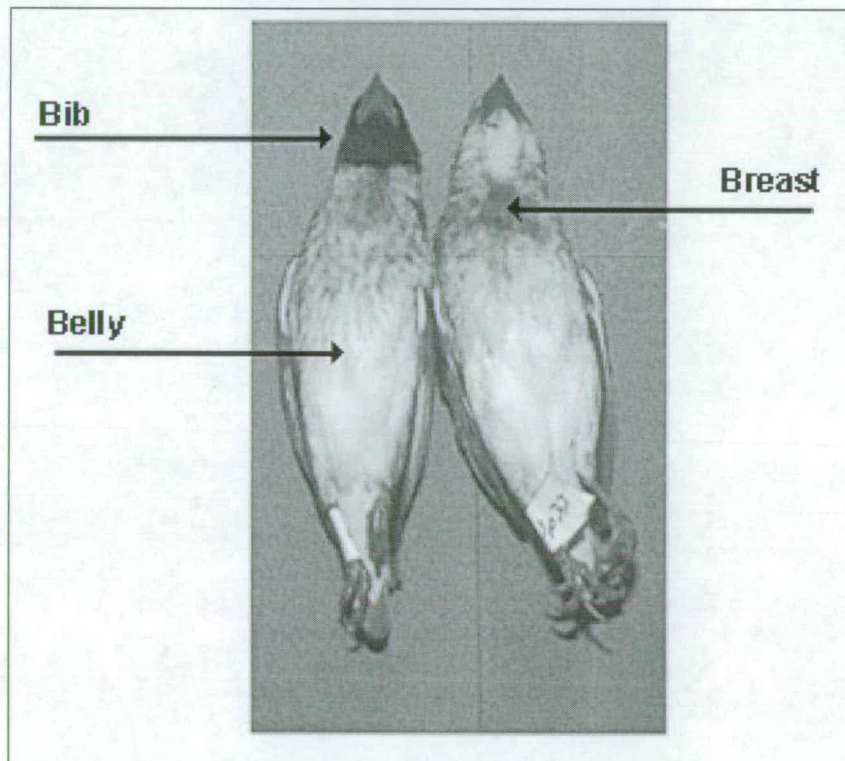
Plumage variation data from 1,397 male quelea from 19 sites were collected during three field seasons from March 1998 to February 2000. The characters that were scored are listed in Table 4.2, and the features examined are illustrated in Figure 4.2

Table 4.2 Plumage characters scored on male redbilled quelea. Figure 4.2 illustrates the location of each character

Character	Scoring
Mask Index	Scored from 0 to 7
Reduced Mask Index	Scored from 0 to 3
Breast Colour	Scored from 1 (buff) to 4 (pink)
Breast Buff	When the breast colour score was 1, the depth of buff was scored from 1 (light) to 3 (dark)
Belly Grey	The extent of the grey, scaly feathers on the belly. Scored from 1 (no grey) to 4 (all grey)
Belly Colour	The underlying feather colour on the belly. Scored from 0 (no colour) through 1 (buff), 2 (mix), 3 (pink)
Belly Colour Intensity	Scored from 0 (pale) to 3 (dark)
Crown Colour	As Breast Colour
Crown Buff	As Breast Buff
Mask Width	In mm using dial callipers
Bib Width	In mm using dial callipers
Subspecies Class	The colour of the mantle dorsal plumage (Clancey 1960). Scored from 1 (cold, grey = <i>spoliator</i> ) to 4 (warm, buff = <i>lathamii</i> ). Scores 2 and 3 are intermediate.



(a) Dorsal side



(b) Ventral side

Figure 4.2 Plumage measurements on male redbilled quelea.

Plumage characters were selected to include all the main areas of quelea plumage that showed colour variation. Characters were defined for the crown, breast, belly and mantle. The main colour variations were from deep pink to mixed pink and buff plumage, to dark brown buff to yellow or light buff (Ward 1973; Sinclair et al. 1993; Maclean 1993). Crown, breast and belly all showed this type of variation, and while the colour of each for a given individual could be similar, it was rarely the same. In addition there was variation in the extent of grey, scaly feathers on the belly and also the amount of belly covered by any colour that was present. The colour of the mantle feathers on the back was also assessed through the character 'Subspecies Class' which varied from 'cold grey' to 'warm brown' (Clancey 1960). Colour variation was assessed with reference to quelea specimens held at the Natural History Museum of Zimbabwe, Bulawayo, and the Durban Natural Science Museum, South Africa. Both museums held specimens that Clancey had labelled *spoliator*. Jones et al. (in press) gives details of the specimens examined.

#### 4.2.3.2 Mask and Bib Width

Mask and Bib Width were measured directly from the specimens using dial callipers, as shown in Figure 4.3. Mask Width is defined as the width (in mm) that the face mask extends onto the crown above the base of the bill. In practice the measurement was taken from the base of the bill to where the mask colour stopped and the crown colour began. In many white masked individuals, there was no contrast between crown colour and mask colour, and hence it was not possible to measure Mask Width. Conversely, for some individuals with mask indices of 5 and below, the mask did not extend above the bill at all. In these cases, Mask Width was measured from the base of the bill to where the mask started below the bill and recorded as a negative number. After all data had been collected, Mask Width was transformed by shifting the zero point so all measurements were positive. White-faced individuals whose mask could not be measured were given a Mask Width of zero.

Bib Width is defined as the width (in mm) that the face mask extends onto the throat from the chin. The measurement was taken from where the bill meets the chin to where the bib colour stopped and the breast colour began. Again in many white

masked individuals there was no contrast between bib colour and breast colour, and hence the bib width was zero.

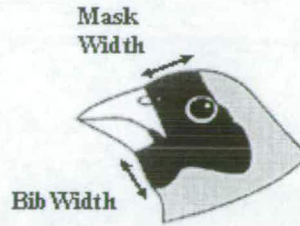


Figure 4.3. Mask and Bib Width for a black-faced male quelea.

#### 4.2.3.3 Mask Index

The Mask Index of Ward (1966) (Figure 4.4) included too many categories for southern Africa where the majority of quelea have Mask Indices of 0, 6 and 7. That is a quelea is white faced (MI = 0), or has the mask extending above the bill (MI = 6 or 7). The remaining categories 1 to 5 were collapsed into a single category that included all individuals whose mask did not extend above the bill. Mask Index was therefore transformed into the character Reduced Mask Index which was scored: 0 (white faced individual), 1 (mask not extending above bill), 2 (MI=6) and 3 (MI=7). Reduced Mask Index was used in all subsequent analysis.

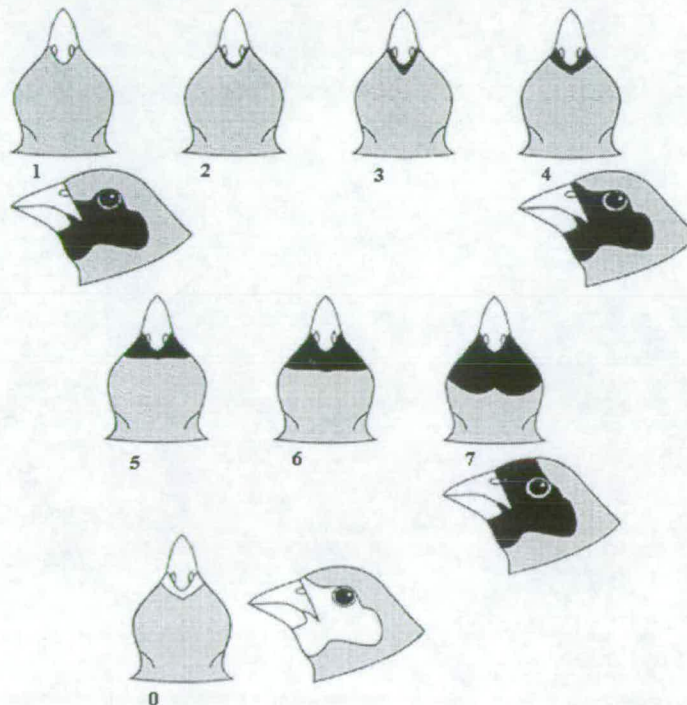


Figure 4.4. The eight mask indices (Ward 1966). From Allan 1996.

#### 4.2.3.4 Photographs and digital images

In addition to assessing the plumage of each specimen using scores and direct measurements, photographs were taken of the dorsal and ventral surfaces. Plumage colour was measured from photographs of the specimens that had been digitised and analysed with Adobe Photoshop 5.02. Photoshop is an imaging software package that gives quantitative scores for any colour in terms of hue, saturation and brightness. Appendix B gives the protocol for photography, digitising the photos, standardising images and taking readings of plumage colour. All males were photographed. Photoshop colour measurements were collected for 393 males from five sites, as shown in Figure 4.1.

#### 4.2.3.5 The hue, saturation and brightness colour model.

The hue, saturation and brightness (HSB) colour model is based on the human perception of colour (Wyszecki & Stiles 1967). The HSB model describes three characteristics of colour. Hue is the colour reflected from or transmitted through an object. It is expressed as a degree between  $0^\circ$  and  $360^\circ$ , measured as a location on the standard colour wheel as shown in Figure 4.5. Hue is identified by the name of the colour such as red, orange, or green. Pure red has a hue of  $0^\circ$  and pure yellow a hue of  $60^\circ$ . Hue is therefore an excellent way to measure the differences in colour on quelea which vary between deep pink and light buff/yellow.

Saturation is the strength or purity of the colour. Saturation represents the amount of grey in proportion to the hue, measured as a percentage from 0% (grey) to 100% (fully saturated), as shown in Figure 4.6. For example red is a more saturated colour than pink. Brightness is the relative lightness or darkness of the colour, usually measured as a percentage from 0% (black) to 100% (white), as shown in Figure 4.7. Photoshop can convert colour images to greyscale images composed entirely of shades of grey. Such an image allows colour variation that is essentially black to white to be expressed as a single number, K, the amount of blackness, with 0% representing pure white and 100% pure black.



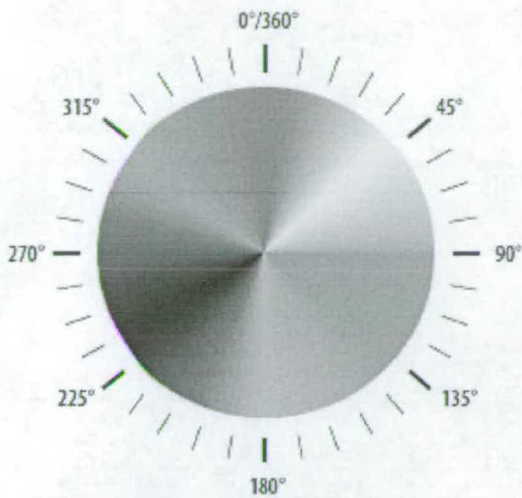


Figure 4.5. The standard colour wheel. Values for hue are given as degrees.

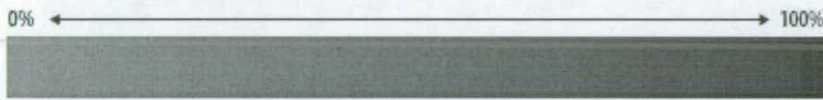


Figure 4.6. A saturation scale running from 0 (grey) to 100 (purple).



Figure 4.7. A brightness scale running from 100 (white) to 0 (black).

#### 4.2.3.6 Colour and shade measurements

Colour readings were taken from the crown and mantle on the dorsal surface, and breast and belly on the ventral surface using the hue, saturation and brightness colour model. Median and standard deviation blackness (K) readings were taken for mantle feathers, mask shade and bib shade.

Photoshop defines pure red as 0 and pure yellow as 60. Since quelea colour variation is between red and yellow, hue can be treated as a linear measurement with low values representing red plumage, and high values representing yellow plumage. Hue, saturation and brightness values were recorded, using the Color Sampler Photoshop tool, for the centre of the crown, mantle, breast and belly.

A further feature of plumage variation is the shade of the mask and bib. Categorized as white or black (e.g. Maclean 1993), the actual variation includes intermediate mask shades, from cream to brown to black (Manikowski et al. 1989; Whittington-Jones 1999; Dale 2000). On greyscale images, blackness, or K, was therefore

recorded using the Color Sampler Photoshop tool to capture the variation in mask and bib shade as varying shades of grey.

The colour of the mantle feathers has been used to classify quelea in southern Africa into separate subspecies (Clancey 1960). Clancey reported that mantle feathers vary from 'warm buff' to 'cold grey'. The variation in colour is adequately described by the hue of the mantle feathers. However 'warmth' and 'coldness' of the plumage is less a feature of colour, and more a feature of the contrast in colour between the central, darker portion of quelea mantle feathers and the lighter feather edge. 'Coldness' is characterised by lighter feather edges compared to feather centres, while 'warmth' is characterised by the contrast between edge and centre being less marked. Hence a measure of the contrast of the mantle feathers gave a good indication of the relative warmth of the plumage. To assess the variation in contrast, the standard deviation of blackness, K, for the mantle feathers was recorded. Median and standard deviation for K were recorded using the Marquee Photoshop tool in a 20 x 20 pixel area of the image, which represented about 1.25 cm<sup>2</sup> of the mantle plumage. High standard deviation indicates a high degree of contrast between feather edge and centre, and hence 'cold' plumage, while low standard deviation indicates less contrast and therefore 'warm' plumage.

The effect of wear on feather colour is a major confounding factor in using 'warmth' of plumage as a diagnostic subspecies feature. The warmth of the plumage depends on the contrast between feather edge and centre. Feather edges progressively wear after moult. The contrast, and hence 'warmth' of the plumage will therefore be affected by the age of the plumage. Feather wear will therefore affect the score, Subspecies Class, as well as colour measurements from the mantle feathers.

In total the plumage colouration in six areas of the quelea was quantified using hue, saturation, brightness and blackness (K) as appropriate. Fifteen pieces of data described the variation in colour for one quelea.

#### 4.2.3.7 Standardising images

Every effort was made to take photographs in standard conditions and known colour standards (Kodak Q13 standard colour cards) were included in each image

(Appendix B). This was done so that a colour reading on one image from one bird could be directly compared to the colour of another specimen on a different photograph. Such procedures were successful for hue, saturation and blackness (K). However, even after standardisation, it was apparent that measures of brightness were not sufficiently standardised. Different images were obviously more or less bright than each other. It was therefore decided to exclude brightness measures from further analysis.

#### *4.2.4 Analysis*

##### *4.2.4.1 Geographic variation in individual characters*

The overall aim of the analysis of plumage pattern variation in the redbilled quelea is to examine two null hypotheses: first that there is no relationship between plumage patterns and geographic location, and second, that there is no relationship between plumage patterns and Subspecies Class. The latter hypothesis is designed to investigate whether describing quelea on the basis of mantle feather colour provides any indication of systematic variation in other plumage characters. If such a relationship is not found then there can be no basis for using mantle feather colour as an indication of subspecies. It is merely one of many plumage variations present in quelea.

Each hypothesis was tested using different statistical techniques depending on the data type. For the categorical characters (plumage scores and mask index) a Kruskal-Wallis test was used to test the significance of the relationship between the character and either geographic location or subspecies class. Where the data were linear and continuous (Photoshop colour measurements, mask width, bib width) an ANOVA was used. Both Kruskal-Wallis and ANOVAs test for significant variation between treatment (in this case site or subspecies class) means. They require the data from each treatment to have equal variances. However in circumstances where sample sizes are large ( $n > 6$ ) and there are five or more treatments the requirement for equal variances can be safely ignored (Underwood 1997). Nonetheless, heterogeneity of variances can lead to lower p-values and an increased chance of type one error – rejecting a null hypothesis that is true. It is therefore appropriate to be cautious where

p-values are close to the chosen critical value,  $\alpha$ , and also for the Subspecies Class analysis when there are only four treatments.

Data are described as *continuous* for all Photoshop measures, mask width and bib width; *Photoshop* for the colour measures taken using that software package, and *categorical scores* for visual assessments of plumage colour. Continuous and categorical characters are given in Table 4.3.

#### 4.2.4.2 Multivariate data analysis

As several characters are likely to show some form of significant variation, it is not possible to describe the variation adequately using univariate statistical techniques. Characters that showed significant differences were used in a principal components analysis (PCA). PCA is performed in order to simplify the description of a set of related characters. It is an ordination technique that rearranges the between- and within-character variances to produce axes that describe a greater proportion of the observed variation. The new axes are orthogonal and hence any intercorrelation of the existing characters is removed. It is possible to distinguish between members of a given treatment or population with fewer variables. Complex multi-dimensional patterns of variation can be described with few principal components and without losing a great deal of information (Afifi & Clark 1996). One of the major advantages of PCA is that few uncorrelated principal components can be used in further analysis instead of many characters that have complex interrelationships. PCA is essentially a descriptive technique that can be used to visualise complex patterns of variation. The categorical scored characters and continuous variables were analysed separately, as listed in Table 4.3.

Table 4.3. The categorical and continuous variables analysed separately. Categorical variables are plumage colours scored by eye. Continuous characters are colours measured using Photoshop and physical measurements taken from individuals.

Categorical Character	Continuous Character
Mask Index	Mask Width (mm)
Breast Colour	Bib Width (mm)
Breast Buff	Breast Hue (H)
Belly Grey	Breast Saturation (S)
Belly Colour	Belly Hue (H)
Belly Colour Intensity	Belly Saturation (S)
Crown Colour	Crown Hue (H)
Crown Buff	Crown Saturation (S)
Subspecies Class	Mantle Hue (H)
	Mantle Saturation (S)
	Mantle Contrast (SD of K)
	Bib shade (K)
	Mask shade (K)

### 4.3 Results

#### 4.3.1 Geographic variation in plumage patterns

##### 4.3.1.1 Categorical scores of plumage characters

The mean and standard deviations for categorical plumage colour scored visually are shown in Table 4.4. The categorical variables were tested for the presence of significant variation among sites using Kruskal-Wallis tests. Four of the nine characters showed significant variation at the 0.05 level after sequential Bonferroni correction for multiple tests (Table 4.5). However, the significant variation in one of the characters, 'Subspecies Class', was entirely due to data collected in the first field season (March 1998) from four sites in Zimbabwe (XA, XB, XC, XX). These sites had lower average 'Subspecies Class' scores than the other sites (1.97-2.00 compared to a minimum of 2.39 elsewhere). When the four sites were removed, there was no significant among site variation ( $H=12.33$ ,  $DF=14$ ,  $p=0.580$ ). Only three significant relationships remained, namely: Belly Grey, Belly Colour and Belly Colour Intensity. Belly Grey was highest, at 1.96, for White Kopjes (WK), and lowest, at 1.10, for both Nokoneng North (NN) and Nokoneng South (NS). Belly Colour and Belly Colour Intensity were both low for Lichtenberg (LT) at 0.46 and 0.36 respectively. The highest values were for Klawervallei (KW) at 1.06 and 0.98.

Table 4.4 Mean and standard deviation for each categorical scored plumage character for each site

Site	N	Belly Grey		Belly Colour		Belly Colour Intensity		Breast Colour		Breast Buff		Crown Colour		Crown Buff		Reduced Mask Index		Subspecies Class	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Alwyn Farm	AF 53	1.19	0.94	0.94	0.86	0.85	0.74	2.15	1.01	0.75	1.07	1.62	0.79	0.98	0.99	2.32	1.14	2.40	0.77
Eden Farm	ED 43	1.35	1.00	0.93	1.03	0.60	0.66	2.35	1.11	0.79	1.19	1.86	0.91	0.88	1.05	2.28	1.08	2.77	0.90
Gumare	GU 63	1.38	0.89	0.89	1.11	0.65	0.83	2.33	1.03	0.59	0.93	1.87	0.87	0.67	0.84	2.54	0.89	2.44	0.71
Kroonstad	KR 57	1.65	0.86	0.46	0.66	0.39	0.53	2.39	1.10	0.51	0.78	1.81	0.91	0.68	0.78	2.40	1.05	2.40	0.53
Klawervallei	KW 62	1.19	0.97	1.06	0.74	0.98	0.71	2.03	1.01	1.02	1.29	1.68	0.84	1.19	1.21	2.40	1.09	2.39	0.69
Lichtenberg	LT 70	1.40	0.86	0.46	0.72	0.36	0.54	2.21	1.10	0.64	0.92	1.79	0.81	0.73	0.88	2.74	0.53	2.44	0.63
Maitengwane	MA 79	1.42	0.94	0.63	0.75	0.57	0.65	2.43	1.00	0.52	0.92	2.00	0.99	0.73	0.93	2.34	1.07	2.43	0.67
Nokoneng North	NN 80	1.10	0.87	1.16	1.17	0.69	0.69	2.39	1.04	0.59	0.96	1.86	0.91	0.76	0.89	2.60	0.87	2.44	0.67
Nokoneng South	NS 59	1.10	0.88	1.32	1.18	0.85	0.83	2.61	1.05	0.34	0.73	2.02	0.84	0.56	0.84	2.39	1.03	2.41	0.62
Riverside Farm	RF 90	1.24	0.77	1.11	0.99	0.81	0.72	2.46	1.06	0.44	0.78	2.03	0.91	0.59	0.86	2.22	1.19	2.53	0.72
Reata Ranch	RR 98	1.37	0.92	0.74	0.78	0.65	0.64	2.41	1.08	0.63	1.02	2.01	0.91	0.63	0.88	2.39	1.08	2.52	0.80
Shirville Farm	SH 96	1.60	0.89	0.72	1.03	0.48	0.66	2.25	0.93	0.58	1.00	1.78	0.85	0.94	1.03	2.40	1.04	2.57	0.74
Terminus	TE 92	1.45	0.93	0.88	0.92	0.70	0.72	2.43	1.14	0.50	0.82	2.02	1.01	0.59	0.79	2.41	1.07	2.58	0.63
Tuinplaas	TU 55	1.56	0.92	0.53	0.86	0.40	0.63	2.13	0.98	0.67	1.00	1.80	0.80	0.71	0.90	2.40	1.08	2.51	0.74
White Kopjes	WK 33	1.94	0.70	0.70	0.73	0.58	0.50	2.45	0.97	0.55	1.00	1.85	0.80	0.79	1.05	2.36	1.17	2.55	0.79
Senulo	XA 88	1.69	0.79	1.05	1.12	0.66	0.76	2.27	0.92	0.53	0.92	1.99	0.92	0.72	0.93	2.56	0.91	2.00	0.61
Bumi Hills	XB 97	1.43	0.88	1.03	1.03	0.71	0.71	2.22	0.90	0.57	0.92	1.90	0.90	0.81	0.97	2.37	1.05	2.16	0.72
Maitengwe Dam	XC 90	1.26	0.84	0.86	1.02	0.66	0.75	2.34	0.93	0.49	0.86	1.94	0.87	0.67	0.91	2.41	0.97	1.99	0.63
Malilangwe	XX 92	1.68	0.77	0.74	1.09	0.49	0.78	2.34	0.84	0.38	0.77	1.99	0.88	0.66	0.89	2.51	0.92	1.97	0.46

Table 4.5. Significant variation among sites for categorical scored plumage characters using Kruskal-Wallis test. P-values in bold indicate a significant result after sequential Bonferroni correction for multiple tests.

Character	H	DF	P
Belly Grey	63.64	18	<b>&lt;0.001</b>
Belly Colour	62.63	18	<b>&lt;0.001</b>
Belly Colour Intensity	61.32	18	<b>&lt;0.001</b>
Breast Colour	19.33	18	0.372
Breast Buff	14.22	18	0.714
Crown Colour	19.16	18	0.382
Crown Buff	21.01	18	0.279
Reduced Mask Index	9.60	18	0.944
Subspecies Class	116.77	18	<b>&lt;0.001</b>

#### 4.3.1.2 Geographic variation in categorical plumage characters

Despite only three of the scored characters showing significant among site variation, all characters were included in a principal component analysis. There were two reasons for this. First, the categorical plumage scores represent the larger of the two plumage data sets (N=1397). Second, as described in Section 4.3.1.4, the majority of the variation in the principal components for the quantitative plumage colour data was due to the variation in pink and buff plumage. If the categorical scores that are equivalent to this variation were not included in the PCA, then the importance of the pink-buff variation in the larger data set cannot be assessed, nor can any comparison of the two techniques be made. Only individuals with no missing data are included in a PCA. As the Subspecies Class score for four sites (XA, XB, XC, XX) were not comparable with the other sites, Subspecies Class was not included in this analysis.

The PCA was also performed using the four removed sites (XA, XB, XC, XX) alone (details not shown, see Jones et al. in press). The pattern of plumage variation matched that revealed by the PCA using the rest of the sites, indicating that the four sites were not different in any other way from the majority of sites except in Subspecies Class. Indeed, when the four sites were included in the PCA (details not shown), the variation in PC1 was mostly due to Subspecies Class. The four sites clustered together and other inter-site relationships were not interpretable.

Eight scored characters were included in the PCA. The first two principal components contained 66.9% of the total variance (Table 4.6). Scatter plots of the

first two principal components for each individual and for site means are shown in Figure 4.8. Individuals have a different shaped symbol depending on site. The colour of the symbol indicates the region. The site means and standard errors for the first two principal components are given in Table 4.7.

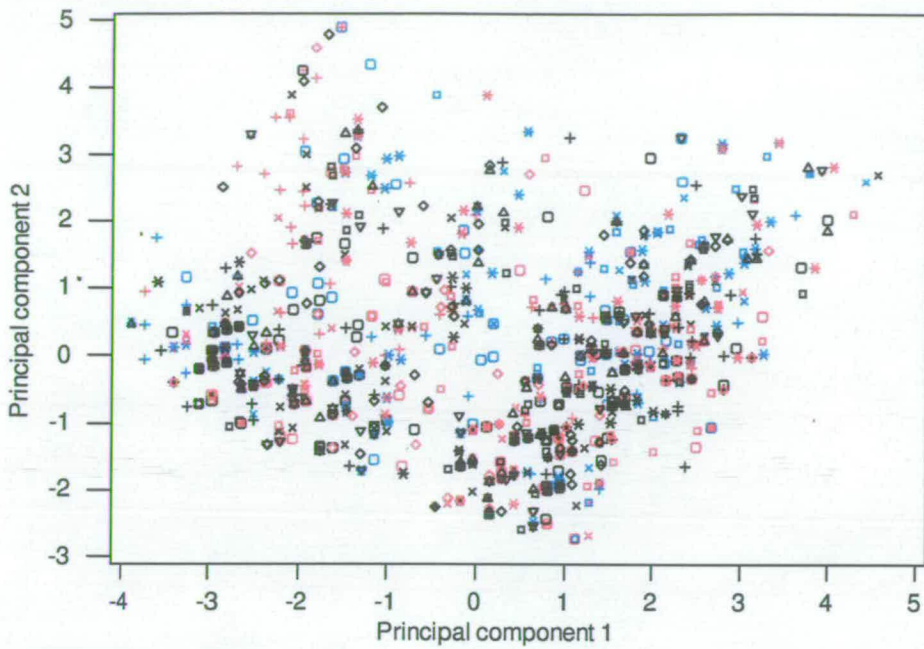
Table 4.6. Principal component analysis of eight plumage scores in relation to site (N=1397). (a) gives the eigenvalues for the principal components and the proportion of the total variance that they represent. (b) gives the eigenvectors.

(a)	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	
Eigenvalue	3.584	1.771	1.014	0.682	0.428	0.244	0.177	0.101	
Proportion	0.448	0.221	0.127	0.085	0.053	0.030	0.022	0.013	
Cumulative	0.448	0.669	0.796	0.881	0.935	0.965	0.987	1.000	
(b)	Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
	Belly grey	-0.129	-0.492	0.162	0.840	0.085	0.000	-0.038	-0.001
	Belly colour	0.293	0.530	0.131	0.360	-0.008	-0.241	0.655	0.018
	Belly colour intensity	0.310	0.525	0.115	0.298	0.027	0.079	-0.720	-0.028
	Breast colour	0.477	-0.168	-0.020	0.006	-0.207	0.563	0.112	0.610
	Breast buff	-0.419	0.254	0.048	0.004	0.724	0.229	0.051	0.424
	Crown colour	0.459	-0.168	-0.003	-0.077	0.539	0.352	0.124	-0.570
	Crown buff	-0.434	0.271	0.058	0.124	-0.366	0.665	0.145	-0.350
	Reduced mask index	0.003	-0.084	0.968	-0.233	-0.033	-0.015	-0.006	0.012

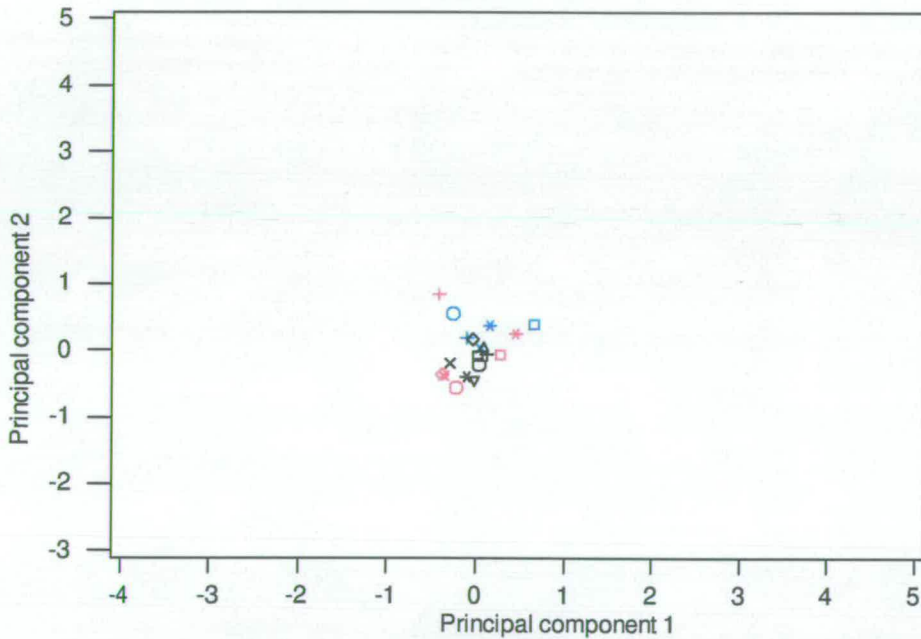
Variation in a principal component is determined by the characters that have the highest absolute eigenvectors for that component. The components of the eigenvector, whether positive or negative, determine the direction of influence of each character. For example an individual or site with a high positive principal component will be distinguished by the characters that have high positive eigenvectors for that principal component. Similarly, the individual will lack those characters that have high negative eigenvectors. These characters will, however, be present in individuals with a high negative value of the principal component.

PC1 was mainly determined by a combination of four characters: Breast Colour, Breast Buff, Crown Colour and Crown Buff. A positive PC1 indicated high values for the colour scores on the breast and crown. High colour scores indicate that the birds are pink as opposed to buff. A negative PC1 therefore indicated birds that are more buff, and as both Crown and Breast Buff had high negative eigenvalues, where birds were scored as buff, they were also a deeper shade of buff than birds with a positive PC1. Hence PC1 can be viewed as an axis expressing the variation in colour around the mask from dark buff to pink.





(a) Individuals



(b) Site means

Figure 4.8. Scatter plot showing the first two principal components of categorical scored plumage character variation between individuals (a) and site means (b). PC1 indicates colour variation from buff (-ve values) to pink (+ve values). PC2 indicates variation from not deeply coloured (-ve) to coloured (+ve). West sites (blue): Circle – AF; Plus – ED; Cross – GU; Star – NN; Square – NS. South sites (red): Circle – KR; Plus – KW; Cross – LT; Star – RF; Square – TE; Diamond – TU. Central sites (black): Circle – MA; Plus – RR; Cross – SH; Star – WK; Square – XA; Diamond – XB; Triangle – XC; Down Triangle – XX.

Table 4.7. Mean and standard error (SE) by site for the first two principal components (PC1 and PC2) for categorical scored plumage characters.

Site	Region	N	PC1		PC2		
			Mean	SE	Mean	SE	
Alwyn Farm	AF	West	53	-0.266	0.243	0.539	0.196
Eden Farm	ED	West	43	-0.102	0.346	0.187	0.153
Gumare	GU	West	63	0.045	0.262	0.026	0.172
Kroonstad	KR	South	57	-0.226	0.227	-0.552	0.146
Klawervallei	KW	South	62	-0.414	0.243	0.837	0.195
Lichtenberg	LT	South	70	-0.366	0.227	-0.379	0.119
Maitengwane	MA	Central	79	0.035	0.216	-0.215	0.130
Nokoneng North	NN	Central	80	0.158	0.226	0.371	0.147
Nokoneng South	NS	Central	59	0.657	0.242	0.394	0.185
Riverside Farm	RF	South	90	0.450	0.199	0.256	0.134
Reata Ranch	RR	Central	98	0.117	0.204	-0.062	0.110
Shirville Farm	SH	Central	96	-0.304	0.189	-0.187	0.142
Terminus	TE	South	92	0.270	0.203	-0.057	0.129
Tuinplaas	TU	South	55	-0.381	0.230	-0.354	0.176
White Kopjes	WK	Central	33	-0.104	0.305	-0.411	0.192
Senuko	XA	Central	88	0.019	0.201	-0.088	0.152
Bumi Hills	XB	Central	97	-0.036	0.181	0.173	0.148
Maitengwe Dam	XC	Central	90	0.075	0.200	0.029	0.137
Malilangwe	XX	Central	92	-0.027	0.180	-0.439	0.144

Principal component two (PC2) was mainly determined by variation in the colour of the belly (Belly Grey, Belly Colour and Belly Colour Intensity). A positive PC2 indicated individuals with pinker, more deeply coloured bellies, while a negative PC2 indicated individuals with more grey on the belly and less colour. The axis can therefore be seen as expressing the variation in colouration on the underside of birds.

The scatter plot of individuals (Figure 4.8) reveals no clusters. There is a more or less continuous cloud. The site means are tightly clumped in the centre of the plot. There is no clear pattern, with very little separation achieved. Concentrating on the first principal component, four of the six southern sites have low values (relatively buff). Sites from the Central and West regions have values around zero. Finally, Terminus (TE), Riverside Farm (RF) and Nokoneng South (NS) have the highest values. For PC2, Klawervallei (KW) had the highest value (relatively pink). Sites from the West region tend to have higher values of PC2, indicating birds with more colour on the belly, and birds from the South region, excluding TE and RF, tend to have low values of PC2. There is then a middle cluster of sites from the Central region and TE and RF.

In summary, there is no clear pattern of geographic variation in the principal components, and hence no clear pattern of variation in plumage. However, most of the characters did not show significant variation among sites. A more sensitive method of assessing colour variation may reveal more characters that vary geographically and hence may have a higher chance of revealing geographic patterns in plumage variation. To examine this possibility, a study involving a smaller number of sites was performed using the continuous plumage characters described in Section 4.2.3.4.

#### 4.3.1.3 Continuous plumage characters.

Table 4.8 gives the mean, standard deviation and number of specimens analysed by site for each continuous character. Continuous variables as listed in Table 4.3 were then tested for the presence of significant variation among sites using a one way ANOVA. Two variables (mask shade and bib shade) were log-transformed before analysis to remove extreme heterogeneity of variance. As shown in Table 4.9, 11 of the 13 characters showed significant differences at the 0.05 level after sequential Bonferroni correction for multiple tests. No single site consistently differed across plumage characters from the others. Alwyn Farm (AF) specimens had higher hue for crown and breast, showing that the birds there were more yellow than elsewhere. Terminus (TE) specimens had higher values for mask and bib shade, indicating that the facial mask above and below the bill is, on average, lighter at that site. Lichtenberg (LT) had the lowest mean mantle hue, indicating that specimens have more red in the colour of the mantle feathers, denoting a brown colour as opposed to a grey colour. Birds from Nokoneng North (NN) and Terminus (TE) had high values for mantle contrast, indicating that the plumage is 'colder' in appearance, which is supposedly a diagnostic feature of the *spoliator* subspecies.

Table 4.8. Mean and standard deviation for each continuous character measured using Photoshop or direct from the specimen for each site.

Site	N	Crown Hue		Crown Saturation		Mantle Hue		Mantle Saturation		Mantle Shade Contrast		Mask Shade (K)		Mask Width+6 (mm)		
		mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	
Alwyn Farm	AF	54	26.05	10.73	52.78	10.54	21.80	6.46	33.15	11.94	20.31	3.37	18.17	12.86	7.65	3.32
Lichtenberg	LT	69	18.38	10.86	59.07	6.63	17.96	7.12	30.17	6.97	17.18	2.56	16.04	2.39	8.50	0.97
Nokoneng North	NN	80	19.23	9.90	64.65	10.33	26.65	3.25	38.85	5.29	23.73	3.55	12.17	10.56	8.46	2.02
Reata Ranch	RR	98	16.27	6.85	53.09	5.94	20.18	5.33	29.51	3.86	17.59	2.90	18.66	7.73	7.67	3.13
Terminus	TE	92	19.87	13.63	65.11	10.27	27.58	7.73	39.35	12.09	20.88	3.58	21.15	13.28	7.48	3.20

Site	N	Breast Hue		Breast Saturation		Belly Hue		Belly Saturation		Bib Shade (K)		Bib Width (mm)		
		mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	
Alwyn Farm	AF	54	24.29	10.89	55.85	9.59	43.37	8.36	24.13	8.75	32.91	20.75	14.88	6.01
Lichtenberg	LT	69	18.31	11.15	56.74	6.81	47.95	5.03	28.04	5.03	20.63	5.31	16.51	1.62
Nokoneng North	NN	80	16.23	9.48	63.23	10.75	32.17	6.14	20.59	6.98	21.62	18.78	16.15	3.59
Reata Ranch	RR	98	17.46	8.94	57.43	6.59	43.80	5.09	30.05	4.81	27.74	16.30	16.86	5.73
Terminus	TE	92	18.93	14.67	62.28	10.48	44.05	8.74	25.96	9.17	32.17	21.20	15.25	4.75

Table 4.9. Significant variation of continuous plumage characters among sites using ANOVA. Degrees of freedom = 4,388 for each character. P-values in bold indicate a significant result after sequential Bonferroni correction for multiple tests.

Character	F	P
Crown Hue	7.72	<b>&lt;0.001</b>
Crown Saturation	36.69	<b>&lt;0.001</b>
Mantle Hue	37.05	<b>&lt;0.001</b>
Mantle Saturation	26.13	<b>&lt;0.001</b>
Mantle Contrast	55.66	<b>&lt;0.001</b>
Log (Mask Shade)	26.48	<b>&lt;0.001</b>
Breast Hue	4.62	<b>0.001</b>
Breast Saturation	10.78	<b>&lt;0.001</b>
Belly Hue	59.54	<b>&lt;0.001</b>
Belly Saturation	22.00	<b>&lt;0.001</b>
Log (Bib shade)	12.47	<b>&lt;0.001</b>
Mask Width+6	2.54	0.039
Bib width	2.45	0.046

#### 4.3.1.4 Geographic variation in continuous plumage data

The 11 characters that showed significant among site variation were used in a principal component analysis. The first two principal components represent 56% of the total variation in plumage characters, as shown in Table 4.10.

The eigenvectors for PC1, which represented 32% of the variance, showed that PC1 was mainly determined by variation in saturation and hue readings on the crown, mantle, breast and belly, and by variation in the mask shade. A positive PC1 indicated an individual with saturated colour on the crown, mantle and breast. A negative PC1 indicates an individual with high readings for hue, i.e., birds that are yellow or buff. Hence PC1 can be thought of as an axis from buff birds (high hue, little saturated colour) to pink/red birds with a deep coloured mantle (low hue, high saturation). This is similar to the interpretation of PC1 from the categorical plumage scores data which represented the variation in colour around the mask from dark buff to pink.

PC 2 represented 24% of the total variation and was mainly determined by crown and breast hue, and saturation on the belly and breast. A positive PC2 indicated an individual with a high hue reading around the head on the crown and breast. A negative PC2 indicated an individual with a highly saturated belly and breast. Hence

PC2 can be thought of as an axis from birds with deeply saturated undersides which are generally pink around the head (have low hue readings) to birds that are generally buff around the head (have high hue readings) and have pale undersides. This is again in some way similar to the second principal component for the categorical plumage scored that represented the variation in colour on the underside of birds.

Table 4.10. Principal component analysis of 11 continuous measures of plumage colour polymorphism related to site (N=393). (a) gives the eigenvalues for the principal components and the proportion of the variance that they represent. (b) gives the eigenvectors for the principal components.

