

Cooperative breeding and density regulation
in small island populations of the Seychelles warbler



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**Cooperative breeding and density regulation
in small island populations of the Seychelles warbler**

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Chapter

Introduction



Currently, one eighth of all bird species, one quarter of all mammals, and one third of all amphibians are threatened with extinction (Beissinger & McCullough 2002). Biodiversity threats are especially prominent in the tropics, where biodiversity is highest and habitat loss and fragmentation is proceeding at an ever increasing pace. As a result, conservation biology in the tropics often has to deal with small isolated populations. Understanding the factors that influence the number of individuals within such populations and determining how we can manipulate such factors is one of the main challenges ecologists now face.

Population regulation

Survival, reproduction and dispersal are the key-processes in regulating numbers and thereby population dynamics (Murdoch 1994; Sæther *et al.* 2002). An important discussion in population ecology focuses on the question of how, and to what extent, density-dependent and density-independent processes affect fluctuations in numbers (Sibly & Hone 2003). Density independent factors, e.g. climate, affect reproduction and survival at all population sizes in contrast to density dependent factors, which change in effect as populations grow. Understanding what determines changes in numbers, especially when population sizes are small, is crucial not only for our understanding of population regulation and persistence but also for the development of a predictive theory of how to manage and conserve threatened populations. The mechanism by which density dependence occurs determines to a large extent the outcome of evolutionary life history models (Mylius & Diekmann 1995), quantitative models of cooperative breeding (Pen & Weissling 2000), but also population viability analyses, which provide a quantitative assessment of the probability of a population to decline to extinction (Beissinger & Westphal 1998).

Studying what causes demographic rates (and therefore population numbers) to change requires long-term data sets with complete knowledge about individual life histories. Dispersal is an interesting phenomenon and important for the persistence of many populations, for example, by maintaining genetic variation (Keller *et al.* 2001), however incomplete knowledge of dispersal in most study systems causes local survival to be confounded by dispersal away from the study site. This problem is avoided by studying effects of density dependence in closed populations.

Although stable natural populations may be regulated in a strong density dependent manner, density dependence will often be impossible to detect, or confounded because density covaries with density independent factors like climate (Murdoch 1994; Sæther 1997). Therefore, experiments are essential to assess the causality of density dependence on demographic rates. The detection of density dependence may also depend on the spatial scale at which such effects are investigated (e.g. Ray & Hastings 1996; Wilkin *et al.* 2006). Intra-specific competition for resources, one of the main density dependent processes (Newton 1992), will work mainly on a local scale in territorial species. The size of the territory and number of

individuals living in it will, therefore, be the appropriate measure of density with which to investigate such effects (Both & Visser 2000).

With increasing densities competition will increase and the strength of selection will also increase. Furthermore, the traits selected under high densities might differ from those selected for under low densities, e.g. under high densities selection will favour cooperative breeding behaviour by increasing reproductive success of groups compared to pairs or individuals are selected to be relatively large at high density because at high competition it is more advantageous to be larger (Both *et al.* 1999).

Tropical environment

A characteristic of many tropical islands is that their faunas are particularly rich in endemic species and form a disproportional large part of global biodiversity (Collar & Stuart 1985; Stattersfield & Capper 2000). However, in the last few centuries these faunas have become the most restricted and threatened in the world, mainly because of anthropogenic factors such as habitat loss, disturbance and the introduction of predators or competitor species (Stattersfield & Capper 2000). Nonetheless, although many studies have investigated population regulation in temperate species there is little knowledge about how factors, such as environmental variation, affect survival and fecundity in tropical species (Martin 1996). In tropical areas, environmental factors are generally considered to vary relatively little between seasons (Karr 1976; Martin & Karr 1986; Loiselle & Blake 1991, 1992). Life histories are typically characterised by small clutches, long developmental periods and the extended parental care of juveniles associated with high survival probabilities for both juveniles and adults (Skutch 1949; Lack 1954; Martin 1996). Furthermore cooperatively breeding species are more prevalent in (sub)tropical areas than in temperate zones (Arnold & Owens 1999a). All these factors suggest that population regulation may differ considerably between tropical and temperate regions.