(a)

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Eigenvalue	3.480	2.632	1.556	0.858	0.678	0.498	0.443	0.342	0.237	0.178	0.098
Proportion	0.316	0.239	0.141	0.078	0.062	0.045	0.040	0.031	0.022	0.016	0.009
Cumulative	0.316	0.556	0.697	0.775	0.837	0.882	0.922	0.953	0.975	0.991	1.000

(b)

Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Crown hue	-0.178	0.524	-0.046	-0.025	0.313	-0.108	0.012	-0.323	-0.245	-0.060	-0.643
Crown saturation	0.444	-0.037	0.161	0.286	0.133	-0.398	-0.094	0.135	0.560	-0.295	-0.300
Mantle hue	0.318	0.282	0.259	-0.070	0.184	0.462	0.637	0.173	-0.002	-0.239	0.086
Mantle saturation	0.418	0.221	0.131	0.293	0.121	-0.251	-0.029	0.232	-0.384	0.616	0.117
Mantle contrast	0.327	0.304	0.093	-0.113	-0.137	0.545	-0.660	-0.089	0.132	0.042	-0.025
Log(Mask shade)	-0.287	-0.052	0.611	-0.018	-0.022	0.076	0.145	-0.260	0.417	0.511	-0.104
Breast Hue	-0.229	0.489	-0.054	0.058	0.357	-0.210	-0.110	-0.138	0.274	-0.097	0.644
Breast saturation	0.380	-0.294	0.116	0.086	0.107	-0.045	0.042	-0.792	-0.222	-0.133	0.198
Belly hue	-0.274	-0.040	0.016	0.885	-0.019	0.340	-0.041	-0.003	-0.077	-0.127	-0.017
Belly saturation	-0.077	-0.415	0.075	-0.115	0.811	0.202	-0.236	0.202	-0.067	0.041	-0.041
Log(Bib shade)	-0.170	0.035	0.695	-0.083	-0.141	-0.218	-0.237	0.179	-0.390	-0.405	0.089

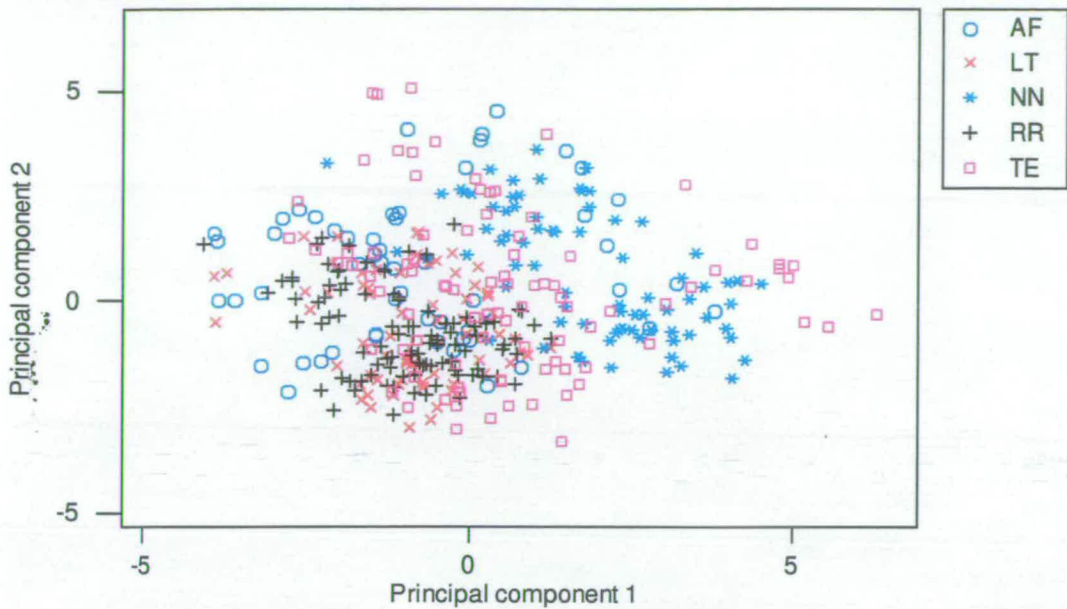
#### 4.3.1.5 Is the observed variation among sites or photographs?

Both principal components reveal an interpretable pattern of variation in the characters based on continuous, quantitative measures of colour. However, despite the efforts taken to standardise the data gathered from digitised images, the possibility remains that the pattern revealed by the principal components is due to noise from variation between photographs. The situation is exacerbated as each photograph only contains specimens from a single site. Any between photograph variation could be due to variation among sites, among photographs, or both. It is therefore important to test for significant variation among sites independent of variation among photographs. A generalised linear model (GLM) was used with the

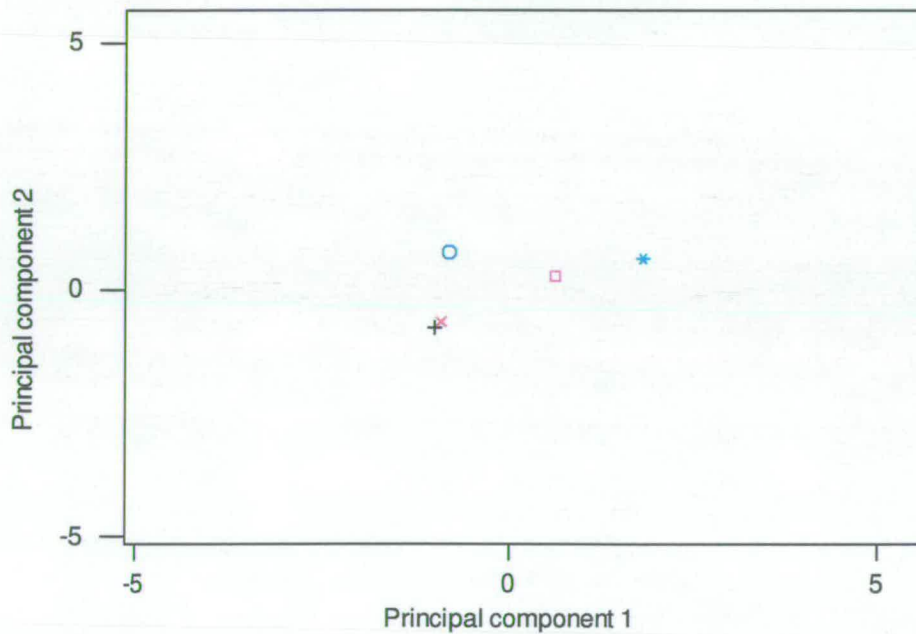
principal component as the response, and the model: Principal Component = Site + Photo(Site). PC1 differed significantly between sites ( $F_{4,53} = 12.627$ ,  $p < 0.001$ ) as did PC2 ( $F_{4,53} = 6.623$ ,  $p < 0.001$ ). However neither PC1 ( $F_{4,53} = 0.079$ ,  $p > 0.05$ ) nor PC2 ( $F_{4,53} = 0.154$ ,  $p > 0.05$ ) differed significantly between the nested terms Photo(Site). This indicates that there is significant variation among sites for both principal components that is independent of the variation among photographs.

#### 4.3.1.6 Geographic variation in principal components of continuous plumage characters

The first two principal components represented 56% of the total variation in characters. Figure 4.9 shows scatter plots for the first two principal components of plumage colour variation for individuals and site means. The scatter plot of individuals showed no divisions in a general cloud of points. However, individuals from Reata Ranch (RR) and Lichtenberg (LT) were clustered towards the negative side of both axes, indicating birds that are more buff. Individuals from Nokoneng North (NN) and Terminus (TE) were concentrated in the positive areas of both principal components, indicating pinker, less deeply coloured birds. There were therefore some differences between sites in plumage patterns. The scatter plot of site means for PC1 and PC2 shows all the sites clustered centrally although some of the pattern in the individuals is present. PC1 separated Reata Ranch (RR), Lichtenberg (LT) and Alwyn Farm (AF) from Terminus (TE) and Nokoneng North (NN). The second principal component achieved some, less marked, separation between the Reata Ranch/Lichtenberg cluster and Alwyn Farm, Nokoneng North and Terminus.



(a) Individuals



(b) Site means

Figure 4.9. Scatter plots showing the first two principal components of continuous plumage colour character variation for individuals (a) and site means (b). PC1 indicates variation from buff (-ve) to pink (+ve). PC2 represents variation from saturated pink (-ve) to pale undersides (+ve).

#### 4.3.1.7 Comparing Photoshop measures with plumage scores

More continuous characters showed significant variation between sites than the categorical plumage scores, suggesting that the Photoshop technique may be a more sensitive method for detecting geographic variation in plumage patterns. There are



also similarities in which plumage patterns were responsible for variation in the two most significant principal components. In both cases PC1 represents variation from buff to pink, and PC2 represents some elements of the colour on the underside of birds. However, there are few similarities in the principal component scatter plots. Alwyn Farm (AF) and Nokoneng North (NN) are reasonably well separated by both data types. The Photoshop continuous data group Lichtenberg (LT) and Reata Ranch (RR) which are well separated by the categorical plumage scores.

As there were no divisions revealed by either technique, it was not possible to judge which technique was more effective at capturing the variation that is present. There was no clear-cut advantage to using the substantially more laborious Photoshop method. The Photoshop study was therefore not extended to include any more sites. Nor will it be used in the following examination of the subspecies differences between *lathamii* and *spoliator*.

### **4.3.2 Is mantle feather colour a good indicator of variation in plumage characters?**

If the scores describing mantle feather colour ('Subspecies Class') are an accurate reflection of the division in the appearance of redbilled quelea in southern Africa, then other plumage characters should vary in a consistent way with Subspecies Class. Correlation would be one way to measure such consistent variation. However, as many of the characters are bimodal (black or white mask, pink or buff crown), correlations are unsuitable (Fowler et al. 1998). Additionally, performing correlations on several relationships generates the problem of multiple tests. Bonferroni corrections can ameliorate this problem but a multivariate approach would be more suitable. Hence, as for analysing the geographic variation, PCA was used for analysing variation among Subspecies Class. The aim was to identify suites of plumage characters that varied consistently with the Subspecies Class score.

#### **4.3.2.1 Variation of categorical plumage scores between Subspecies Class**

The categorical scored plumage characters were analysed for consistent patterns of variation among Subspecies Class using PCA. The four sites sampled in 1998 (XA, XB, XC and XX) were left out of this analysis as the Subspecies Class scores are not

comparable with other sites (see Section 4.3.1). However a similar analysis to the one below based solely on those four sites (Jones et al. in press) revealed no discrete clusters of individuals that could be identified with respect to Subspecies Class. The mean and standard deviation for each scored character by Subspecies Class is shown in Table 4.11, for the remaining 15 populations. Four of the eight characters showed significant variation among Subspecies Class, as shown in Table 4.12. These were Belly Colour, Belly Colour Intensity, Breast Colour and Crown Colour. All four were highest in Subspecies Class 4, and lowest in Class 1.

The four characters were included in a PCA. The first two principal components represented 91% of the total variation (Table 4.13). PC1 represented 63% of the total variance. A negative PC1 indicated a specimen with an overall pink colouration, while a positive PC1 indicated a specimen with overall buff colouration. PC2 is mainly defined by Belly and Crown Colour. A positive PC2 indicated an individual with a pink crown, and a negative PC2 indicating an individual with a pink belly. Figure 4.10 shows a scatter plot of the subspecies means for PC1 and PC2. Ellipses represent two standard deviations of each principal component, an area that contains >95% of the individuals from each Subspecies Class. The class means were all grouped together in the centre of the plot. Some minimal separation was achieved by PC1, with classes 3 and 4 marginally pinker than classes 1 and 2.

If it were possible to discriminate between individuals from each Subspecies Class, then the ellipses representing two standard deviations should be substantially separate. An individual from one class would then be unlikely to look like an individual from another. However, there is almost complete overlap for each class, indicating that it is virtually impossible to reliably assign an individual to a Subspecies Class based on other plumage characters. PC2 achieved almost no separation.

Table 4.11. Mean and standard deviation for each categorical scored plumage character for each Subspecies Class.

Class	N	Belly Grey		Belly Colour		Belly Colour Intensity		Breast Colour		Breast Buff	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
1	124	1.38	0.95	0.73	1.01	0.51	0.69	2.10	0.99	0.74	0.99
2	699	1.44	0.88	0.81	1.01	0.58	0.71	2.23	0.98	0.59	0.94
3	506	1.40	0.89	0.93	0.93	0.72	0.70	2.48	1.03	0.51	0.93
4	68	1.35	0.93	1.10	0.98	0.85	0.78	2.63	0.98	0.44	0.95

Class	N	Crown Colour		Crown Buff		Reduced Mask Index	
		Mean	sd	Mean	sd	Mean	sd
1	28	1.69	0.88	0.88	0.86	2.36	1.08
2	166	1.83	0.87	0.78	0.93	2.39	1.06
3	178	2.02	0.91	0.68	0.95	2.48	0.96
4	20	2.18	0.85	0.50	0.91	2.54	0.85

Table 4.12. Results of Kruskal-Wallis tests for significant variation among Subspecies Class. P-values in bold indicate a significant difference after sequential Bonferroni correction for multiple tests.

Character	H	DF	P
Belly Grey	3.56	3	0.313
Belly Colour	17.67	3	<b>0.001</b>
Belly Colour Intensity	20.98	3	<b>&lt;0.001</b>
Breast Colour	18.09	3	<b>&lt;0.001</b>
Breast Buff	6.46	3	0.091
Crown Colour	19.62	3	<b>&lt;0.001</b>
Crown Buff	9.59	3	0.022
Reduced Mask Index	1.41	3	0.704

Table 4.13. Eigenvalues and eigenvectors for the PCA of categorical scored plumage data related to Subspecies Class (N=1030).

	PC1	PC2	PC3	PC4
Eigenvalue	2.528	1.104	0.198	0.170
Proportion	0.632	0.276	0.050	0.042
Cumulative	0.632	0.908	0.958	1.000

Character	Eigenvector			
	PC1	PC2	PC3	PC4
Belly Colour	-0.495	-0.519	0.027	0.696
Belly Colour Intensity	-0.514	-0.471	0.014	-0.717
Breast Colour	-0.503	0.483	-0.716	0.030
Crown Colour	-0.487	0.525	0.698	0.018

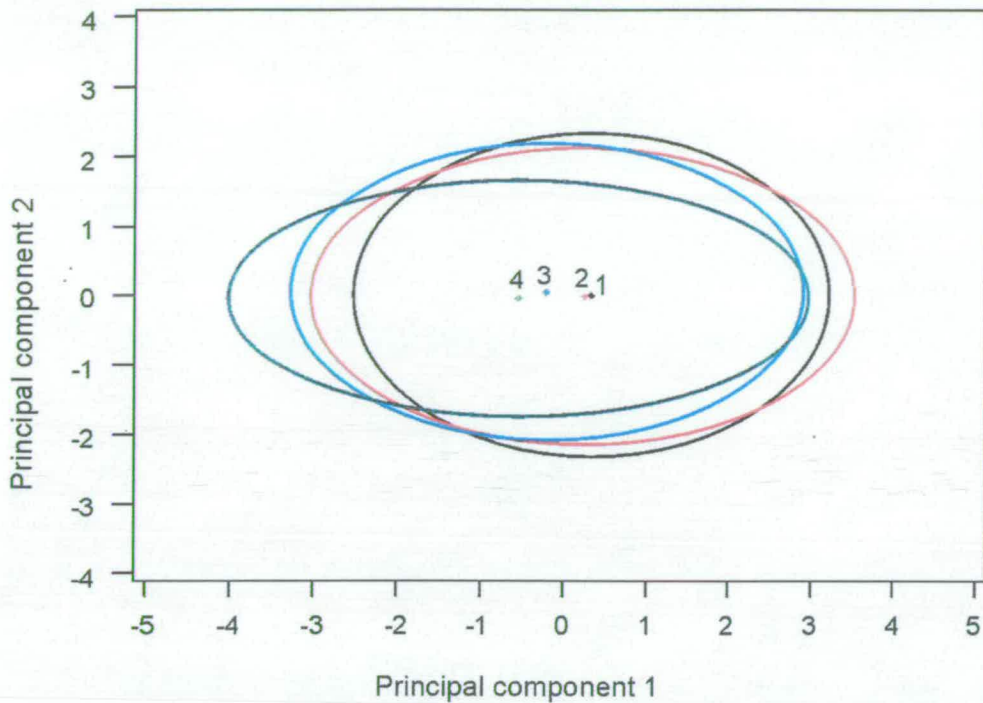


Figure 4.10. Scatter plot showing the first two principal components of plumage scores variation for Subspecies Class group means with ellipses indicating 2 SDs. 1 (black) – *spoliator* like, 2 (red) and 3 (blue) – intermediates, 4 (green) – *lathamii* like. PC1 represents variation from pink (-ve) to buff (+ve). PC2 achieved no separation.

#### 4.4 Discussion

The patterns of geographic variation in plumage in the redbilled quelea have been studied throughout Africa and have led to the definition of three accepted subspecies (Ward 1966). In East and West Africa, various authors have disagreed with the rigidity of the subspecies definition (Jaeger et al. 1989b; Manikowski et al. 1989) and whether colour variation was a true indicator of phylogenetic division. Nonetheless plumage patterns are inherited (Dale 2000) and appear to remain the same in a given area over a number of years (Manikowski et al. 1989). Variations have been used in the past to suggest inter-population relationships, and recording plumage patterns remains a recommended technique for monitoring quelea populations by pest management teams (Allan 1996). This is the first wide-ranging study of the regional variation of quelea plumage patterns in southern Africa.

##### 4.4.1 Plumage pattern variation in southern Africa

In this study there was little evidence for geographically interpretable variation in colour patterns in the redbilled quelea in southern Africa. Both methods that were

used, subjective categorical plumage scores, and quantitative continuous colour measurements from digital images, showed some minor multivariate inter-population variability, but the patterns revealed by the principal components analysis were not identical. Both methods did agree on the most important characters that were responsible for the variation, namely colour (buff – pink) for the first principal component and saturation, or depth of colour, for the second. However the two different techniques used to assess variation between sites did not agree on which sites showed particular traits.

There were some differences between the appearance of an average bird at each site, although the site means were all tightly clumped in the centre of a cloud of individuals. The differences between sites are therefore small compared to the variation that occurred within the samples collected at each site. In other words the vast majority of the variation in plumage patterns in quelea in southern Africa is not dependent on where individuals were sampled. Quelea simply show extensive polymorphisms in plumage patterns. The similarity of quelea plumage polymorphisms across southern Africa meant that it was not possible to infer inter-population relationships that could be indicative of migration between locations.

Patterns of variation were seen in the PCA of the plumage scores. For example, birds from Eastern Cape province in the far south of South Africa (sites Terminus (TE) and Riverside Farm (RF)) together with the site Nokoneng South (NS) in Botswana were the pinkest. Birds from the north of South Africa, such as Tuinplaas (TU) and Lichtenberg (LT) were slightly more buff. Some pairs of sites that were geographically very close or even part of the same colony (for example Nokoneng North (NN) and Nokoneng South (NS)) were some of the most different. Others that are geographically separate (such as Eden Farm (ED) and Bumi Hills (XB)) were very similar in plumage characteristics. In summary, all sites were very similar in their plumage and any differences were only of degree.

Similarly, the variation in the appearance of birds within each of the four Subspecies Classes was more marked than any variation between the Classes. It was near impossible to predict reliably to which Class an individual belonged based on other

plumage characters. This suggests that Subspecies Class does not act as a reliable way of dividing quelea based on appearance.

Subspecies Class as an individual character does not show significant geographic variation. Therefore, although there is variation in the mantle plumage colour, as shown by the continuous colour readings, there was little evidence that other plumage characteristics varied systematically with mantle colour as assessed by Subspecies Class. There was no evidence that mantle colour can be used to separate subspecies of quelea in southern Africa. Mantle colour is one of many plumage characteristics that vary, sometimes in conjunction with other plumage patterns, among individuals. In addition, individuals from Riverside Farm (RF) and Terminus (TE), the two sites in the supposed range of *spoliator*, are pinker than other specimens, and have relatively high Subspecies Class scores. Thus individuals sampled in the *spoliator* region are less *spoliator*-like than individuals from other regions. This confirms the results of a previous study (Jones et al. in press) and further emphasises the meaninglessness of the *spoliator* subspecies classification.

Mantle feather colour does vary as do other plumage characters. Although it can be argued that Subspecies Class does allow the division of quelea into groups based on appearance, it is no more a signal of real phylogenetic information than any of the other characters, and possibly less so. Mantle feather colour variation is probably more a feature of the age of the feathers since last moult as much as a feature that varies geographically among populations.

The variation in mantle colour of the museum samples defined as *spoliator* by Clancey has been assessed (Jones et al. in press). The entire range of mantle colour variation was present in specimens referred to as either *lathamii* or *spoliator*, including Clancey's own paratypes, indicating that the cold grey to warm buff descriptions are simply two extremes of a continuum.

In conclusion, plumage variation in southern Africa does not imply that any structure exists in the colour variation of male redbilled quelea in breeding plumage. There are no definitive divisions or clines. Perhaps the best way to interpret the variation is in terms of a multivariate cloud of plumage types for southern Africa. Individuals have

one of these types, and the individuals that breed in the same place and therefore share a flock are no more likely to be found in the same plumage space as each other. Birds that breed in different places do not show noticeably different plumage patterns, with all the main plumage polymorphisms present in each sample studied. There is therefore no evidence of regional variations in plumage patterns of quelea in southern Africa such as has been found in West and East Africa.

Continuous quantitative colour measures taken using the software package Photoshop indicated a similar lack of differences in plumage patterns between the sites. However, more individual characters did show significant variation between sites. Visual scores are likely to be subjective, unrepeatable and potentially open to larger causes of error than the quantitative colour measures. It is therefore possible that a Photoshop technique could be more sensitive to plumage pattern variation than scores taken with the human eye. However, as no divisions or clusters were identified in this study, there is no way to test this idea further.

#### *4.4.2 Comparison with molecular data*

The lack of consistent variation in plumage matches the lack of significant variation found using microsatellite molecular markers. With both sets of data, the variation found within any one site was greater than the variation found between sites. The conclusion must be that quelea in southern Africa show no population structure, and each sampled flock is simply part of a single large panmictic population.

Quelea in southern Africa carry out long distance migrations which are believed to be related to the progress of rainfronts in the subcontinent (Ward 1971). Where and when quelea breeding colonies form depends on the pattern of rainfall and food availability providing suitable conditions. Such conditions are often short-lived in any particular region. Even though there is some evidence that flocks can remain cohesive during migration (Jaeger et al. 1986), the ephemeral nature of the resources that quelea require to breed mean simply that quelea breed whenever and wherever suitable conditions exist. There is little evidence that individual quelea routinely return to the same locations to breed. Hence a given colony is likely to contain

individuals originating from many sources. There is thus no mechanism for maintaining differences between populations in allele frequency or plumage patterns.

Quelea can and do breed more than once in a given season (Jaeger et al. 1986).

Itinerant breeding means that quelea who have already bred in different colonies could come together in a third colony and reproduce, which is a further force for mixing the population. Colonies could be sufficiently synchronous to allow all the birds from one breeding attempt to remain together for a second attempt later in the season, as has been suggested (Jaeger et al. 1986). However flocks have to follow rainfronts to find suitable conditions for further breeding. Any variation that may be present between flocks will therefore be independent of where they were sampled. Flocks could show molecular or plumage differences, but such variations are unlikely to be related to geography. This could allow patterns of variation to be interpreted in terms of migration pathways. However as this chapter has revealed that there are no differences between sites it is not possible to interpret the similarities as evidence of inter-population relationships and migration pathways.

#### 4.4.3 Summary

In this chapter evidence has been presented suggesting that there is no geographic variation in plumage patterns for male redbilled quelea in southern Africa. What variation there is exists within each sampled breeding colony. The variation can be interpreted as a cloud of possible plumage patterns. Each individual has its own position, which is not necessarily related to other individuals from the same site. In some cases, individuals from geographically close sites also occupy neighbouring positions in the cloud of variability, but this is not common. There are no obvious breaks in the cloud of points. Instead there is continuous variation, with most variation occurring within sites, and all plumage polymorphisms likely to occur at any of the sites. Such a picture provides no support for dividing quelea in southern Africa into two separate subspecies. Additionally individuals from the suggested *spoliator* range are not more likely to be *spoliator*-like in plumage patterns. The lack of geographic variation in plumage patterns agrees with the lack of variation in microsatellites outlined in Chapter Three, which is further evidence that there are no population divisions in southern Africa.





## 5 Migration orientation behaviour of the redbilled quelea

### 5.1 Introduction

Quelea perform regular seasonal migrations which are dictated by the availability of grass seeds that make up the major component of their diet. This, in turn, is controlled by the rainfall patterns in the region. The arrival of the rains allows the grass seed to germinate, which subjects the quelea to a severe food shortage. At this point they must move to an area where grass seed is available (Ward 1971).

In central southern Africa and Zimbabwe the rains arrive in November. By this time, the only areas of suitable habitat lie on the far side of the advancing rainfront in an area that received its first rains six to eight weeks previously. Quelea therefore have to migrate over the rainfront to reach areas of suitable habitat. In southern Africa these areas are on opposite sides of the continent: - in KwaZulu-Natal/southern Mozambique in the east and in southern Angola in the west, as shown in Figure 1.8. In the past it has been assumed that all quelea flocks migrate to the south-east, to KwaZulu-Natal. However the north-west migration route is theoretically available (Jones 1989a).

Despite the lack of population structure in quelea in southern Africa, the existence of separate migration pathways in southern Africa would provide important background for effectively managing quelea as a pest species. A migratory divide could provide a mechanism to maintain some degree of separation between populations. The two groups of populations that followed the different migration patterns could, in turn, correspond to the two proposed subspecies of quelea in southern Africa *lathamii* and *spoliator* (Clancey 1960). The description of *spoliator* as a subspecies remains controversial (e.g. Jones et al. in press).

This Chapter investigates whether there is a migratory divide for quelea in southern Africa. As Zimbabwe lies at the heart of the region under study, it is a sensible place to test quelea migration direction preferences especially as the Zimbabwean Highveld could act as an additional reproductive barrier. Quelea tend not to breed above 1000m (Vernon 1989; Mundy & Herremans 1997). It might be expected that

quelea populations that bred to the north west of the Highveld of Zimbabwe would migrate to the north west when the November rains arrive. Similarly quelea populations that bred to the south east of the Highveld would migrate in that direction. The predicted directions of migration for the site tested in this Chapter, Lake Manyame (LM), are shown in Figure 5.1. If migration direction is acting as a mechanism for population separation, as suggested by the subspecies definition, then those birds migrating to the north-west should be *lathamii*-like while those migrating to the south-east should be *spoliator*-like.

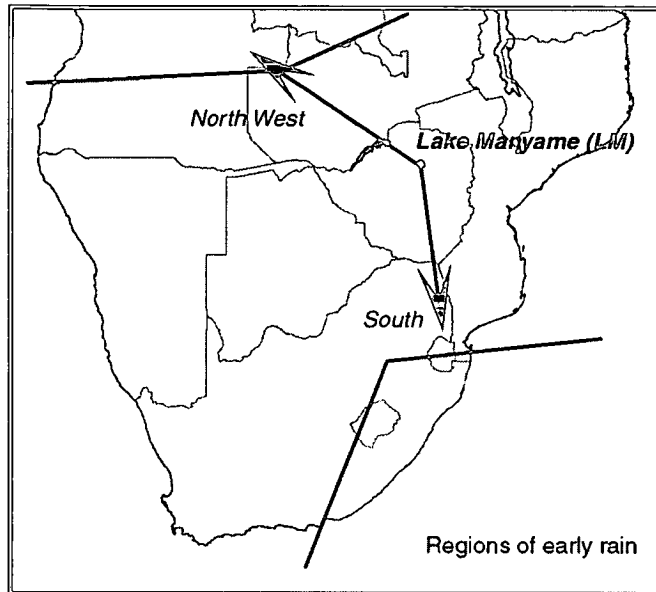


Figure 5.1. Regions of early rain and predicted migration direction for quelea roosting at Lake Manyame (LM), Zimbabwe.

It is still appropriate to test the migratory divide hypothesis even though there is no evidence for population genetic structure in southern Africa. Work on blackcaps *Sylvia atricapilla* in Europe has indicated a genetic basis for migration direction and distance (Berthold et al. 1992). The recently established over-wintering blackcaps in the British Isles migrate from central Europe. Previously all blackcaps over-wintered in southern Europe. The change in the migration direction was shown to be heritable. Indeed changes in migration direction and distance can be selected for over a few generations (Berthold & Pulido 1994). It is therefore possible that a migratory divide could exist without being detected by the genetic markers used. Recent range expansion reported in South Africa (Whittington-Jones 1998) may be an example of quelea rapidly altering their migratory habits in response to changing environmental factors in a similar way to European blackcaps.

### 5.1.1 Testing Migratory Direction

Emlen funnels (Emlen & Emlen 1966) have been used to test a wide variety of migrating bird species for preference in migratory direction. The funnels are designed to test orientation ability based on the observation that birds kept in small cages during their migratory period generally tend to orient towards the side of the cage corresponding to the normal migration direction. Individuals placed in the centre of a funnel try to escape from the cage by hopping up the walls in their preferred direction of migration. The walls are coated with a suitable material that records the hops as a pattern of scratches.

Most tested species have been nocturnal migrants in the Northern Hemisphere. Some examples include the previously mentioned work on blackcaps (Berthold et al. 1992), also robins *Erithacus rubecula* (Sandberg 1991) and the indigo bunting *Passerina cyanea* (Emlen et al. 1976). Non-passerines, such as the dunlin *Calidris alpina* (Sandberg & Gudmundsson 1996), have also been tested.

The technique works equally well with diurnal species. Examples include the starling *Sturnus vulgaris* (Wiltschko & Wiltschko 1985), the meadow pipit *Anthus pratensis* (Helbig et al. 1987) and the yellow-faced honeyeater *Lichenostomus chrysops* from Australia (Munro & Wiltschko 1992; Munro et al. 1993; Munro & Wiltschko 1993). Weindler (1994) tested freshly caught goldcrests *Regulus regulus* and showed that they were able to express orientation behaviour in funnels. In contrast, the migration preferences of the diurnally migrating chaffinch *Fringilla coelebs* could not be tested due to the strong phototaxis that the species showed (Muheim et al. 1999). The time of day when quelea migrate is not known.

Timing is a major factor in determining the likely success of the experiments. First, it is important to test quelea at the right time in relation to when they migrate. In other passerines only birds that have undergone pre-migratory fattening tend to show an orientation preference for the direction of migration (Sandberg 1994; Sandberg & Moore 1996). As quelea only lay down a small amount of fat in the two weeks before they migrate (Ward & Jones 1977) there is only a short window of opportunity in which to test the birds. Unfattened, or lean, birds of other species have often been

found to behave in a different way from fattened birds. A further difficulty is that it is likely that quelea over a large area will migrate at a similar time which would reduce the opportunities for testing.

Another problem concerns the time of day when quelea should be tested. In other studies with diurnal migrants (e.g. Munro & Wiltschko 1992) the time of day when the study species was most likely to migrate was known, which is not the case in quelea. It is also unknown whether quelea only migrate during the day, or if they continue at night. It would therefore be reasonable to test quelea at the time(s) of their peak in daily activity of early morning. Ward (1965a) described two peaks in activity at quelea roosts, the first of 2 to 3 hours at dawn and a second, shorter peak in the lead up to dusk.

## 5.2 Methods

### 5.2.1 Data Collection

Fifty-one quelea from a single non-breeding roost (Lake Manyame (LM), Zimbabwe) were tested for preferences in orientation direction just prior to the onset of rains in November 1998. Quelea were caught in mist nets in the evenings of 15<sup>th</sup> and 16<sup>th</sup> November 1998. Significant rainfall (cumulative 62.0 mm) fell 2 - 3 days after the quelea were tested. At this time quelea abandoned the sampled roost and I observed quelea leave other nearby dry-season roosts suggesting that the tested individuals should have been preparing to migrate. Each bird was kept overnight and tested for preference in orientation direction the following morning. Twenty quelea were tested at a time in individual Emlen funnels for 90 minutes. Testing started at 5.30 am and finished at 7.00 am for the first batch. Remaining birds were tested between 7.30 am and 9.00 am.

Emlen funnels consisted of a funnel of aluminium with an internal height of 15 cm. At the widest point the funnel diameter was 35 cm, narrowing to 10 cm at the base. Funnels were made of a non-magnetic material to reduce the risk that the funnel would interfere with the orientation abilities of the birds. The top of the funnel was covered with a 3 mm thick sheet of opaque Perspex, as shown in Figure 5.2. The

opaque Perspex prevented quelea from seeing anything outside the funnel that may have influenced the direction they wished to fly while still allowing light to enter the funnel. Prior to testing, each funnel was lined with a piece of Tipp-Ex *Vogelpapier* (BIC, Liederbach, Germany) that had been cut to the correct size. Funnels were then placed upside down on the ground. Single birds were introduced to all 20 funnels through the base, which was then covered. When all quelea were in place, the funnels were inverted and placed the correct way up in plastic bowls ('small washing up bowls', OK Stores, Harare, Zimbabwe) that were the correct size to prevent the funnels falling over. Testing began when all funnels were in the correct position. As quelea are highly sociable, funnels were kept close together. All tests were carried out in the shade away from the roost site.

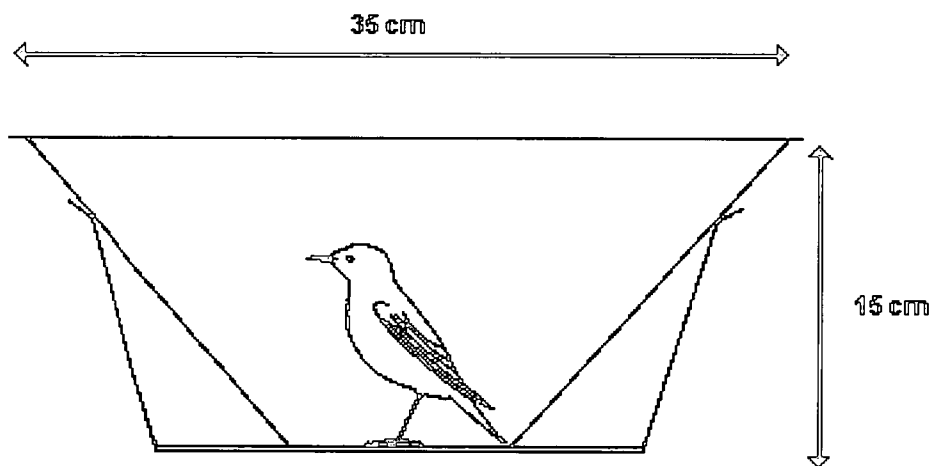


Figure 5.2. A schematic drawing of an Emlen Funnel

At the end of the test period, the Tipp-Ex paper lining the funnel was labelled with the same number as the quelea and the position of magnetic north was marked. The amount of fat carried by each individual was scored on a scale of 0 (lean) to 5 (maximum fat carried). Wing and mask moult (Ward 1973) were also recorded. Quelea were assigned to one of four categories on the basis of the colour of the mantle feathers ('Subspecies Class' in Chapter Four). Birds were identified as male if head moult had begun to reveal a noticeable facial mask. Otherwise birds were not sexed. Finally, blood samples were taken (see Chapter Two) and birds were photographed (see Chapter Four).

### 5.2.2 Data Analysis

Each *Vogelpapier* was subdivided into 24 sectors, and the number of scratches within each 15° sector was counted over a light table (Helbig 1991). When single scratches were not visible due to too much activity, their number was estimated by comparison with scratch density in areas of paper where scratches were visible. This process could lead to an underestimate of the activity. Only birds that left at least 50 scratches on the paper were included in subsequent analyses (Emlen et al. 1976; Sandberg & Gudmundsson 1996). The mean orientation direction of individual birds was calculated by vector addition (Batschelet 1981). Vector addition calculates a mean angle of orientation ( $\alpha$ ), representing the direction in which the scratches on the paper are concentrated, and a mean vector length ( $r$ ), which gives an indication of the degree of concentration of the scratches.

A potentially confounding issue with circular data is axuality. Axuality is defined as a bimodal distribution of with two modes each 180° apart. In such circumstances the mean angle of orientation does not reflect either of the actual directions where scratches are concentrated and can be misleading. It is therefore important to test for axuality using the method of doubling of angles. By doubling the angles, the bimodal distribution is transformed into a unimodal one. For example, if an individual has an axial distribution with modes at 90° and 270°, doubling the angles produces a unimodal distribution at 180° ( $2 \times 90^\circ = 180^\circ$ ,  $2 \times 270^\circ = 540^\circ = 180^\circ$ ).

The scratches left by an individual were axially distributed when the mean vector length of the scratch distribution with doubled angles was larger than the mean vector length without doubled angles (Batschelet 1981). If an individual showed an axial distribution, then the mean vector has two components – one for each of the modes. For subsequent analysis only one of these components was used. The component closest to the mean vector of the unimodal distribution of the same individual was used in further analysis (Muheim et al. 1999). The distribution of scratches for each individual was tested for significance at the 0.05 and 0.01 level using the Rayleigh test (Batschelet 1981). Significance was corrected for multiple tests using the sequential Bonferroni procedure (Rice 1989). A significant result indicates that there is directionality in the distribution of scratches.

The mean orientation directions for all individuals sampled from the population were tested for directionality using the same technique of vector addition, including testing for axiality and significance. This gives an indication of the preferred orientation direction of the population sample as a whole.

The vector addition process calculates a mean angle and a mean vector. The mean angle is a circular measure and hence is unsuitable for use with linear statistics. However, the mean vector varies from 0 to 1, and hence it is appropriate to use linear statistics. Four characteristics of quelea were examined in order to investigate their effect on either preferred direction (mean angle) or degree of directionality in the migrating birds (mean vector length). The characters used were degree of fat deposition (0-5), mantle feather colour score (1-4), the amount of mask moult (0-4), and the assignment index calculated for each individual in Chapter Three which gave the likelihood that an individual had come from the population in which it was sampled. Wing feathers for all individuals were fresh and not in moult.

Tests for influence on preferred direction of orientation, mean angle  $\alpha$ , were performed using pairwise Watson-Williams tests, which are the circular statistic equivalent of t-tests. Individuals were characterised as fat (fat deposit score 1-5) or lean (fat deposit score 0), as *lathamii* (mantle feather score 3 or 4) or *spoliator* (mantle feather score 1 or 2). In mask moult (score 1 – 4), or not (score 0). Finally individuals were classified as assigned to population LM ( $p > 0.05$ ) or not ( $p < 0.05$ ) based on the probability of belonging as calculated in Chapter Three.

Individual mean vector length decreases with increasing number of scratches (Batschelet 1981). Mean vector length cannot therefore be used as a direct measure of concentration of scratches. The residuals from the regression equation of mean vector length on the logarithm of the number of scratches were used instead ( $y = 0.425 - 0.047x$ ,  $n=48$ ,  $r=0.5$ ,  $p=0.068$ ) (Muheim et al. 1999). Tests for influence on the strength of preference (the mean vector length) were carried out using the residuals from the regression equation. A general linear model (GLM) was performed using the software package Minitab with the transformed mean vector length residuals as the response. Fat score, mantle feather score and assignment likelihood were factors, and fat score\*assignment likelihood was an interaction term.



### 5.3 Results

Of the 51 quelea tested, 48 left more than 50 scratches, indicating that they had been sufficiently active in the funnels to be used in further analysis. Thirty-seven birds had a significant directional preference, 10 of these showed a significantly axial distribution of scratches, as shown in Table 5.1. For individuals showing axial distribution, only the mean vector closest to its unimodal distribution was used in further analysis. An example of the pattern of scratches left by a single bird (LM50) showing a unimodal distribution is shown in Figure 5.3. The pattern of scratches left by a bird showing an axial distribution (LM45) is shown in Figure 5.4. Figure 5.5 shows the population wide distribution of individual mean angles of orientation. The population distribution shows a significant axial distribution, with the mean angle,  $\alpha = 117^\circ - 297^\circ$  and the mean vector,  $r = 0.296$ . Variation in the direction preferences of individual quelea ranged from  $2.2^\circ$  for LM17 to  $347.7^\circ$  for LM11. Nine individuals showed a direction preference to the north-west, and seven to the south-east.

The results of the paired Watson-Williams test showed that there was no significant relationship between mean angle and either fat score, mask moult, sex, assignment index or mantle feather score, as shown in Table 5.2. Similarly, the GLM showed no significant relationships between mean vector length and fat score ( $F_{4,42}=0.33$ ,  $p=0.854$ ), mantle feather score ( $F_{3,42}=2.15$ ,  $p=0.115$ ), assignment index ( $F_{1,42}=0.49$ ,  $p=0.487$ ) or the interaction term fat\*assignment index ( $F_{4,42}=0.36$ ,  $p=0.836$ ).

Table 5.1 No of scratches (N), Mean direction (a), mean vector length (r) for each quelea tested in Emlen funnels. Both unimodal and axial distribution of scratches were tested for significance. The *Final Data* used in subsequent analysis is given. Sex (where known), Fat Score, Mantle feather score, mask moult and Assignment Index (AI) is given for each individual

Sample	Sex	Fat	Mantle	Mask	Moult	AI	p-value	N	Unimodal Distribution				Axial Distribution				Final data					
									a	r	p	sig	a	r	p	sig	a	r	p	sig		
LM1	M	0	2	1		22.91	0.000	200	241.4	0.448	<0.001	**	40.7	220.7	0.271	<0.001	**	unimodal	241.4	0.448	<0.001	**
LM2	-	0	2	0		16.90	0.020	97	200.5	0.338	<0.001	**	61.8	241.8	0.335	<0.001	**	unimodal	200.5	0.338	<0.001	**
LM3	-	0	3	1		15.63	0.119	159	133.3	0.580	<0.001	**	154.0	334.0	0.103	0.186	NS	unimodal	133.3	0.580	<0.001	**
LM4	M	0	3	1		18.44	0.001	345	239.1	0.273	<0.001	**	100.8	280.8	0.164	<0.001	**	unimodal	239.1	0.273	<0.001	**
LM5	M	0	3	2		16.16	0.055	271	157.2	0.221	<0.001	**	113.1	293.1	0.101	0.063	NS	unimodal	157.2	0.221	<0.001	**
LM6	-	0	3	0		13.39	0.727	322	290.1	0.164	<0.001	**	116.5	296.5	0.268	<0.001	**	axial	296.5	0.268	<0.001	**
LM7	M	0	2	1		12.96	0.831	121	297.1	0.205	0.006	NS	44.1	224.1	0.132	0.122	NS	unimodal	297.1	0.205	0.006	NS
LM9	-	0	3	0		-	-	290	344.5	0.205	<0.001	**	5.3	185.3	0.282	<0.001	**	axial	5.3	0.282	<0.001	**
LM10	-	0	3	0		17.80	0.004	586	319.1	0.114	0.001	*	155.3	335.3	0.150	<0.001	**	axial	335.3	0.150	<0.001	**
LM11	-	0	4	0		17.35	0.007	188	347.7	0.325	<0.001	**	168.3	348.3	0.101	0.145	NS	unimodal	347.7	0.325	<0.001	**
LM12	-	0	3	0		17.55	0.005	196	289.4	0.372	<0.001	**	99.4	279.4	0.226	<0.001	**	unimodal	289.4	0.372	<0.001	**
LM13	M	0	1	2		15.29	0.176	207	90.6	0.558	<0.001	**	9.2	189.2	0.093	0.170	NS	unimodal	90.6	0.558	<0.001	**
LM14	-	0	3	0		15.55	0.128	120	235.0	0.514	<0.001	**	113.1	293.1	0.158	0.050	NS	unimodal	235.0	0.514	<0.001	**
LM15	M	0	2	1		15.61	0.122	181	148.6	0.323	<0.001	**	158.0	338.0	0.282	<0.001	**	unimodal	148.6	0.323	<0.001	**
LM16	-	0	2	1		12.68	0.891	310	302.1	0.278	<0.001	**	1.5	181.5	0.129	0.006	NS	unimodal	302.1	0.278	<0.001	**
LM17	-	0	3	0		14.85	0.268	129	42.8	0.330	<0.001	**	2.2	182.2	0.497	<0.001	**	axial	2.2	0.497	<0.001	**
LM18	-	0	4	0		15.91	0.081	148	15.0	0.220	0.001	*	114.1	294.1	0.132	0.077	NS	unimodal	15.0	0.220	0.001	*
LM19	-	0	3	0		15.15	0.211	35	212.2	0.906	<0.001	**	31.9	211.9	0.661	<0.001	**	N<50				
LM20	-	0	4	0		14.74	0.301	309	142.2	0.253	<0.001	**	77.2	257.2	0.228	<0.001	**	unimodal	142.2	0.253	<0.001	**
LM21	-	0	2	0		12.68	0.893	222	233.8	0.521	<0.001	**	17.3	197.3	0.276	<0.001	**	unimodal	233.8	0.521	<0.001	**
LM22	-	0	4	0		20.31	0.000	155	107.5	0.091	0.274	NS	102.7	282.7	0.126	0.086	NS	unimodal	107.5	0.091	0.274	NS
LM23	M	0	2	1		13.91	0.560	140	254.0	0.323	<0.001	**	59.3	239.3	0.245	<0.001	*	unimodal	254.0	0.323	<0.001	**
LM24	M	0	2	3		18.05	0.003	142	292.5	0.223	0.001	*	26.3	206.3	0.201	0.003	NS	unimodal	292.5	0.223	0.001	*
LM25	M	1	3	2		19.03	0.000	248	139.2	0.119	0.030	NS	109.9	289.9	0.138	0.009	NS	axial	109.9	0.138	0.009	NS
LM26	M	2	3	2		18.65	0.001	76	231.6	0.245	0.010	NS	56.2	236.2	0.346	<0.001	**	axial	236.2	0.346	<0.001	**
LM27	-	2	2	0		15.50	0.138	139	64.2	0.682	<0.001	**	77.5	257.5	0.349	<0.001	**	unimodal	64.2	0.682	<0.001	**
LM28	M	2	2	2		15.51	0.131	81	281.9	0.176	0.082	NS	84.2	264.2	0.113	0.355	NS	unimodal	281.9	0.176	0.082	NS
LM29	M	0	2	2		13.05	0.811	170	211.6	0.217	<0.001	*	24.9	204.9	0.442	<0.001	**	axial	204.9	0.442	<0.001	**
LM30	-	3	3	0		-	-	73	296.8	0.295	0.002	NS	54.4	234.4	0.144	0.219	NS	unimodal	296.8	0.295	0.002	NS

Table 5.1 continued

Sample	Sex	Fat	Mantle	Mask	Moult	AI	p-value	N	Unimodal Distribution				Axial Distribution				Final Data				
									a	r	p-value	sig	a	r	p-value	sig	a	r	p-value	sig	
LM31	M	0	2	1	16.53	0.036	66	146.1	0.179	0.120	NS	57.8	237.8	0.085	0.620	NS	unimodal	146.1	0.179	0.120	NS
LM32	M	2	4	0	15.86	0.084	89	107.4	0.156	0.115	NS	71.5	251.5	0.255	0.003	NS	unimodal	107.4	0.156	0.115	NS
LM33	-	3	3	0	16.94	0.019	320	5.3	0.302	<0.001	**	133.3	313.3	0.170	0.000	**	unimodal	5.3	0.302	<0.001	**
LM34	M	1	2	1	13.38	0.727	305	88.7	0.191	<0.001	**	74.2	254.2	0.130	0.006	NS	unimodal	88.7	0.191	<0.001	**
LM35	M	2	2	1	18.32	0.001	196	77.5	0.103	0.127	NS	137.3	317.3	0.112	0.085	NS	axial	137.3	0.112	0.085	NS
LM36	-	1	2	0	14.92	0.258	60	49.1	0.428	<0.001	**	143.7	323.7	0.037	0.920	NS	unimodal	49.1	0.428	<0.001	**
LM37	-	0	2	0	-	-	134	199.7	0.147	0.055	NS	75.3	255.3	0.051	0.702	NS	unimodal	199.7	0.147	0.055	NS
LM38	-	3	2	0	-	-	38	68.2	0.492	<0.001	**	64.9	244.9	0.273	0.059	NS	N<50				
LM39	-	2	2	0	-	-	188	287.9	0.431	<0.001	**	105.6	285.6	0.210	0.000	*	unimodal	287.9	0.431	<0.001	**
LM40	-	-	-	0	-	-	49	28.3	0.310	0.009	NS	154.4	334.4	0.084	0.708	NS	N<50				
LM41	-	3	2	0	-	-	258	322.5	0.415	<0.001	**	146.9	326.9	0.126	0.017	NS	unimodal	322.5	0.415	<0.001	**
LM42	M	1	1	1	16.98	0.016	223	95.5	0.018	0.928	NS	169.5	349.5	0.248	0.000	**	unimodal	95.5	0.018	0.928	NS
LM43	-	2	4	0	16.23	0.051	397	312.0	0.200	<0.001	**	60.9	240.9	0.056	0.294	NS	unimodal	312.0	0.200	<0.001	**
LM44	-	4	3	0	17.70	0.004	268	327.4	0.475	<0.001	**	159.8	339.8	0.336	0.000	**	unimodal	327.4	0.475	<0.001	**
LM45	-	2	2	0	18.15	0.002	222	95.0	0.135	0.017	NS	111.9	291.9	0.377	0.000	**	axial	111.9	0.377	<0.001	**
LM46	-	1	2	0	13.28	0.756	146	160.9	0.175	0.011	NS	67.2	247.2	0.219	0.001	*	unimodal	160.9	0.175	0.011	NS
LM47	-	2	2	0	19.94	0.000	155	292.9	0.193	0.003	NS	65.4	245.4	0.279	0.000	**	axial	245.4	0.279	<0.001	**
LM48	-	4	3	0	13.50	0.680	98	154.0	0.620	<0.001	**	142.4	322.4	0.364	0.000	**	unimodal	154.0	0.620	<0.001	**
LM49	M	2	1	3	16.15	0.056	27	264.3	0.401	0.013	NS	77.4	257.4	0.494	0.001	NS	N<50				
LM50	-	0	3	0	17.06	0.016	107	279.9	0.504	<0.001	**	105.0	285.0	0.508	0.000	**	axial	285.0	0.508	<0.001	**
LM51	-	2	3	0	15.27	0.179	109	265.7	0.397	<0.001	**	100.8	280.8	0.444	0.000	**	axial	280.8	0.444	<0.001	**
LM52	-	1	3	0	14.64	0.336	141	110.7	0.292	<0.001	**	81.0	261.0	0.361	0.000	**	unimodal	110.7	0.292	<0.001	**
LM53	M	3	3	1	14.47	0.388	183	312.0	0.236	<0.001	**	112.6	292.6	0.009	0.986	NS	unimodal	312.0	0.236	<0.001	**

NS - Not significant

\* - significant at 0.05 level, after correction for multiple tests

\*\* - significant at 0.01 level, after correction for multiple tests

Table 5.2. The results of the Wallis-Williams pairwise tests between the categories of individuals listed, giving the test statistic, F, the angular difference between the medians, and 95% confidence intervals. No tests were significant at the 0.05 level.

Test performed	N1	N2	F		Difference between medians		
			F	95% C.I.	angle	95% C.I.	Sig
Fat (N1) vs Lean (N2)	22	26	2.92	0.01 - 10.24	131.56	-174.7 - 174.3	NS
Mask moult (N1) vs No mask moult (N2)	19	29	6.20	0.01 - 11.49	-54.58	-172.0 - 174.9	NS
Lathamii (N1) vs Spoliator (N2)	25	23	5.13	0.01 - 11.65	62.30	-174.1 - 171.5	NS
Assigned (N1) vs Not assigned (N2)	25	23	1.79	0.01 - 12.67	177.91	-170.6 - 170.5	NS

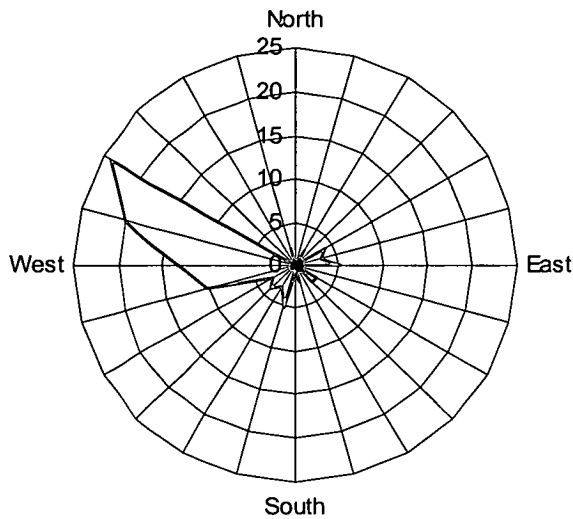


Figure 5.3. Distribution of scratches left by an individual quelea (LM50) in an Emlen funnel. Radial axis gives the number of scratches. The distribution shows significant directionality. Mean angle,  $\alpha = 279.9^\circ$ , Mean vector length,  $r=0.504$ .  $p<0.001$ .

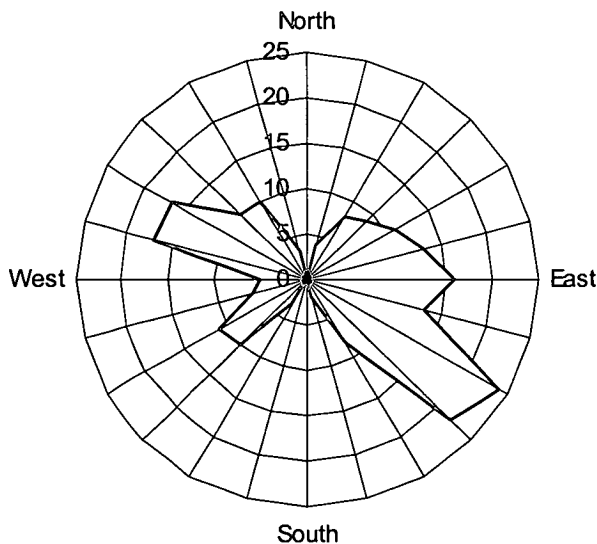


Figure 5.4. Distribution of scratches for a quelea in an Emlen funnel (LM45) showing a significant axial distribution. Radial axis gives the number of scratches. Axial distribution: Mean angle,  $\alpha = 111.9 - 291.1$ , mean vector,  $r = 0.377$ ,  $p<0.001$ .

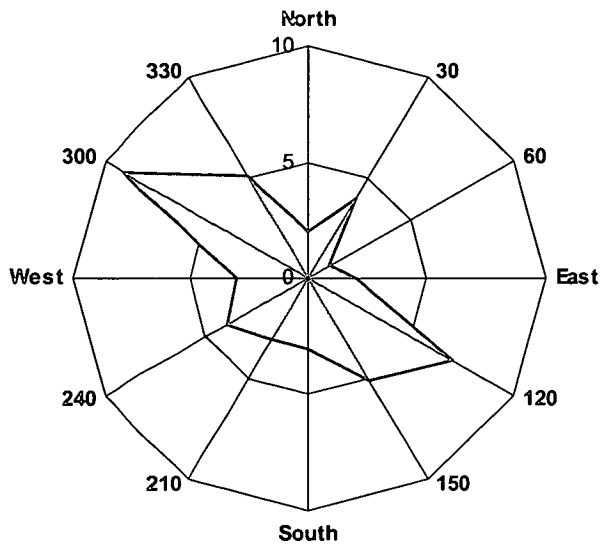


Figure 5.5. Mean orientation direction of 48 quelea at Lake Manyame showing significant axial distribution. Radial axis gives the number of scratches. Axial distribution:  $117^{\circ} - 297^{\circ}$ , Mean vector,  $r = 0.296$ . Rayleigh test,  $p = 0.029$ .

#### 5.4 Discussion

This is the first time that the technique of using Emlen funnels to test for migration direction preferences has been used on an intra-African migrant. The redbilled quelea was capable of exhibiting its preferred migration direction in a funnel. When compared with the direction of approach of the rainfronts, the directions that quelea chose to migrate were remarkably close to those predicted, as shown in Figure 5.6. The quelea tested at Lake Manyame were from a non-breeding roost at the end of the dry season prior to the onset of the rains. Shortly after the site was tested, heavy rain fell in the area. Quelea were subsequently observed to leave the area completely. Hence, the directions that quelea expressed in the Emlen funnels were highly likely to have been the directions that they would initially have chosen for migration over the incoming rainfronts and away from an area where food was shortly to become scarce. The only previous explanation for how quelea orientate for migration at the start of the rains suggested that the birds navigate by flying upwind (Ward 1979) as the prevailing wind direction comes from the approaching rains.

Previous studies using Emlen funnels have found that it is only birds that have laid down fat reserves in preparation for migration that show a significant preference in the direction of migration (Sandberg 1994; Sandberg & Moore 1996; Sandberg et al.

1998). Quelea do deposit fat in preparation for migration, and the amount of fat deposited is proportional to the distances that quelea then migrate (Ward & Jones 1977). However, there was no relationship between whether the individual birds had fat reserves and either the direction of migration, or the strength with which that direction was expressed. This is in contrast to previous experiments.

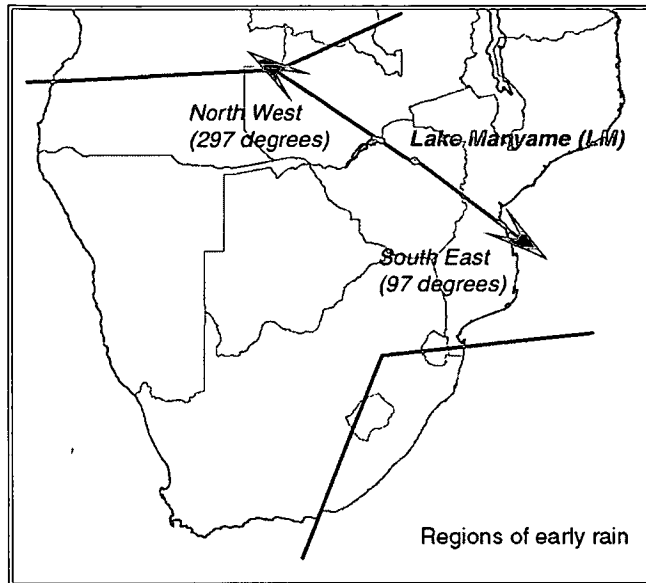


Figure 5.6. Direction of migration of quelea from Lake Manyame, as indicated by Emlen funnels.

Many birds while migrating have to cross areas of inhospitable habitat, or stretches of sea. Some tested individuals orientate in the opposite direction from their destination, such as robins *Erithacus rubecula* (Sandberg 1994) and red-eyed vireos *Vireo olivaceus* (Sandberg & Moore 1996). The explanation proposed is that some individuals may not be in the correct physiological condition to cross the ecological barrier in question, which is backed up by the observation that birds with fat reserves continue on migration, while those without do not. Lean individuals backtrack to find a suitable area to regain condition before resuming migration. In queleas, because there was no relationship between fat and lean birds in the strength and direction of orientation, the explanation for the axial distribution of direction preferences in quelea is unlikely to depend on the energetic condition of the individual involved.

If quelea behaviour in funnels is not mediated by energetic considerations, it is likely that the two opposite directions in which quelea choose to migrate at the onset of the rains reflect their actual migration behaviour. Some birds fly towards one rainfront

advancing from the north west, and others fly towards the second rainfront arriving from the south east, as illustrated in Figure 5.6. There is therefore evidence for a migratory divide in quelea. However, as direction preferences for both migration patterns occur in the same population, it is unlikely that the two migration patterns could act as an isolating mechanism leading to genetic differentiation and subspeciation. Instead individual quelea in the same population can respond to the changing environmental conditions in opposite ways. This implies that individual quelea have a plastic, opportunistic response to the approach of rain and potential food shortage. Such flexibility does not allow population division to develop, and hence no genetic or plumage differentiation would be expected. There was no evidence that mantle feather score, which is an indication of proposed subspecies classification, was related to direction or strength of migration orientation. Similarly, there was no relationship between assignment index and migration orientation.

Variation in migration patterns does not have to be under genetic control, or be inherited at all. There is evidence that migrating birds are able to react to ecological conditions and change migratory behaviour accordingly. Yellow-rumped warblers *Dendroica coronata* in the south-western United States show facultative north-south migration that depends on the food resource abundance in their desert riparian habitats (Terrill & Ohmart 1984). A facultative pattern could exist whereby quelea take local conditions into account before migrating along either of the possible routes.

#### 5.4.1 Summary

At a Zimbabwean site, quelea showed a behavioural preference to migrate in one of two opposite directions as revealed by Emlen funnels. The directions of the preferences (north-west and south-east) are very close to the directions from which the rainfronts approach (north-west and south). There was no evidence for the difference in migration directions being caused by energetic condition of the individuals, or for it being related to the supposed subspecies or assignment index of the individual.

Further conclusions are limited because individuals from only one site were tested. Quelea were observed to abandon dry season roosts over a large area of central Zimbabwe at the onset of the heavy rain that followed the completion of this study. The synchronous abandonment is in agreement with the Ward hypothesis (Ward 1971), however it also prevented more than one roost from being tested.

If Emlen funnels accurately reflect the directions in which quelea migrate at the onset of the rains, then it would be predicted that the directions preferred by quelea would change at different locations. Hence any future work should test quelea in different places. A future observation could also be that quelea tested closer to one advancing rainfront as opposed to the other may show a higher preference for migrating over the nearest rainfront. In other words, there could be geographic variation in migration direction preference depending on where the tested quelea are at the onset of rains.



## 6 Sex-biased dispersal in the redbilled quelea

### 6.1 Introduction

Sex-biased dispersal can be defined as movement in which one sex characteristically disperses and breeds at a greater distance from the natal site than the other sex (Wolff & Plissner 1998). Which sex disperses more varies among taxonomic groups, with a general pattern that in mammals males tend to disperse more frequently and farther than females, while in birds, female-biased dispersal is seen (Greenwood 1980; Dobson 1982; Clarke et al. 1997). In this Chapter evidence that dispersal patterns differ between males and females in the redbilled quelea will be examined. First the theory of why patterns of dispersal should vary between the sexes will be discussed.

#### 6.1.1 Sex-biased dispersal

##### 6.1.1.1 Theories

There must be costs associated with moving away from a known natal area through potentially inhospitable habitat in order to find a new home range (Dobson 1982). Equally therefore there must be an evolutionary driving force behind such movements. Several theories have been presented to account for the differences between the sexes in dispersal, including inbreeding avoidance, resource competition and local mate competition.

The *inbreeding hypothesis* (Wolff 1993; Wolff 1994) states that dispersal away from the natal area is a mechanism to avoid inbreeding. The sex at greatest risk from inbreeding should disperse. The inbreeding risk is specifically given as likelihood of mating with the opposite-sex parent. In polygynous species only mothers live with progeny, so daughters do not have to disperse and a male biased dispersal pattern is predicted. In monogamous species, there should be no bias in which sex disperses.

Philopatry increases the amount of competition among kin for mates with consequent fitness costs. The *local mate competition hypothesis* states that the sex with the highest reproductive potential should avoid such competition and therefore disperse more readily (Dobson 1982). Again in polygynous species, males have a higher

reproductive potential and should disperse. In monogamous species there is no difference between the sexes.

Greenwood's (1980) *resource competition hypothesis* was the first to explain different dispersal patterns between males and females, which he believed to be concerned with the different breeding systems common in mammals and birds. In the generally monogamous bird systems, males will often acquire and defend a territory, and will take an active part in feeding the young. Males therefore benefit more from philopatry through familiarity with the local resources. In contrast, in the polygynous systems that characterise breeding in mammals, males play little or no role in parental investment. Females have more to gain from territory acquisition and so benefit more from philopatry.

The *first choice hypothesis* (Wolff & Plissner 1998) combines many of the general principles outlined above. As dispersal through unknown and potentially inhospitable habitat has attached costs, philopatry is the preferred default option (Dobson 1982; Perrin & Mazalov 1999). Which sex gets to stay, and which has to disperse depends then on which has the 'first choice' of breeding sites. For example, where there is a polygynous system, males have a short-lived period of reproductive dominance. On reaching sexual maturity daughters do not find themselves surrounded by related males as their father has long-since lost reproductive dominance. Therefore as daughters do not have to disperse, they stay. As a consequence sons must disperse to avoid inbreeding and find unrelated mates. Many of the cases in birds and mammals that do not fit the general taxon pattern are found instead to represent examples of the resource competition hypothesis or the first choice hypothesis. For instance among the Anatidae where mate defence is the mating system, there is a pattern of male biased dispersal (Clarke et al. 1997). However not all instances of sex-biased dispersal fit neatly into a single hypothesis.

For migratory species, the dispersal distance can be thought of as the distance from the natal site to the breeding site when returning to the breeding area. In a survey of 24 migratory northern hemisphere passerines, males left the wintering grounds and arrived in the breeding grounds first in all cases. Males are philopatric, and there is female biased dispersal. Male biased dispersal was not recorded for any migratory

passerine (Clarke et al. 1997). Similarly in non-migratory passerines, males tend to defend breeding territories, and females choose a combination of mate and territory. Males dispersing away from the natal area should choose the first available territory. Females therefore disperse farther to avoid the chance of breeding with relatives.

The theories of sex-biased dispersal are based mainly on surveys of the available literature. Simulation studies have been performed to test the various hypotheses, such as whether inbreeding avoidance (Perrin & Mazalov 1999), or local competition avoidance (Perrin & Mazalov 2000) is the driving force. However the simulations only provide further predictions, such as that inbreeding avoidance can only rarely be the sole reason to disperse (Perrin & Mazalov 1999), and that some combination of resource competition and local mate competition should produce a pattern of male biased dispersal in polygynous species (Perrin & Mazalov 2000). Such hypotheses and simulations need field observations to corroborate or refute their predictions.

#### 6.1.1.2 Studies of sex-biased dispersal

It is often difficult to gather unbiased data on dispersal patterns. Direct observations through mark-recapture techniques are limited in geographical scope, and many long-distance dispersal events are inevitably missed (Koenig et al. 1996; Crochet 1996; Gauthreaux 1996). Genetic techniques provide an opportunity to describe long distance dispersal patterns. Whilst it is no doubt dangerous to base conclusions entirely on data gathered using molecular markers (Taylor & Dizon 1996; Waples 1998; Bossart & Pashley Prowell 1998a; Taylor & Dizon 1999), they can provide invaluable data where extensive field observations are impossible or logistically difficult, as with a wide ranging, abundant migratory species such as the redbilled quelea.

Several recent studies have used molecular markers to distinguish between the sexes in natal dispersal patterns. Based on six polymorphic microsatellites, the relatedness between spatially adjacent cichlid fish from the *Pseudotropheus* complex in Lake Malawi was calculated (Knight et al. 1999). Females that were found close together were more related than those farther apart. For males, spatially close individuals

were not closely related. This pattern revealed dispersal to be male biased for these fish species.

The dispersal patterns of the monogamous European shrew *Crocidura russula* were investigated using both direct field observations and indirect genetic analysis using microsatellites (Favre et al. 1997). Although levels of natal dispersal were low, and migration occurred mainly over short distances, there was a detectable pattern of female biased dispersal. The assignment index of Paetkau et al. (1995) was used to determine the probability that individuals were originally from the population in which they were sampled. The assignment index is an indication of the frequency with which an individual's genotype occurs in a given population. A lower index indicates the individual is less likely to have come from that population and could be an immigrant. The assignment index for females was significantly lower than that for males, indicating that females were more likely to be immigrants than males. The finding was consistent with the field observations that all individuals that emigrated from the natal territory were females.

Mossman and Waser (Mossman & Waser 1999) followed the method of Favre to examine the natal dispersal of white-footed mice *Peromyscus leucopus*. In this species dispersal is male biased, but both sexes do disperse. Previous studies showed that 69% of males left their natal range, but so did 41% of females (Keane 1990). Despite both sexes dispersing, the authors found that males had significantly lower assignment indices than females, indicating that males dispersed more widely than females. Favre's method can therefore be used to detect sex-biased dispersal in species where the differences between the sexes are marginal.

In this Chapter Favre's (1997) method was used to search for sex-biased dispersal patterns in the quelea. In the absence of good behavioural data on dispersal patterns away from natal breeding colonies and flocks, the genetic evidence was used to add to what is known about the different behaviour of the sexes in quelea.

## 6.2 Methods

Genotypes for male and female quelea from eight different populations were obtained for six microsatellite loci (Esc4, Hru5, Mcyu4, Phtr2, WBSW2, WBSW4).

Loci were chosen based on ease of amplification. Locus Phtr3 was not used in this Chapter as it is sex-linked (Fridolfsson et al. 1997). The populations used for this Chapter and the number of male and female samples from each are given in Table 6.1. The methods presented in this Chapter are based on Favre et al. (1997) and Mossman and Waser (1999).

Table 6.1. The number of samples genotyped for each of eight sub-populations. For site locations see Map 2.

Site	Full Name	Country	Date	Type	No of Genotypes	
					Males	Females
AF	Alwyn Farm	Namibia	22/04/99	Breeding	45	28
BU	Bulawayo	Zimbabwe	27/11/97	Non-Breeding	45	34
JD	JDMalilangwe97	Zimbabwe	03/97	Breeding	37	22
KR	Kroonstad	South Africa	24/02/99	Breeding	45	21
NS	Nokoneng South	Botswana	14/03/99	Breeding	45	22
RF	Riverside Farm	South Africa	16/02/99	Breeding	45	29
XB	Bumi Hills	Zimbabwe	18/03/98	Breeding	47	27
XX	Malilangwe	Zimbabwe	08/03/98	Breeding	48	24
					357	207

Genotypes were obtained following the protocol in Appendix A. Females from seven of the eight sites were genotyped by Camilla Blackburn. Allele frequencies, pairwise  $F_{ST}$ , and deviations from Hardy-Weinberg were all performed as outlined in Chapter Two. Assignment indices were calculated for the pooled male and female data according to the Bayesian method. Only individuals with genotypes from five or six loci were included. The assignment indices for males were recalculated using the same six loci for which the females had been typed. As previously discussed, the Bayesian method for calculating assignment indices as performed in GeneClass calculates a probability of belonging for each individual in each population.

The assignment index for each individual was then  $\log_{10}$  transformed. As there were several probabilities of zero, one was added to each value before transformation. Differences between populations were not of interest, so, following Favre et al. (1997) the assignment indices of each individual to their own population were standardised to remove the effect of population. An average assignment index was calculated for all individuals in a population. The corrected assignment index (AIC) values were calculated by subtracting the mean assignment index for a given population from the assignment index of an individual. Therefore the average AIC is

zero for a population. Individuals with negative AIC values are less likely than average to belong to that population, while individuals with positive AIC values are more likely than average to have come from that population. The AIC value gives an indication of how likely, compared to the average, an individual is to have come from the population in which it was actually sampled.

### 6.3 Results

1075 additional genotypes were obtained from 207 female quelea from eight populations at six polymorphic microsatellite loci. The number of genotypes for females at each locus and population and the allele frequencies for each population are presented in Appendix E. Male allele frequencies by locus and population are given in Appendix C.

#### 6.3.1 Population differentiation

Observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity and the inbreeding coefficient,  $F_{IS}$ , for the pooled male and female data from each population are given in Table 6.2.  $H_o$  was generally high, ranging from 0.625 for Phtr2-JD up to 0.909 for WBSW4-NS. In many cases,  $H_o$  was lower than  $H_e$  and the mean  $F_{IS}$  by locus was greater than zero in all cases. The pooled male and female data were tested for deviation from Hardy-Weinberg equilibrium. After correction for multiple tests using the sequential Bonferroni correction (Rice 1989), only one population-locus combination (Hru5-JD) differed significantly from Hardy-Weinberg expectations at the 0.05 level (Table 6.3). As there was no evidence of consistent deviations from equilibrium within a single population or locus this single incidence can probably be safely ignored.

Evidence of genetic differentiation between the eight populations was examined using overall and pairwise  $F_{ST}$ .  $F_{ST}$  was chosen in preference to  $R_{ST}$  as it is a more accurate estimator of differentiation for microsatellites when the sample sizes and number of loci used is small (Gaggiotti et al. 1999). Using data from both sexes, across all populations, small but significant sub-structuring was detected ( $F_{ST}=0.002$ ,  $p=0.001$ ). This was the same level of sub-structuring found in the main

Table 6.2. Observed and expected heterozygosity, and  $F_{IS}$  by locus and population.

		AF	BU	JD	KR	NS	RF	XB	XX	Mean	S.E.
Esc4	N	65	51	54	62	62	61	67	66	488	
	He	0.909	0.910	0.900	0.910	0.912	0.924	0.902	0.910	0.909	0.003
	Ho	0.862	0.882	0.889	0.887	0.887	0.885	0.836	0.818	0.868	0.010
	Fis	0.059	0.040	0.021	0.033	0.035	0.050	0.081	0.108	0.053	0.010
Hru5	N	72	60	57	66	67	72	62	72	528	
	He	0.891	0.910	0.903	0.910	0.899	0.914	0.905	0.902	0.904	0.003
	Ho	0.764	0.783	0.772	0.894	0.821	0.903	0.807	0.819	0.820	0.019
	Fis	0.149	0.148	0.154	0.025	0.094	0.019	0.117	0.098	0.101	0.019
Mcyu4	N	73	43	55	57	58	71	43	62	462	
	He	0.877	0.874	0.871	0.854	0.857	0.895	0.873	0.871	0.872	0.004
	Ho	0.699	0.884	0.782	0.877	0.759	0.831	0.907	0.871	0.826	0.026
	Fis	0.210	0.001	0.111	-0.018	0.123	0.078	-0.027	0.009	0.061	0.030
Phtr2	N	73	72	56	66	67	63	69	71	537	
	He	0.692	0.695	0.668	0.674	0.688	0.700	0.739	0.699	0.694	0.008
	Ho	0.658	0.708	0.625	0.652	0.716	0.714	0.768	0.676	0.690	0.016
	Fis	0.057	-0.012	0.073	0.040	-0.034	-0.012	-0.032	0.040	0.015	0.015
WBSW2	N	73	74	57	66	64	73	74	71	552	
	He	0.849	0.864	0.780	0.858	0.829	0.859	0.845	0.862	0.843	0.010
	Ho	0.740	0.892	0.737	0.818	0.906	0.808	0.838	0.789	0.816	0.022
	Fis	0.136	-0.025	0.064	0.054	-0.086	0.065	0.015	0.092	0.039	0.025
WBSW4	N	73	75	56	66	66	73	74	70	553	
	He	0.936	0.928	0.932	0.929	0.931	0.942	0.935	0.939	0.934	0.002
	Ho	0.795	0.880	0.857	0.849	0.909	0.904	0.811	0.871	0.859	0.014
	Fis	0.157	0.058	0.089	0.095	0.031	0.047	0.139	0.079	0.087	0.015

Table 6.3. Deviations from Hardy-Weinberg equilibrium by population and locus. P-values in italics indicate significance at the 0.05 level. P-values in bold italics indicate significance after Bonferroni correction for multiple tests.

Site	Esc4		Hru5		Mcyu4		Phtr2		WBSW2		WBSW4	
	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.	P	S.E.
AF	0.261	0.012	<i>0.015</i>	0.003	<i>0.000</i>	0.000	0.379	0.011	<i>0.010</i>	0.003	<i>0.010</i>	0.004
BU	0.077	0.008	<i>0.011</i>	0.002	0.214	0.010	0.986	0.001	0.557	0.015	0.135	0.010
JD	0.022	0.005	<b><i>0.000</i></b>	0.000	0.070	0.007	<i>0.029</i>	0.003	0.443	0.017	<i>0.050</i>	0.007
KR	0.013	0.003	0.190	0.012	0.658	0.014	0.356	0.009	0.272	0.013	0.104	0.009
NS	0.378	0.015	0.336	0.014	<i>0.004</i>	0.001	0.102	0.006	0.668	0.014	0.318	0.016
RF	0.421	0.012	0.474	0.015	0.230	0.013	0.177	0.008	0.248	0.014	<i>0.037</i>	0.005
RR	0.773	0.012	0.169	0.009	0.901	0.005	0.191	0.008	0.425	0.015	<i>0.010</i>	0.003
XB	0.311	0.013	0.103	0.009	0.237	0.011	0.916	0.005	0.481	0.017	0.080	0.009
XX	<i>0.043</i>	0.005	<i>0.006</i>	0.002	0.306	0.011	0.723	0.009	0.555	0.016	0.052	0.007

population genetics data. Pairwise  $F_{ST}$  values (Table 6.4) were generally low, ranging from -0.036 for the BU-AF comparison, to 0.008 for the NS-JD comparison. Only the latter pairwise comparison was significant at the 0.05 level after correction for multiple tests. There was therefore little evidence of significant population genetic

structure as indicated by pairwise  $F_{ST}$  values. This result was expected in the light of the lack of population differentiation in quelea described in Chapter 2. Female overall  $F_{ST}$  ( $F_{ST}=0.0038$ , 95% CI 0.0018-0.0058) was higher than male overall  $F_{ST}$  ( $F_{ST}=0.0018$ , 95% CI: 0.0008-0.0028).

Table 6.4. Pairwise  $F_{ST}$  estimates (lower matrix) and significance values (upper matrix). Significant values at the 0.05 level are in italics, those significant after Bonferroni correction for multiple tests are in bold italics.

	AF	BU	JD	KR	NS	RF	XB	XX
AF	-	0.9999	<i>0.0022</i>	0.2997	0.6017	0.7677	0.9999	0.9370
BU	-0.0365	-	0.9999	0.9999	0.9999	0.9999	0.9867	0.9999
JD	0.0077	-0.0261	-	<i>0.0013</i>	<b><i>0.0008</i></b>	<i>0.0191</i>	0.9994	<i>0.0027</i>
KR	0.0012	-0.0295	0.0082	-	0.0885	0.9804	0.9961	0.6783
NS	-0.0002	-0.0268	0.0080	0.0024	-	0.9707	0.9647	0.3006
RF	-0.0009	-0.0313	0.0045	-0.0031	-0.0031	-	0.9999	0.9993
XB	-0.0160	-0.0067	-0.0084	-0.0057	-0.0034	-0.0163	-	0.9998
XX	-0.0018	-0.0279	0.0067	-0.0007	0.0008	-0.0049	-0.0089	-

### 6.3.2 Assignment Index

The frequency distribution for AIC values for males and females is shown in Figure 6.1. Population by population frequency distributions are shown in Figure 6.2. Male AIC values ranged from  $-0.177$  to  $0.174$ , with a mean ( $\pm$ SE) value of  $-0.0121$  ( $\pm 0.006$ ). Female values ranged from  $-0.178$  to  $0.191$ , with a mean ( $\pm$ SE) of  $0.0242$  ( $\pm 0.008$ ). Both sexes showed a bimodal distribution and this pattern was strongest in females. In males the highest peak was at  $-0.12$ , with a second peak at  $0.16$ . In females the highest peak was at  $0.16$ , with a second peak at  $-0.08$ . The bimodal distributions for both sexes indicated that there could be two sources of sampled individuals: immigrants with AIC values below zero and non-immigrants, with AIC values above zero. The variance for male AIC was  $0.011$ , which was not significantly different ( $F=1.064$ ,  $p=0.369$ ) from the female variance ( $0.012$ ). Previous studies (Favre et al 1997; Mossman et al. 1999) found that the dispersing sex had a negative skew in the frequency distribution of AIC values. The dispersing sex also had a higher variance, although not necessarily significantly so. There was no evidence for skew for either sex in quelea, although a higher proportion of male quelea had negative AIC values than female quelea. Conversely, the variance in AIC values was slightly higher for females.



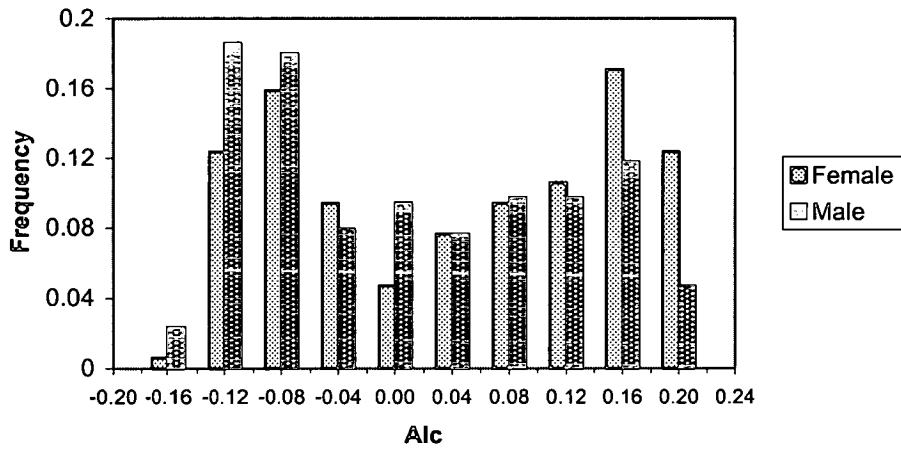


Figure 6.1. Frequency distribution of corrected assignment indices (Aic) for male and female quelea.

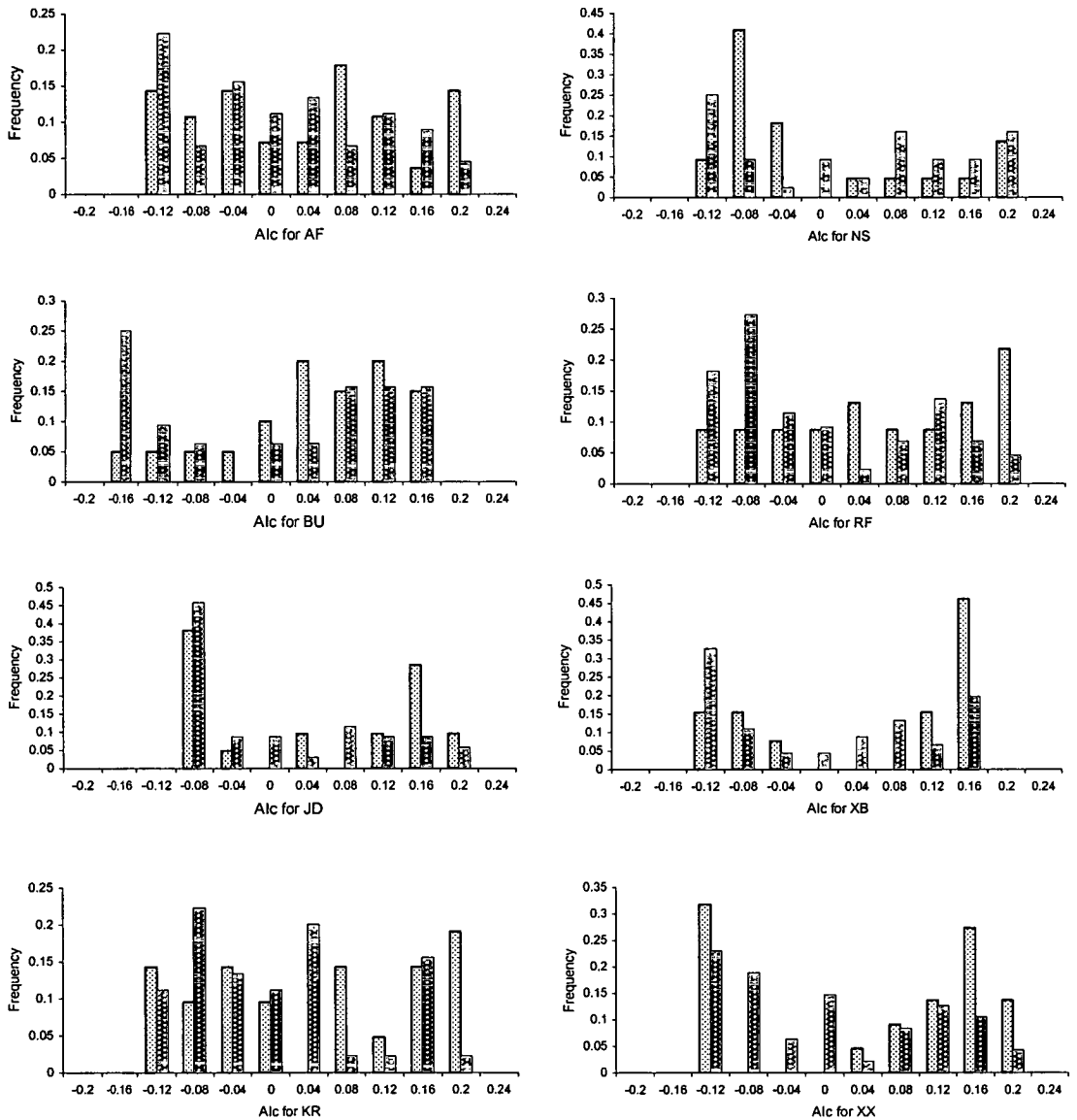


Figure 6.2. Frequency distribution of corrected assignment indices (Aic) for male and female quelea by population. Females – white bars; Males – black bars.

Population by population differences in AIC showed that mean male AIC was negative and mean female AIC was positive (Figure 6.3 and Table 6.5), except for Nokoneng South (NS), where mean male AIC was 0.014, and female AIC was -0.029. Mean male AIC was lowest for Riverside Farm (RF) (AIC = -0.023). The highest mean female AIC was for Bumi Hills (XB) (AIC = 0.052). On a population-by-population basis, only the comparison between the sexes for Riverside Farm (RF) was significant ( $t=2.53$ ,  $p=0.015$ ), as shown in Table 6.5. Over all populations, male AIC was significantly lower than female AIC ( $t=3.55$ ,  $p<0.001$ ).

The significance of the population-wide difference between the sexes was further confirmed by a permutation test. Across all populations, sex was randomised keeping the number of males and females the same. One hundred randomisations were performed and t-tests carried out on each new data set. The distribution of the t statistics from the randomised data and the t-statistic from the actual data are shown in Figure 6.4. The t statistic for all randomised data sets was less than the actual statistic, indicating a significance of approximately  $p<0.01$ .