Small populations

Chance effects on birth and death rates become a particular risk in small populations (Diamond 1984; Pimm *et al.* 1988). Furthermore, at very low densities an increase in population size can have a positive effect on population growth, i.e. inverse density dependence, known as the Allee effect (Allee 1951; for review see: Courchamp *et al.* 1999). Another important feature of small population sizes is that with decreasing population sizes the chances of mating with relatives will increase and detrimental effects of inbreeding might threaten the persistence of small populations (Keller & Waller 2002).

The translocations of individuals to new suitable habitat is a powerful tool in the fight to save endangered species and could potentially become a major component

of conservation management worldwide (Fischer & Lindenmayer 2000; Bell & Merton 2002). Such (re)introductions provide a great opportunity to study density dependence as these populations usually start at very low densities, after which changes in vital rates can be studied as the population grows (Nicoll *et al.* 2003; Armstrong *et al.* 2005). Interestingly, increasing population densities can also have a large impact on behaviour: as density increases, breeding vacancies become rare and individuals have to delay reproduction, which is thought to facilitate the evolution of cooperative breeding (Koenig & Pitelka 1981; Emlen 1982).

Cooperative breeding

In cooperatively breeding species individuals refrain from reproduction and instead help to rear kin instead of reproducing themselves. This intriguing behaviour occurs in at least 3% of bird and mammal species (Brown 1987) and in some fishes (Taborsky 1994). Increasing density may not only promote cooperative breeding, but interestingly, cooperative breeding might in turn affect the way that density dependence shapes demographic rates; higher density may be advantageous if individuals survive or reproduce better in larger cooperative groups (Woolfenden 1975; Brown 1987; Balshine *et al.* 2001; Magrath 2001; Clutton-Brock 2002; Hatchwell *et al.* 2004; Heg *et al.* 2005). Although an increase in reproductive success with large group sizes could be the consequence of helping itself (Brown *et al.* 1982; Mumme 1992; Shreeves & Field 2002; Brouwer *et al.* 2005), long-term effects on fitness caused by the presence of helpers has rarely been shown (but see: Hatchwell *et al.* 2004).

Numerous adaptive hypotheses have been suggested to explain the evolution of cooperative breeding, e.g. kin-selection (Komdeur & Hatchwell 1999; Griffin & West 2003), pay to stay (Balshine-Earn *et al.* 1998b), group augmentation (Clutton-Brock 2002) and the role of ecological (Arnold & Owens 1999b; Hatchwell & Komdeur 2000) and life history factors (Arnold & Owens 1998). However, the proximate mechanisms underlying this behaviour have received considerably less attention. To gain insight into the mechanisms that contribute to delayed breeding and helping hormones levels of breeders and non-breeders within cooperatively breeding species can be investigated (e.g. Schoech *et al.* 1996). Differences in blood hormone levels, e.g. testosterone, would indicate that there are fundamental differences between breeders and non-breeders in their reproductive physiology. Consideration of both functional and mechanistic explanations can improve our knowledge of what determines which individuals show cooperative breeding behaviour and it might contribute to our understanding of the large variation in helping behaviour both within and between species.