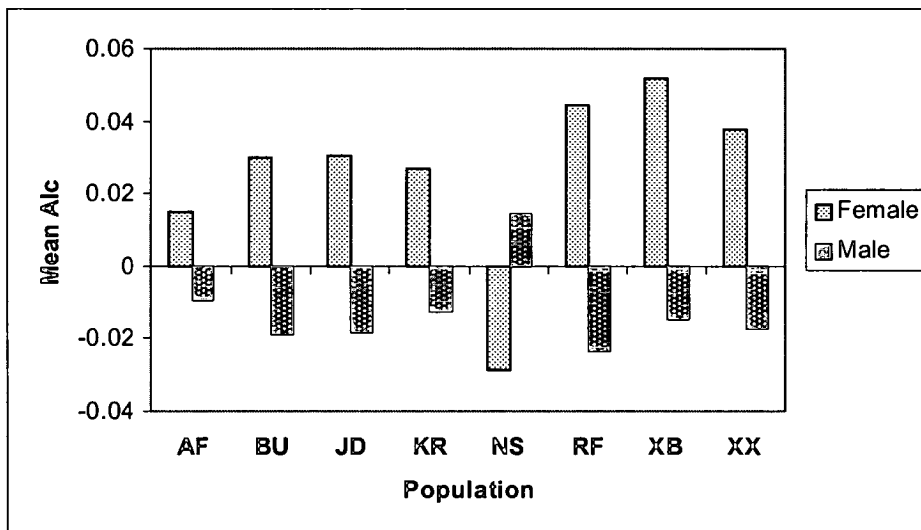


Figure 6.3. Mean AIC for males and females by population

Table 6.5. Mean AIC values for male and female quelea from eight populations. N gives the sample size (males:females). Significance was calculated using a two-sample t-test. Values in bold show significance at 0.05 level after Bonferroni correction for multiple tests.

Site	N	Female		Male		Significance		
		Mean	S.E.	Mean	S.E.	t	d.f.	p
AF	45:28	0.015	0.020	-0.009	0.015	0.98	55	0.330
BU	32:20	0.030	0.019	-0.019	0.022	1.67	49	0.100
JD	35:21	0.030	0.025	-0.018	0.017	1.61	37	0.120
KR	45:21	0.027	0.025	-0.012	0.014	1.38	32	0.180
NS	44:22	-0.029	0.023	0.014	0.017	-1.5	44	0.140
RF	44:23	0.045	0.022	-0.023	0.015	2.53	43	<b>0.015</b>
XB	46:13	0.052	0.035	-0.015	0.017	1.71	17	0.110
XX	48:22	0.038	0.027	-0.017	0.015	1.79	34	0.083
overall	339:170	0.024	0.008	-0.012	0.006	3.55	323	<b>&lt;0.001</b>

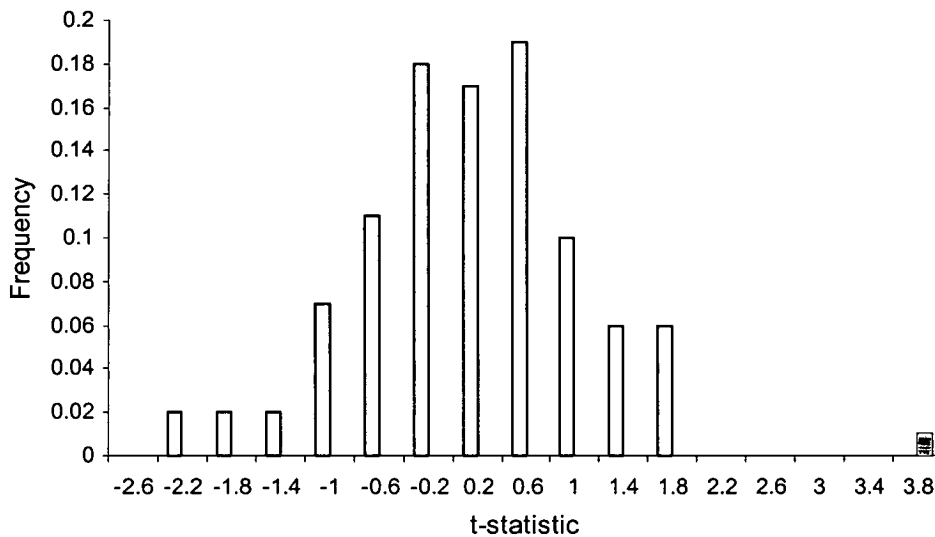


Figure 6.4. Distribution of t-statistics from permuted datasets (grey) which represent the null distribution of values of t under the null hypothesis that there is no difference between male and female AIC across all populations. The observed t-statistic (black) is shown on the right hand side.

## 6.4 Discussion

The results presented in this Chapter provide evidence that there is a sex-bias in dispersal pattern away from the natal flock/breeding colony in the redbilled quelea. Mean male AIC was significantly different from female mean AIC. Individual male AIC were more likely to be negative than female AIC. The result from the assignment index test was supported by F statistics, as male  $F_{ST}$  was lower than female  $F_{ST}$ . This is what would be expected if males moved more widely than females. However, one

other statistic that indicates the dispersing sex, variance of individual AIC, was higher for females than males. It was also clear from the frequency distribution (Figure 6.1) that, as in most bird species, both sexes disperse to some extent (Wolff & Plissner 1998), but in quelea males disperse to a greater extent than females.

This is the first time that the Bayesian method of calculating assignment indices has been used as the basis for calculating corrected assignment indices (AIC) to examine differential dispersal between sexes. The two other studies that have calculated AIC (Favre et al. 1997; Mossman & Waser 1999) have both used the frequency method. The advantage of the Bayesian method is that it deals more effectively with the rare and unique alleles, which characterise quelea microsatellite genotypes. As in this study, Mossman and Waser (1999) were also able to show a significant difference in dispersal between the sexes even though traditional F statistics showed no significant differences. It is therefore likely that the assignment index technique could be more sensitive than previous methods for detecting patterns in dispersal.

Other studies have also used genetic techniques to disentangle the different dispersal behaviours of the sexes. Using  $F_{ST}$  estimates to show that male marine iguanas *Amblyrhynchus cristatus* in the Galapagos were shown to move between islands more than females (Rassman et al. 1997). Male  $F_{ST}$  estimates ( $\theta=0.09$ ) were significantly lower than those for females ( $\theta=0.12$ ). The shrew *Crocidura russula*, showed an extreme bias in dispersal towards females, as recorded in field observations (Favre et al. 1997). Females also had an  $F_{ST}$  of 0.03 which was significantly lower than that of males ( $F_{ST} = 0.15$ ) (Balloux et al. 1998). In marine turtles females are strongly philopatric. The frequency of mtDNA haplotypes in the green turtle *Chelonia mydas* were used to show that males must also show equal levels of philopatry to females (Fitzsimmons et al. 1997).

Most previous studies that have used genetic techniques to examine sex-differences in dispersal have used the genetic tests to confirm observations made in the field. Male white-footed mice had already been shown to disperse more than females. There was strong field evidence in the shrew *Crocidura russula* showing that dispersal was both rare and solely involved females (Favre et al. 1997). Nonetheless, genetic evidence does still reveal novel patterns that had thus far not been seen in

field observations. In common with this study, neither previous study (Rassman et al. 1997 and FitzSimmons et al. 1997) could confidently say that they had adequately sampled the supposed sources and sinks for their dispersing individuals, but both were able to throw new light on previous observations of behaviour.

#### 6.4.1 *Behavioural differences between the sexes in the redbilled quelea*

Research into the different movement and migration patterns of males and females in the redbilled quelea has been limited. Quelea movements regardless of sex have remained unclear despite several decades of research using direct techniques of observation and mark-recapture. The same difficulties of studying movement in a highly mobile, abundant widespread species would also face researchers wanting to ask specific questions about gender differences. Nonetheless, there is a body of evidence that suggests that males and females may have different life-history and behaviour patterns. First, male and females have different activity patterns in and around breeding colonies, and second, there are several studies showing seasonal sex-ratio imbalances in quelea flocks.

During the period after the early-rains migration when quelea start to breed, males establish breeding colonies by selecting suitable breeding sites and begin to build nests (Crook & Butterfield 1970; Craig 1989). It is quite possible that groups of males leave the non-breeding flock in groups to establish colonies (Jones 1989b). After the males have begun nest construction, they display to attract females who choose a suitable nest site. Many colonies have a number of abandoned nests on their periphery indicating that males who are unsuccessful in attracting mates probably move on to another colony, or a different part of the same colony to attempt to breed (Jarvis & Vernon 1989a). Both sexes feed the young, but males and females have different activity patterns at different times of the day (Crook 1960b). Males also leave a colony before fledglings are fully independent, and before females finally abandon their brood (Ward 1971; Jones 1989b). Further, if a colony is unsuccessful, males leave the breeding attempt earlier than females (Jones & Ward 1979). Males also initiate and end breeding attempts first, which could allow them more breeding opportunities in more locations than females. The likely mobility of males is further

illustrated by the occurrence of male-only non-breeding roosts that can establish near to breeding colonies (Jaeger et al. 1979).

Males build more nests at a colony than are later used by females. A given flock of males that tries to establish a breeding colony will not therefore all breed at that colony. Males therefore have to be more mobile and move between colonies to find one in which they can breed. As females choose their nest site, they are likely to breed at the first establishing colony in an area of sufficient quality that they encounter. Flocks can form cohesive units (Jaeger et al. 1986), and as colonies are synchronous (Jarvis & Vernon 1989a), it is possible that females from the same natal flock breed in the same place, while males do not.

Different activity patterns within a breeding colony do not necessarily mean that the long-distance migratory movements should also show a bias due to sex. Such biases could be shown by different sex ratios in quelea flocks. An increase in the number of males found in flocks as the dry season progresses has been found in Senegal (Morel & Bourliere 1955), the Lake Chad basin (Ward 1965c) and Ethiopia (Jaeger et al. 1979). Intraspecific food competition at times of food scarcity has been suggested as the explanation for this phenomenon. Larger, more aggressive males are thought to out-compete females for the available food, leading to increased female mortality and therefore a male biased sex ratio (Ward 1965c; Yom-Tov & Ollason 1976). The disturbed sex ratio is restored to unity in breeding colonies (Morel & Bourliere 1955) (Ward 1965c), and the sex ratio at hatching and of recruited nestlings is also equal (Morel & Bourliere 1955). In contrast there have been observations of female biased flocks in Lake Chad (Manikowski 1980), and Botswana (Jones 1989c).

There is therefore some equivocal evidence that quelea do separate by sex for at least part of the year. Again this is not the same as having different dispersal strategies between the sexes, but combined with the observations of quelea behaviour at colonies a picture of the differences between the sexes is starting to emerge.

Females may be more likely to show some degree of natal philopatry because they are more dependent on adequate resources than males. Egg production is a nutritionally demanding process. A clutch weighs 30% of female body weight and is

produced in a week (Jones 1989d). Females stop red blood cell production, leading to low haematocrit levels when producing eggs, which indicates the level of nutritional stress that females are under. In a colony in Nigeria that was abandoned because of poor nutrition levels, females showed lower than normal pre-breeding haematocrit levels, while males did not (Jones 1983). Later at the same colony, females had deteriorated to such an extent that many were found dead after laying. The males had already abandoned the colony and made up only 5% of the dead birds (Jones & Ward 1979).

Females therefore put more of their resources into breeding than males. As food resources for quelea are highly ephemeral, females are more likely to return to their natal breeding site or region than males, as it is the only place they know that has been suitable in the past. Males are not as nutritionally limited as females (Jones 1989d). They are more flexible and do not necessarily have the same requirement to go somewhere familiar. Even if males did return to their natal site, the many incomplete nests at almost every colony show that not all of them breed successfully. Some give up and move on, inevitably meaning that they mix with other males in other breeding colonies.

The general pattern of sex-biased dispersal in socially monogamous bird species, where the male defends a resource or territory, is that philopatry should benefit males, and therefore there should be female biased dispersal. Certain features of quelea behaviour fit in with the model. Males do establish a territory or nest on the breeding sites before females arrive. Males also exhibit territoriality in the early stages of colony establishment (Crook & Butterfield 1970) indicating that there must be differences in the quality of nest sites, and therefore advantages in knowing what signals a good nest site. However there is little evidence that quelea exhibit any form of philopatry or habitat patch familiarity, and would not therefore build up the knowledge of surrounding habitat that is a key to the supposed benefits of philopatry. In contrast, quelea males show very plastic behaviour when choosing colony sites, and are prepared to abandon a site at short notice if the conditions are not right, with a high chance of finding a better nesting site in a different part of the breeding range.

This plastic behaviour could be the main reason why quelea show the opposite pattern to what is expected, rather than no pattern at all.

#### 6.4.2 Summary

The qualitative difference in dispersal patterns between birds, where dispersal is mainly female biased, and mammals, where it is mainly male biased, was first described two decades ago (Greenwood 1980). Based on a resource competition hypothesis, philopatry benefits the sex that chooses the breeding area, maybe forcing the other to disperse for reasons of inbreeding avoidance (Wolff & Plissner 1998). There are many exceptions to the general bird/mammal division. Nonetheless all migratory passerines so far studied have a female biased dispersal pattern (Clarke et al. 1997). The quelea is the first migratory passerine to show a male biased pattern of dispersal. Although quelea males choose the breeding area, they also show behavioural plasticity and a lack of breeding site philopatry.

Despite a lack of genetic differentiation among quelea populations, a pattern of male biased dispersal has been shown using a technique based on the probability of being able to assign individuals to populations based on their multilocus genotype (Favre et al. 1997). This is the first time that the assignment index technique has been used in a bird species, and the first time that clear evidence of a sex-biased pattern of dispersal has been shown for the quelea. However, it is important not to base statements of ecology and behaviour solely on genetic evidence (Waser & Strobeck 1998; Bossart & Pashley Prowell 1998a; Taylor & Dizon 1999). The pattern is consistent with the available behavioural evidence, which suggests that male quelea have a more plastic pattern of movement and breeding than females. Males from a wider geographic area, and hence from a wider range of genetic sources, therefore have the potential to come together to initiate breeding colonies.



## 7 Parentage analysis in the redbilled quelea

### 7.1 Introduction

The redbilled quelea breeds in massive, synchronous colonies often containing millions of birds (Bruggers & Elliott 1989). In such a situation, the potential for social parents to raise offspring that are not actually theirs is huge. However, in common with 95% of bird species (Schwagmeyer & Ketterson 1999) quelea are characterised as being socially monogamous (Crook 1960b). In this Chapter, the level of extra pair fertilisation in a single breeding colony of redbilled quelea will be quantified using polymorphic microsatellite loci.

The development of genetic markers has revealed that socially monogamous bird species are rarely genetically monogamous. As shown in Figure 7.1, the frequency of extra-pair fertilisations (EPF) varies among species, ranging from zero to 76% (Petrie et al. 1998). An EPF is the result of an extra-pair copulation (EPC), which is defined as a mating between a female and a male other than her pair-bonded mate (Westneat et al. 1990). EPFs have been documented in all passerine species studied so far (Westneat & Sherman 1997). It is therefore highly unlikely that quelea are monogamous as initially believed.

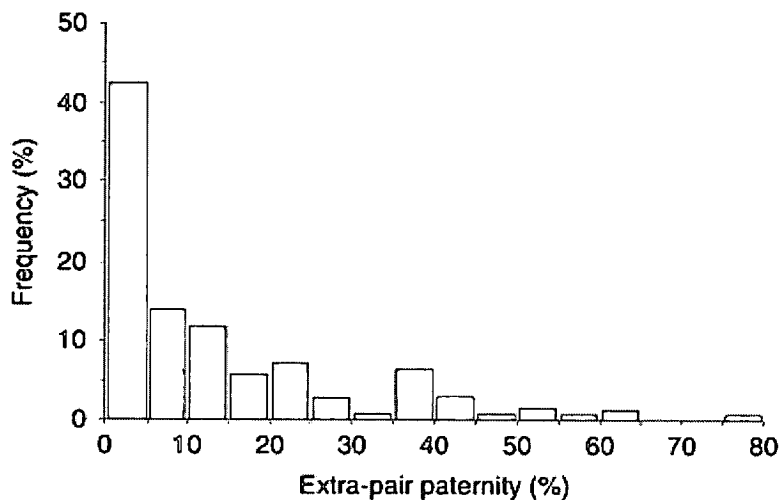


Figure 7.1. Frequency distribution for species levels of extra-pair paternity (measured as % extra-pair offspring,  $n=136$  species) (Petrie & Kempenaers 1998).

Birds show an enormous range in the rates of EPFs both between and within species. Several explanations have been proposed for this variation. It is likely that females

control the success of copulation attempts in most bird species (Birkhead & Møller 1993). If this is true, then the proportion of EPFs will vary according to the costs and benefits of EPFs to the female. The variation in extra pair paternity frequency in birds could be explained by the variation in benefits to females and variation in the costs and constraints to females seeking extra pair copulations (Petrie & Kempenaers 1998).

In some species females do get direct benefits from seeking extra-pair partners (e.g. dunnocks *Prunella modularis* (Davies et al. 1996), red-winged blackbird *Agelaius phoeniceus* (Gray 1997)). However, the main benefit females are expected to gain is to improve the quality of their offspring by seeking out high quality extra-pair mates with 'good genes' (e.g. Kempenaers et al. 1992). If males vary in quality, females paired with low quality males will benefit from EPCs with males of better quality. The proportion of EPOs (extra-pair offspring) will therefore be associated with the amount of variation in male quality, and could be expected to be positively correlated with the amount of genetic variation in fitness present (Petrie & Kempenaers 1998). Rates of EPOs have been shown to be highest in species that had greater levels of genetic diversity (Petrie et al. 1998).

There are also costs to a female from seeking extra pair copulations. The variation in costs will therefore also influence the proportion of extra pair offspring. Where costs are low it will pay more females to seek EPCs. Costs to females could be of at least two sorts, there could be a cost associated with seeking the EPC in the first place, and there could be a cost imposed by the social mate. If males alter their level of parental care based on the levels of certainty of parentage (Xia 1992; Petrie et al. 1998; Schwagmeyer et al. 1999), the main cost to the female is likely to be loss of help at the nest from the social mate.

Additionally, there may be costs associated with finding an additional mate. Where males are dispersed over a wide area, females will find it increasingly difficult to find extra-pair mates. Differences in breeding density may well account for some variation in EPOs. Reproductive synchrony could act in a similar way. Where breeding is synchronous, the costs to a female of finding and assessing the quality of breeding males are low so EPOs could be more likely. Where breeding is

asynchronous, assessing the suitability of mates could become harder (Stutchbury & Morton 1995), and the costs of seeking EPCs would therefore increase.

EPFs have been observed to occur more frequently in birds that nest colonially as opposed to those that are more dispersed (Møller & Birkhead 1992). Synchrony in breeding is the feature of colonial breeders that enables females to have a wider choice of extra-pair partners. Males engage in sexual display simultaneously allowing females to choose among a wide variety of mates apart from their social mate (Stutchbury 1998a; Stutchbury 1998b). However, in a comprehensive literature survey, there was no relationship between the frequency of extra-pair fertilisation and either colonial nesting or synchronicity of breeding. The correlation that did exist between synchronous breeding and rates of EPF disappeared when confounding effects such as phylogenetic relationships were removed (Westneat & Sherman 1997). There is therefore no evidence that breeding synchrony and coloniality account for interspecific differences in levels of EPF.

Despite the evidence that synchrony does not play a role in determining levels of EPO, and that genetic variability does, there is still controversy over the relative roles of each factor (Petrie & Kempenaers 1998; Weatherhead & Yezerinac 1998; Cordero 1998; Stutchbury 1998a; Schwagmeyer & Ketterson 1999). Genetic variability played a key role in the great reed warbler *Acrocephalus arundinaceus*; the frequency of EPOs was higher in breeding populations with greater genetic variability (Leisler et al. 2000). A similar pattern was shown for the tree swallow *Tachycineta bicolor* (Kempenaers et al. 1999) a species in which more than half of all offspring result from an EPF, one of the highest levels recorded. Synchrony was found to be important in the barn swallow *Hirundo rustica* (Saino et al. 1999) where increased synchrony led to a decrease in the number of EPOs. The opposite result was found for black-throated blue warblers *Dendroica caerulescens* (Chuang et al. 1999). Nests in areas of high local synchrony were more likely to contain extra-pair young, with variation in synchrony accounting for 22% of the variation in rates of EPFs. Further, the frequency of occurrence of intraspecific brood parasitism, which occurs when a female lays eggs in the nest of a conspecific and leaves without providing parental care (Zink 2000), has been related to the degree of synchrony and

coloniality in breeding (Reyer et al. 1997; Bjornstad & Lifjeld 1997). There is therefore likely to be a role for synchrony and breeding density in the level of EPOs observed (Westneat & Sherman 1997).

In quelea, synchronous breeding is preceded by rapid nest building by males and settlement by females. One possible scenario is therefore that females have only a short time-span for comparisons among males. They may therefore leave the choice of a genetic mate, if such a choice takes place, until after they have chosen a nest site and accompanying social mate. Quelea breed in huge colonies that can number many millions of birds (Ward 1965b). Within any individual colony, there is a high degree of synchrony, with most, if not all nests, tending to be at the same stage of development (Jaeger et al. 1989a; Jones 1989b). Up to 90% of eggs are laid and hatched within a few days of each other (Crook 1960b; Ward 1965b). The most likely reason for this synchrony is the short window of opportunity in any one region when conditions are suitable for quelea to breed (Jones 1989b). Quelea nest at high density, with up to 1500 nests in each tree, although on average there are 100 to 300 nests (Morel & Bourliere 1955; Haylock 1959). An average hectare of breeding quelea in Namibia in 1999 was home to 76,000 adults (pers obs; Simmons 1999). Such a high density of mating birds could allow females to seek extra pair copulations, and must call into question Crook's (1960b) assertion that quelea are 'strictly monogamous'. There are therefore likely to be benefits for females that seek EPFs, and low costs in finding and assessing the quality of extra-pair mates as there are likely to be many opportunities in a dense, synchronous breeding colony. It would therefore be predicted that quelea will show a high proportion of EPOs.

Microsatellites are good markers for use in parentage studies. Alleles are co-dominant and discrete allowing precise genotypes to be determined. The offspring inherits one allele from each parent, so simply by matching the alleles in the offspring to those in the parent at enough loci, precise information can be gathered about the true identity of parents (Queller et al. 1993; Schlötterer & Pemberton 1994; McDonald & Potts 1997). However, microsatellites are not infallible. Scoring error, new mutations and null alleles can all lead to errors in excluding potential parents.

It is common for microsatellites to have non-amplifying or null alleles. Their presence can lead to misleading conclusions from genetic data, especially when markers are used for parentage studies (Pemberton et al. 1995). They can reach frequencies of up to 15% (Paetkau & Strobeck 1994) in individual loci, and can be found in up to 25% of microsatellite loci (Callen et al. 1993; Jarne & Lagoda 1996). Null alleles are believed to be caused by primers failing to bind at the annealing sites flanking the microsatellite repeat region due to nucleotide sequence variation (Callen et al. 1993). PCR then fails to amplify alleles, leading to a heterozygote individual appearing as a homozygote with the second allele failing to show. The presence of null alleles is therefore usually inferred from a significant heterozygote deficit (Brookfield 1996).

Scoring errors and new mutations are additional sources of difficulty in parentage studies. Even small errors in scoring microsatellite allele sizes can lead to incorrect exclusion of parental genotypes (Marshall et al. 1998). Mean mutation rates in microsatellites can be as high as  $10^{-3}$  events per locus per generation (Weber & Wong 1993). Mutation rates can be much higher in the cases of individual loci. A new mutation rate of 3.6% was detected in one locus isolated in the swallow *Hirundo rustica* (Primmer et al. 1996a; Primmer et al. 1998).

Each of the three processes, null alleles, scoring errors and mutation, leads to an increase in the number of mismatches between parent and offspring. Therefore the overall percentage estimate of number of mismatches is likely to be higher than in a perfect data set where no mistakes, mutations or null alleles were present.

## 7.2 Methods

118 individuals (Table 7.1) were screened using five microsatellite loci. The individuals were pre-assigned to families on the basis of behavioural data gathered by Jim Dale at a wild colony in Zimbabwe (Malilangwe (XX) 21°05'S 31°55'E), the population 'JD' in this thesis. Parents were assigned to nests using binoculars. Chicks and putative parents were trapped and bled (Dale 2000). Nests were sampled from different locations within the breeding colony making it unlikely that any of the adults could be the parents of offspring other than those to which they were

putatively assigned (Jim Dale pers comm). There were a total of 92 putative relationships in 37 families containing 61 offspring and 57 putative parents.

The five microsatellite loci used were Hru5, Hru7, Lox8, Mcyu4 and Phtr2. Hru7 and Lox8 were chosen as they showed high levels of polymorphism ( $H_o = 0.961$  and  $0.973$  respectively) and a large number of alleles (41 and 52 respectively). Loci Hru5, Mcyu4 and Phtr2 were chosen as they showed no evidence of null alleles in the population study in Chapter Three. With neither parent known, these loci provided an overall exclusionary power,  $P(e)$ , of 0.9998, as shown in Table 7.2. This value was calculated using Cervus 1.0 (Marshall et al. 1998) from a formula in Chakravati and Li (1983), and represents the average probability that a randomly chosen individual from the population will be excluded from being a parent. Putative parents were considered to be true parents if they shared at least one allele with the offspring for all five loci. Although the overall exclusionary power  $P(e)=0.9998$ , for any one relationship the exclusionary power may be lower, especially where there are missing genotypes.

Table 7.1. The number of individuals and broods analysed from population JD for parent offspring relationships.

Genotyped parents	Chicks	Broods
Putative mother only	3	2
Putative father only	27	15
Both parents	31	20
Total	61	37

Table 7.2. Loci used for parental exclusion. No. alleles, observed heterozygosity ( $H_o$ ) and exclusionary power  $P(e)$  were calculated for all the samples from population JD used in the parentage analysis. Across all loci overall  $P(e)= 0.998$ .

Locus	No alleles	$H_o$	$P(e)$
Hru5	17	0.909	0.6760
Mcyu4	16	0.876	0.5940
Phtr2	9	0.665	0.2530
Hru7	41	0.961	0.8420
Lox8	52	0.973	0.8830
Mean	27	0.877	0.6496

### 7.3 Results

The number of loci successfully screened for each parent-offspring relationship and the number of mismatches observed are shown in Table 7.3. Of the 92 relationships, there were 47 with no mismatches, indicating cases where the observed parent is not excluded as the actual parent. There are 45 cases where putative parents and offspring mismatch for at least one locus. In 20 cases parent-offspring pairs mismatch at more than one locus.

If the pairs of individuals were a random set, then the total number of matches between pairs for the five loci would be related to the probability that two unrelated individuals would match at a given locus. In other words it would be related to the  $P(e)$  of the loci. Hence a binomial expansion of  $p=0.6496$ , the average  $P(e)$  across all five loci, will construct a null distribution of the number of matches expected under a model where no individuals are related. The observed and predicted number of mismatches at zero to five loci is shown in Figure 7.2. The binomial expansion predicts that 0.53% of pairs of individuals mismatch at no loci and 4.9% mismatch at a single locus by chance.

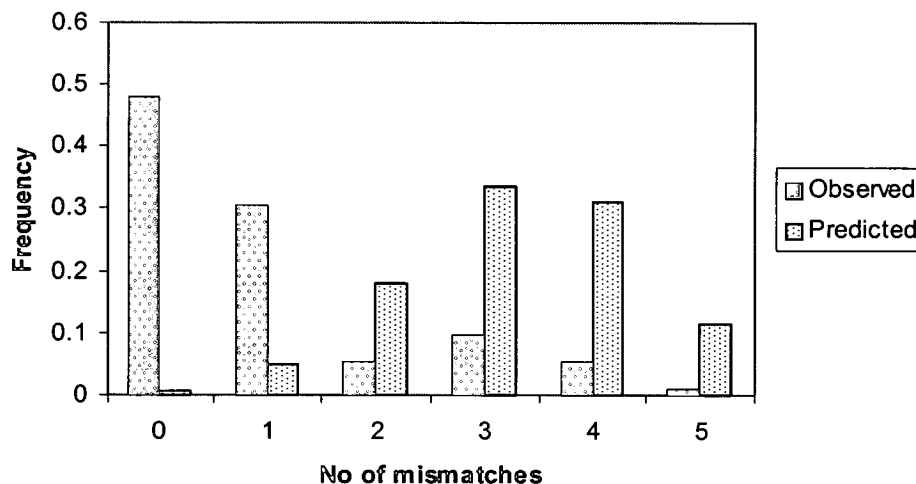


Figure 7.2. Observed and predicted number of mismatches at zero to five loci. The predicted number of mismatches is based on a binomial expansion of  $p=0.6496$  assuming a null hypothesis that there is no relationship between putative parents and offspring.

The number of relationships that mismatch at a single locus is therefore substantially higher than would be expected if there were no relationships and the individuals matched at four out of five loci by chance under a binomial expansion. It is also

Table 7.3 Number of loci scored ('Scored') and 'Mismatches' for each putative parent offspring combination. 'Correct' indicates whether the putative parent is not excluded as a parent. For each offspring 'Event' lists whether an extrapair fertilisation (EPF) or intraspecific brood parasitism (IBP) occurred. Where only the father is genotyped, it is not possible to distinguish between them, and the event is indicated ?. Broods are separated with lines.

Offspring	Parent	Scored	Mismatch	Correct	Event	Offspring	Parent	Scored	Mismatch	Correct	Event	Offspring	Parent	Scored	Mismatch	Correct	Event
JD193X	JD191M	5	0	Yes		JD307X	JD302M	5	0	Yes		JD398X	JD377M	5	4	No	?
	JD187F	5	0	Yes			JD303F	5	1	Yes#		JD400X	JD377M	5	4	No	?
JD231X	JD229M	5	2	No	IBP	JD306X	JD302M	5	1	Yes#		JD388X	JD386M	5	1	Yes	
	JD228F	5	4	No	IBP		JD303F	5	0	Yes			JD385F	5	0	Yes	
JD243X	JD241M	5	0	Yes		JD305X	JD302M	5	3	No	EPF	JD397X	JD389M	5	0	Yes	
	JD240F	5	0	Yes			JD303F	5	0	Yes		JD396X	JD389M	5	3	No	?
JD252X	JD249M	5	0	Yes		JD312X	JD309M	5	1	Yes#		JD395X	JD389M	5	0	Yes	
	JD250F	5	0	Yes			JD308F	5	0	Yes		JD391X	JD402M	5	5	No	?
JD258X	JD254M	5	0	Yes		JD311X	JD309M	5	3	No	IBP	JD407X	JD404M	5	0	Yes	
	JD256F	5	1	Yes#			JD308F	5	2	No	IBP		JD403F	5	0	Yes	
JD266X	JD255M	5	1	No#	EPF	JD330X	JD316M	5	0	Yes		JD411X	JD409M	5	4	No	IBP
JD271X	JD265M	5	1	Yes			JD323F	5	0	Yes			JD408F	5	4	No	IBP
JD297X	JD273M	5	1	Yes		JD329X	JD316M	5	0	Yes		JD412X	JD409M	5	1	Yes#	
JD276X	JD274F	4	2	No	IBP		JD323F	5	0	Yes			JD408F	5	0	Yes	
JD282X	JD278M	5	1	Yes#		JD328X	JD316M	5	0	Yes		JD416X	JD414F	5	0	Yes	
	JD280F	5	2	No	IBP*		JD323F	5	0	Yes		JD415X	JD414F	5	1	Yes#	
JD281X	JD278M	5	3	No	EPF	JD320X	JD319M	5	1	Yes#		JD430X	JD417M	5	1	Yes	
	JD280F	5	1	Yes			JD318F	5	0	Yes		JD429X	JD417M	5	3	No	?
JD295X	JD279M	5	0	Yes		JD325X	JD324M	5	0	Yes		JD421X	JD418M	5	0	Yes	
JD294X	JD279M	5	1	Yes#		JD327X	JD324M	5	0	Yes			JD420F	5	1	Yes	
JD296X	JD279M	5	0	Yes		JD326X	JD324M	5	0	Yes		JD428X	JD419M	5	1	Yes#	
JD286X	JD283M	5	0	Yes		JD340X	JD334M	5	3	No	?		JD425F	5	0	Yes	
	JD284F	5	0	Yes		JD365X	JD347M	5	0	Yes		JD426X	JD419M	5	1	Yes#	
JD287X	JD283M	5	0	Yes		JD366X	JD347M	5	0	Yes			JD425F	5	0	Yes	
	JD284F	5	1	Yes		JD358X	JD348M	5	0	Yes		JD427X	JD419M	5	1	Yes#	
JD293X	JD288M	5	0	Yes		JD357X	JD348M	5	1	Yes#			JD425F	5	0	Yes	
JD291X	JD288M	5	3	No	?	JD370X	JD368M	5	0	Yes		JD435X	JD433M	5	3	No	IBP
JD299X	JD290M	5	1	Yes#		JD374X	JD372M	5	0	Yes			JD434F	4	1	No#	IBP
	JD289F	5	1	Yes#			JD373F	5	0	Yes		JD436X	JD433M	5	0	Yes	
JD313X	JD301M	5	3	No	?	JD381X	JD376M	5	1	Yes			JD434F	5	0	Yes	
JD315X	JD301M	5	3	No	?		JD379F	5	0	Yes							

IBP\* - the putative mother has been excluded from parentage but the putative father has not. This is an example of pseudo-brood parasitism.

No# - The relationship revealed further mismatches when tested with four additional loci.

Yes# - The relationship revealed no further mismatches when tested with four additional loci.



higher than would be expected if the individuals were related, and a single mismatch occurred due to a rare mutation, a scoring error or the presence of a null allele. 17 of the 25 relationships that mismatched at a single locus were screened at a further four loci (Esc4, WBSW1, WBSW2, WBSW4). Only two mismatched at further loci (JD435X – JD434F and JD266X – JD255M). Therefore with the exception of these two relationships, in all the cases where a single mismatch was observed across five loci, the putative parent was considered to be the genetic parent.

Assuming pairs that mismatched at zero or one locus were relatives, 70 of the putative relationships were correctly identified in the field. Of the 92 relationships, 22 (23.9%) putative parent-offspring pairs were incorrect. Nineteen offspring (31.1% of offspring) from 16 broods (43.2% of broods) were not related to at least one putative parent. In five cases (13.5% of broods, 8.2% of offspring), neither parent was correct for at least one offspring, indicating IBP. In two cases the father was excluded, but the mother was not. In a further ten cases the father was excluded but the mother was not genotyped. There are therefore up to 12 examples (32.4% of broods) of EPF, although where the mother has not been genotyped it is not possible to distinguish between EPF and IBP. In a single case the mother was excluded, but the father was not, which is a possible example of pseudo-brood parasitism.

25 cases out of 92 relationships (27.2% of cases) mismatched at a single locus. If these cases are assumed to be an indication of the genotyping error rate, then  $(0.272)^2 = 0.074$  of the observations with two mismatches will also be genotyping errors. There are four cases that mismatch at two loci, therefore  $0.074 * 4 = 0.295$  of these cases are likely to be incorrect. A similar calculation shows that  $(0.272)^3 = 0.020$  of the cases that mismatch at three loci are genotyping errors.. There are ten cases that mismatch at three loci, indicating that the number of incorrect relationships has been overestimated by 0.2 of a case. A similar crude estimate of error rates for the cases that mismatch at four and five loci can also be performed. It can therefore be assumed that there has been a slight overestimate of the number of mismatches detected. However this does not substantially alter the overall results.

## 7.4 Discussion

Over 40% of the broods of quelea contained at least one chick, an EPO, which was the result of either an EPF or IBP. Nearly a third of all offspring were not related to at least one of their putative parents. Even though quelea are classified as socially monogamous, 31.1% of offspring were extra-pair.

Quelea exhibit extreme synchronicity in their breeding colonies. It could therefore be predicted that quelea would have high levels of EPOs. The rate of EPOs for quelea is towards the higher end of the distribution of EPO rates across all bird species (Figure 7.1), but many species show higher levels still. For example the highest rate of EPO is over 50% in the tree sparrow (Kempnaers et al. 1999) and 76% of offspring for the superb fairy wren (Double et al. 1997).

Quelea are therefore not the strictly monogamous bird that early researchers believed. They have a high proportion of extra-pair offspring. The levels shown are consistent with the hypothesis that attempts to explain the huge variation in levels of extra-pair young among bird species in terms of breeding synchrony. However the result presented here does not further illuminate the debate as to the relative roles of synchrony and genetic variability in fitness in determining levels of EPFs.

8.5% of all offspring are the result of IBP. In waterbirds 28 species show IBP, and over 20 of these suffer nest parasitism rates greater than 20% (Zink 2000). From the passerines some examples of IBP include the corvids, where it is common (Sandell & Diemer 1999; Yamaguchi 2000); IBP occurs at a low frequency (1.8% of chicks) in sand martins *Riparia riparia* (Alves & Bryant 1998); it does not occur at all even in the highly unfaithful black-throated blue warbler (Chuang et al. 1999). IBP is believed to be promoted by higher fecundity and reduced costs of parental care in the parasitising female, a limited number of nest sites (Zink 2000), high risk of nest predation (Poysa 1999), and communal, synchronous breeding (Reyer et al. 1997; Bjornstad & Lifjeld 1997; Hotker 2000). Just as for EPFs therefore, the degree of communal and synchronous nesting could be a causative factor in the rate of IBPs observed in quelea.

There is considerable variation in the number of eggs laid by quelea. The modal clutch size for quelea is three, but only up to 85% of the nests in a colony contain three eggs (Ward 1965b). The actual number of eggs in a nest varies from one to 13 (Haylock 1959), but clutches of more than four are uncommon (Jones & Ward 1976). Jones and Ward (1976) determined the expected number of eggs a female would lay by the number of yolky follicles each female produced. Fifty-two percent of females developed a fourth yolky follicle even though only 13% of nests in the colony later contained four eggs. Three-quarters of the females with four follicles showed signs of resorption. The authors concluded that female quelea customarily develop one more follicle than they lay. In exceptionally good years this allows females to lay and raise more young. The female does not determine the actual clutch size until laying has started. Hence females have some control over their clutch size with respect to feeding conditions.

Females attempt to lay as many eggs as their physiological condition allows. However, males only make one nest (Crook 1960b); in certain scenarios more than one female has been observed laying in the same nest (e.g. Jones 1979). Therefore the finding that 13.5% of broods contain an IBP is entirely consistent with known patterns of egg-laying and clutch size in the redbilled quelea. Given the opportunity, females have the physiological potential to lay eggs in a nest other than their own.

#### *7.4.1 Summary*

Exceptional breeding density and synchrony in quelea colonies offer the opportunity, at minimal cost, for females to find and assess the quality of extra-pair mates.

Despite the status of quelea as socially monogamous, a high proportion of extra pair offspring would therefore be predicted. The level of EPOs (31%) in quelea is high compared to other bird species, and is consistent with predictions.

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## 8 Discussion

### 8.1 Introduction

A variety of different techniques have been used in this study in order to develop an understanding of the migration patterns of the redbilled quelea *Quelea quelea* in southern Africa. This thesis presents the first investigation of genetic variation in the redbilled quelea. It also describes for the first time the geographic variation in plumage polymorphisms for quelea across all of southern Africa. Emlen funnels were used for the first time in an intra-African migrant to assess migration orientation behaviour. Finally, this thesis is the first time the assignment method for determining sex-biased dispersal patterns has been used in a migratory bird.

Detailed discussions of the main findings have been presented at the end of each Chapter. In this Chapter, the general conclusions of the thesis as a whole will be summarised. The implications for managing quelea as a serious pest of grain crops in southern Africa will also be discussed, as well as the need for future work.

### 8.2 Genetic, morphological and behavioural analysis of the migration patterns of the redbilled quelea in southern Africa

A variety of genetic and morphological techniques showed no evidence for population division of quelea in southern Africa. Polymorphic microsatellites showed no evidence of population structure, and there was no substantive geographic variation in the breeding plumage patterns of males across the subcontinent. Neither technique provided evidence that quelea follow regular migration pathways or that a migratory divide was present.

However, despite the lack of differentiation in redbilled quelea across the subcontinent, there was behavioural evidence that a migratory divide could exist for quelea in central southern Africa. Additionally there was genetic evidence that males dispersed more widely from natal flocks than females.

### 8.3 Implications for quelea management

The lack of genetic and morphological variation in quelea in southern Africa strongly suggests that there is no need to define separate management units for quelea within the region as there is no evidence of population structure in southern Africa.

However, the discovery of a potential migratory divide could complicate quelea management. Despite the evidence that two migration direction preferences occur in the same population of quelea, it remains unknown whether individual quelea show a fixed or variable response to the changing environmental conditions. Individual birds may have a plastic, opportunistic response in migratory direction to the approach of rain and potential food shortage or, alternatively, within a single roost there are flocks following fixed, separate migration pathways. Additionally, the description of sedentary populations of quelea in southern South Africa (Whittington-Jones 1998) suggests if the conditions are habitable, quelea will stay in one place year round and not migrate at all. Even recognised migratory populations will, under favourable conditions, breed in the same place twice running (Thompson 1993), leading to temporarily sedentary behaviour. Modern agriculture provides year round food and water. In such a situation migration, which is an inherently risky strategy (Berthold 1993), need not be undertaken at all.

Such a conclusion makes predicting where and when quelea are likely to pose a threat to crops difficult. The flexibility that quelea show means that they are unlikely to fit into any but the most basic model. Current control policies whereby quelea are only controlled if deemed a sufficient imminent threat to crop production are therefore likely to remain the most effective way to manage quelea as a pest.

### 8.4 Further study

Quelea are in many ways an ideal study organism. They have huge population sizes and fast generation times. Large sample sizes from many populations are easy to obtain from control operations and the close attention farmers and government agriculture departments pay to the presence of quelea helps in identifying suitable sampling sites. This study has concentrated on attempting to answer questions about quelea in southern Africa that are of direct relevance to its control and management

as a pest. However, the lack of any population differentiation described here precludes any immediate use of molecular and morphological markers for quelea management. In other regions in Africa, where the migration routes and morphological variation have been more intensively studied, the techniques outlined in this thesis could be used to answer more esoteric questions pertaining to morphological and molecular variation across hybrid zones.

#### *8.4.1 Southern Africa*

The central aim of this study was the description of migration patterns in quelea in southern Africa. Molecular markers revealed a lack of population structure. However this did not rule out the possibility that more than one migration strategy is followed by quelea in different parts of the subcontinent, as shown in the Emlen funnel experiments.

Within southern Africa, further experiments would be of use to support the conclusions of this thesis. First, the existence of a migratory divide needs to be confirmed. Quelea preparing to undertake the early rains migration from other parts of the region could be tested for their direction preferences. Research could attempt to determine the exact location where the two migration pathways meet, and hence the location of the migratory divide. Such information would be of interest in the construction of a predictive model of quelea movements. It would allow the migration pathways based on both converging rainfronts to be addressed. Any model therefore would be more likely to provide accurate predictions in the regions where the rainfronts, and hence migratory pathways, converge.

Additionally, direct observations of quelea movements based on ringing studies, seasonal quelea abundance and the locations of breeding colonies could provide more information as to the extent of sedentary behaviour of quelea in southern Africa. It is unlikely that further indirect molecular techniques would throw any light on such issues.

### 8.4.2 *The rest of Africa*

Although the genetic and morphological techniques presented in this thesis revealed no evidence of substantive differentiation in southern Africa, variation in quelea morphology is well documented in the rest of Africa. Applying the techniques developed in this study to different regions would provide further information as to the extent of population divisions and differentiation in the redbilled quelea across its range.

The most promising region is the Sahel where migration patterns and variation in plumage coincide. There is already the suggestion of some population structure that could be detected using molecular markers. Additionally, 'hybrid swarms' have been identified where the typical appearance of the subspecies of one region melts into the appearance of the subspecies of another. The Sahel offers a good opportunity to study the way in which genetics and morphology vary across hybrid zones.

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## A Appendix. Laboratory methods and software sources

### A.1 DNA extraction

DNA was extracted from blood or liver using Chelex extraction (Walsh 1991). 2 $\mu$ l of blood (or 2 mm x 2 mm piece of liver) was transferred to a 1.5ml eppendorf tube containing 200 $\mu$ l of 5% w/v Chelex resin (Chelex@100, Instagene). The sample was transferred to a 65°C water bath overnight. The next day the tube was mixed vigorously, placed in boiling water for 8 minutes and centrifuged at 13,000 rpm for 4 minutes. The aqueous phase contained the DNA sample.

### A.2 PCR amplification

DNA samples were transferred from the 1.5 ml eppendorf tubes to 96 well storage plates. Samples were diluted 1:1 with BDH ultrapure H<sub>2</sub>O. A drop of mineral oil was added to each well to reduce evaporation of the sample. Plates were wrapped in parafilm and stored at -20°C. DNA samples were stored in this state for several months.

The per sample PCR mix was as follows: 2 $\mu$ l genomic DNA template was used in a 10 $\mu$ l reaction mix containing 0.1mM dATP, dGTP and dTTP; 0.01mM dCTP; 2pmol each primer; 1X 'Parr' buffer containing 1.5mM MgCl<sub>2</sub> (Cambio); 0.25 unit Taq polymerase (Advanced Biotechnologies) and <1 $\mu$ Ci [ $\alpha$ -<sup>32</sup>P] dCTP. Where Tmac was used, the conditions included 0.5mM MgCl<sub>2</sub> (total MgCl<sub>2</sub> 2.0mM), and 60mM tetramethylammonium chloride/2.5% formamide (TmacIDE) added to the reaction mix (Gemmell 1997). Table A.1 gives the mix used for 100 reactions, which is enough for a microtitre plate with a small amount of PCR mix left over to account for pipetting errors.

Table A.1. PCR mixes for 100 samples. The PCR mix with Tmac was only used with locus Mcyu4.

Chemicals	No Tmac	Tmac
PCR Buffer	100 $\mu$ l	100 $\mu$ l
dNTP	100 $\mu$ l	100 $\mu$ l
MgCl <sub>2</sub> (50mM)	10 $\mu$ l	30 $\mu$ l
Primers upper	40 $\mu$ l	40 $\mu$ l
lower	40 $\mu$ l	40 $\mu$ l
Tmac (1M)	None	60 $\mu$ l
Formamide	None	25 $\mu$ l
BDH H <sub>2</sub> O	710 $\mu$ l	600 $\mu$ l
Taq	12.4 $\mu$ l	12.4 $\mu$ l
p <sup>32</sup>	1 $\mu$ l	1 $\mu$ l

Reactions were overlaid with one drop of mineral oil and amplified in a Hybaid Omnigene Temperature Cycler. PCR was performed as follows: 2 min denaturing step at 93°C; 7 cycles of 93°C for 30 s denaturation, 50°C for 1 min annealing and 72°C for 30 s extension; then 25 cycles of 93°C for 30 s denaturation, followed by 52°C for 1 min annealing and 72°C for 30 s extension. The temperature of the two annealing steps depended on the locus being amplified, and is given in Table A.2.

Table A.2. Annealing temperatures and gel loading parameters for 12 microsatellite loci. *Run* gives the length of time that the locus was electrophoresed. Two times are given for loci that could be double loaded. *Double Load* gives the gap between the first and second set of samples loaded.

Locus	Temp	Run	Double load	
			Distance (cm)	Time (mins)
Esc4	50/52	2hrs05	-	-
Hru5	54/56	1hr30/2hr	6cm	30 mins
Hru7	50/52	3hrs	-	-
Lox8	50/52	3hrs	-	-
Mcyu4	50/52	2hr15/2hr45	6cm	30 mins
Pdou3	54/56	1hr15	-	-
Phtr2	54/56	1hr45/2hr15	6cm	30 mins
Phtr3	54/56	2hrs/2hrs30	8cm	40 mins
WBSW1	50/52	2hrs/2hrs40	7cm	35 mins
WBSW2	50/52	2hr20/3hrs	8cm	40 mins
WBSW4	50/52	2hrs30	-	-
WBSW11	50/52	2hrs20	-	-

After PCR was completed 5µl of loading 'stop' buffer (95% formamide, 20mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) was added to the product. 2µl of the product/dye mixture was loaded on standard 6% polyacrylamide sequencing gels using a multichannel pipette and 96-well shark's tooth combs, and electrophoresed (power at 140W, temperature at 50°C) for up to 3 hours. The exact time for each locus is given in Table A.2. As indicated, some loci were double-loaded, with the second set of samples loaded up to 35 minutes after the first. The gel was dried under a vacuum drier and exposed to X-ray film for between one hour and one week. A <sup>35</sup>S-labelled M13 sequencing reaction was also loaded on each gel as a size marker.

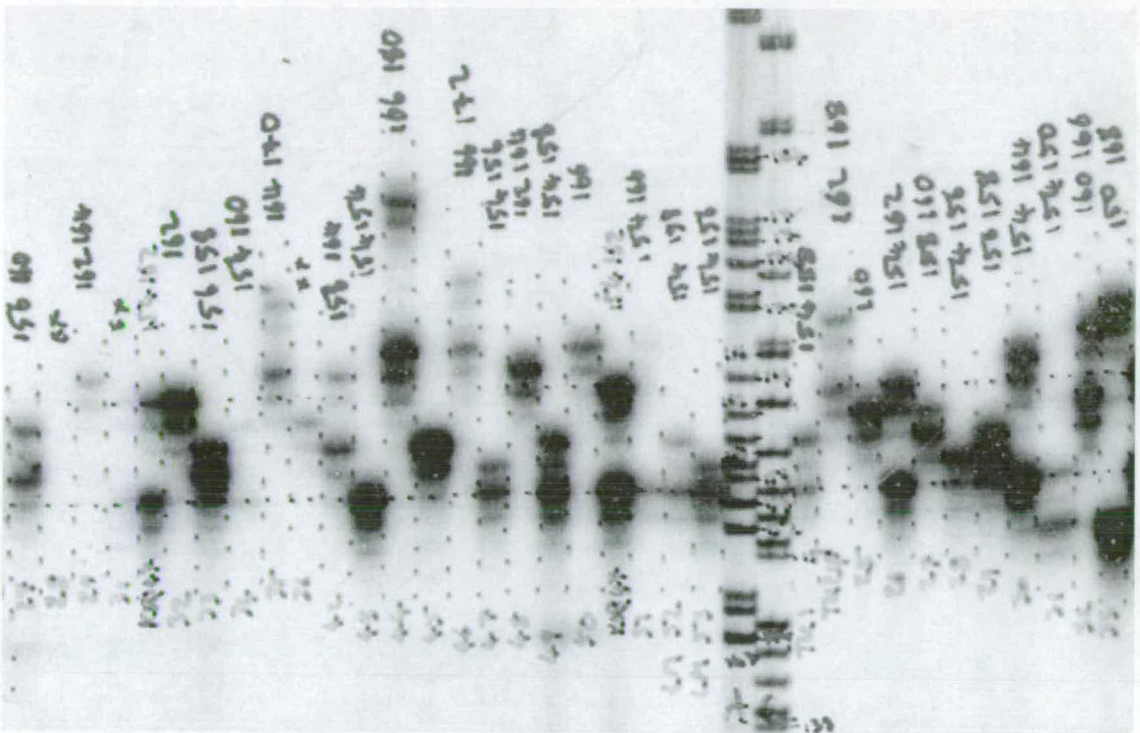


Figure A.1. Autoradiogram of locus Mcyu4. The gel was loaded with samples from Lake Manyame (LM) and Tuinplaas (TU). Bird identities and genotypes were marked on the autorad prior to data entry in a Microsoft Access (Microsoft Corporation) database. Two bases (A and C) of the size ladder are shown towards the right-hand side.

### A.3 Software sources

Programme	website (as at Nov 2000)
Arlequin	<a href="http://lgb.unige.ch/arlequin/">http://lgb.unige.ch/arlequin/</a>
Brzostowski programmes	<a href="http://www.biology.ualberta.ca/jbrzusto/index.html">http://www.biology.ualberta.ca/jbrzusto/index.html</a>
Cervus	<a href="http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html">http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html</a>
Fstat	<a href="http://www.unil.ch/izea/software/fstat.html">http://www.unil.ch/izea/software/fstat.html</a>
GeneClass	<a href="http://www.ensam.inra.fr/URLB/">http://www.ensam.inra.fr/URLB/</a>
Genepop	<a href="http://wbiomed.curtin.edu.au/genepop/">http://wbiomed.curtin.edu.au/genepop/</a>
Genetix	<a href="http://www.univ-montp2.fr/~genetix/intro.htm">http://www.univ-montp2.fr/~genetix/intro.htm</a>
Treeview	<a href="http://taxonomy.zoology.gla.ac.uk/rod/treeview.html">http://taxonomy.zoology.gla.ac.uk/rod/treeview.html</a>



## **B Appendix. Photography and Digitised Images**

### **B.1 Camera Equipment**

A Minolta X700 camera with a 50 mm lens and a Minolta Auto 132PX flash unit were used throughout. Photographs were taken without any lens filter, with shutter speed 1/60<sup>th</sup> second ("Flash Sync" setting) and an aperture of 16. Four layers of tissue paper were placed over the flash head to reduce glare. Fuji Provia 100 ISO slide film was used throughout.

### **B.2 Photographs**

A standard grey card (Kodak R 27) was used as the background. Kodak Q13 colour standards and grey scales were included in each frame. Up to seven specimens were included in each frame. Photographs were taken in the shade or indoors, with the camera held vertical above the specimens at a distance of 0.7m. Dorsal and ventral sides of each specimen were photographed and labelled with a frame code and a list of which birds were included in the frame.

### **B.3 Scanning**

Photographs were scanned using a Nikon LS-2000 slide scanner and NikonScan 2.2 Electronic Imaging software. Scale was set to 200% and resolution to 28.3 pixels per cm (72 pixels per inch). Images were saved in JPEG format with quality set at 80%.

### **B.4 Standardising images**

Grey cards, grey scale and colour standards were included in each frame so that each image could be standardised with respect to known colours and shades.

### **B.5 Taking Readings**

All colour readings were taken using the software package Photoshop 5.02 (Adobe Systems Inc.). Brightness was adjusted using the 'Adjust Brightness' menu so that the grey background had a Brightness of at least 50. Image resolution was reduced using the 'Image Size' menu so that the Color Sampler tool sampled a larger area of plumage for each measurement. Resolution was reduced to 5 pixels per cm. The 5x5 average option on the Color Sampler tool then sampled 1 cm<sup>2</sup> of the image, roughly 0.5 cm<sup>2</sup> of plumage. Colour readings were taken of red and yellow standard colour boxes and the black and white standard greyscale boxes using the 5x5 average option on the Color Sampler tool.

Readings of colour were taken using the 5x5 average option on the Color Sampler tool in HSB colour mode. Readings of mask shade, bib shade and mantle contrast were taken using a greyscale version of the image, using the Marquee tool. Values for K were obtained using the Histogram function, which gives both median and standard deviation of the colour in the area selected. Each reading was standardised with respect to the measurements taken from the pure red, yellow, black and white colour standards.

Appendix C. Microsatellite allele frequency distribution by population and locus.

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH
Esc4	146	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	148	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0111	0.0152	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	150	0.0000	0.0192	0.0000	0.0128	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0114	0.0119	0.0111	0.0111	0.0000	0.0000
	152	0.0222	0.0577	0.0349	0.0256	0.0455	0.0429	0.0667	0.0303	0.0444	0.0833	0.0455	0.0357	0.0222	0.0667	0.0222	0.0000
	154	0.0222	0.0000	0.0116	0.0256	0.0114	0.0143	0.0111	0.0000	0.0556	0.0000	0.0000	0.0000	0.0111	0.0111	0.0778	0.0517
	156	0.0333	0.0962	0.0349	0.0769	0.0568	0.0286	0.0556	0.0152	0.0667	0.0000	0.0341	0.0714	0.0444	0.0556	0.0333	0.0862
	158	0.0778	0.0000	0.0930	0.1282	0.0795	0.0857	0.0333	0.1515	0.0444	0.0833	0.1477	0.0357	0.0222	0.0778	0.0444	0.0862
	160	0.0222	0.0192	0.0581	0.0256	0.0227	0.0286	0.0444	0.0152	0.0111	0.0278	0.0000	0.0476	0.0556	0.0778	0.0556	0.0000
	162	0.0111	0.0577	0.1628	0.0769	0.0568	0.0429	0.0222	0.0000	0.0556	0.0556	0.0114	0.0238	0.0889	0.0556	0.0444	0.0345
	164	0.0667	0.0385	0.1279	0.1154	0.0568	0.1714	0.1222	0.1970	0.1000	0.0833	0.1705	0.0714	0.0778	0.1333	0.0778	0.0690
	166	0.1333	0.0962	0.0698	0.0641	0.1136	0.1286	0.1222	0.0606	0.0889	0.1944	0.0341	0.0833	0.0667	0.0889	0.1111	0.0690
	168	0.1333	0.1731	0.1163	0.0897	0.1023	0.1571	0.1111	0.1667	0.1000	0.2222	0.2045	0.1667	0.1333	0.0667	0.0667	0.0690
	170	0.0333	0.1731	0.0698	0.1282	0.0568	0.0714	0.1444	0.0606	0.1222	0.0556	0.1136	0.0952	0.1667	0.1000	0.1111	0.1724
	172	0.0889	0.0962	0.0581	0.1026	0.1023	0.1429	0.1000	0.0303	0.1556	0.1111	0.0455	0.1190	0.1111	0.0444	0.1222	0.1034
	174	0.1222	0.0769	0.0814	0.0256	0.1250	0.0143	0.0444	0.1061	0.0444	0.0556	0.0341	0.0595	0.0444	0.0889	0.0889	0.1207
	176	0.0444	0.0577	0.0581	0.0385	0.0341	0.0000	0.0333	0.0455	0.0333	0.0000	0.0795	0.0476	0.0889	0.0444	0.0556	0.0172
	178	0.0889	0.0000	0.0000	0.0256	0.0795	0.0286	0.0444	0.0606	0.0333	0.0278	0.0114	0.0357	0.0111	0.0444	0.0444	0.0517
	180	0.0667	0.0192	0.0233	0.0385	0.0000	0.0143	0.0000	0.0152	0.0222	0.0000	0.0114	0.0714	0.0000	0.0000	0.0111	0.0345
	182	0.0222	0.0000	0.0000	0.0000	0.0455	0.0000	0.0000	0.0152	0.0000	0.0000	0.0341	0.0119	0.0000	0.0333	0.0111	0.0172
	184	0.0111	0.0000	0.0000	0.0000	0.0000	0.0143	0.0000	0.0152	0.0222	0.0000	0.0000	0.0119	0.0333	0.0000	0.0111	0.0172
186	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0143	0.0111	0.0000	0.0000	0.0114	0.0000	0.0111	0.0000	0.0000	0.0000	
188	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	

Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
Esc4	146	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0004	0.0000
	148	0.0111	0.0833	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0058	0.0000
	150	0.0000	0.0000	0.0000	0.0152	0.0000	0.0000	0.0000	0.0000	0.0313	0.0104	0.0060	0.0476
	152	0.0111	0.0000	0.0000	0.0455	0.0714	0.0000	0.0227	0.0213	0.0208	0.0208	0.0331	0.0238
	154	0.0556	0.0000	0.0000	0.0152	0.0357	0.0000	0.0114	0.0532	0.0521	0.0313	0.0215	0.0000
	156	0.0222	0.0833	0.0000	0.0758	0.0000	0.0000	0.0795	0.0426	0.0104	0.0625	0.0448	0.0000
	158	0.0556	0.0833	0.0000	0.0606	0.0714	0.0000	0.0455	0.0638	0.0521	0.0625	0.0648	0.0000
	160	0.0111	0.0833	0.0000	0.0455	0.0000	0.0000	0.0114	0.0745	0.0729	0.0208	0.0320	0.0238
	162	0.0667	0.0833	0.0000	0.0455	0.1429	0.0000	0.0568	0.0213	0.0521	0.0938	0.0524	0.2381
	164	0.1444	0.0000	0.1667	0.1970	0.0357	0.0000	0.1250	0.1809	0.0938	0.0833	0.1041	0.2381
	166	0.1333	0.0000	0.0000	0.0455	0.1071	0.0000	0.1818	0.1277	0.0833	0.1146	0.0892	0.0000
	168	0.1333	0.0000	0.0000	0.0909	0.2143	0.0000	0.1250	0.0745	0.1250	0.1042	0.1133	0.0714
	170	0.1889	0.1667	0.3333	0.0758	0.1429	0.0000	0.1705	0.0532	0.0833	0.1563	0.1171	0.0238
	172	0.0444	0.2500	0.5000	0.0909	0.0357	0.0000	0.0909	0.1596	0.0938	0.0625	0.1101	0.0238
	174	0.0222	0.0833	0.0000	0.1061	0.0357	0.0000	0.0341	0.0426	0.0521	0.0833	0.0612	0.0476
	176	0.0556	0.0833	0.0000	0.0152	0.0000	0.0000	0.0114	0.0106	0.0417	0.0313	0.0357	0.0714
	178	0.0222	0.0000	0.0000	0.0455	0.0000	0.0000	0.0114	0.0213	0.0208	0.0521	0.0293	0.0238
	180	0.0111	0.0000	0.0000	0.0152	0.1071	0.0000	0.0000	0.0213	0.0729	0.0000	0.0214	0.0238
	182	0.0000	0.0000	0.0000	0.0152	0.0000	0.0000	0.0114	0.0213	0.0417	0.0000	0.0108	0.0952
	184	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0061	0.0476
186	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0023	0.0000	
188	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE
Hru5	106	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	108	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000
	109	0.0000	0.0000	0.0000	0.0000	0.0000	0.0139	0.0000	0.0000	0.0000	0.0000	0.0217	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	110	0.0111	0.0333	0.0000	0.0682	0.0000	0.0833	0.0444	0.0366	0.0114	0.0455	0.0000	0.0109	0.0000	0.0114	0.0233	0.0111	0.0222
	111	0.0556	0.0833	0.0610	0.0455	0.0349	0.0278	0.0111	0.0366	0.0568	0.0795	0.0870	0.0109	0.0889	0.0795	0.0581	0.0889	0.0667
	112	0.1000	0.1333	0.0732	0.0909	0.0581	0.0278	0.0333	0.0732	0.0909	0.1023	0.1196	0.0326	0.0667	0.0682	0.0814	0.0889	0.0667
	113	0.0667	0.0667	0.0610	0.0341	0.1163	0.0694	0.0889	0.1220	0.0568	0.0341	0.1087	0.0870	0.1222	0.1364	0.0233	0.1333	0.1000
	114	0.0444	0.1000	0.0488	0.0568	0.0349	0.1667	0.0444	0.0610	0.0568	0.0568	0.0217	0.0978	0.0889	0.0909	0.0930	0.0556	0.0667
	115	0.0778	0.0833	0.0854	0.1136	0.1279	0.0694	0.0778	0.0854	0.1136	0.0909	0.0870	0.1196	0.0778	0.1250	0.0930	0.1444	0.0889
	116	0.1222	0.0667	0.1220	0.1250	0.1047	0.1111	0.1111	0.1341	0.1477	0.0909	0.1196	0.0761	0.1667	0.1023	0.1163	0.0889	0.1444
	117	0.2556	0.1500	0.1463	0.1705	0.1047	0.1389	0.1444	0.0976	0.1477	0.1364	0.0761	0.1413	0.1667	0.1364	0.1744	0.1000	0.1667
	118	0.0667	0.0833	0.0854	0.1364	0.0814	0.1250	0.1667	0.1220	0.0795	0.1705	0.1739	0.0870	0.1111	0.1023	0.1279	0.1000	0.0778
	119	0.0333	0.0833	0.1098	0.0682	0.0814	0.0833	0.0778	0.0732	0.1023	0.0568	0.0870	0.1304	0.0333	0.0227	0.0698	0.0333	0.0778
	120	0.0333	0.0333	0.0488	0.0227	0.0698	0.0694	0.0444	0.0732	0.0000	0.0227	0.0217	0.0543	0.0111	0.0341	0.0465	0.0444	0.0333
	121	0.0556	0.0000	0.0244	0.0114	0.0581	0.0000	0.0667	0.0122	0.0455	0.0114	0.0217	0.0543	0.0111	0.0227	0.0116	0.0222	0.0000
	122	0.0111	0.0167	0.0244	0.0114	0.0233	0.0000	0.0111	0.0122	0.0000	0.0114	0.0000	0.0109	0.0444	0.0000	0.0233	0.0000	0.0222
	123	0.0333	0.0167	0.0244	0.0114	0.0581	0.0000	0.0000	0.0122	0.0341	0.0114	0.0000	0.0326	0.0000	0.0114	0.0349	0.0111	0.0111
	124	0.0333	0.0000	0.0244	0.0114	0.0116	0.0139	0.0222	0.0122	0.0341	0.0682	0.0000	0.0109	0.0111	0.0341	0.0233	0.0222	0.0333
	125	0.0000	0.0500	0.0244	0.0114	0.0116	0.0000	0.0111	0.0122	0.0000	0.0000	0.0217	0.0109	0.0000	0.0114	0.0000	0.0000	0.0111
	126	0.0000	0.0000	0.0122	0.0000	0.0000	0.0000	0.0111	0.0000	0.0114	0.0000	0.0000	0.0109	0.0000	0.0114	0.0000	0.0000	0.0111
	127	0.0000	0.0000	0.0122	0.0114	0.0000	0.0000	0.0222	0.0000	0.0114	0.0114	0.0109	0.0109	0.0000	0.0000	0.0000	0.0111	0.0000
	128	0.0000	0.0000	0.0122	0.0000	0.0000	0.0000	0.0111	0.0122	0.0000	0.0000	0.0109	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000
	129	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	130	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000
	131	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	133	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	135	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	137	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0122	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Locus	Allele	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
Hru5	106	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0476
	108	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000
	109	0.0000	0.0000	0.0000	0.0357	0.1000	0.0104	0.0122	0.0000	0.0104	0.0079	0.0000
	110	0.0000	0.1429	0.0465	0.0000	0.0000	0.0521	0.0244	0.0938	0.0833	0.0329	0.0476
	111	0.1000	0.0714	0.1279	0.2143	0.1000	0.0729	0.1098	0.0833	0.0521	0.0732	0.0000
	112	0.3000	0.0714	0.0349	0.0000	0.2000	0.0625	0.1098	0.0729	0.0521	0.0850	0.0238
	113	0.2000	0.0000	0.0465	0.0357	0.0000	0.1042	0.0732	0.0833	0.0833	0.0790	0.0000
	114	0.1000	0.0000	0.1047	0.0000	0.1000	0.0729	0.0976	0.0417	0.0833	0.0687	0.0000
	115	0.0000	0.0000	0.0698	0.0357	0.1000	0.0625	0.0976	0.1146	0.0729	0.0852	0.3571
	116	0.0000	0.0714	0.1279	0.2500	0.1000	0.1771	0.0732	0.0729	0.1667	0.1150	0.0000
	117	0.1000	0.2857	0.1395	0.2500	0.1000	0.1250	0.1463	0.1146	0.1146	0.1473	0.0000
	118	0.1000	0.2143	0.1047	0.0000	0.1000	0.0729	0.0854	0.1250	0.0729	0.1066	0.0000
	119	0.1000	0.0714	0.0814	0.0357	0.0000	0.0625	0.0732	0.1146	0.1042	0.0718	0.0000
	120	0.0000	0.0000	0.0233	0.0357	0.1000	0.0104	0.0000	0.0417	0.0313	0.0348	0.0000
	121	0.0000	0.0000	0.0233	0.0357	0.0000	0.0521	0.0488	0.0104	0.0521	0.0251	0.0000
	122	0.0000	0.0714	0.0000	0.0000	0.0000	0.0208	0.0122	0.0208	0.0000	0.0134	0.0000
	123	0.0000	0.0000	0.0233	0.0000	0.0000	0.0104	0.0000	0.0000	0.0000	0.0129	0.0000
	124	0.0000	0.0000	0.0233	0.0714	0.0000	0.0104	0.0122	0.0104	0.0000	0.0190	0.0000
	125	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0072	0.0000
	126	0.0000	0.0000	0.0116	0.0000	0.0000	0.0104	0.0122	0.0000	0.0000	0.0039	0.0476
	127	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0122	0.0000	0.0104	0.0048	0.0714
	128	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0000	0.0000	0.0000	0.0026	0.0000
	129	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0476
130	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	
131	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0476	
133	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.1667	
135	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0238	
137	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.0000	
139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238	
141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238	
143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0714	

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH
Mcyu4	138	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	140	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0278	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000
	142	0.0000	0.0000	0.0000	0.0000	0.0116	0.0143	0.0000	0.0000	0.0000	0.0455	0.0000	0.0000	0.0000	0.0116	0.0143	0.0000
	144	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0119	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	146	0.0000	0.0000	0.0000	0.0000	0.0000	0.0143	0.0139	0.0000	0.0000	0.0227	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	148	0.0000	0.0000	0.0128	0.0000	0.0000	0.0000	0.0139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0233	0.0000	0.0000
	150	0.0111	0.0116	0.0256	0.0000	0.0000	0.0429	0.0000	0.0476	0.0222	0.0227	0.0256	0.0000	0.0139	0.0116	0.0000	0.0469
	152	0.0111	0.0465	0.0000	0.0610	0.0465	0.0143	0.0139	0.0357	0.0000	0.0227	0.0256	0.0135	0.0278	0.0233	0.0429	0.0625
	154	0.2444	0.2093	0.3205	0.2439	0.1512	0.0571	0.2639	0.1667	0.1778	0.2500	0.1538	0.1622	0.2778	0.1860	0.1286	0.1719
	156	0.0556	0.1047	0.0641	0.0610	0.0698	0.2429	0.0833	0.1429	0.1222	0.0682	0.0769	0.1081	0.0833	0.1512	0.1429	0.0625
	158	0.0778	0.1512	0.2051	0.0732	0.1279	0.0714	0.1528	0.1190	0.1667	0.1477	0.1667	0.1216	0.1667	0.0814	0.1571	0.0938
	160	0.1556	0.1395	0.1026	0.1220	0.0930	0.1286	0.0972	0.0952	0.0889	0.0795	0.1026	0.1351	0.0556	0.1163	0.0714	0.1406
	162	0.2000	0.1163	0.1154	0.1829	0.1860	0.1429	0.1528	0.1071	0.1000	0.0341	0.2051	0.1081	0.2083	0.1628	0.1571	0.1719
	164	0.0667	0.0814	0.0769	0.0610	0.0930	0.1143	0.0972	0.1190	0.0889	0.1364	0.1154	0.0676	0.0556	0.0698	0.1429	0.0625
	166	0.0778	0.0698	0.0769	0.0854	0.0814	0.0857	0.0417	0.0476	0.0778	0.0568	0.0256	0.1486	0.0139	0.0465	0.0571	0.0781
	168	0.0222	0.0116	0.0000	0.0610	0.0698	0.0000	0.0417	0.0595	0.0444	0.0341	0.0385	0.0676	0.0139	0.0465	0.0429	0.0156
	170	0.0444	0.0349	0.0000	0.0122	0.0349	0.0286	0.0000	0.0000	0.0333	0.0114	0.0513	0.0405	0.0417	0.0349	0.0143	0.0625
	172	0.0111	0.0116	0.0000	0.0244	0.0349	0.0143	0.0000	0.0238	0.0444	0.0000	0.0000	0.0135	0.0278	0.0116	0.0286	0.0156
	174	0.0000	0.0000	0.0000	0.0122	0.0000	0.0143	0.0000	0.0238	0.0000	0.0341	0.0000	0.0000	0.0139	0.0000	0.0000	0.0000
	176	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0156
178	0.0000	0.0000	0.0000	0.0000	0.0000	0.0143	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
180	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0128	0.0135	0.0000	0.0000	0.0000	
192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	

Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
Mcyu4	138	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000
	140	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0015	0.0682
	142	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0042	0.0455
	144	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0227
	146	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0024	0.0455
	148	0.0000	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0116	0.0000	0.0000	0.0037	0.3409
	150	0.0119	0.0000	0.0000	0.0233	0.0357	0.1000	0.0465	0.0233	0.0217	0.0435	0.0226	0.0909
	152	0.0476	0.0000	0.0833	0.0233	0.0714	0.0000	0.0349	0.0116	0.0435	0.0217	0.0302	0.0682
	154	0.2143	0.3333	0.0000	0.2326	0.0714	0.2000	0.2442	0.1744	0.1739	0.2500	0.1946	0.0682
	156	0.1071	0.0833	0.2500	0.1163	0.1429	0.0000	0.1047	0.1279	0.0978	0.1196	0.1073	0.0682
	158	0.1786	0.2500	0.0833	0.1279	0.1071	0.0000	0.1395	0.1977	0.2283	0.1413	0.1359	0.1818
	160	0.1071	0.0833	0.1667	0.1279	0.0714	0.2000	0.0930	0.1395	0.1304	0.1522	0.1152	0.0000
	162	0.1310	0.1667	0.1667	0.0814	0.1429	0.1000	0.1047	0.0698	0.0761	0.1087	0.1346	0.0000
	164	0.0714	0.0000	0.0833	0.0930	0.1429	0.2000	0.0698	0.0465	0.1087	0.0652	0.0896	0.0000
	166	0.0595	0.0833	0.0000	0.0581	0.0714	0.2000	0.0349	0.0930	0.0870	0.0217	0.0684	0.0000
	168	0.0357	0.0000	0.1667	0.0349	0.0357	0.0000	0.0581	0.0581	0.0109	0.0435	0.0390	0.0000
	170	0.0000	0.0000	0.0000	0.0349	0.0357	0.0000	0.0233	0.0349	0.0109	0.0109	0.0229	0.0000
	172	0.0238	0.0000	0.0000	0.0233	0.0357	0.0000	0.0349	0.0000	0.0109	0.0217	0.0158	0.0000
	174	0.0119	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0042	0.0000
	176	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0019	0.0000
178	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000	
180	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0027	0.0000	
192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	

Locus Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH
Pdou3 80	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000
84	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
88	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
92	0.0000	0.0114	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114
96	0.0233	0.0000	0.0476	0.0000	0.0111	0.0000	0.0238	0.0233	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000
100	0.0233	0.0000	0.0238	0.0000	0.0111	0.0000	0.0000	0.0349	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0116	0.0000
104	0.0000	0.0000	0.0119	0.0222	0.0000	0.0000	0.0000	0.0233	0.0227	0.0000	0.0000	0.0000	0.0000	0.0444	0.0233	0.0341
108	0.0116	0.0227	0.0238	0.0333	0.0444	0.0270	0.0238	0.0116	0.0000	0.0111	0.0000	0.0000	0.0000	0.0222	0.0116	0.0227
112	0.0349	0.0341	0.0238	0.0000	0.0556	0.0135	0.0119	0.0116	0.0114	0.0333	0.0444	0.0000	0.0000	0.0333	0.0349	0.0000
116	0.0581	0.0341	0.0595	0.0111	0.0444	0.0811	0.0119	0.0698	0.0909	0.0889	0.0444	0.0581	0.0556	0.0222	0.0465	0.0227
120	0.0116	0.1250	0.0952	0.0889	0.0333	0.0676	0.1190	0.1047	0.1023	0.0222	0.0889	0.0698	0.0667	0.0444	0.1279	0.0455
124	0.0930	0.1705	0.1429	0.1000	0.1444	0.0811	0.0476	0.1047	0.0455	0.1111	0.0667	0.1279	0.1111	0.1111	0.1047	0.1364
128	0.1628	0.1591	0.0833	0.1889	0.1000	0.1486	0.1310	0.2093	0.1250	0.2222	0.0778	0.1977	0.1222	0.2111	0.1395	0.1364
132	0.1279	0.0909	0.1071	0.1222	0.1556	0.1892	0.1667	0.0581	0.2386	0.1667	0.1444	0.1628	0.1778	0.1667	0.1047	0.1705
136	0.1977	0.0909	0.1190	0.1000	0.1111	0.0811	0.0833	0.0698	0.0341	0.1444	0.1889	0.1047	0.1111	0.0667	0.1047	0.1136
140	0.1163	0.0795	0.0595	0.0889	0.0444	0.0811	0.1786	0.0233	0.1023	0.0556	0.1111	0.0465	0.0778	0.0778	0.0465	0.0341
144	0.0581	0.0455	0.0357	0.0444	0.1444	0.0405	0.0595	0.0930	0.0341	0.0556	0.0444	0.0581	0.0667	0.0444	0.0698	0.0455
148	0.0581	0.0341	0.0357	0.0556	0.0111	0.0676	0.0357	0.0465	0.0568	0.0333	0.0667	0.0349	0.0667	0.0333	0.0698	0.0341
152	0.0000	0.0909	0.0952	0.0889	0.0222	0.0135	0.0357	0.0233	0.0568	0.0222	0.0667	0.0233	0.0333	0.0667	0.0581	0.0341
156	0.0116	0.0114	0.0000	0.0222	0.0111	0.0541	0.0119	0.0349	0.0227	0.0222	0.0000	0.0233	0.0222	0.0000	0.0349	0.1136
160	0.0000	0.0000	0.0238	0.0111	0.0222	0.0000	0.0238	0.0349	0.0000	0.0000	0.0444	0.0349	0.0222	0.0111	0.0000	0.0114
164	0.0000	0.0000	0.0119	0.0111	0.0222	0.0000	0.0119	0.0000	0.0341	0.0000	0.0000	0.0349	0.0222	0.0333	0.0000	0.0227
168	0.0116	0.0000	0.0000	0.0000	0.0000	0.0405	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
172	0.0000	0.0000	0.0000	0.0000	0.0000	0.0135	0.0000	0.0116	0.0000	0.0000	0.0111	0.0233	0.0000	0.0000	0.0000	0.0000
176	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0114	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114
184	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0111	0.0000	0.0000
200	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000



Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
Pdou3	80	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	84	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	88	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	92	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0217	0.0000	0.0026	0.0000
	96	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217	0.0000	0.0000	0.0213	0.0071	0.0000
	100	0.0222	0.0000	0.0000	0.0116	0.0000	0.0000	0.0217	0.0000	0.0000	0.0000	0.0066	0.0000
	104	0.0000	0.0000	0.0833	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217	0.0000	0.0110	0.0000
	108	0.0111	0.0000	0.0000	0.0465	0.0000	0.0000	0.0000	0.0000	0.0000	0.0319	0.0137	0.0000
	112	0.0111	0.0000	0.0000	0.0233	0.0357	0.0000	0.0109	0.0543	0.0109	0.0213	0.0196	0.0000
	116	0.0333	0.0000	0.0833	0.0233	0.0000	0.2000	0.0652	0.0543	0.0217	0.0638	0.0517	0.0000
	120	0.0444	0.0000	0.0833	0.0698	0.1071	0.0000	0.0435	0.0652	0.0870	0.0426	0.0675	0.0000
	124	0.1000	0.2500	0.1667	0.1047	0.1429	0.1000	0.0978	0.0978	0.1413	0.0957	0.1152	0.0000
	128	0.1889	0.2500	0.1667	0.1512	0.2500	0.2000	0.2174	0.1304	0.1304	0.1596	0.1638	0.0000
	132	0.1444	0.0000	0.0833	0.1628	0.1071	0.2000	0.1196	0.1304	0.1739	0.1809	0.1405	0.0000
	136	0.1556	0.1667	0.0833	0.0930	0.1071	0.2000	0.0978	0.1848	0.1522	0.0957	0.1176	0.0000
	140	0.1111	0.0833	0.1667	0.0930	0.0714	0.0000	0.1196	0.1196	0.0761	0.0957	0.0831	0.0000
	144	0.0333	0.0833	0.0000	0.0581	0.0000	0.0000	0.0543	0.0109	0.0543	0.0426	0.0491	0.0000
	148	0.0444	0.0000	0.0000	0.0465	0.1071	0.0000	0.0326	0.0652	0.0543	0.0851	0.0452	0.0000
	152	0.0000	0.0833	0.0000	0.0233	0.0357	0.0000	0.0761	0.0109	0.0326	0.0426	0.0398	0.0000
	156	0.0444	0.0833	0.0000	0.0698	0.0000	0.1000	0.0000	0.0217	0.0000	0.0213	0.0283	0.0000
160	0.0222	0.0000	0.0833	0.0000	0.0000	0.0000	0.0109	0.0109	0.0217	0.0000	0.0150	0.0000	
164	0.0000	0.0000	0.0000	0.0233	0.0000	0.0000	0.0000	0.0326	0.0000	0.0000	0.0100	0.0000	
168	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0029	0.0000	
172	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0027	0.0000	
176	0.0111	0.0000	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0000	0.0000	0.0036	0.0000	
184	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000	
200	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000	

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH
Phtr2	107	0.0111	0.0000	0.0000	0.0000	0.0000	0.0143	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000
	109	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	115	0.0222	0.0366	0.0114	0.0114	0.0444	0.0000	0.0333	0.0349	0.0222	0.0227	0.0109	0.0870	0.0000	0.0111	0.0233	0.0233
	117	0.4556	0.4390	0.4091	0.3523	0.4333	0.4286	0.4667	0.5233	0.5111	0.5341	0.4891	0.4348	0.4778	0.4556	0.5465	0.3721
	119	0.1222	0.0610	0.0795	0.1364	0.0556	0.1286	0.1444	0.0698	0.1111	0.1250	0.0543	0.1413	0.1333	0.0778	0.0581	0.1860
	121	0.3111	0.3171	0.2727	0.2500	0.2556	0.2857	0.2222	0.2442	0.2556	0.2500	0.3370	0.1848	0.2889	0.3000	0.2326	0.2442
	123	0.0000	0.0000	0.0114	0.0341	0.0556	0.0429	0.0444	0.0116	0.0111	0.0000	0.0435	0.0109	0.0111	0.0111	0.0349	0.0116
	125	0.0222	0.0732	0.1250	0.1477	0.1333	0.0714	0.0556	0.0698	0.0667	0.0227	0.0326	0.1196	0.0444	0.1111	0.0698	0.1163
	127	0.0444	0.0488	0.0682	0.0455	0.0111	0.0143	0.0222	0.0116	0.0000	0.0114	0.0109	0.0109	0.0000	0.0222	0.0233	0.0233
	129	0.0111	0.0244	0.0114	0.0227	0.0111	0.0000	0.0111	0.0233	0.0111	0.0114	0.0109	0.0000	0.0333	0.0111	0.0116	0.0116
	131	0.0000	0.0000	0.0000	0.0000	0.0000	0.0143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	137	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0000
	141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116
	143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000
	151	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	159	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	163	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
169	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
179	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
181	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
183	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
Phtr2	107	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	0.0000
	109	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000
	111	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0013	0.0000
	115	0.0111	0.0000	0.0000	0.0341	0.0000	0.0000	0.0106	0.0319	0.0104	0.0208	0.0198	0.0000
	117	0.4222	0.4167	0.5000	0.3750	0.4643	0.1667	0.4468	0.4043	0.5313	0.5208	0.4453	0.0000
	119	0.1333	0.0833	0.2000	0.0795	0.2143	0.0000	0.0957	0.1702	0.1250	0.1250	0.1120	0.0000
	121	0.2778	0.1667	0.2000	0.3523	0.1786	0.5000	0.2553	0.2234	0.2083	0.2083	0.2624	0.0357
	123	0.0222	0.0833	0.0000	0.0227	0.0000	0.0000	0.0319	0.0319	0.0625	0.0104	0.0230	0.0000
	125	0.0444	0.1667	0.0000	0.0909	0.0714	0.3333	0.1383	0.0532	0.0417	0.0625	0.0878	0.0357
	127	0.0556	0.0833	0.1000	0.0114	0.0714	0.0000	0.0106	0.0745	0.0208	0.0208	0.0314	0.0000
	129	0.0000	0.0000	0.0000	0.0341	0.0000	0.0000	0.0000	0.0000	0.0000	0.0313	0.0108	0.1786
	131	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.2857
	137	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0000	0.0008	0.0000
	151	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0357
	159	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0714
	161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0357
	163	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0714
167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0357	
169	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0357	
179	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0714	
181	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0714	
183	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0357	