A long-term study on the Seychelles warbler

Here we studied cooperative breeding and population regulation in the Seychelles warbler (*Acrocephalus sechellensis*), a species endemic to just a few small islands in the Indian Ocean. This species went through a severe bottleneck, and the total world population consisted of only 30 individuals remaining on the island of Cousin between 1920 and 1968 (Crook 1960; Loustau-Lalanne 1968). Although the species must have formerly inhabited at least three, but probably more, islands in the Praslin group of the Seychelles archipelago (Diamond 1980; Collar & Stuart 1985), habitat loss caused by planting of coconuts (*Cocos nucifera*) and the introduction of predators such as rats and cats was responsible for the near extinction of this species. With the purchase of Cousin Island in 1968 by the International Council for Bird Protection (ICBP, now BirdLife International) a recovery programme was started. Restoration of the original native habitat allowed the warbler population to recover. Studies on warbler habitat use on Cousin suggested that continuous dense scrubby vegetation would allow the highest numbers of warblers (Bathe & Bathe 1982), which can explain why the species was also known as the Seychelles brush warbler (Penny 1974). However, at that time scrubby vegetation was the only suitable habitat available and further regeneration of the habitat showed that mature *Pisonia* woodland did not form a closed canopy as had been thought. The Cousin Island population recovered and stabilized at around 320 adult individuals by 1982. The vulnerability of a single small population to catastrophic events raised the idea of establishing new populations. Three translocation were performed by transferring birds from the founding island of Cousin to the islands of Aride (68ha; 1988), Cousine (26ha; 1990) (Komdeur 1994a), and Denis (2004) (Figure 1.1). In 2000 the world population of Seychelles warblers was estimated at 2060 (BirdLife International 2000) and the IUCN Threat Status changed from “endangered” into “vulnerable” (BirdLife International 2000). The Seychelles warbler has been the focus of intense study since 1985, first by Jan Komdeur and since 1997 also by David Richardson in collaboration with Terry Burke (Komdeur 1992; Richardson *et al.* 2003).

Seychelles warblers are facultative cooperative breeders; instead of breeding independently many individuals become subordinate within their natal territory (Komdeur 1992). Some of these subordinates help with incubating or feeding of the nestling. Group size varies from two to seven birds per territory. The development of microsatellite markers for this species (Richardson *et al.* 2000) has enabled accurate genetic parentage analyses and shown that the breeding system is even more complex than previously thought. Richardson *et al.* (2001) showed that in 44% of cases female subordinates were co-breeding and that 40% of all offspring were fathered by a male from outside the territory. As the population is a closed system, with virtually no dispersal on or off the island (Komdeur *et al.* 2004), almost all parentage can be assigned and importantly, accurate, unbiased survival probabilities can be calculated.

Here we study population dynamics in four populations of Seychelles warblers. Translocations to previously uninhabited islands allow us to test the causality of den-

sity dependence, using the change of numbers in the populations from early establishment through to saturation as a natural experiment. Furthermore, we investigate the proximate mechanisms involved in helping behaviour and the long-term fitness consequences of group living.

Thesis outline

In **chapter 2** we investigate whether survival was density dependent in a saturated population of Seychelles warblers. Furthermore, we investigate whether the reductions in the population density on Cousin, caused by translocating birds to other islands results in an increase in juvenile and adult survival. As resource competition will mainly work on a local scale, i.e. the territory, we also investigate whether survival probabilities are associated with local density, measured as the average group size an individual lived in during its life. In addition, we investigate the effect environmental factors like rainfall and temperature have upon survival. We show that in a territorial species, although density dependent effects might not be detectable at the population level they can be detected at the individual territory level - the scale at which individuals directly compete.

As only experiments can test the causality of density dependence, we use the change in numbers during the process of saturation in the newly established populations on Aride, Cousine and Denis as a natural experiment to investigate this in **chapter 3**. We determine how reproduction and survival changed over the course of the populations' development and, in addition, study the effect of territory quality and food abundance on reproduction and survival. We show that the regulation of numbers takes place in a density dependent way and that the mechanism causing this is probably competition for food. Although the translocations to Aride and Cousine can be considered as being very successful, this does not guarantee success for future translocations. In **Box A** we investigate whether the recent translocation to Denis Island has, so far, been successful, by analysing the survival of warblers during the first two years after the establishment of the population.