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH
Phtr3	126	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	128	0.0000	0.0250	0.0000	0.0000	0.0222	0.0139	0.0000	0.0116	0.0000	0.0000	0.0227	0.0000	0.0111	0.0000	0.0000	0.0256
	130	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	132	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0111	0.0000	0.0000	0.0000	0.0114	0.0114	0.0000	0.0000	0.0000	0.0000
	134	0.0000	0.0000	0.0114	0.0000	0.0111	0.0139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0385
	136	0.0111	0.0250	0.0000	0.0000	0.0000	0.0000	0.0222	0.0116	0.0111	0.0000	0.0000	0.0000	0.0000	0.0111	0.0122	0.0000
	138	0.0111	0.0250	0.0227	0.0222	0.0111	0.0278	0.0111	0.0116	0.0000	0.0000	0.0341	0.0000	0.0222	0.0111	0.0122	0.0513
	140	0.0222	0.0125	0.0000	0.0000	0.0000	0.0556	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0244	0.0128
	142	0.0889	0.1000	0.0455	0.0556	0.0778	0.0278	0.0333	0.0930	0.0778	0.1000	0.0682	0.0795	0.0556	0.1000	0.0854	0.1026
	144	0.1111	0.0500	0.0568	0.1000	0.0444	0.0417	0.0444	0.0116	0.0333	0.0667	0.0455	0.0682	0.0444	0.0111	0.0732	0.0513
	146	0.0444	0.0375	0.0227	0.0778	0.0778	0.0556	0.0333	0.0814	0.1111	0.0556	0.0341	0.1136	0.0778	0.0556	0.0610	0.0769
	148	0.1222	0.1000	0.1818	0.1000	0.1556	0.2500	0.2000	0.0930	0.0889	0.2333	0.2045	0.1250	0.1111	0.0778	0.1707	0.1667
	150	0.0444	0.0375	0.0682	0.0333	0.0333	0.0278	0.0667	0.0581	0.0111	0.0333	0.0227	0.0227	0.0667	0.1000	0.0366	0.0256
	152	0.0667	0.0250	0.0455	0.0444	0.0000	0.0417	0.0556	0.0233	0.0667	0.0778	0.0455	0.0568	0.0222	0.0222	0.1098	0.0128
	154	0.0556	0.0250	0.0568	0.0556	0.0556	0.0278	0.0333	0.0930	0.1000	0.0222	0.0909	0.0455	0.0556	0.0222	0.0732	0.0513
	156	0.1000	0.1000	0.0682	0.1000	0.0556	0.1111	0.0889	0.0930	0.0556	0.0778	0.1136	0.0795	0.0556	0.1333	0.0610	0.0385
	158	0.0333	0.0750	0.0795	0.0556	0.0444	0.0833	0.0444	0.0465	0.0778	0.0556	0.0000	0.0341	0.0667	0.1000	0.0488	0.0897
	160	0.0444	0.0750	0.1136	0.0556	0.0889	0.0556	0.0667	0.0465	0.0667	0.0667	0.0795	0.0568	0.1000	0.0778	0.0000	0.0385
	162	0.0444	0.0875	0.0227	0.0778	0.0556	0.0417	0.0333	0.0814	0.0778	0.0556	0.0568	0.0227	0.0333	0.0889	0.0122	0.0641
	164	0.0222	0.0250	0.0568	0.0444	0.0556	0.0417	0.0222	0.0698	0.0778	0.0333	0.0909	0.0568	0.0444	0.0778	0.0244	0.0641
	166	0.0556	0.0625	0.0455	0.0667	0.0556	0.0417	0.0778	0.0465	0.0556	0.0444	0.0455	0.0909	0.1444	0.0556	0.1098	0.0256
	168	0.0556	0.0375	0.0341	0.0667	0.0889	0.0278	0.0444	0.0000	0.0444	0.0111	0.0341	0.0568	0.0333	0.0000	0.0366	0.0000
	170	0.0333	0.0750	0.0114	0.0222	0.0222	0.0000	0.0222	0.0581	0.0222	0.0333	0.0000	0.0341	0.0222	0.0333	0.0366	0.0128
	172	0.0111	0.0000	0.0341	0.0222	0.0333	0.0139	0.0111	0.0349	0.0222	0.0111	0.0000	0.0114	0.0222	0.0000	0.0000	0.0385
	174	0.0222	0.0000	0.0114	0.0000	0.0000	0.0000	0.0111	0.0233	0.0000	0.0000	0.0000	0.0114	0.0111	0.0111	0.0122	0.0000
	176	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0222	0.0116	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000
178	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	
180	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0128	
182	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
Phtr3	126	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	128	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0106	0.0059	0.0000
	130	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0013	0.0000
	132	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0000	0.0021	0.0000
	134	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0106	0.0111	0.0000	0.0000	0.0046	0.0000
	136	0.0222	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0053	0.0208
	138	0.0222	0.0000	0.0000	0.0114	0.0000	0.1667	0.0213	0.0111	0.0114	0.0000	0.0199	0.0000
	140	0.0222	0.0000	0.0000	0.0000	0.0000	0.1667	0.0106	0.0111	0.0114	0.0213	0.0147	0.0000
	142	0.0333	0.0833	0.0000	0.0455	0.1071	0.0000	0.0319	0.0111	0.0795	0.1064	0.0650	0.0000
	144	0.0222	0.0833	0.1250	0.0455	0.0000	0.0000	0.0532	0.0444	0.0455	0.0638	0.0514	0.0625
	146	0.0778	0.0000	0.0000	0.1023	0.0714	0.0000	0.0532	0.0556	0.1023	0.0532	0.0589	0.0208
	148	0.1333	0.1667	0.0000	0.1591	0.1071	0.0000	0.1809	0.1000	0.1818	0.2128	0.1393	0.3125
	150	0.0556	0.0833	0.0000	0.0341	0.0714	0.0000	0.0426	0.1111	0.0909	0.0532	0.0473	0.0417
	152	0.0778	0.0000	0.0000	0.0455	0.0714	0.1667	0.0106	0.1333	0.0682	0.0319	0.0508	0.1458
	154	0.0556	0.0000	0.3750	0.0455	0.0714	0.1667	0.0532	0.0444	0.0341	0.0106	0.0662	0.0208
	156	0.0444	0.2500	0.0000	0.1136	0.0714	0.0000	0.0638	0.0778	0.0341	0.0638	0.0789	0.0208
	158	0.0778	0.0000	0.1250	0.0909	0.0357	0.0000	0.0426	0.0333	0.0455	0.0851	0.0566	0.0208
	160	0.1222	0.1667	0.0000	0.0568	0.0357	0.1667	0.1170	0.0667	0.0795	0.0319	0.0721	0.1042
	162	0.0333	0.0000	0.0000	0.0455	0.0357	0.0000	0.0213	0.0667	0.0455	0.0532	0.0445	0.1458
	164	0.0556	0.0000	0.1250	0.0341	0.1429	0.0000	0.0745	0.0444	0.0568	0.0638	0.0540	0.0625
	166	0.0778	0.0833	0.0000	0.0795	0.0714	0.0000	0.0957	0.0556	0.0114	0.0426	0.0593	0.0000
	168	0.0444	0.0000	0.0000	0.0114	0.0357	0.0000	0.0532	0.0667	0.0114	0.0532	0.0326	0.0208
	170	0.0222	0.0000	0.1250	0.0000	0.0357	0.0000	0.0426	0.0333	0.0341	0.0106	0.0286	0.0000
	172	0.0000	0.0833	0.0000	0.0227	0.0000	0.0000	0.0000	0.0222	0.0000	0.0106	0.0156	0.0000
	174	0.0000	0.0000	0.0000	0.0227	0.0357	0.0000	0.0106	0.0000	0.0000	0.0106	0.0074	0.0000
	176	0.0000	0.0000	0.1250	0.0000	0.0000	0.1667	0.0000	0.0000	0.0000	0.0000	0.0134	0.0000
	178	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0017	0.0000
	180	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0227	0.0000	0.0018	0.0000
	182	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0004	0.0000

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	
WBSW1	141	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000	
	143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
	145	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
	149	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	
	151	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	163	0.0222	0.0116	0.0114	0.0111	0.0111	0.0270	0.0222	0.0114	0.0333	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	165	0.0444	0.0930	0.0341	0.0222	0.0222	0.0135	0.0222	0.0568	0.0111	0.0222	0.0326	0.0435	0.0222	0.0778	0.0444	0.0000	0.0000
	167	0.0889	0.0581	0.0455	0.0778	0.0778	0.1216	0.0778	0.1250	0.1333	0.0556	0.1196	0.0435	0.0889	0.0889	0.0667	0.0778	0.0000
	169	0.1000	0.0930	0.0341	0.0333	0.1222	0.0541	0.0889	0.0227	0.0444	0.0333	0.0652	0.0435	0.0667	0.0444	0.0889	0.1000	0.0000
	171	0.4556	0.4070	0.5455	0.5444	0.3667	0.4595	0.3778	0.4659	0.4000	0.4778	0.4130	0.5435	0.4000	0.4333	0.4333	0.5000	0.0000
	173	0.0778	0.0698	0.1136	0.0333	0.0889	0.0676	0.1556	0.0682	0.0667	0.1333	0.0543	0.1413	0.0222	0.0778	0.0889	0.0556	0.0000
	175	0.0333	0.0465	0.0000	0.0333	0.0667	0.0541	0.0444	0.0000	0.0222	0.0556	0.0761	0.0217	0.0667	0.0222	0.0222	0.0222	0.0000
	177	0.0222	0.0233	0.0000	0.0000	0.0444	0.0000	0.0333	0.0114	0.0111	0.0111	0.0000	0.0217	0.0222	0.0222	0.0111	0.0111	0.0000
	179	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	181	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0111	0.0227	0.0000	0.0222	0.0217	0.0000	0.0111	0.0000	0.0444	0.0000	0.0000
	183	0.0444	0.0000	0.0455	0.0111	0.0333	0.0541	0.0111	0.0341	0.0444	0.0222	0.0109	0.0109	0.0222	0.0444	0.0222	0.0333	0.0000
	185	0.0111	0.0116	0.0227	0.0444	0.0222	0.0135	0.0111	0.0455	0.0111	0.0222	0.0326	0.0000	0.0111	0.0111	0.0000	0.0000	0.0000
	187	0.0000	0.0116	0.0114	0.0222	0.0111	0.0000	0.0111	0.0114	0.0000	0.0000	0.0000	0.0109	0.0111	0.0000	0.0111	0.0111	0.0000
	189	0.0000	0.0000	0.0114	0.0111	0.0111	0.0000	0.0000	0.0000	0.0111	0.0111	0.0000	0.0000	0.0111	0.0444	0.0000	0.0000	0.0000
	191	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	193	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	195	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0111	0.0111	0.0000	0.0000	0.0000
	197	0.0000	0.0349	0.0568	0.0111	0.0444	0.0135	0.0111	0.0000	0.0333	0.0333	0.0435	0.0217	0.0444	0.0000	0.0000	0.0000	0.0000
	199	0.0111	0.0814	0.0568	0.0333	0.0222	0.0811	0.0667	0.0682	0.0889	0.0667	0.0543	0.0652	0.1444	0.0778	0.0667	0.0667	0.0000
	201	0.0444	0.0581	0.0114	0.0667	0.0111	0.0405	0.0444	0.0455	0.0667	0.0111	0.0435	0.0326	0.0333	0.0222	0.0778	0.0889	0.0000
	203	0.0444	0.0000	0.0000	0.0000	0.0222	0.0000	0.0111	0.0114	0.0000	0.0000	0.0109	0.0000	0.0111	0.0222	0.0111	0.0222	0.0000
	205	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111
213	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
WBSW1	141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0013	0.0000
	143	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0000	0.0000	0.0000	0.0008	0.0000
	145	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0000	0.0000	0.0000	0.0004	0.0000
	149	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	151	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5000
	163	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0319	0.0000	0.0111	0.0087	0.1053
	165	0.0333	0.0000	0.0000	0.0227	0.0357	0.0000	0.0625	0.0319	0.0208	0.0111	0.0300	0.0263
	167	0.1333	0.0833	0.1000	0.0682	0.0714	0.0000	0.1458	0.0638	0.0625	0.1444	0.0854	0.0000
	169	0.1222	0.0833	0.2000	0.1250	0.0714	0.2000	0.0208	0.0426	0.0521	0.0111	0.0755	0.2632
	171	0.4111	0.1667	0.6000	0.4432	0.4286	0.3000	0.4167	0.4149	0.4688	0.3889	0.4332	0.1053
	173	0.0222	0.2500	0.0000	0.0795	0.1429	0.2000	0.0938	0.1277	0.0938	0.1667	0.0958	0.0000
	175	0.0111	0.0833	0.0000	0.0114	0.0000	0.1000	0.0521	0.0106	0.0417	0.0111	0.0349	0.0000
	177	0.0222	0.0000	0.0000	0.0227	0.0357	0.0000	0.0104	0.0426	0.0208	0.0333	0.0166	0.0000
	179	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0017	0.0000
	181	0.0111	0.0000	0.0000	0.0227	0.0000	0.0000	0.0417	0.0000	0.0000	0.0444	0.0102	0.0000
	183	0.0222	0.0000	0.0000	0.0000	0.0357	0.0000	0.0208	0.0106	0.0313	0.0000	0.0217	0.0000
	185	0.0222	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0213	0.0417	0.0222	0.0159	0.0000
	187	0.0111	0.0000	0.0000	0.0000	0.0000	0.1000	0.0000	0.0000	0.0208	0.0000	0.0098	0.0000
	189	0.0222	0.0000	0.0000	0.0227	0.0000	0.0000	0.0000	0.0106	0.0104	0.0111	0.0072	0.0000
	191	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0208	0.0000	0.0000	0.0000	0.0021	0.0000
	193	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	195	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000
	197	0.0111	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0319	0.0208	0.0333	0.0185	0.0000
	199	0.0667	0.1667	0.0000	0.0795	0.0357	0.1000	0.0104	0.0745	0.0208	0.0556	0.0639	0.0000
	201	0.0444	0.1667	0.1000	0.0795	0.0714	0.0000	0.0313	0.0745	0.0729	0.0222	0.0524	0.0000
	203	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0521	0.0106	0.0208	0.0000	0.0100	0.0000
	205	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000
	213	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH
WBSW2	201	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0111	0.0119	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0122	0.0000
	203	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	205	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0116	0.0000	0.0000
	207	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000
	209	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000
	211	0.0556	0.0500	0.0682	0.0444	0.0333	0.0417	0.0889	0.0119	0.0333	0.0000	0.0217	0.0109	0.0349	0.0333	0.0854	0.0227
	213	0.1111	0.1000	0.1136	0.0667	0.0778	0.0833	0.0778	0.1190	0.0667	0.0111	0.0870	0.0761	0.1279	0.1222	0.0976	0.1136
	215	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0109	0.0217	0.0116	0.0000	0.0114
	217	0.0222	0.0125	0.0341	0.0333	0.0444	0.0000	0.0000	0.0357	0.0222	0.0222	0.0543	0.0761	0.0233	0.0333	0.0122	0.0455
	219	0.0000	0.0625	0.0341	0.0333	0.0556	0.0556	0.0444	0.0238	0.0222	0.0222	0.0217	0.0326	0.0349	0.0667	0.0488	0.0000
	221	0.1333	0.0750	0.0682	0.0889	0.1778	0.1250	0.1444	0.1429	0.2000	0.0778	0.1522	0.1304	0.0930	0.1778	0.1585	0.1932
	223	0.0333	0.0250	0.0341	0.0222	0.0111	0.0278	0.0333	0.0000	0.0667	0.1000	0.0217	0.0217	0.0814	0.0111	0.0000	0.0455
	225	0.3222	0.3375	0.2955	0.4000	0.3333	0.3611	0.2444	0.3810	0.2889	0.3000	0.3043	0.2935	0.3488	0.2556	0.3780	0.3295
	227	0.0444	0.0875	0.1477	0.0111	0.0778	0.1250	0.1111	0.0476	0.0556	0.0667	0.0326	0.0543	0.0814	0.1111	0.0488	0.0682
	229	0.1111	0.1250	0.1136	0.1111	0.0778	0.0556	0.1889	0.1310	0.1222	0.1000	0.1522	0.0435	0.0930	0.0778	0.1098	0.0795
	231	0.0111	0.0125	0.0227	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0217	0.0870	0.0233	0.0111	0.0122
	233	0.0889	0.0250	0.0227	0.0000	0.0222	0.0417	0.0444	0.0238	0.0222	0.0222	0.0109	0.0652	0.0116	0.0333	0.0366	0.0227
	235	0.0111	0.0125	0.0114	0.0111	0.0444	0.0000	0.0111	0.0238	0.0111	0.0000	0.0217	0.0217	0.0000	0.0333	0.0000	0.0000
	237	0.0222	0.0250	0.0227	0.0444	0.0111	0.0278	0.0000	0.0357	0.0333	0.0222	0.0326	0.0109	0.0116	0.0111	0.0000	0.0114
	239	0.0000	0.0125	0.0000	0.0000	0.0000	0.0278	0.0000	0.0000	0.0000	0.0111	0.0222	0.0109	0.0109	0.0000	0.0111	0.0000
	241	0.0111	0.0000	0.0000	0.0222	0.0111	0.0000	0.0000	0.0000	0.0000	0.0111	0.0111	0.0109	0.0000	0.0000	0.0000	0.0114
	243	0.0000	0.0125	0.0000	0.0111	0.0111	0.0139	0.0000	0.0119	0.0000	0.0000	0.0217	0.0109	0.0000	0.0000	0.0000	0.0114
	245	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0109	0.0000	0.0000	0.0114
	247	0.0000	0.0250	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0109	0.0109	0.0000	0.0000	0.0000
	249	0.0111	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0116	0.0000	0.0000
	251	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0139	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0111	0.0000
	253	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
257	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	



Locus	Allele	TE	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
WBSW2	201	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0026	0.0278
	203	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	205	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000
	207	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0047	0.0000
	209	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0208	0.0319	0.0000	0.0208	0.0037	0.0278
	211	0.0222	0.0000	0.0000	0.0341	0.0714	0.0000	0.0729	0.0638	0.0417	0.0208	0.0370	0.0000
	213	0.1222	0.2500	0.0000	0.1250	0.1071	0.0000	0.1042	0.0213	0.0729	0.0521	0.0887	0.0000
	215	0.0000	0.0000	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0208	0.0104	0.0089	0.0000
	217	0.0778	0.0833	0.0000	0.0341	0.0000	0.1000	0.0208	0.0532	0.0000	0.0313	0.0335	0.1389
	219	0.0333	0.0833	0.2000	0.0000	0.0357	0.0000	0.0313	0.0000	0.0000	0.0833	0.0394	0.1111
	221	0.1444	0.0833	0.1000	0.1477	0.1786	0.1000	0.1458	0.1702	0.1146	0.1458	0.1334	0.0556
	223	0.0111	0.0000	0.0000	0.0341	0.0357	0.2000	0.0313	0.0532	0.0417	0.0104	0.0366	0.1111
	225	0.3222	0.4167	0.4000	0.3409	0.2857	0.5000	0.2813	0.3085	0.3958	0.2708	0.3344	0.1389
	227	0.0556	0.0000	0.0000	0.0795	0.0357	0.0000	0.1042	0.0213	0.1146	0.0833	0.0640	0.0000
	229	0.1222	0.0833	0.2000	0.0795	0.1429	0.1000	0.0521	0.0745	0.0625	0.1458	0.1060	0.0000
	231	0.0111	0.0000	0.0000	0.0114	0.0357	0.0000	0.0313	0.0319	0.0104	0.0313	0.0170	0.0556
	233	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0208	0.0638	0.0208	0.0417	0.0251	0.0000
	235	0.0111	0.0000	0.0000	0.0114	0.0000	0.0000	0.0417	0.0426	0.0521	0.0000	0.0143	0.2778
	237	0.0111	0.0000	0.0000	0.0455	0.0000	0.0000	0.0104	0.0106	0.0208	0.0000	0.0162	0.0000
	239	0.0111	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0106	0.0104	0.0000	0.0058	0.0556
	241	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0208	0.0000	0.0000	0.0208	0.0055	0.0000
	243	0.0111	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0000	0.0104	0.0000	0.0062	0.0000
	245	0.0111	0.0000	0.0000	0.0114	0.0000	0.0000	0.0104	0.0213	0.0000	0.0208	0.0046	0.0000
	247	0.0111	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0000	0.0000	0.0000	0.0053	0.0000
	249	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0104	0.0025	0.0000
	251	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0014	0.0000
	253	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	257	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0104	0.0000	0.0004	0.0000

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE
WBSW4	131	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0227	0.0000
	133	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	135	0.0222	0.0122	0.0000	0.0222	0.0222	0.0143	0.0333	0.0341	0.0341	0.0222	0.0227	0.0238	0.0227	0.0444	0.0233	0.0000	0.0000
	137	0.0778	0.0488	0.0227	0.0444	0.0111	0.0286	0.0444	0.0455	0.0568	0.0111	0.0114	0.0238	0.0341	0.0778	0.0581	0.0568	0.0444
	139	0.0000	0.0122	0.0227	0.0000	0.0000	0.0429	0.0000	0.0114	0.0114	0.0222	0.0227	0.0000	0.0114	0.0111	0.0000	0.0341	0.0000
	141	0.0778	0.0366	0.0227	0.0444	0.0222	0.0143	0.0222	0.0227	0.0455	0.0333	0.0227	0.0357	0.0227	0.0333	0.0233	0.0341	0.0333
	143	0.0444	0.0488	0.0455	0.0111	0.0778	0.0714	0.0889	0.0568	0.0682	0.0333	0.0568	0.0476	0.0568	0.0444	0.0349	0.0114	0.0333
	145	0.1556	0.1707	0.1136	0.0778	0.1222	0.1000	0.1444	0.1136	0.1023	0.1222	0.1591	0.1190	0.1364	0.1111	0.1047	0.1591	0.1000
	147	0.0556	0.0854	0.0568	0.0222	0.0111	0.0714	0.0667	0.0568	0.0568	0.1000	0.0455	0.0833	0.0909	0.0444	0.0581	0.0909	0.0778
	149	0.1000	0.0976	0.0909	0.0667	0.1000	0.0714	0.0556	0.1136	0.0682	0.1000	0.0568	0.1190	0.1364	0.0778	0.1047	0.0795	0.0889
	151	0.0333	0.0610	0.0227	0.0444	0.0111	0.0429	0.0333	0.0568	0.0227	0.0333	0.0227	0.0476	0.0341	0.0444	0.0698	0.0227	0.0222
	153	0.0444	0.0732	0.0568	0.0667	0.0444	0.0286	0.1111	0.0682	0.0568	0.0333	0.0682	0.0833	0.0114	0.0444	0.0116	0.0455	0.0444
	155	0.0444	0.0244	0.0909	0.1333	0.0667	0.0857	0.1000	0.0909	0.0227	0.0444	0.0568	0.0357	0.1023	0.0556	0.0930	0.0568	0.1000
	157	0.0444	0.0488	0.0795	0.0889	0.1111	0.0286	0.0333	0.0227	0.1023	0.0889	0.0909	0.0476	0.0341	0.0444	0.0116	0.0795	0.0778
	159	0.0333	0.0610	0.0682	0.0667	0.0667	0.0714	0.0556	0.0568	0.0455	0.1000	0.0114	0.0595	0.0227	0.0667	0.0814	0.0682	0.0333
	161	0.1000	0.0854	0.0568	0.0889	0.0111	0.1143	0.0333	0.0568	0.1023	0.0444	0.1023	0.0357	0.0341	0.0444	0.0465	0.0455	0.0778
	163	0.0444	0.0488	0.0114	0.0333	0.0556	0.0429	0.0444	0.0455	0.0455	0.0556	0.0341	0.0595	0.0455	0.0444	0.0581	0.0455	0.0889
	165	0.0222	0.0366	0.0909	0.0667	0.0889	0.0571	0.0000	0.0682	0.0341	0.0333	0.0909	0.0714	0.0568	0.0556	0.0814	0.0341	0.0778
	167	0.0333	0.0244	0.0795	0.0667	0.0889	0.0143	0.0333	0.0568	0.0568	0.1000	0.0455	0.0238	0.0682	0.0556	0.0349	0.0227	0.0556
	169	0.0222	0.0122	0.0227	0.0222	0.0444	0.0571	0.0333	0.0227	0.0227	0.0111	0.0227	0.0476	0.0455	0.0556	0.0465	0.0455	0.0111
	171	0.0222	0.0000	0.0341	0.0111	0.0222	0.0286	0.0333	0.0000	0.0000	0.0000	0.0455	0.0119	0.0000	0.0111	0.0233	0.0227	0.0222
	173	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0111	0.0000	0.0114	0.0000	0.0114	0.0238	0.0000	0.0111	0.0116	0.0114	0.0000
	175	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0222	0.0116	0.0000	0.0000
	177	0.0111	0.0122	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0116	0.0000	0.0111
	179	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	181	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000
	187	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0111	0.0000	0.0000	0.0114	0.0000	0.0000	0.0114	0.0000
	189	0.0111	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	191	0.0000	0.0000	0.0000	0.0000	0.0000	0.0143	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	193	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Locus	Allele	TS	TTQ	TU	UP	VVQ	XA	XB	XC	XX	Overall	JRB
WBSW4	131	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000
	133	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0109	0.0009	0.0000
	135	0.0000	0.0000	0.0114	0.0000	0.0000	0.0109	0.0000	0.0455	0.0543	0.0183	0.0000
	137	0.0000	0.0000	0.0568	0.0714	0.0000	0.0109	0.0532	0.0568	0.0543	0.0385	0.0000
	139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0435	0.0000	0.0227	0.0000	0.0103	0.0000
	141	0.0833	0.0000	0.0568	0.0357	0.0000	0.0326	0.0106	0.0568	0.0435	0.0333	0.0000
	143	0.0000	0.1429	0.0341	0.0357	0.1000	0.0761	0.0532	0.0568	0.0109	0.0516	0.0000
	145	0.0833	0.1429	0.1023	0.1071	0.4000	0.0870	0.0957	0.1477	0.1413	0.1315	0.0000
	147	0.0000	0.0000	0.0341	0.1786	0.2000	0.0435	0.0851	0.0114	0.0326	0.0638	0.0000
	149	0.0833	0.1429	0.0909	0.0000	0.1000	0.0326	0.0638	0.0682	0.0761	0.0840	0.0000
	151	0.0000	0.1429	0.0227	0.1071	0.0000	0.0543	0.0426	0.0455	0.0217	0.0408	1.0000
	153	0.0000	0.1429	0.0568	0.1429	0.0000	0.0761	0.0213	0.0227	0.0435	0.0538	0.0000
	155	0.2500	0.0000	0.0682	0.0357	0.0000	0.0326	0.0532	0.0909	0.0109	0.0671	0.0000
	157	0.0833	0.0000	0.0795	0.0357	0.1000	0.1522	0.1064	0.0227	0.0652	0.0646	0.0000
	159	0.0833	0.0714	0.0227	0.0714	0.0000	0.0870	0.0957	0.1023	0.1196	0.0624	0.0000
	161	0.0833	0.0000	0.1136	0.0000	0.0000	0.0652	0.0638	0.0682	0.0543	0.0588	0.0000
	163	0.0000	0.0000	0.0455	0.1429	0.0000	0.0543	0.0426	0.0114	0.0435	0.0440	0.0000
	165	0.0000	0.0714	0.0341	0.0357	0.0000	0.0326	0.0426	0.0341	0.0543	0.0489	0.0000
	167	0.0833	0.0714	0.0568	0.0000	0.0000	0.0109	0.0638	0.0795	0.0761	0.0501	0.0000
	169	0.1667	0.0000	0.0682	0.0000	0.1000	0.0217	0.0213	0.0227	0.0435	0.0380	0.0000
	171	0.0000	0.0714	0.0227	0.0000	0.0000	0.0217	0.0532	0.0000	0.0217	0.0184	0.0000
	173	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0227	0.0109	0.0057	0.0000
	175	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217	0.0000	0.0000	0.0000	0.0030	0.0000
	177	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0031	0.0000
	179	0.0000	0.0000	0.0114	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0013	0.0000
	181	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217	0.0106	0.0000	0.0000	0.0021	0.0000
	185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0109	0.0009	0.0000
	187	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0106	0.0000	0.0000	0.0022	0.0000
	189	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000
	191	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006	0.0000
	193	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000

Locus	Allele	AF	BU	ED	GU	HU	JD	KR	KW	LM	LT	MA	NN	NS	RF	RR	SH	TE	TS	TTQ	TU	UP	VVQ
WBSW11	151	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	155	0.0333	0.0000	0.0227	0.0000	0.0333	0.0000	0.0111	0.0233	0.0222	0.0000	0.0000	0.0326	0.0000	0.0000	0.0125	0.0000	0.0000	0.1667	0.0000	0.0116	0.0000	0.0000
	157	0.0222	0.0000	0.0114	0.0114	0.0111	0.0769	0.0000	0.0000	0.0556	0.0444	0.0326	0.0000	0.0111	0.0000	0.0000	0.0000	0.0227	0.0000	0.0000	0.0465	0.0000	0.0000
	159	0.0444	0.0349	0.0227	0.0114	0.0000	0.0192	0.0000	0.0233	0.0111	0.0000	0.0217	0.0435	0.0000	0.0111	0.0250	0.0222	0.0227	0.0000	0.0000	0.0116	0.0000	0.0000
	161	0.0111	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217	0.0111	0.0000	0.0250	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000
	163	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0222	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	165	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0000	0.0109	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0116	0.0000	0.0000
	167	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0233	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	169	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0217	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	175	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	177	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	179	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0125	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	181	0.0111	0.0116	0.0341	0.0000	0.0111	0.0192	0.0111	0.0116	0.0000	0.0111	0.0217	0.0217	0.0111	0.0000	0.0125	0.0333	0.0114	0.0000	0.0000	0.0116	0.0000	0.0000
	183	0.0556	0.0000	0.0000	0.0227	0.0111	0.0577	0.0222	0.0465	0.0111	0.0444	0.0326	0.0000	0.0111	0.0333	0.0375	0.0556	0.0341	0.0833	0.0000	0.0349	0.0000	0.0000
	185	0.0444	0.0814	0.0568	0.0227	0.0556	0.0769	0.0222	0.0116	0.0778	0.0889	0.0326	0.0435	0.0778	0.0667	0.0750	0.0667	0.0568	0.1667	0.0000	0.0465	0.0000	0.0000
	187	0.0778	0.1395	0.0682	0.0795	0.0778	0.0769	0.0778	0.0698	0.1111	0.1111	0.0978	0.0761	0.0889	0.1222	0.1250	0.1111	0.1136	0.0000	0.0833	0.0930	0.0714	0.2000
	189	0.0667	0.1163	0.0568	0.1023	0.0444	0.0577	0.0556	0.0814	0.0444	0.1000	0.0978	0.1413	0.1111	0.1111	0.0625	0.1333	0.0568	0.2500	0.2500	0.0581	0.0357	0.2000
	191	0.1556	0.1977	0.2841	0.2841	0.2889	0.2500	0.2000	0.2093	0.2222	0.2333	0.2500	0.1957	0.2333	0.2111	0.1750	0.1333	0.2500	0.0000	0.1667	0.2093	0.1429	0.1000
	193	0.1333	0.0465	0.0909	0.0909	0.1000	0.0962	0.1111	0.0930	0.1222	0.1000	0.1087	0.0870	0.1000	0.1111	0.0375	0.0889	0.0909	0.0000	0.0833	0.1279	0.1071	0.3000
	195	0.0556	0.0581	0.0341	0.0227	0.0444	0.0769	0.0556	0.0930	0.0333	0.0222	0.0326	0.0870	0.0667	0.0333	0.0875	0.0667	0.0682	0.0833	0.0000	0.0581	0.1071	0.0000
	197	0.0000	0.0000	0.0341	0.0227	0.0333	0.0000	0.0111	0.0233	0.0000	0.0333	0.0652	0.0217	0.0111	0.0556	0.0500	0.0333	0.0114	0.0000	0.1667	0.0000	0.0714	0.0000
	199	0.0222	0.0000	0.0227	0.0568	0.0222	0.0000	0.0222	0.0233	0.0222	0.0333	0.0326	0.0435	0.0333	0.0222	0.0375	0.0889	0.0227	0.0000	0.0833	0.0233	0.0000	0.0000
	201	0.0333	0.0465	0.0114	0.0000	0.0333	0.0577	0.0444	0.0581	0.0111	0.0222	0.0109	0.0000	0.0000	0.0222	0.0000	0.0111	0.0114	0.0000	0.0000	0.0465	0.0357	0.0000
	203	0.0111	0.0465	0.0227	0.0114	0.0111	0.0192	0.0111	0.0349	0.0111	0.0222	0.0326	0.0326	0.0222	0.0222	0.0500	0.0000	0.0114	0.0000	0.0833	0.0581	0.0000	0.0000
	205	0.0222	0.0349	0.0114	0.0455	0.0111	0.0192	0.0778	0.0233	0.0111	0.0111	0.0000	0.0000	0.0222	0.0111	0.0250	0.0333	0.0114	0.1667	0.0833	0.0000	0.0357	0.0000
	207	0.0333	0.0233	0.0227	0.0114	0.0444	0.0000	0.0667	0.0116	0.0556	0.0444	0.0000	0.0217	0.0111	0.0556	0.0375	0.0222	0.0227	0.0000	0.0000	0.0233	0.2500	0.0000
	209	0.0111	0.0116	0.0795	0.1023	0.0556	0.0385	0.0111	0.0465	0.0556	0.0556	0.0000	0.0109	0.0333	0.0444	0.0625	0.0333	0.0568	0.0833	0.0000	0.0233	0.0000	0.0000
	211	0.0000	0.0581	0.0341	0.0341	0.0556	0.0000	0.0556	0.0465	0.0333	0.0111	0.0326	0.0109	0.0667	0.0000	0.0000	0.0556	0.0455	0.0000	0.0000	0.0116	0.0357	0.2000
	213	0.0333	0.0349	0.0455	0.0341	0.0222	0.0385	0.0333	0.0116	0.0222	0.0000	0.0326	0.0217	0.0222	0.0222	0.0250	0.0000	0.0114	0.0000	0.0000	0.0000	0.0714	0.0000
	215	0.0333	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0217	0.0000	0.0111	0.0125	0.0111	0.0114	0.0000	0.0000	0.0233	0.0000	0.0000
	217	0.0000	0.0116	0.0114	0.0114	0.0222	0.0000	0.0444	0.0233	0.0000	0.0000	0.0326	0.0435	0.0333	0.0000	0.0125	0.0000	0.0114	0.0000	0.0000	0.0465	0.0357	0.0000
	219	0.0333	0.0233	0.0000	0.0000	0.0000	0.0000	0.0222	0.0000	0.0000	0.0111	0.0217	0.0000	0.0000	0.0222	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000
	221	0.0333	0.0116	0.0114	0.0227	0.0000	0.0000	0.0000	0.0116	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0000	0.0000	0.0000	0.0000
	223	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	225	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	227	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0233	0.0000
	231	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	233	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0111	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Locus	Allele	XA	XB	XC	XX	Overall	JRB
WBSW11	151	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000
	155	0.0208	0.0000	0.0000	0.0000	0.0150	0.0000
	157	0.0208	0.0000	0.0213	0.0104	0.0153	0.0000
	159	0.0521	0.0106	0.0000	0.0417	0.0165	0.0000
	161	0.0000	0.0000	0.0000	0.0000	0.0038	0.0000
	163	0.0000	0.0106	0.0106	0.0000	0.0025	0.0000
	165	0.0000	0.0000	0.0000	0.0000	0.0026	0.0000
	167	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000
	169	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000
	175	0.0000	0.0000	0.0000	0.0000	0.0000	0.0238
	177	0.0000	0.0000	0.0000	0.0000	0.0000	0.0952
	179	0.0000	0.0000	0.0000	0.0000	0.0005	0.0000
	181	0.0000	0.0106	0.0106	0.0000	0.0102	0.0000
	183	0.0208	0.0213	0.0106	0.0417	0.0265	0.1667
	185	0.0625	0.0532	0.0426	0.1354	0.0563	0.1905
	187	0.1042	0.0745	0.0957	0.0208	0.0910	0.0714
	189	0.0521	0.1170	0.0638	0.0521	0.0969	0.0238
	191	0.2604	0.2766	0.2340	0.2708	0.2090	0.1429
	193	0.0313	0.0957	0.1064	0.1042	0.0986	0.0714
	195	0.0729	0.0638	0.0319	0.0625	0.0545	0.1190
	197	0.0104	0.0106	0.0106	0.0208	0.0268	0.0952
	199	0.0000	0.0000	0.0319	0.0313	0.0260	0.0000
	201	0.0417	0.0319	0.0000	0.0000	0.0204	0.0000
	203	0.0313	0.0106	0.0000	0.0000	0.0214	0.0000
	205	0.0104	0.0532	0.0426	0.0000	0.0293	0.0000
	207	0.0208	0.0000	0.0000	0.0521	0.0319	0.0000
	209	0.0208	0.0532	0.0426	0.0625	0.0382	0.0000
	211	0.0417	0.0106	0.0957	0.0000	0.0360	0.0000
	213	0.0521	0.0638	0.0319	0.0208	0.0250	0.0000
	215	0.0208	0.0213	0.0213	0.0208	0.0089	0.0000
	217	0.0104	0.0000	0.0532	0.0208	0.0163	0.0000
	219	0.0417	0.0106	0.0319	0.0104	0.0092	0.0000
	221	0.0000	0.0000	0.0000	0.0104	0.0056	0.0000
223	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000	
225	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	
227	0.0000	0.0000	0.0000	0.0104	0.0004	0.0000	
229	0.0000	0.0000	0.0000	0.0000	0.0009	0.0000	
231	0.0000	0.0000	0.0106	0.0000	0.0004	0.0000	
233	0.0000	0.0000	0.0000	0.0000	0.0004	0.0000	

Hru7		Lox8	
Allele	JD	Allele	JD
137	0.0000	190	0.0000
139	0.0093	194	0.0439
141	0.0370	202	0.0175
143	0.0556	206	0.0088
145	0.0926	218	0.0175
146	0.0093	220	0.0088
147	0.0278	222	0.0263
149	0.0278	224	0.0263
151	0.0185	226	0.0175
153	0.0093	228	0.0263
155	0.0185	230	0.0175
157	0.0185	232	0.0175
161	0.0093	234	0.0263
165	0.0278	236	0.0088
167	0.0370	238	0.0263
169	0.0463	240	0.0526
171	0.0093	244	0.0088
181	0.0093	246	0.0088
183	0.0278	248	0.0175
185	0.0741	250	0.0088
187	0.0093	258	0.0175
189	0.0370	260	0.0263
191	0.0370	262	0.0175
193	0.0463	264	0.0263
195	0.0185	266	0.0175
199	0.0185	268	0.0175
201	0.0185	270	0.0614
203	0.0278	272	0.0088
205	0.0463	274	0.0877
215	0.0093	276	0.0088
227	0.0278	280	0.0175
229	0.0185	282	0.0175
231	0.0278	286	0.0088
233	0.0185	288	0.0439
235	0.0093	290	0.0263
237	0.0093	300	0.0439
241	0.0185	304	0.0263
243	0.0093	306	0.0088
245	0.0093	308	0.0175
251	0.0093	310	0.0351
259	0.0093	318	0.0263
261	0.0000	326	0.0088
		332	0.0088
		334	0.0088
		346	0.0088
		350	0.0175
		354	0.0000

Appendix D. Bayesian assignment index. Mean likelihood and mean probability of belonging for each population-population comparison.