Although environmental factors faced at some point can obviously affect an individual's fitness immediately, awareness is growing that conditions during the rearing period also strongly affect important fitness components later in life. (e.g. Magrath 1991; Sedinger *et al.* 1995; Lindström 1999; Green & Cockburn 2001; Cam *et al.* 2003; van de Pol *et al.* 2006). In cooperative breeding species, the presence of helpers adds an important component to these rearing conditions. For example, the presence of helpers has been shown to positively affect offspring survival and/or weight (Brown 1987; Emlen 1991). However, the long-term fitness consequences of helping on offspring have rarely been investigated (but see: Hatchwell *et al.* 2004). In **chapter 4** we investigate, using multi-state capture-recapture models, how conditions during the rearing period of Seychelles warblers affect future survival and recruitment to a breeding position. Furthermore, we use data from a cross-fostering

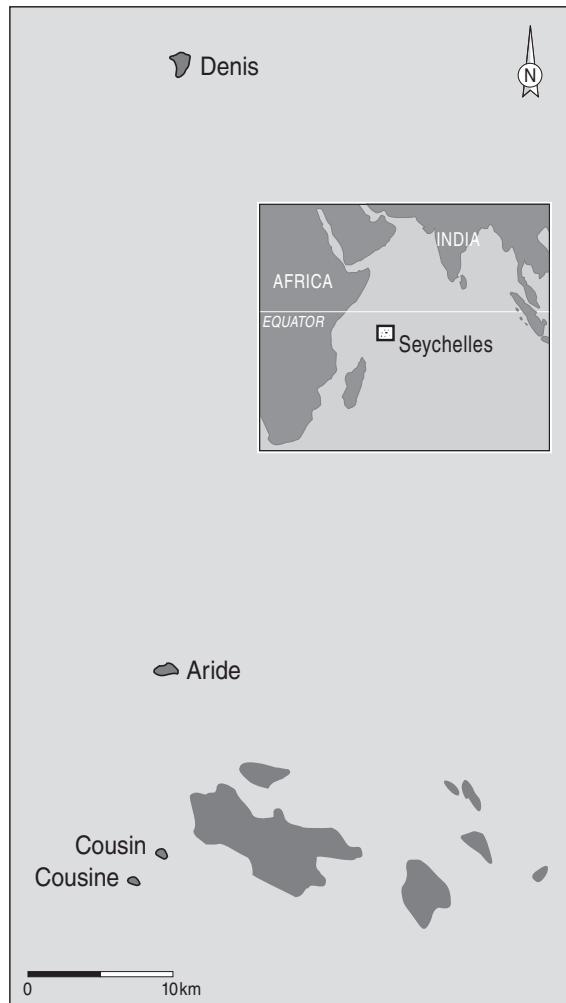


Figure 1.1 A map showing the four islands on which the Seychelles warbler occurs nowadays. In the 1960's the ca. 30 last remaining Seychelles warblers were confined to Cousin. New populations were established by translocations to Aride, Cousine and Denis.

experiment to determine whether effects were caused by the rearing factors per se rather than genetic or parental quality. Our results show that helping by subordinates within a territory can have long-term fitness consequences for the offspring helped.

The translocation of Seychelles warblers to new islands has shown that individuals of this species are able to reproduce in their first year of life, but that a lack of suitable habitat in a saturated population drives them to become subordinate in their natal territory (Komdeur 1992). Although habitat saturation seems the ultimate explanation for this behaviour, the proximate mechanisms involved are

unknown. In chapter 5 we determine the natural variation in plasma testosterone level and cloacal protuberance size - an indicator of sperm storage - in successfully reproducing primary males, and in apparently sexually inactive subordinate male Seychelles warblers. Testosterone has been known to play a role in male reproductive behaviour (for review see: Nelson 2000). Differences in testosterone levels between primaries and subordinates might indicate that subordinates are suppressed, however experiments are needed to test whether any relation between testosterone levels and status is really due to status per se rather than age, sexual immaturity or a lack of breeding opportunities for subordinate males. We experimentally promote subordinate males to primary status by permanently removing primary males from their territories. We then investigate testosterone levels and cloacal protuberance sizes of the promoted males. We show that primary and subordinate males do differ in their testosterone levels and size of cloacal protuberances, and that subordinate males are able to elevate their testosterone levels once social suppression is removed, although they do not appear to reach levels as high as primary males in the initial season after promotion.