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
AF	AF	45	15.73	2.21	11.58	20.34	0.24	0.28	0.00	0.97
	BU	32	15.32	2.68	10.79	24.00	0.30	0.34	0.00	1.00
	ED	44	15.57	2.18	11.18	21.25	0.24	0.30	0.00	0.99
	GU	44	15.78	2.37	10.10	22.45	0.22	0.29	0.00	1.00
	HU	44	16.53	2.60	13.04	23.20	0.18	0.22	0.00	0.71
	JD	35	16.37	2.01	13.02	20.47	0.15	0.20	0.00	0.71
	JRB	24	23.87	6.17	11.50	33.54	0.05	0.20	0.00	0.97
	KR	45	15.93	2.40	10.90	21.83	0.23	0.28	0.00	0.99
	KW	39	15.09	2.30	10.96	23.61	0.31	0.31	0.00	0.99
	LM	45	15.93	2.19	11.83	20.61	0.21	0.27	0.00	0.94
	LT	44	15.38	2.60	10.27	20.60	0.32	0.35	0.00	1.00
	MA	44	15.78	2.48	11.18	21.44	0.26	0.30	0.00	0.99
	NN	45	15.51	2.57	11.59	22.00	0.30	0.36	0.00	0.97
	NS	44	15.45	2.47	11.15	22.29	0.30	0.35	0.00	0.99
	RF	44	16.26	2.01	12.10	20.89	0.16	0.21	0.00	0.91
	RR	37	15.26	2.01	10.83	19.23	0.26	0.30	0.00	1.00
	SH	34	15.47	1.79	11.72	19.25	0.23	0.25	0.00	0.96
	TE	45	15.85	2.16	10.91	19.35	0.23	0.30	0.00	0.99
	TS	6	15.82	2.04	12.38	18.32	0.20	0.34	0.00	0.86
	TU	44	15.11	2.28	10.25	20.25	0.31	0.33	0.00	1.00
	UP	14	15.94	2.06	12.85	21.71	0.16	0.23	0.00	0.75
	XA	48	15.93	2.43	11.50	22.84	0.22	0.28	0.00	0.97
	XB	46	15.76	2.55	11.38	21.40	0.26	0.32	0.00	0.98
	XC	47	15.78	2.25	10.18	19.50	0.21	0.32	0.00	1.00
	XX	47	16.29	2.39	11.27	22.55	0.18	0.25	0.00	0.98
	BU	AF	45	16.56	2.36	12.76	21.87	0.15	0.23	0.00
BU		32	15.42	2.23	11.70	21.11	0.27	0.35	0.00	0.97
ED		44	15.57	2.14	11.41	21.14	0.22	0.28	0.00	0.99
GU		44	16.45	2.49	10.43	21.49	0.16	0.26	0.00	1.00
HU		44	17.34	2.68	12.78	24.51	0.08	0.18	0.00	0.79
JD		35	16.62	2.54	12.78	22.51	0.15	0.24	0.00	0.79
JRB		24	22.61	5.53	12.28	30.54	0.07	0.22	0.00	0.90
KR		45	16.28	2.51	11.40	22.39	0.18	0.28	0.00	0.99
KW		39	16.14	2.44	11.77	23.57	0.19	0.27	0.00	0.96
LM		45	16.74	2.60	12.50	22.88	0.14	0.23	0.00	0.86
LT		44	15.51	2.88	10.03	21.68	0.32	0.37	0.00	1.00
MA		44	16.22	2.42	11.09	21.51	0.19	0.30	0.00	1.00
NN		45	16.21	2.88	11.88	22.39	0.25	0.33	0.00	0.95
NS		44	15.51	2.67	11.02	21.35	0.31	0.36	0.00	1.00
RF		44	16.90	2.07	12.12	20.77	0.10	0.22	0.00	0.92
RR		37	16.23	2.59	10.81	21.58	0.20	0.30	0.00	1.00
SH		34	16.55	2.30	12.08	22.48	0.13	0.25	0.00	0.93
TE		45	16.16	2.06	12.55	20.38	0.16	0.24	0.00	0.84
TS		6	15.19	1.83	12.79	17.30	0.27	0.33	0.00	0.79
TU		44	15.95	2.86	10.20	22.59	0.26	0.33	0.00	1.00
UP		14	16.63	1.58	13.76	19.38	0.08	0.15	0.00	0.48
XA		48	16.42	2.51	12.25	22.22	0.17	0.27	0.00	0.91
XB		46	16.90	2.87	11.75	23.02	0.17	0.27	0.00	0.96
XC		47	16.30	2.31	10.81	20.30	0.16	0.31	0.00	1.00
XX		47	16.61	2.66	12.15	23.82	0.16	0.27	0.00	0.92

Population			Likelihood				Probability				
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max	
ED	AF	45	16.44	2.57	11.85	22.45	0.17	0.26	0.00	0.93	
	BU	32	15.39	2.73	10.96	22.64	0.27	0.30	0.00	0.99	
	ED	44	15.13	2.09	10.77	21.08	0.27	0.29	0.00	1.00	
	GU	44	16.06	2.61	11.13	21.54	0.21	0.30	0.00	0.98	
	HU	44	17.08	2.88	12.33	25.02	0.13	0.24	0.00	0.84	
	JD	35	16.50	2.33	12.32	21.43	0.14	0.20	0.00	0.84	
	JRB	24	22.80	6.11	11.01	32.05	0.08	0.23	0.00	0.99	
	KR	45	16.09	2.45	11.74	21.97	0.20	0.30	0.00	0.94	
	KW	39	15.63	2.41	11.72	22.13	0.23	0.29	0.00	0.94	
	LM	45	16.38	2.63	11.76	22.28	0.17	0.25	0.00	0.94	
	LT	44	15.45	2.51	10.65	20.70	0.28	0.34	0.00	1.00	
	MA	44	15.85	2.61	11.25	23.53	0.23	0.32	0.00	0.98	
	NN	45	15.73	2.95	11.32	22.54	0.29	0.33	0.00	0.97	
	NS	44	15.36	2.97	11.20	23.71	0.33	0.36	0.00	0.98	
	RF	44	16.45	2.26	12.55	22.46	0.14	0.22	0.00	0.79	
	RR	37	16.06	2.45	11.09	21.35	0.19	0.26	0.00	0.99	
	SH	34	16.16	2.47	11.19	20.61	0.18	0.29	0.00	0.98	
	TE	45	15.81	2.32	11.14	21.34	0.21	0.27	0.00	0.98	
	TS	6	14.73	1.10	13.36	16.07	0.25	0.22	0.04	0.56	
	TU	44	15.65	2.93	10.29	23.09	0.28	0.32	0.00	1.00	
	UP	14	16.14	2.46	13.12	21.47	0.17	0.22	0.00	0.63	
	XA	48	16.12	3.09	11.32	24.89	0.23	0.31	0.00	0.97	
	XB	46	16.17	2.51	11.09	21.65	0.19	0.28	0.00	0.99	
	XC	47	16.16	3.18	9.88	22.42	0.21	0.33	0.00	1.00	
	XX	47	16.49	2.59	11.06	23.09	0.17	0.27	0.00	0.99	
	GU	AF	45	16.10	2.13	11.82	21.68	0.21	0.25	0.00	0.96
		BU	32	15.31	2.72	12.04	23.64	0.38	0.33	0.00	0.94
ED		44	15.07	2.15	10.92	22.60	0.36	0.32	0.00	1.00	
GU		44	15.74	2.31	11.56	20.19	0.29	0.33	0.00	0.98	
HU		44	16.49	2.35	11.88	21.39	0.20	0.26	0.00	0.96	
JD		35	16.33	2.10	11.94	20.07	0.20	0.26	0.00	0.95	
JRB		24	23.19	6.14	10.96	31.87	0.08	0.22	0.00	1.00	
KR		45	16.09	2.24	12.08	20.53	0.24	0.30	0.00	0.94	
KW		39	15.02	2.08	10.75	19.79	0.37	0.34	0.00	1.00	
LM		45	15.97	2.38	10.61	20.11	0.27	0.31	0.00	1.00	
LT		44	15.06	2.37	11.06	20.13	0.39	0.36	0.00	0.99	
MA		44	15.34	2.13	10.98	20.39	0.31	0.31	0.00	1.00	
NN		45	15.51	2.44	11.59	23.38	0.32	0.34	0.00	0.98	
NS		44	15.19	2.21	11.11	20.50	0.34	0.34	0.00	0.99	
RF		44	16.42	1.91	13.19	21.05	0.16	0.19	0.00	0.73	
RR		37	15.48	2.19	9.98	20.87	0.27	0.32	0.00	1.00	
SH		34	15.65	2.25	10.70	19.73	0.28	0.31	0.00	1.00	
TE		45	15.53	2.31	10.13	19.93	0.30	0.33	0.00	1.00	
TS		6	14.59	1.61	12.10	16.91	0.39	0.32	0.03	0.94	
TU		44	15.26	2.34	10.52	21.65	0.34	0.33	0.00	1.00	
UP		14	16.09	1.57	13.29	18.48	0.17	0.21	0.00	0.71	
XA		48	15.95	2.40	11.51	21.09	0.26	0.31	0.00	0.98	
XB		46	15.99	2.37	11.22	21.50	0.25	0.29	0.00	0.99	
XC		47	15.53	2.64	10.00	22.30	0.30	0.32	0.00	1.00	
XX		47	15.89	2.03	10.63	20.69	0.22	0.27	0.00	1.00	



Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
HU	AF	45	16.21	1.81	13.13	20.87	0.21	0.25	0.00	0.86
	BU	32	15.40	2.10	11.09	19.11	0.34	0.36	0.00	1.00
	ED	44	15.26	1.89	10.34	20.55	0.35	0.31	0.00	1.00
	GU	44	16.08	2.59	10.24	20.53	0.28	0.36	0.00	1.00
	HU	44	16.28	2.00	13.26	20.75	0.22	0.25	0.00	0.83
	JD	35	16.71	1.82	13.39	21.06	0.14	0.19	0.00	0.79
	JRB	24	24.10	6.19	9.96	35.28	0.04	0.20	0.00	1.00
	KR	45	16.01	2.10	12.73	21.02	0.27	0.29	0.00	0.92
	KW	39	15.15	2.32	10.97	21.16	0.39	0.37	0.00	1.00
	LM	45	16.43	2.65	12.52	23.89	0.25	0.29	0.00	0.95
	LT	44	15.82	2.74	10.79	22.52	0.35	0.38	0.00	1.00
	MA	44	15.41	1.80	10.94	18.97	0.32	0.32	0.00	1.00
	NN	45	15.78	2.53	11.77	21.95	0.34	0.36	0.00	0.99
	NS	44	15.36	1.92	12.23	20.64	0.35	0.33	0.00	0.97
	RF	44	15.88	1.52	12.42	18.62	0.23	0.25	0.00	0.96
	RR	37	15.95	2.11	12.36	19.71	0.28	0.32	0.00	0.96
	SH	34	15.69	2.02	11.86	20.25	0.29	0.33	0.00	0.99
	TE	45	16.23	2.41	11.95	20.76	0.28	0.36	0.00	0.99
	TS	6	14.94	1.80	12.70	17.79	0.42	0.37	0.01	0.93
	TU	44	15.61	2.59	11.16	22.76	0.35	0.34	0.00	1.00
	UP	14	17.13	1.57	15.45	21.19	0.05	0.06	0.00	0.19
	XA	48	15.62	2.50	11.66	24.27	0.35	0.36	0.00	1.00
	XB	46	16.80	2.80	11.85	23.83	0.23	0.32	0.00	0.99
	XC	47	16.34	2.73	10.25	23.22	0.24	0.34	0.00	1.00
	XX	47	16.06	2.25	11.98	23.53	0.26	0.29	0.00	0.99
	JD	AF	45	16.814	3.004	11.9	27.07	0.14	0.20	0.00
BU		32	16.035	2.992	11.15	24.7	0.23	0.31	0.00	0.98
ED		44	15.803	2.183	11.72	20.79	0.21	0.27	0.00	0.94
GU		44	16.378	2.813	10.48	21.55	0.18	0.28	0.00	1.00
HU		44	17.528	2.942	12.96	26.24	0.11	0.19	0.00	0.68
JD		35	16.277	2.289	11.95	20.87	0.17	0.26	0.00	0.91
JRB		24	23.3	6.01	11.95	33.61	0.07	0.19	0.00	0.91
KR		45	16.405	2.723	11.27	23.69	0.18	0.29	0.00	0.97
KW		39	15.809	2.854	10.34	25.25	0.23	0.34	0.00	1.00
LM		45	16.615	2.678	11.61	24.51	0.15	0.25	0.00	0.95
LT		44	15.45	2.754	11.08	23.08	0.29	0.36	0.00	0.98
MA		44	16.046	2.353	10.85	21.11	0.19	0.27	0.00	0.99
NN		45	16.831	2.947	10.68	22.7	0.18	0.29	0.00	0.99
NS		44	15.878	2.732	10.32	22.64	0.24	0.30	0.00	1.00
RF		44	16.825	2.081	11.69	20.91	0.10	0.20	0.00	0.94
RR		37	15.992	2.549	11.7	21.48	0.21	0.30	0.00	0.94
SH		34	15.992	2.121	11.6	21.54	0.17	0.25	0.00	0.95
TE		45	16.388	2.563	11.15	21.31	0.17	0.26	0.00	0.98
TS		6	16.087	2.042	13.38	18.78	0.14	0.21	0.00	0.56
TU		44	15.851	2.856	10.63	23.36	0.24	0.29	0.00	1.00
UP		14	16.299	2.04	13.43	20.24	0.13	0.19	0.00	0.54
XA		48	16.382	2.444	12.21	22.59	0.16	0.23	0.00	0.86
XB		46	16.812	3.094	11	23.48	0.18	0.31	0.00	0.99
XC		47	15.99	3.03	9.96	21.49	0.24	0.34	0.00	1.00
XX		47	16.702	2.563	12.53	20.99	0.16	0.24	0.00	0.79

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
JRB	AF	45	32.25	3.34	23.22	38.24	0.00	0.00	0.00	0.00
	BU	32	30.59	4.05	20.78	37.62	0.00	0.00	0.00	0.00
	ED	44	30.09	3.56	22.59	36.05	0.00	0.00	0.00	0.00
	GU	44	30.96	3.86	21.50	39.69	0.00	0.00	0.00	0.00
	HU	44	31.99	3.88	24.78	38.38	0.00	0.00	0.00	0.00
	JD	35	31.36	3.72	24.41	38.82	0.00	0.00	0.00	0.00
	JRB	24	11.71	3.28	6.82	18.95	0.38	0.45	0.00	1.00
	KR	45	31.67	4.32	20.63	41.66	0.00	0.00	0.00	0.00
	KW	39	31.14	3.60	22.62	39.13	0.00	0.00	0.00	0.00
	LM	45	31.90	3.46	24.74	39.77	0.00	0.00	0.00	0.00
	LT	44	29.34	3.78	21.51	38.10	0.00	0.00	0.00	0.00
	MA	44	30.63	4.23	18.15	38.39	0.00	0.00	0.00	0.00
	NN	45	29.96	4.22	19.64	38.32	0.00	0.00	0.00	0.00
	NS	44	31.05	3.22	25.42	37.42	0.00	0.00	0.00	0.00
	RF	44	31.44	3.19	23.25	37.42	0.00	0.00	0.00	0.00
	RR	37	31.72	3.67	24.88	40.29	0.00	0.00	0.00	0.00
	SH	34	30.90	4.07	23.83	40.89	0.00	0.00	0.00	0.00
	TE	45	31.05	4.28	22.92	39.55	0.00	0.00	0.00	0.00
	TS	6	32.29	4.74	27.57	37.81	0.00	0.00	0.00	0.00
	TU	44	30.10	4.03	22.44	38.32	0.00	0.00	0.00	0.00
	UP	14	32.20	4.02	21.73	39.41	0.00	0.00	0.00	0.00
	XA	48	30.80	3.55	23.84	38.41	0.00	0.00	0.00	0.00
	XB	46	30.51	3.53	22.29	37.67	0.00	0.00	0.00	0.00
	XC	47	30.48	4.15	21.85	37.90	0.00	0.00	0.00	0.00
	XX	47	31.49	3.20	24.65	38.05	0.00	0.00	0.00	0.00
	KR	AF	45	16.15	2.30	11.96	21.05	0.23	0.29	0.00
BU		32	15.26	2.38	10.82	19.56	0.35	0.38	0.00	1.00
ED		44	15.49	2.23	11.41	20.07	0.32	0.34	0.00	0.99
GU		44	16.25	2.06	11.21	20.70	0.18	0.28	0.00	0.99
HU		44	16.82	3.07	11.78	24.55	0.22	0.29	0.00	0.98
JD		35	16.34	1.92	12.49	19.38	0.19	0.27	0.00	0.90
JRB		24	22.70	6.10	11.12	33.39	0.12	0.29	0.00	1.00
KR		45	15.75	2.00	12.03	20.06	0.25	0.29	0.00	0.95
KW		39	15.39	2.75	10.95	25.41	0.35	0.33	0.00	1.00
LM		45	16.53	2.16	11.60	20.46	0.18	0.28	0.00	0.98
LT		44	15.51	2.73	10.21	22.29	0.32	0.37	0.00	1.00
MA		44	16.24	2.77	10.87	21.01	0.26	0.34	0.00	1.00
NN		45	15.98	2.68	10.33	22.10	0.28	0.35	0.00	1.00
NS		44	15.44	2.56	11.46	20.72	0.36	0.36	0.00	0.99
RF		44	16.31	1.86	11.77	20.28	0.16	0.26	0.00	0.98
RR		37	15.53	1.95	12.07	19.85	0.29	0.30	0.00	0.96
SH		34	16.27	2.47	11.00	21.98	0.22	0.30	0.00	1.00
TE		45	16.17	2.20	12.39	22.21	0.20	0.25	0.00	0.92
TS		6	14.83	1.84	11.33	16.14	0.31	0.37	0.07	0.99
TU		44	15.71	2.44	9.93	22.22	0.28	0.31	0.00	1.00
UP		14	16.59	1.92	13.46	19.49	0.15	0.25	0.00	0.68
XA		48	15.81	2.79	11.64	24.18	0.33	0.37	0.00	0.98
XB		46	16.23	2.83	11.73	21.55	0.28	0.34	0.00	0.98
XC		47	15.75	2.49	10.37	21.11	0.27	0.32	0.00	1.00
XX		47	15.93	2.13	11.37	22.58	0.21	0.30	0.00	0.99

Population			Likelihood				Probability				
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max	
KW	AF	45	16.31	2.82	11.24	24.48	0.19	0.26	0.00	0.98	
	BU	32	15.57	2.60	10.75	20.91	0.26	0.33	0.00	1.00	
	ED	44	16.01	1.84	10.82	19.44	0.15	0.23	0.00	0.99	
	GU	44	16.18	2.42	11.15	20.32	0.19	0.30	0.00	0.98	
	HU	44	16.94	2.69	12.26	22.77	0.13	0.22	0.00	0.85	
	JD	35	16.31	2.27	12.63	22.62	0.14	0.20	0.00	0.76	
	JRB	24	23.61	5.94	12.21	32.68	0.06	0.19	0.00	0.86	
	KR	45	16.22	2.22	12.51	22.72	0.16	0.23	0.00	0.80	
	KW	39	15.34	2.68	9.99	26.70	0.26	0.32	0.00	1.00	
	LM	45	16.63	2.77	11.66	22.95	0.17	0.28	0.00	0.95	
	LT	44	15.77	2.95	9.83	23.53	0.26	0.34	0.00	1.00	
	MA	44	15.85	2.68	11.92	21.65	0.25	0.30	0.00	0.91	
	NN	45	16.02	3.15	11.10	22.80	0.28	0.33	0.00	0.99	
	NS	44	16.02	2.98	11.07	24.30	0.25	0.32	0.00	0.99	
	RF	44	16.02	1.99	11.71	20.79	0.16	0.22	0.00	0.94	
	RR	37	15.62	2.01	10.45	20.70	0.20	0.26	0.00	1.00	
	SH	34	16.10	2.31	11.63	20.75	0.18	0.27	0.00	0.95	
	TE	45	16.05	2.16	11.58	20.19	0.18	0.26	0.00	0.96	
	TS	6	15.62	1.45	14.62	18.33	0.13	0.09	0.00	0.22	
	TU	44	15.54	2.29	11.63	21.65	0.24	0.27	0.00	0.95	
	UP	14	16.68	1.71	13.56	19.19	0.08	0.14	0.00	0.50	
	XA	48	16.34	3.09	11.78	22.79	0.22	0.30	0.00	0.93	
	XB	46	17.04	3.24	11.08	26.04	0.15	0.26	0.00	0.99	
	XC	47	15.95	2.91	9.47	23.12	0.22	0.33	0.00	1.00	
	XX	47	16.38	2.53	11.53	23.70	0.17	0.25	0.00	0.96	
	LM	AF	45	15.87	2.00	12.70	20.00	0.25	0.29	0.00	0.88
		BU	32	15.20	2.41	11.75	21.87	0.38	0.34	0.00	0.99
		ED	44	15.43	2.16	10.47	21.42	0.30	0.31	0.00	1.00
		GU	44	15.86	2.47	10.83	21.67	0.27	0.33	0.00	1.00
		HU	44	16.56	2.53	12.56	21.45	0.21	0.27	0.00	0.91
JD		35	16.16	2.32	12.28	22.19	0.24	0.29	0.00	0.94	
JRB		24	23.56	6.02	11.30	32.58	0.07	0.23	0.00	1.00	
KR		45	15.99	2.43	11.52	21.57	0.26	0.32	0.00	0.99	
KW		39	15.34	2.74	10.70	26.22	0.35	0.34	0.00	1.00	
LM		45	16.10	2.21	12.65	22.89	0.22	0.29	0.00	0.90	
LT		44	15.07	2.41	10.81	22.20	0.39	0.36	0.00	1.00	
MA		44	15.64	2.26	12.28	21.17	0.31	0.33	0.00	0.94	
NN		45	15.55	2.48	11.13	20.36	0.32	0.37	0.00	1.00	
NS		44	15.03	2.35	10.23	20.89	0.39	0.37	0.00	1.00	
RF		44	16.19	1.96	12.35	20.27	0.20	0.28	0.00	0.94	
RR		37	15.80	2.58	11.13	22.14	0.28	0.35	0.00	1.00	
SH		34	15.87	2.39	11.38	22.46	0.28	0.34	0.00	1.00	
TE		45	15.83	2.59	11.15	23.31	0.29	0.34	0.00	1.00	
TS		6	15.42	2.48	12.13	19.78	0.30	0.34	0.00	0.96	
TU		44	15.04	2.41	11.11	21.61	0.41	0.36	0.00	1.00	
UP		14	16.01	2.11	13.07	20.54	0.22	0.24	0.00	0.80	
XA		48	15.80	2.78	12.26	25.73	0.32	0.32	0.00	0.95	
XB		46	15.92	2.77	10.62	22.90	0.29	0.36	0.00	1.00	
XC		47	15.87	2.92	11.06	22.58	0.30	0.35	0.00	1.00	
XX		47	15.87	2.31	11.68	21.10	0.27	0.31	0.00	0.99	

Population			Likelihood				Probability				
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max	
LT	AF	45	17.03	2.60	11.73	22.18	0.14	0.25	0.00	0.92	
	BU	32	16.12	3.13	11.37	25.79	0.22	0.28	0.00	0.96	
	ED	44	15.98	2.70	10.51	24.04	0.20	0.28	0.00	1.00	
	GU	44	16.63	2.89	11.68	24.99	0.16	0.22	0.00	0.92	
	HU	44	17.48	3.02	12.68	24.80	0.12	0.19	0.00	0.74	
	JD	35	16.33	2.03	12.12	20.58	0.13	0.22	0.00	0.86	
	JRB	24	23.34	6.27	10.73	34.28	0.07	0.23	0.00	0.99	
	KR	45	16.78	2.70	10.82	21.72	0.15	0.25	0.00	0.99	
	KW	39	15.95	2.65	11.24	25.54	0.20	0.27	0.00	0.96	
	LM	45	16.79	2.48	11.68	23.13	0.13	0.24	0.00	0.92	
	LT	44	14.75	2.73	10.95	21.76	0.40	0.38	0.00	0.98	
	MA	44	16.39	2.54	11.94	22.13	0.18	0.25	0.00	0.89	
	NN	45	16.32	3.02	11.34	23.61	0.21	0.29	0.00	0.96	
	NS	44	15.90	2.79	11.66	22.50	0.25	0.30	0.00	0.93	
	RF	44	17.26	2.21	13.60	22.82	0.08	0.13	0.00	0.49	
	RR	37	16.55	2.55	10.44	23.13	0.15	0.25	0.00	1.00	
	SH	34	16.64	2.45	12.84	22.98	0.13	0.19	0.00	0.70	
	TE	45	16.64	2.12	10.60	20.49	0.11	0.21	0.00	0.99	
	TS	6	16.74	2.67	11.54	18.81	0.16	0.38	0.00	0.94	
	TU	44	16.16	2.70	12.64	23.41	0.20	0.22	0.00	0.75	
	UP	14	16.98	1.43	14.40	19.56	0.05	0.08	0.00	0.28	
	XA	48	16.60	2.84	11.90	28.55	0.15	0.22	0.00	0.89	
	XB	46	16.53	3.04	10.95	21.70	0.22	0.30	0.00	0.98	
	XC	47	16.37	2.70	9.77	22.73	0.18	0.30	0.00	1.00	
	XX	47	16.19	2.35	11.16	22.51	0.18	0.28	0.00	0.97	
	MA	AF	45	16.59	2.36	12.53	22.31	0.13	0.20	0.00	0.78
		BU	32	15.72	2.79	11.17	22.79	0.25	0.30	0.00	0.97
		ED	44	15.86	2.29	10.38	21.81	0.19	0.24	0.00	1.00
		GU	44	16.14	2.40	11.30	20.51	0.20	0.30	0.00	0.96
		HU	44	16.69	2.65	12.46	23.21	0.15	0.22	0.00	0.79
		JD	35	16.62	2.08	12.37	21.58	0.10	0.17	0.00	0.82
		JRB	24	23.70	5.64	12.61	31.52	0.04	0.16	0.00	0.76
KR		45	16.46	2.32	11.65	20.90	0.15	0.27	0.00	0.93	
KW		39	15.58	2.54	10.43	22.76	0.25	0.32	0.00	1.00	
LM		45	16.76	2.67	11.66	23.98	0.13	0.22	0.00	0.93	
LT		44	15.82	2.67	10.48	22.91	0.24	0.29	0.00	1.00	
MA		44	15.41	2.08	11.71	19.66	0.25	0.28	0.00	0.93	
NN		45	16.14	2.84	11.74	22.20	0.23	0.30	0.00	0.92	
NS		44	15.74	3.09	10.54	22.62	0.29	0.33	0.00	1.00	
RF		44	17.12	2.14	13.74	23.21	0.07	0.12	0.00	0.45	
RR		37	16.78	2.49	12.19	22.13	0.14	0.23	0.00	0.85	
SH		34	16.24	1.72	10.72	20.14	0.11	0.19	0.00	0.99	
TE		45	16.39	2.83	11.07	24.18	0.20	0.30	0.00	0.98	
TS		6	15.46	1.78	12.48	17.32	0.21	0.31	0.01	0.79	
TU		44	15.96	2.62	10.70	24.83	0.19	0.26	0.00	0.99	
UP	14	16.68	1.50	14.09	19.06	0.06	0.09	0.00	0.36		
XA	48	15.99	2.37	12.19	23.25	0.20	0.25	0.00	0.85		
XB	46	16.92	2.83	11.71	23.39	0.14	0.24	0.00	0.93		
XC	47	16.38	3.17	9.81	22.42	0.22	0.32	0.00	1.00		
XX	47	16.64	2.49	11.76	25.48	0.13	0.21	0.00	0.92		

Population			Likelihood				Probability				
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max	
NN	AF	45	15.80	2.12	11.61	20.11	0.27	0.30	0.00	0.99	
	BU	32	15.14	2.34	10.26	19.97	0.37	0.35	0.00	1.00	
	ED	44	15.21	2.13	11.28	20.11	0.35	0.31	0.00	1.00	
	GU	44	15.73	2.23	10.32	21.02	0.27	0.29	0.00	1.00	
	HU	44	16.02	2.07	12.68	20.55	0.24	0.27	0.00	0.89	
	JD	35	16.30	1.98	13.05	19.58	0.21	0.27	0.00	0.81	
	JRB	24	23.15	6.09	11.23	32.42	0.07	0.22	0.00	1.00	
	KR	45	15.52	2.02	11.42	19.31	0.30	0.29	0.00	0.99	
	KW	39	15.17	2.03	10.47	21.17	0.33	0.30	0.00	1.00	
	LM	45	15.98	2.05	11.60	21.08	0.23	0.28	0.00	0.99	
	LT	44	15.41	2.51	11.29	20.55	0.37	0.35	0.00	1.00	
	MA	44	15.76	2.38	10.04	22.33	0.29	0.33	0.00	1.00	
	NN	45	15.81	3.13	10.46	23.00	0.35	0.39	0.00	1.00	
	NS	44	15.39	2.46	11.20	21.33	0.37	0.36	0.00	1.00	
	RF	44	16.18	1.78	13.30	20.25	0.19	0.23	0.00	0.75	
	RR	37	15.57	2.32	10.72	21.41	0.30	0.34	0.00	1.00	
	SH	34	15.68	2.12	12.31	20.19	0.30	0.31	0.00	0.94	
	TE	45	16.00	2.23	12.02	21.55	0.25	0.31	0.00	0.97	
	TS	6	15.12	2.02	12.80	18.05	0.37	0.36	0.00	0.87	
	TU	44	15.33	2.13	11.01	21.94	0.33	0.31	0.00	1.00	
	UP	14	15.95	2.02	13.12	21.00	0.23	0.24	0.00	0.80	
	XA	48	15.92	2.64	11.35	24.02	0.29	0.33	0.00	1.00	
	XB	46	15.89	2.81	11.47	21.51	0.32	0.36	0.00	0.99	
	XC	47	15.75	2.39	10.46	20.96	0.26	0.33	0.00	1.00	
	XX	47	16.05	2.41	11.74	25.27	0.23	0.29	0.00	0.98	
	NS	AF	45	16.36	2.32	11.73	22.03	0.16	0.25	0.00	0.93
		BU	32	15.47	2.60	10.67	21.03	0.28	0.32	0.00	0.99
		ED	44	15.40	1.90	11.83	19.69	0.23	0.25	0.00	0.92
		GU	44	16.09	2.47	10.49	21.97	0.18	0.27	0.00	1.00
		HU	44	16.74	2.64	12.36	23.12	0.13	0.19	0.00	0.82
		JD	35	16.33	2.13	12.40	21.10	0.15	0.23	0.00	0.81
		JRB	24	24.07	6.21	10.97	34.00	0.05	0.20	0.00	0.99
KR		45	16.21	3.06	11.04	23.32	0.22	0.29	0.00	0.98	
KW		39	15.46	2.63	11.71	26.41	0.26	0.31	0.00	0.93	
LM		45	16.05	2.30	12.53	21.79	0.18	0.24	0.00	0.78	
LT		44	15.21	2.33	10.52	20.05	0.29	0.31	0.00	1.00	
MA		44	15.54	1.93	12.34	19.97	0.22	0.25	0.00	0.82	
NN		45	16.25	2.84	10.82	24.06	0.21	0.30	0.00	0.99	
NS		44	15.28	2.80	11.33	20.41	0.34	0.38	0.00	0.98	
RF		44	16.16	1.81	12.55	19.24	0.14	0.21	0.00	0.78	
RR		37	15.57	2.14	10.59	19.71	0.22	0.30	0.00	0.99	
SH		34	15.79	2.24	11.16	19.97	0.21	0.26	0.00	0.98	
TE		45	15.67	2.22	11.68	20.49	0.23	0.30	0.00	0.93	
TS		6	14.74	2.98	11.33	19.61	0.41	0.40	0.00	0.97	
TU		44	15.51	2.80	10.73	22.73	0.29	0.33	0.00	0.99	
UP		14	16.43	1.84	12.70	19.37	0.10	0.19	0.00	0.74	
XA		48	15.93	2.75	11.94	23.18	0.24	0.29	0.00	0.90	
XB		46	16.79	3.08	10.86	22.68	0.18	0.27	0.00	0.99	
XC		47	16.14	2.73	9.80	23.35	0.19	0.31	0.00	1.00	
XX	47	16.16	2.55	11.90	25.50	0.18	0.23	0.00	0.91		

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
RF	AF	45	15.97	2.13	12.62	21.02	0.25	0.28	0.00	0.91
	BU	32	15.15	2.23	11.13	19.59	0.36	0.38	0.00	1.00
	ED	44	15.26	1.79	11.60	19.23	0.33	0.30	0.00	0.99
	GU	44	16.10	2.27	10.94	22.07	0.23	0.31	0.00	1.00
	HU	44	16.29	2.28	12.76	23.25	0.22	0.27	0.00	0.89
	JD	35	15.93	1.74	12.64	20.71	0.21	0.25	0.00	0.91
	JRB	24	21.89	5.46	11.15	31.78	0.09	0.24	0.00	1.00
	KR	45	15.74	2.24	12.31	20.13	0.31	0.32	0.00	0.95
	KW	39	14.72	2.49	10.95	25.56	0.45	0.36	0.00	1.00
	LM	45	16.15	2.40	12.51	21.66	0.26	0.32	0.00	0.93
	LT	44	15.46	2.52	10.39	20.44	0.35	0.36	0.00	1.00
	MA	44	15.63	2.11	11.50	22.37	0.29	0.31	0.00	1.00
	NN	45	15.78	2.68	10.56	23.33	0.31	0.35	0.00	1.00
	NS	44	15.25	2.53	11.10	20.89	0.38	0.37	0.00	1.00
	RF	44	16.15	1.94	12.04	20.43	0.20	0.26	0.00	0.98
	RR	37	15.47	2.04	10.93	19.34	0.29	0.31	0.00	1.00
	SH	34	15.51	2.20	11.11	21.69	0.31	0.33	0.00	1.00
	TE	45	15.72	2.04	11.52	21.07	0.28	0.33	0.00	1.00
	TS	6	15.03	2.53	12.36	19.41	0.44	0.39	0.00	0.95
	TU	44	14.93	2.28	10.46	21.26	0.40	0.33	0.00	1.00
	UP	14	16.22	1.52	14.08	19.47	0.13	0.14	0.00	0.52
	XA	48	15.62	2.60	11.25	23.43	0.33	0.35	0.00	1.00
	XB	46	16.19	2.44	11.81	21.19	0.25	0.33	0.00	0.99
	XC	47	15.62	2.18	10.17	21.75	0.27	0.32	0.00	1.00
	XX	47	15.93	2.29	11.57	24.37	0.26	0.29	0.00	0.99
	RR	AF	45	15.99	2.75	11.76	22.60	0.24	0.31	0.00
BU		32	15.77	3.05	11.33	23.54	0.29	0.35	0.00	0.99
ED		44	16.26	2.35	13.41	22.24	0.16	0.20	0.00	0.57
GU		44	16.46	2.46	9.79	21.77	0.14	0.26	0.00	1.00
HU		44	17.32	2.93	12.02	24.61	0.12	0.22	0.00	0.93
JD		35	16.33	2.50	11.84	21.26	0.18	0.26	0.00	0.95
JRB		24	23.68	6.53	10.17	34.35	0.07	0.23	0.00	1.00
KR		45	16.31	2.72	10.48	21.95	0.20	0.28	0.00	1.00
KW		39	15.29	2.90	9.96	25.92	0.30	0.35	0.00	1.00
LM		45	17.04	2.67	12.25	23.67	0.12	0.23	0.00	0.89
LT		44	16.20	3.27	10.92	24.65	0.26	0.35	0.00	1.00
MA		44	16.90	3.00	11.75	26.73	0.16	0.26	0.00	0.96
NN		45	16.01	3.03	11.31	23.15	0.28	0.35	0.00	0.99
NS		44	15.97	2.85	10.98	23.16	0.24	0.34	0.00	1.00
RF		44	16.65	2.28	13.01	22.37	0.11	0.17	0.00	0.70
RR		37	15.39	2.60	11.05	24.25	0.27	0.31	0.00	1.00
SH		34	16.34	2.54	10.38	21.80	0.16	0.29	0.00	1.00
TE		45	16.18	2.37	11.92	21.44	0.19	0.29	0.00	0.94
TS		6	16.08	1.32	13.63	17.30	0.10	0.19	0.00	0.50
TU		44	15.72	2.61	9.54	21.48	0.25	0.31	0.00	1.00
UP		14	16.27	2.34	13.32	21.80	0.14	0.20	0.00	0.60
XA		48	16.91	2.62	12.16	23.01	0.13	0.23	0.00	0.90
XB		46	16.94	3.46	11.55	25.95	0.21	0.33	0.00	0.97
XC		47	16.15	2.98	9.78	23.67	0.21	0.33	0.00	1.00
XX		47	16.36	2.71	11.16	21.84	0.19	0.31	0.00	0.99

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
SH	AF	45	16.07	2.54	12.74	22.77	0.24	0.25	0.00	0.83
	BU	32	16.09	2.86	10.66	22.28	0.26	0.38	0.00	1.00
	ED	44	15.99	2.60	10.69	22.38	0.25	0.31	0.00	1.00
	GU	44	16.15	2.16	11.50	20.85	0.20	0.28	0.00	0.98
	HU	44	17.08	2.70	11.93	25.68	0.13	0.22	0.00	0.96
	JD	35	16.38	1.91	12.35	21.01	0.15	0.23	0.00	0.91
	JRB	24	24.20	6.21	10.96	34.41	0.05	0.20	0.00	1.00
	KR	45	16.72	2.57	11.62	22.80	0.18	0.29	0.00	0.98
	KW	39	15.73	2.52	11.18	22.43	0.27	0.32	0.00	0.99
	LM	45	16.44	2.61	11.39	24.06	0.20	0.30	0.00	0.99
	LT	44	15.65	2.24	12.31	21.11	0.29	0.30	0.00	0.91
	MA	44	16.02	2.06	11.85	20.54	0.21	0.26	0.00	0.96
	NN	45	15.97	2.62	11.67	25.41	0.25	0.31	0.00	0.97
	NS	44	15.76	2.43	11.89	20.74	0.29	0.34	0.00	0.96
	RF	44	16.97	2.09	13.41	22.74	0.11	0.18	0.00	0.65
	RR	37	16.24	2.18	11.17	20.07	0.19	0.31	0.00	0.99
	SH	34	16.03	2.13	12.20	19.91	0.22	0.28	0.00	0.93
	TE	45	16.16	2.49	11.95	21.98	0.23	0.31	0.00	0.96
	TS	6	15.17	1.64	12.94	16.72	0.29	0.32	0.02	0.78
	TU	44	15.51	2.51	10.72	22.09	0.31	0.32	0.00	1.00
	UP	14	16.51	2.31	13.52	23.60	0.11	0.16	0.00	0.62
	XA	48	16.23	2.45	11.27	21.37	0.21	0.31	0.00	0.99
	XB	46	16.48	2.77	10.89	23.20	0.21	0.30	0.00	1.00
	XC	47	15.87	2.51	10.17	21.55	0.25	0.31	0.00	1.00
	XX	47	16.38	2.62	12.37	22.67	0.23	0.30	0.00	0.90
	TE	AF	45	16.31	2.12	13.35	21.48	0.17	0.20	0.00
BU		32	14.85	2.36	11.29	20.04	0.41	0.35	0.00	0.99
ED		44	14.99	2.07	11.26	21.68	0.34	0.32	0.00	0.99
GU		44	15.49	2.44	10.68	20.52	0.30	0.34	0.00	1.00
HU		44	16.63	2.50	11.72	24.26	0.16	0.23	0.00	0.97
JD		35	15.96	2.26	12.66	22.37	0.22	0.25	0.00	0.83
JRB		24	23.35	6.32	11.66	32.90	0.08	0.24	0.00	0.97
KR		45	16.13	2.44	11.12	21.37	0.23	0.27	0.00	0.99
KW		39	15.04	2.18	11.03	22.38	0.34	0.33	0.00	1.00
LM		45	16.02	2.60	12.02	22.88	0.26	0.30	0.00	0.94
LT		44	15.33	2.46	10.34	20.61	0.34	0.35	0.00	1.00
MA		44	15.49	2.26	11.19	22.69	0.28	0.30	0.00	0.99
NN		45	15.92	3.04	11.24	23.14	0.31	0.36	0.00	0.99
NS		44	14.94	2.37	10.78	19.36	0.38	0.36	0.00	1.00
RF		44	16.29	2.01	12.95	20.80	0.17	0.23	0.00	0.76
RR		37	15.07	2.40	10.32	21.51	0.35	0.33	0.00	1.00
SH		34	15.87	2.33	11.15	21.12	0.25	0.29	0.00	0.99
TE		45	15.89	2.19	12.21	22.32	0.24	0.29	0.00	0.93
TS		6	15.11	1.78	12.36	17.13	0.30	0.36	0.02	0.89
TU		44	15.24	2.76	10.74	23.30	0.36	0.34	0.00	1.00
UP		14	15.50	1.74	13.25	19.01	0.25	0.24	0.00	0.69
XA		48	15.62	2.50	11.60	21.71	0.30	0.34	0.00	0.97
XB		46	16.07	2.91	10.76	21.89	0.27	0.33	0.00	1.00
XC		47	15.65	2.55	9.77	23.16	0.27	0.33	0.00	1.00
XX		47	16.22	2.21	11.67	21.93	0.19	0.27	0.00	0.97

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
TS	AF	45	19.72	2.79	13.62	26.78	0.00	0.00	0.00	0.00
	BU	32	18.38	3.08	12.73	24.56	0.00	0.01	0.00	0.03
	ED	44	18.35	2.73	13.78	25.12	0.00	0.00	0.00	0.00
	GU	44	18.65	2.78	12.78	24.61	0.00	0.00	0.00	0.03
	HU	44	20.00	2.93	13.58	27.81	0.00	0.00	0.00	0.00
	JD	35	19.83	2.75	12.38	24.26	0.00	0.01	0.00	0.08
	JRB	24	23.63	5.66	11.57	32.86	0.01	0.06	0.00	0.31
	KR	45	19.30	2.86	14.76	26.50	0.00	0.00	0.00	0.00
	KW	39	19.39	2.58	14.25	27.82	0.00	0.00	0.00	0.00
	LM	45	19.77	3.09	13.12	27.94	0.00	0.00	0.00	0.01
	LT	44	18.08	3.07	11.78	27.04	0.01	0.04	0.00	0.24
	MA	44	18.73	2.40	13.45	23.74	0.00	0.00	0.00	0.00
	NN	45	18.84	3.06	12.63	26.12	0.00	0.01	0.00	0.04
	NS	44	17.93	3.16	10.94	24.14	0.02	0.10	0.00	0.62
	RF	44	19.86	2.56	13.62	25.79	0.00	0.00	0.00	0.00
	RR	37	19.24	2.28	14.18	24.12	0.00	0.00	0.00	0.00
	SH	34	18.46	3.14	11.26	25.47	0.02	0.08	0.00	0.46
	TE	45	19.40	2.83	11.95	24.11	0.00	0.03	0.00	0.18
	TS	6	21.26	3.17	17.68	25.89	0.00	0.00	0.00	0.00
	TU	44	18.19	3.64	9.94	25.12	0.04	0.19	0.00	0.94
	UP	14	21.63	2.18	17.32	25.10	0.00	0.00	0.00	0.00
	XA	48	19.29	3.21	12.87	29.47	0.00	0.00	0.00	0.02
	XB	46	19.22	2.53	13.59	22.80	0.00	0.00	0.00	0.00
	XC	47	18.87	2.81	12.84	24.55	0.00	0.00	0.00	0.02
	XX	47	18.87	2.64	14.44	26.12	0.00	0.00	0.00	0.00
	TU	AF	45	15.77	2.16	12.06	21.72	0.26	0.29	0.00
BU		32	15.59	2.83	11.07	23.05	0.32	0.33	0.00	1.00
ED		44	15.31	2.38	11.30	24.36	0.34	0.31	0.00	0.99
GU		44	15.67	2.30	11.24	22.75	0.27	0.31	0.00	0.99
HU		44	16.52	2.54	12.31	21.88	0.21	0.29	0.00	0.91
JD		35	16.11	2.02	12.21	19.39	0.21	0.28	0.00	0.92
JRB		24	23.15	5.64	11.20	31.17	0.08	0.25	0.00	0.99
KR		45	15.84	2.33	11.53	21.00	0.26	0.31	0.00	0.98
KW		39	15.25	2.49	11.48	26.07	0.32	0.32	0.00	0.98
LM		45	15.87	2.39	11.81	22.59	0.27	0.31	0.00	0.96
LT		44	15.39	2.81	10.75	22.70	0.36	0.33	0.00	1.00
MA		44	15.55	2.19	11.15	19.97	0.28	0.34	0.00	0.99
NN		45	15.42	2.66	10.86	21.38	0.35	0.37	0.00	1.00
NS		44	15.33	2.69	9.91	22.68	0.35	0.32	0.00	1.00
RF		44	15.95	1.85	12.46	19.40	0.21	0.27	0.00	0.89
RR		37	15.25	1.95	11.26	19.49	0.31	0.32	0.00	0.99
SH		34	15.39	2.18	10.24	20.73	0.30	0.30	0.00	1.00
TE		45	15.66	2.23	11.22	20.49	0.28	0.32	0.00	0.99
TS		6	14.79	1.55	12.44	16.93	0.35	0.31	0.02	0.89
TU		44	15.55	2.93	10.23	23.81	0.34	0.34	0.00	1.00
UP		14	16.38	2.19	13.93	22.67	0.14	0.14	0.00	0.52
XA		48	15.75	2.29	12.11	20.70	0.28	0.31	0.00	0.93
XB		46	15.90	2.59	11.19	21.05	0.27	0.31	0.00	0.99
XC		47	15.66	2.43	10.58	20.28	0.28	0.33	0.00	1.00
XX		47	15.82	2.22	12.00	23.36	0.24	0.27	0.00	0.95



Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
UP	AF	45	18.16	2.39	12.75	23.03	0.03	0.10	0.00	0.49
	BU	32	17.46	3.32	12.72	24.90	0.07	0.14	0.00	0.50
	ED	44	17.28	2.72	11.26	22.21	0.07	0.20	0.00	0.93
	GU	44	18.28	3.26	12.14	24.81	0.04	0.13	0.00	0.71
	HU	44	18.94	2.76	13.30	24.84	0.01	0.05	0.00	0.30
	JD	35	18.16	2.80	13.63	22.88	0.02	0.04	0.00	0.21
	JRB	24	21.35	5.48	11.28	29.62	0.10	0.26	0.00	0.93
	KR	45	17.92	3.23	11.45	26.95	0.05	0.16	0.00	0.89
	KW	39	17.22	2.74	10.05	22.96	0.07	0.20	0.00	1.00
	LM	45	17.76	2.77	12.29	23.99	0.05	0.14	0.00	0.66
	LT	44	17.15	2.85	12.65	23.22	0.05	0.12	0.00	0.53
	MA	44	17.94	3.38	9.19	23.48	0.08	0.22	0.00	1.00
	NN	45	17.64	3.24	11.12	27.42	0.08	0.23	0.00	0.95
	NS	44	17.70	2.84	11.53	22.25	0.05	0.16	0.00	0.88
	RF	44	18.44	2.36	13.39	24.02	0.02	0.06	0.00	0.27
	RR	37	17.86	3.10	11.73	24.14	0.06	0.17	0.00	0.83
	SH	34	17.68	3.43	11.63	25.63	0.08	0.21	0.00	0.85
	TE	45	17.31	2.64	11.30	24.10	0.06	0.18	0.00	0.92
	TS	6	19.70	2.25	16.62	22.10	0.00	0.00	0.00	0.00
	TU	44	17.92	3.11	12.01	26.82	0.06	0.17	0.00	0.75
	UP	14	18.67	3.43	12.60	24.80	0.06	0.17	0.00	0.59
	XA	48	17.33	2.90	11.91	24.75	0.05	0.15	0.00	0.78
	XB	46	18.17	2.95	13.47	24.35	0.02	0.05	0.00	0.25
	XC	47	17.88	3.11	11.49	23.74	0.05	0.15	0.00	0.89
	XX	47	17.91	2.70	12.46	22.88	0.04	0.11	0.00	0.59
	XA	AF	45	16.18	1.91	12.58	19.82	0.20	0.26	0.00
BU		32	15.63	2.63	10.38	22.61	0.31	0.35	0.00	1.00
ED		44	15.50	2.23	11.37	23.29	0.29	0.30	0.00	0.99
GU		44	16.00	2.57	10.56	23.69	0.25	0.33	0.00	1.00
HU		44	16.33	2.09	12.45	20.76	0.19	0.25	0.00	0.90
JD		35	15.87	1.87	13.10	19.85	0.23	0.24	0.00	0.77
JRB		24	23.51	6.31	10.96	33.38	0.09	0.24	0.00	1.00
KR		45	15.81	2.32	12.01	21.43	0.28	0.30	0.00	0.95
KW		39	15.62	2.80	11.62	27.84	0.32	0.32	0.00	0.98
LM		45	16.25	2.30	12.33	21.30	0.22	0.29	0.00	0.92
LT		44	15.53	2.43	11.36	21.16	0.33	0.33	0.00	0.99
MA		44	15.52	2.19	11.22	19.60	0.32	0.32	0.00	0.99
NN		45	15.76	2.96	10.86	23.94	0.34	0.36	0.00	1.00
NS		44	15.61	2.70	11.09	21.76	0.34	0.34	0.00	1.00
RF		44	16.14	1.67	11.99	20.28	0.16	0.20	0.00	0.95
RR		37	15.56	2.14	10.92	20.49	0.27	0.31	0.00	1.00
SH		34	15.76	2.45	11.31	22.43	0.28	0.32	0.00	0.99
TE		45	15.75	2.28	11.48	20.67	0.29	0.33	0.00	0.99
TS		6	15.34	2.08	11.79	17.73	0.27	0.36	0.01	0.97
TU		44	15.40	2.42	10.66	22.95	0.32	0.30	0.00	1.00
UP		14	16.14	1.31	13.83	19.41	0.13	0.15	0.00	0.57
XA		48	15.64	2.74	11.75	23.75	0.34	0.34	0.00	0.98
XB		46	16.06	2.57	11.99	22.95	0.27	0.31	0.00	0.95
XC		47	15.99	2.18	10.87	23.10	0.20	0.28	0.00	1.00
XX		47	15.71	2.13	11.32	21.75	0.26	0.31	0.00	0.99

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
XB	AF	45	15.90	1.92	12.19	19.88	0.24	0.29	0.00	0.96
	BU	32	15.92	2.48	11.55	23.47	0.29	0.32	0.00	0.99
	ED	44	15.42	2.33	10.92	21.61	0.36	0.30	0.00	1.00
	GU	44	15.88	2.45	10.68	21.63	0.29	0.32	0.00	1.00
	HU	44	17.02	2.75	12.18	22.98	0.19	0.27	0.00	0.96
	JD	35	16.37	2.30	13.16	22.02	0.23	0.27	0.00	0.81
	JRB	24	21.94	6.40	11.37	32.41	0.12	0.27	0.00	1.00
	KR	45	16.12	2.03	11.78	20.91	0.22	0.27	0.00	0.98
	KW	39	15.96	2.65	11.25	25.93	0.28	0.31	0.00	1.00
	LM	45	15.98	2.06	11.53	21.11	0.24	0.28	0.00	0.99
	LT	44	14.98	2.23	11.09	20.12	0.41	0.36	0.00	1.00
	MA	44	15.95	2.45	11.32	22.82	0.29	0.30	0.00	1.00
	NN	45	15.51	2.70	10.10	22.69	0.34	0.36	0.00	1.00
	NS	44	15.71	2.93	10.40	25.32	0.37	0.38	0.00	1.00
	RF	44	16.53	2.00	12.72	21.34	0.16	0.21	0.00	0.89
	RR	37	15.94	2.47	10.93	20.18	0.28	0.32	0.00	1.00
	SH	34	15.75	2.09	10.76	19.56	0.27	0.30	0.00	1.00
	TE	45	15.82	2.22	11.42	20.14	0.29	0.34	0.00	0.99
	TS	6	15.27	1.97	11.65	17.32	0.28	0.37	0.01	0.99
	TU	44	15.46	2.39	11.34	22.17	0.35	0.32	0.00	1.00
	UP	14	16.19	1.74	13.23	20.26	0.17	0.22	0.00	0.79
	XA	48	15.80	2.47	12.06	22.62	0.31	0.33	0.00	0.97
	XB	46	15.79	2.89	11.29	22.77	0.36	0.37	0.00	1.00
	XC	47	15.60	2.51	10.11	21.31	0.31	0.33	0.00	1.00
	XX	47	16.33	2.26	11.26	21.71	0.22	0.27	0.00	1.00
	XC	AF	45	16.17	2.41	12.13	23.09	0.20	0.26	0.00
BU		32	15.65	2.52	11.57	22.29	0.27	0.32	0.00	0.97
ED		44	15.51	2.19	10.80	20.88	0.25	0.27	0.00	1.00
GU		44	16.06	2.38	10.82	21.38	0.20	0.28	0.00	1.00
HU		44	17.01	2.69	12.17	23.08	0.14	0.22	0.00	0.91
JD		35	16.01	1.82	11.94	21.05	0.16	0.24	0.00	0.94
JRB		24	23.39	6.17	10.66	31.32	0.08	0.25	0.00	1.00
KR		45	16.27	2.36	12.22	21.39	0.19	0.28	0.00	0.90
KW		39	15.40	2.53	11.02	21.87	0.31	0.35	0.00	0.99
LM		45	16.53	2.62	11.95	23.60	0.19	0.28	0.00	0.94
LT		44	15.27	2.77	9.85	22.03	0.34	0.34	0.00	1.00
MA		44	15.88	2.20	12.12	22.66	0.22	0.27	0.00	0.92
NN		45	16.25	2.51	11.02	24.04	0.19	0.27	0.00	0.99
NS		44	15.87	2.98	11.00	25.03	0.28	0.33	0.00	0.99
RF		44	16.41	1.97	12.74	22.33	0.13	0.20	0.00	0.80
RR		37	15.88	2.22	10.89	21.00	0.21	0.30	0.00	1.00
SH		34	15.63	1.98	10.40	18.86	0.22	0.30	0.00	1.00
TE		45	16.30	2.38	11.70	22.44	0.18	0.25	0.00	0.96
TS		6	15.92	2.51	11.18	18.10	0.19	0.39	0.00	0.99
TU		44	15.82	2.49	11.17	22.42	0.26	0.31	0.00	0.99
UP		14	16.35	2.03	14.41	22.68	0.10	0.10	0.00	0.33
XA		48	16.35	2.64	12.08	24.17	0.20	0.27	0.00	0.92
XB		46	16.18	2.61	10.53	21.12	0.23	0.30	0.00	1.00
XC		47	15.29	2.28	10.16	20.86	0.27	0.33	0.00	1.00
XX		47	16.30	2.78	11.13	23.74	0.22	0.31	0.00	0.99

Population			Likelihood				Probability			
Original	Target	N	Mean	StDev	Min	Max	Mean	StDev	Min	Max
XX	AF	45	16.50	2.32	11.76	21.12	0.18	0.26	0.00	0.96
	BU	32	15.38	2.41	10.59	20.28	0.31	0.36	0.00	1.00
	ED	44	15.49	2.16	12.08	21.40	0.29	0.29	0.00	0.93
	GU	44	15.79	2.07	11.33	21.16	0.22	0.28	0.00	0.99
	HU	44	16.70	2.76	12.69	25.43	0.18	0.23	0.00	0.83
	JD	35	16.35	2.35	12.86	21.57	0.18	0.22	0.00	0.80
	JRB	24	24.33	6.58	10.99	33.33	0.08	0.25	0.00	0.99
	KR	45	15.74	2.43	12.23	24.34	0.26	0.30	0.00	0.91
	KW	39	15.38	2.39	11.03	25.29	0.27	0.29	0.00	0.99
	LM	45	16.35	2.52	11.91	20.72	0.22	0.31	0.00	0.95
	LT	44	15.28	2.69	9.82	22.06	0.34	0.35	0.00	1.00
	MA	44	15.78	2.16	11.53	20.33	0.24	0.31	0.00	0.98
	NN	45	15.73	2.67	11.23	22.65	0.29	0.35	0.00	0.99
	NS	44	15.43	2.45	11.49	20.82	0.31	0.31	0.00	0.98
	RF	44	16.34	2.01	13.11	22.35	0.16	0.21	0.00	0.73
	RR	37	15.74	2.07	12.00	19.74	0.24	0.30	0.00	0.94
	SH	34	15.96	2.54	11.40	23.52	0.24	0.28	0.00	0.98
	TE	45	16.12	2.38	12.29	21.84	0.23	0.29	0.00	0.91
	TS	6	15.18	2.00	11.91	18.19	0.27	0.34	0.00	0.95
	TU	44	15.45	2.63	11.26	25.94	0.29	0.29	0.00	0.99
	UP	14	16.42	1.56	14.24	19.55	0.10	0.13	0.00	0.40
	XA	48	15.74	2.57	11.07	21.76	0.29	0.33	0.00	0.99
	XB	46	16.05	2.55	11.19	23.28	0.24	0.30	0.00	0.99
	XC	47	15.98	2.73	10.63	21.79	0.24	0.35	0.00	1.00
	XX	47	15.63	2.17	10.77	21.46	0.26	0.31	0.00	1.00

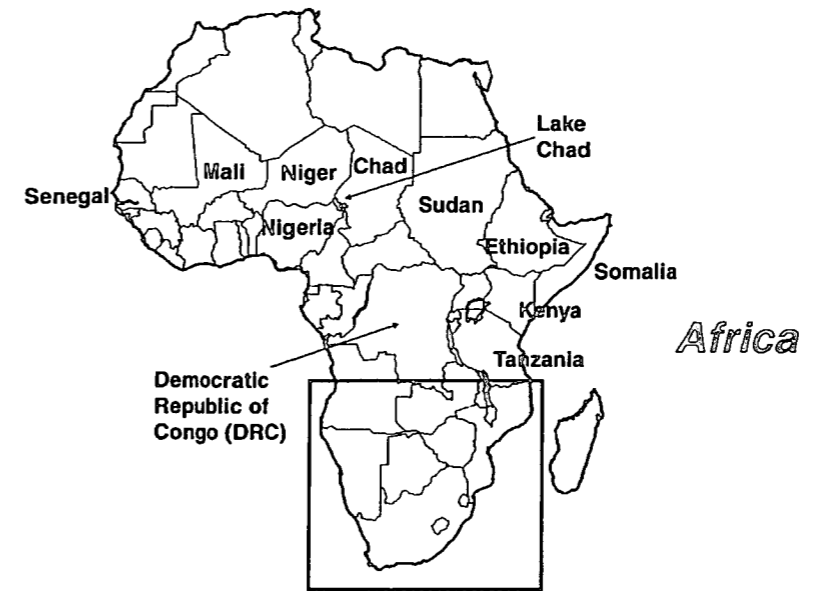
## Appendix E. Microsatellite allele frequencies for female quelea.

Locus	Allele	AF	BU	JD	KR	NS	RF	XB	XX	Overall
Esc4	N	20	25	19	17	17	16	20	18	152
	150	0.000	0.000	0.026	0.000	0.029	0.031	0.000	0.000	0.011
	152	0.025	0.040	0.026	0.000	0.000	0.094	0.025	0.000	0.026
	154	0.025	0.020	0.000	0.000	0.029	0.031	0.000	0.000	0.013
	156	0.025	0.080	0.053	0.088	0.059	0.000	0.175	0.056	0.067
	158	0.025	0.060	0.000	0.147	0.088	0.031	0.075	0.000	0.053
	160	0.025	0.040	0.053	0.029	0.029	0.063	0.025	0.028	0.036
	162	0.075	0.100	0.026	0.029	0.059	0.031	0.025	0.056	0.050
	164	0.100	0.160	0.105	0.206	0.029	0.031	0.225	0.194	0.131
	166	0.125	0.020	0.132	0.059	0.029	0.031	0.075	0.111	0.073
	168	0.250	0.120	0.158	0.118	0.118	0.219	0.125	0.111	0.152
	170	0.050	0.100	0.079	0.118	0.118	0.125	0.075	0.111	0.097
	172	0.075	0.100	0.132	0.059	0.088	0.094	0.025	0.111	0.085
	174	0.100	0.080	0.105	0.029	0.059	0.063	0.075	0.139	0.081
	176	0.025	0.040	0.026	0.088	0.177	0.125	0.000	0.028	0.064
	178	0.050	0.000	0.000	0.029	0.059	0.031	0.000	0.028	0.025
	180	0.025	0.020	0.026	0.000	0.000	0.000	0.000	0.000	0.009
	182	0.000	0.000	0.026	0.000	0.029	0.000	0.075	0.028	0.020
184	0.000	0.020	0.026	0.000	0.000	0.000	0.000	0.000	0.006	
Hru5	N	27	30	21	21	22	28	21	24	194
	110	0.019	0.083	0.095	0.000	0.068	0.036	0.024	0.021	0.043
	111	0.130	0.083	0.000	0.167	0.046	0.071	0.143	0.083	0.090
	112	0.093	0.050	0.071	0.048	0.114	0.036	0.071	0.000	0.060
	113	0.019	0.067	0.095	0.095	0.091	0.054	0.024	0.146	0.074
	114	0.074	0.100	0.095	0.071	0.046	0.125	0.071	0.021	0.075
	115	0.148	0.117	0.048	0.024	0.091	0.054	0.119	0.104	0.088
	116	0.111	0.033	0.119	0.024	0.136	0.161	0.071	0.063	0.090
	117	0.185	0.183	0.048	0.119	0.159	0.054	0.191	0.333	0.159
	118	0.019	0.067	0.143	0.119	0.046	0.125	0.095	0.042	0.082
	119	0.093	0.100	0.119	0.238	0.023	0.107	0.095	0.063	0.105
	120	0.019	0.017	0.095	0.000	0.046	0.071	0.048	0.021	0.039
	121	0.037	0.017	0.024	0.000	0.023	0.018	0.048	0.021	0.023
	122	0.000	0.017	0.048	0.000	0.046	0.018	0.000	0.021	0.019
	123	0.019	0.033	0.000	0.000	0.023	0.000	0.000	0.000	0.009
	124	0.000	0.017	0.000	0.024	0.000	0.018	0.000	0.042	0.013
	125	0.019	0.000	0.000	0.024	0.000	0.000	0.000	0.021	0.008
	126	0.019	0.000	0.000	0.024	0.023	0.018	0.000	0.000	0.010
	127	0.000	0.000	0.000	0.024	0.000	0.018	0.000	0.000	0.005
	128	0.000	0.017	0.000	0.000	0.000	0.018	0.000	0.000	0.004
129	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.003	

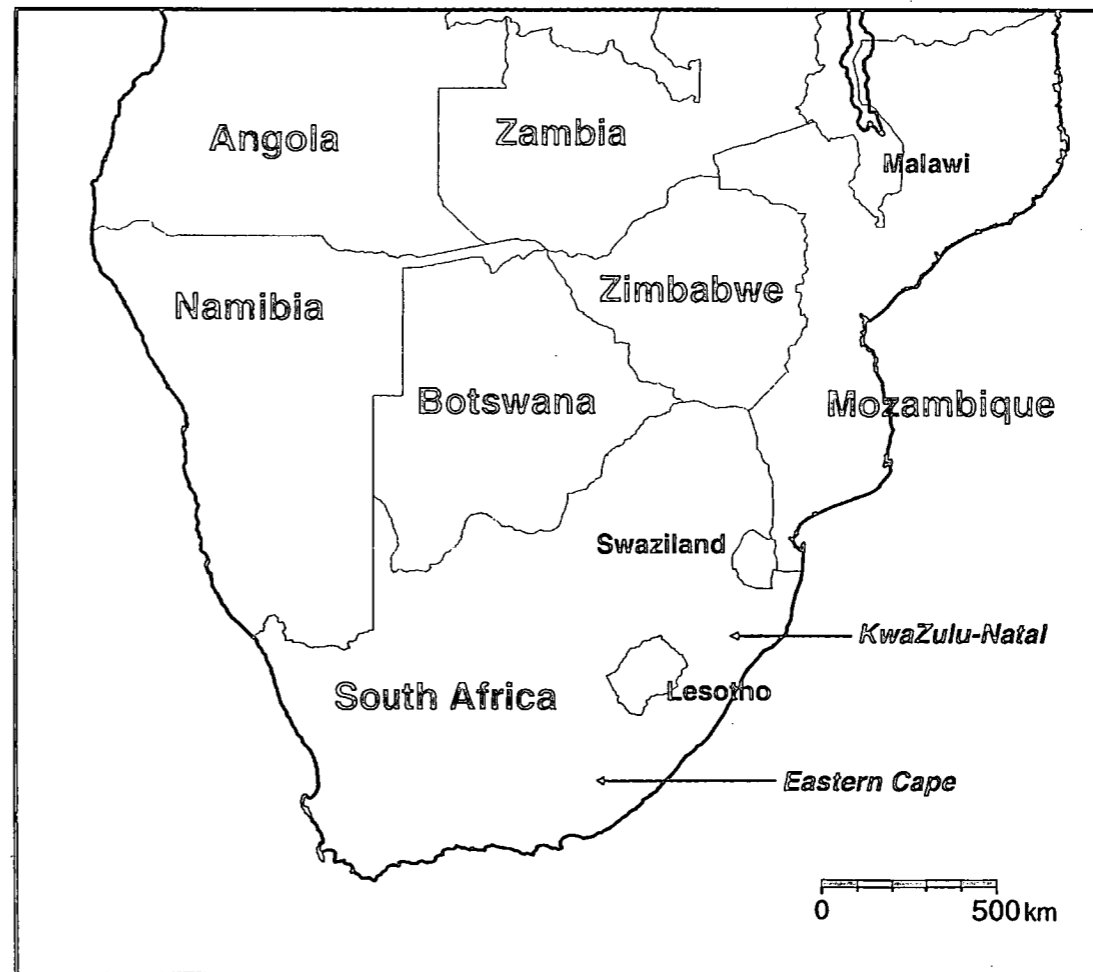
Locus	Allele	AF	BU	JD	KR	NS	RF	XB	XX	Overall
Mcyu4	N	28	0	20	21	22	28	0	16	135
	142	0.000	-	0.025	0.000	0.000	0.000	-	0.000	0.003
	144	0.000	-	0.000	0.024	0.000	0.000	-	0.000	0.003
	146	0.000	-	0.000	0.000	0.000	0.036	-	0.000	0.004
	148	0.000	-	0.000	0.000	0.000	0.018	-	0.031	0.006
	150	0.036	-	0.025	0.024	0.000	0.018	-	0.063	0.021
	152	0.054	-	0.025	0.024	0.068	0.071	-	0.000	0.030
	154	0.089	-	0.000	0.214	0.182	0.179	-	0.125	0.099
	156	0.089	-	0.150	0.071	0.136	0.071	-	0.094	0.077
	158	0.179	-	0.075	0.214	0.068	0.161	-	0.250	0.118
	160	0.161	-	0.275	0.048	0.227	0.143	-	0.063	0.115
	162	0.143	-	0.200	0.214	0.136	0.054	-	0.094	0.105
	164	0.089	-	0.075	0.071	0.114	0.107	-	0.063	0.065
	166	0.054	-	0.050	0.024	0.046	0.036	-	0.094	0.038
	168	0.036	-	0.000	0.024	0.023	0.000	-	0.094	0.022
	170	0.018	-	0.025	0.000	0.000	0.036	-	0.031	0.014
	172	0.054	-	0.025	0.024	0.000	0.000	-	0.000	0.013
	174	0.000	-	0.050	0.000	0.000	0.054	-	0.000	0.013
176	0.000	-	0.000	0.024	0.000	0.000	-	0.000	0.003	
188	0.000	-	0.000	0.000	0.000	0.018	-	0.000	0.002	
Phtr2	N	28	31	21	21	22	18	22	23	186
	107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044	0.005
	115	0.018	0.000	0.000	0.000	0.023	0.028	0.000	0.000	0.009
	117	0.446	0.452	0.619	0.524	0.409	0.389	0.386	0.348	0.447
	119	0.071	0.129	0.143	0.048	0.159	0.194	0.136	0.065	0.118
	121	0.268	0.274	0.167	0.381	0.273	0.278	0.341	0.348	0.291
	123	0.018	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.005
	125	0.125	0.113	0.071	0.024	0.068	0.056	0.068	0.130	0.082
	127	0.036	0.016	0.000	0.024	0.000	0.056	0.046	0.044	0.028
	129	0.018	0.016	0.000	0.000	0.046	0.000	0.023	0.022	0.015
WBSW2	N	28	34	21	21	21	28	27	23	203
	209	0.018	0.000	0.024	0.000	0.000	0.018	0.000	0.000	0.007
	211	0.018	0.103	0.048	0.024	0.000	0.018	0.093	0.022	0.041
	213	0.125	0.132	0.000	0.119	0.214	0.089	0.111	0.109	0.112
	215	0.018	0.000	0.024	0.048	0.000	0.000	0.019	0.022	0.016
	217	0.018	0.029	0.000	0.000	0.024	0.018	0.056	0.065	0.026
	219	0.089	0.000	0.024	0.095	0.048	0.000	0.019	0.022	0.037
	221	0.125	0.132	0.071	0.119	0.024	0.196	0.148	0.196	0.126
	223	0.036	0.074	0.024	0.000	0.071	0.071	0.000	0.087	0.045
	225	0.286	0.177	0.524	0.262	0.310	0.268	0.333	0.283	0.305
	227	0.107	0.088	0.143	0.048	0.048	0.107	0.019	0.044	0.075
	229	0.071	0.191	0.095	0.167	0.167	0.125	0.148	0.087	0.131
	231	0.000	0.000	0.000	0.024	0.048	0.000	0.000	0.022	0.012
	233	0.054	0.015	0.024	0.048	0.000	0.018	0.037	0.022	0.027
	235	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.022	0.006
	237	0.036	0.015	0.000	0.000	0.048	0.036	0.019	0.000	0.019
	239	0.000	0.015	0.000	0.000	0.000	0.018	0.000	0.000	0.004
	241	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.003
245	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.002	
247	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.003	

Locus	Allele	AF	BU	JD	KR	NS	RF	XB	XX	Overall
WBSW4	N	28	34	21	21	22	28	27	24	205
	135	0.054	0.029	0.048	0.000	0.000	0.000	0.019	0.021	0.021
	137	0.054	0.044	0.024	0.048	0.068	0.000	0.000	0.063	0.037
	139	0.000	0.015	0.000	0.000	0.046	0.018	0.000	0.021	0.012
	141	0.018	0.015	0.000	0.048	0.000	0.125	0.037	0.063	0.038
	143	0.089	0.059	0.024	0.000	0.023	0.054	0.056	0.042	0.043
	145	0.054	0.118	0.214	0.095	0.068	0.107	0.093	0.104	0.107
	147	0.054	0.059	0.095	0.095	0.091	0.054	0.056	0.042	0.068
	149	0.107	0.074	0.048	0.119	0.068	0.071	0.111	0.063	0.083
	151	0.089	0.015	0.048	0.048	0.023	0.036	0.037	0.083	0.047
	153	0.071	0.059	0.048	0.071	0.000	0.018	0.019	0.042	0.041
	155	0.000	0.059	0.024	0.048	0.068	0.036	0.019	0.146	0.050
	157	0.036	0.074	0.071	0.119	0.068	0.054	0.093	0.021	0.067
	159	0.089	0.074	0.048	0.024	0.091	0.054	0.074	0.021	0.059
	161	0.054	0.118	0.048	0.191	0.114	0.036	0.056	0.063	0.085
	163	0.054	0.059	0.048	0.000	0.000	0.071	0.019	0.042	0.036
	165	0.036	0.029	0.095	0.000	0.046	0.000	0.148	0.063	0.052
	167	0.071	0.044	0.048	0.071	0.114	0.125	0.037	0.021	0.066
	169	0.018	0.029	0.048	0.024	0.023	0.054	0.074	0.021	0.036
	171	0.000	0.000	0.024	0.000	0.046	0.018	0.019	0.021	0.016
	173	0.054	0.029	0.000	0.000	0.023	0.054	0.019	0.000	0.022
	179	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.002
	181	0.000	0.000	0.000	0.000	0.023	0.018	0.000	0.000	0.005
	187	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.003
	189	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.003





*Southern Africa*



Map 1. Africa and southern Africa. Countries indicated are mentioned in the text. Provinces in South Africa are in italics.



Country/Locality	Host plant	Genotypic class
SE France, St Blaise (Hengy)	Cucumber	A
SE France, St Blaise (Biagini)	Zucchini	D
SE France, Antibes	Cucumber	B
SE France, Pegomas	Cucumber	C
S France, Cambous	Pumpkin	B
S France, Avignon	Melon	A
S France, Navacelles	Pumpkin	A
Algeria	Cucumber	E
La Réunion	Melon	F
Laos, Vientiane	Cucumber	G
Laos, Vientiane	Cotton	H
Burkina Faso	Cotton	I
Ivory Coast, Bouaké	Cotton	J
SE France, Frejus	Chrysanthemum	K
SE France, Porquerolles	Citrus	L
SE France, Corse	Citrus	M
SE France, Golfe Juan	Citrus	M
Spain, Valencia	Citrus	N
SW France, Orx	Potato	D
Spain, Mijas	Potato	O
Portugal	Hibiscus	P

**Table 2** Origin and genotypic class of the 21 clones

All eight loci were polymorphic, having between three and 10 alleles. This number of alleles was encouraging given that only one to three aphids per locality and host plant were assayed. Moreover, we were able to define 16 genotypic classes (combinations of alleles at all eight loci borne by individual aphids) out of the 21 clones (Table 2). Therefore, microsatellite markers should prove to be very useful for assessing the genetic variability within the species *A. gossypii*, the importance and occurrence of sexual reproduction, and the factors leading to genetic differentiation among *A. gossypii* populations.

#### Acknowledgements

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## Cross-species amplification success of avian microsatellites in the redbilled quelea *Quelea quelea*

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Microsatellites are widely used in the study of natural populations (Queller *et al.* 1993; Jarne & Lagoda 1996) and have many positive attributes including hypervariability, abundance, and tolerance to sample quality and quantity. Their main disadvantage is the need to characterize species-specific loci. This is because PCR primers often require a high degree of homology in the flanking regions to allow adequate annealing. Mutations in the flanking regions may prevent amplification, with the probability of mismatch due to mutations related to the evolutionary distance between species. Several avian studies show that microsatellites cloned from one species will amplify homologous products in a closely related species, but not in an evolutionary distant species (Hanotte *et al.* 1994; Primmer *et al.* 1996b; Fields & Scribner 1997; Piertney & Dallas 1997; Hughes *et al.* 1998; Piertney *et al.* 1998b).

The studies cited above tested novel microsatellites from one species on a range of other species. This study takes the opposite approach by testing available avian microsatellite primers in the redbilled quelea *Quelea quelea* (order:

Passeriformes, family: Passeridae). The redbilled quelea is an abundant pest of grain crops in sub-Saharan Africa. Estimates of the total African population range as high as 100 billion (Crook & Ward 1968). Seventy-three primer sets from 16 species representing nine families were tested on quelea DNA. The success of the primers in amplifying homologous products was then compared to the evolutionary distance between quelea and the source species proposed by Sibley & Ahlquist (1990).

Quelea blood samples were collected in Bulawayo, Zimbabwe in November 1997. A volume of 3 µL of blood was extracted in 200 µL of 5% w/v Chelex-100 resin (Walsh *et al.* 1991). Two microlitres of the resulting supernatant containing the DNA was used as a PCR template.

Seventy-three primer sets were tested with two different PCR conditions: (i) a 10-µL reaction mix containing 0.1 mM dATP, dGTP and dTTP; 0.01 mM dCTP; 2 pmol each primer;

1× 'Parr' buffer containing 1.5 mM MgCl<sub>2</sub> (Cambio); 0.5 mM MgCl<sub>2</sub> (final MgCl<sub>2</sub> concentration 2.0 mM), 0.25 U *Taq* polymerase (Advanced Biotechnologies) and < 1 µCi [α<sup>32</sup>P]-dCTP; (ii) as above but containing 1.5 mM MgCl<sub>2</sub> (final MgCl<sub>2</sub> concentration 3.0 mM), and 60 mM tetramethylammonium chloride/2.5% formamide (TMACIDE) (Gemmell 1997). This mix often resulted in gels that were easier to score. Reactions were overlaid with one drop of mineral oil and amplified in a Hybaid Omnigene Temperature Cycler. The PCR profile was as follows: 2 min denaturing step at 93 °C; seven cycles of 93 °C for 30 s denaturation, 50 °C for 1 min annealing and 72 °C for 30 s extension; then 25 cycles of 93 °C for 30 s denaturation, followed by 52 °C for 1 min annealing and 72 °C for 30 s extension. Products were separated on 6% polyacrylamide sequencing gels and visualized on X-ray film (Bancroft *et al.* 1995).

Each marker was tested using between four and 10 quelea

**Table 1** Avian microsatellite loci tested and successes. Families as in Sibley and Ahlquist (1990)

Loci				
Tested	Successful	Species	Family	Source
WBSW1,2,4,7-11	WBSW1	<i>Plocepasser mahali</i>	Passeridae (p)	McRae & Amos (1999)
	WBSW2	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW4	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW9	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW10	<i>Plocepasser mahali</i>	Passeridae (p)	
	WBSW11	<i>Plocepasser mahali</i>	Passeridae (p)	
Pdou3	Pdou3	<i>Passer domesticus</i>	Passeridae (p)	Neumann and Wetton (1996)
ESC4	ESC4	<i>Emberiza schoeniclus</i>	Fringillidae (p)	Hannotte <i>et al.</i> (1994)
LOX1-8	LOX3	<i>Loxia scotia</i>	Fringillidae (p)	Piertney <i>et al.</i> (1998b)
	LOX6	<i>Loxia scotia</i>	Fringillidae (p)	
	LOX8	<i>Loxia scotia</i>	Fringillidae (p)	
HrU1-10	HrU1	<i>Hirundo rustica</i>	Hirundinidae (p)	Primmer <i>et al.</i> (1996b)*
	HrU5	<i>Hirundo rustica</i>	Hirundinidae (p)	
	HrU6	<i>Hirundo rustica</i>	Hirundinidae (p)	
	HrU7	<i>Hirundo rustica</i>	Hirundinidae (p)	
Phtr1-4	Phtr2	<i>Phylloscopus trochilus</i>	Sylviidae (p)	Fridolfsson <i>et al.</i> (1997)
	Phtr3	<i>Phylloscopus trochilus</i>	Sylviidae (p)	
Pocc1,2,5,6,8	Pocc6	<i>Phylloscopus occipitalis</i>	Sylviidae (p)	Bensch <i>et al.</i> (1997)
FhU1-6	FhU2	<i>Ficedula hypoleuca</i>	Muscicapidae (p)	Primmer <i>et al.</i> (1996a)
	FhU3	<i>Ficedula hypoleuca</i>	Muscicapidae (p)	
	FhU5	<i>Ficedula hypoleuca</i>	Muscicapidae (p)	
Mycu1-8	Mycu4	<i>Malurus cyaneus</i>	Maluridae (p)	Double <i>et al.</i> (1997)
PcD2,6	None	<i>Phalacrocorax carbo</i>	Phalacrocoracidae	Piertney <i>et al.</i> (1998a)
PcT3,4	None	<i>Phalacrocorax carbo</i>	Phalacrocoracidae	
LLSD2,7,10	None	<i>Lagopus lagopus</i>	Phasianidae	Piertney & Dallas (1997)
LLST1	None	<i>Lagopus lagopus</i>	Phasianidae	
ADL102,158,172,176	None	<i>Gallus gallus</i>	Phasianidae	United States Poultry Gene Mapping†
Bca5,6,10,11	None	<i>Branta canadensis</i>	Anatidae	Buchholz <i>et al.</i> (1998)
WFG2,8	None	<i>Anser albifrons</i>	Anatidae	Fields and Scribner (1997)
Hhi1,3,5	None	<i>Histrionicus histrionicus</i>	Anatidae	Buchholz <i>et al.</i> (1998)
44 (Sfi)	None	<i>Somateria fischeri</i>	Anatidae	Fields and Scribner (1997)

(p), passerine families.

\*And refs therein.

†'Population Tester Kit' (<http://poultry.mph.msu.edu/index.256.htm>)

Table 2 Amplification success in the redbilled quelea and evolutionary distance from the source family of successful loci. Family names as Sibley and Ahlquist (1990)

Family	$\Delta T_{50}H$	Number of loci			Average Heterozygosity
		Tested	Successful	Proportion success	
Passeridae (p)	6.65	9	7	0.78	0.57
Fringillidae (p)	10	9	4	0.44	0.55
Hirundinidae (p)	11.1	10	4	0.40	0.80
Sylviidae (p)	11.1	9	3	0.33	0.62
Muscicapidae (p)	11.7	6	3	0.50	0.33
Maluridae (p)	12.8	8	1	0.13	0.60
Phalacrocoracidae	21.6	4	0	0.00	–
Phasianidae	28	8	0	0.00	–
Anatidae	28	10	0	0.00	–
Passerines		51	22	0.43	
Nonpasserines		22	0	0.00	
Total		73	22	0.30	

(p), passerine families.

DNA samples. A sample of DNA from the original species was also included when available. A locus that was detected in quelea DNA was considered homologous when major bands occurred similar in size to that expected in the original species. In general positive amplifications also displayed characteristic 'stutter' bands that typify microsatellite loci. A high proportion of amplified loci were polymorphic (see below) which also aided their identification as microsatellite products.

Of the 73 primer pairs tested, 22 pairs produced homologous amplification products in the quelea (Table 1), of which 21 gave a polymorphic product. Loci isolated from species more closely related to the quelea were more likely to amplify successfully (Table 2). Six out of eight markers from the most closely related species (Sibley & Ahlquist 1990), *Plocepasser mahali*, gave a product, and 22 of 51 loci (43%) derived from passerines (maximum  $\Delta T_{50}H = 12.8$ ) gave a product, including at least one locus from each species. In contrast, no loci cloned from nonpasserines yielded a homologous product. There was a negative relationship between evolutionary distance and the proportion of loci that amplified successfully ( $y = 1.45 - 0.0991x$ ;  $r^2 = 0.967$ ). If this linear function is appropriate, it suggests that no loci would amplify from species of evolutionary distance  $\Delta T_{50}H$  14.6 (95% Confidence Interval (CI)  $\pm 1.12$ ) to quelea, which is at the upper end of the range  $\Delta T_{50}H = 10$ –15 suggested by Primmer *et al.* (1996b). However, the lack of species in the evolutionary distance range between *Malurus* ( $\Delta T_{50}H$  12.8 to quelea) and *Phalacrocorax* ( $\Delta T_{50}H$  21.6 to quelea) in this study means this figure is speculative. Perhaps a more useful measure is the evolutionary distance at which 50% of loci tested may be expected to amplify a product. The value predicted by the linear model is  $\Delta T_{50}H = 9.6$  (95% CI  $\pm 0.55$ ), considerably further in evolutionary distance than the previous estimates of  $\Delta T_{50}H = 6.7$  (Primmer *et al.* 1996b) and  $\Delta T_{50}H = 3.9$  (Hughes *et al.* 1998).

As 21 of 22 loci amplified across species were polymorphic, there was no obvious dependence of polymorphism on the evolutionary distance separating the source species from quelea. Nor was there any correlation between heterozygosity in quelea and evolutionary distance to quelea ( $r^2 = 0.128$ ,  $P = 0.344$ ). Although the relationship between evolutionary distance and polymorphism has been shown to apply in many other studies (e.g. Primmer *et al.* 1996b), another possibility in this case is that the high level of polymorphism is consistent with the idea that abundant species maintain more variation at neutral loci (Hartl & Clark 1989) rather than any dependence of polymorphism upon evolutionary distance.

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## Polymorphic microsatellite DNA markers for *Magnolia obovata* Thunb. and their utility in related species

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The genus *Magnolia* is an archaic taxon of angiosperms where most have flowers with primitive morphological characters (Doyle & Donoghue 1986). The flowers do not secrete nectar, and the pollinators are beetles which are thought to be less efficient than bees. The flowers are protogynous which usually close between the female and male period. Most *Magnolia* species possess these floral characteristics in common (Thien 1974). These interesting floral and reproductive characteristics have prompted a number of studies on the reproductive and pollination biology of *Magnolia* (e.g. Thien 1974; Kikuzawa & Mizui 1990; Yasukawa *et al.* 1992; Thien *et al.* 1995; Ishida 1996).

*Magnolia obovata* is a large deciduous tree reaching 30 m in height. Standing density of the adult reproductive trees in forest ecosystems is low: usually a few per hectare. In Japanese temperate forests, such tree species are common and dominate the ecosystem as a whole. Hence, in order to elucidate the structure and maintenance mechanism of biological diversity of forest ecosystems, it is important to analyse their characteristics of pollination, seed dispersal and the regeneration process under the low standing density in forest ecosystems. For this purpose, we developed highly polymorphic, codominant microsatellite markers for *M. obovata*.

Crude genomic DNA of *M. obovata* was extracted from leaves sampled from adult trees growing in Ibaraki Pref., Japan, using the CTAB method (Milligan 1992), and then digested with *Mbo*I. Fragments ranging between 300 and 600 bp were ligated to pUC19 which had been digested with *Bam*HI. Recombinant colonies formed on agar plates were lifted with positively charged nylon membranes (Hybond-N<sup>+</sup>, Amersham), and screened using two synthetic oligonucleotides (GA)<sub>20</sub> and (CA)<sub>20</sub>, labelled by DIG oligonucleotide tailing according to the supplier's instructions (Boehringer Mannheim). Positive clones were sequenced on an ABI377 sequencing instrument and PCR primers were designed with the aid of the computer program OLIGO (National Bioscience). PCR amplifications were performed using a thermal cycler Perkin-Elmer 9600, under the following conditions: initial denaturing at 94 °C for 9 min, then 30 cycles of denaturation at 94 °C for 30 s, annealing for 30 s, and extension at 72 °C for 1 min, followed by a final incubation at 72 °C for 7 min. The volume of the reaction mixture was 10 µL containing 10 ng of DNA from *M. obovata*, 5 pmol of