In chapter 6 we investigate heterozygosity-survival correlations. In small isolated populations the probability that closely related individuals will mate will increase. Such inbreeding will result in decreased heterozygosity in offspring and may lead to the expression of recessive deleterious alleles, or to the loss of the benefits associated with heterozygosity at loci influencing fitness (Hartl & Clark 1997). Consequently, inbred individuals are expected to be less fit than outbred individuals, a phenomenon known as inbreeding depression (Hartl & Clark 1997). Inbreeding depression can have important consequences in wild populations and can contribute toward driving populations to extinction (Saccheri *et al.* 1998). Heterozygosity measured at microsatellite markers is generally assumed to reflect genome-wide inbreeding effects (e.g. Lynch & Walsh 1998). Although this might be true in partially inbred populations and when very large numbers of microsatellites are used, this is often not the case (Slate *et al.* 2004). Such correlations may also arise as a result of local effects with specific markers being closely linked to genes which determine fitness (Lynch & Walsh 1998; David 1998). We show that heterozygosity-fitness correlations can occur in bottlenecked populations but highlight the difficulty in distinguishing between the different hypotheses.

Finally, in chapter 7 we integrate some of the conclusions of the separate chapters and discuss these in a more general framework. Specifically, we discuss what the consequences of the establishment of new populations means for our understanding of population dynamics and conservation.



Top left: a ladder and some acrobatics are needed to check nests, which can be up to 25 m high in the trees. Top right: warbler nests can be hard to find hidden between large *Pisonia* leafs.
Bottom: juvenile warbler with mosquito on his back; mosquitoes can infect warblers with avian malaria.



2

Chapter

The role of group size and environmental factors on survival in a cooperatively breeding tropical passerine

Lyanne Brouwer, David S. Richardson, Cas Eikenaar and Jan Komdeur

Abstract

1. Variation in survival, a major determinant of fitness, may be caused by individual or environmental characteristics. Furthermore, interactions between individuals may influence survival through the negative feedback effects of density dependence. Compared to species in temperate regions, we have little knowledge about population processes and variation in fitness in tropical bird species.
2. To investigate whether variation in survival could be explained by population size or climatic variables we used capture-recapture models in conjunction with a long-term dataset from an island population of the territorial, cooperatively breeding, Seychelles warbler (*Acrocephalus sechellensis*). The lack of migration out of the study population means that our results are not confounded by dispersal.
3. Annual survival was high, both for adults (84%) and juveniles (61%), and did not differ between the sexes. Although there was significant variation in survival between years, this variation could not be explained by overall population size or weather variables.
4. For territorial species, resource competition will mainly work on a local scale. The size of a territory and number of individuals living in it will, therefore, be a more appropriate measure of density than overall population density. Consequently both an index of territory quality per individual (food availability) and local density, measured as group size, were included as individual covariates in our analyses.
5. Local density had a negative effect on survival; birds living in larger groups had lower survival probabilities than those living in small groups. Food availability did not affect survival.
6. Our study shows that, in a territorial species, although density-dependent effects might not be detectable at the population level they can be detected at the individual territory level - the scale at which individuals compete. These results will help provide a better understanding of the small scale processes involved in the dynamics of a population in general, but in particular, in tropical species living in relatively stable environments.

Introduction

Understanding the factors that influence population dynamics is of fundamental importance to many areas in biology. Survival is a key variable in population dynamics as it is often a major source of individual variation in lifetime reproductive success (Clutton-Brock 1988; Newton 1989). Indeed, in many populations a large proportion of individuals will die before having the chance to reproduce (Newton 1989). In long-lived species especially, small differences in survival may have large evolutionary effects and far reaching consequences for the dynamics of the population.

Survival may vary due to individual differences in genotype or phenotype (e.g. sex or mass), but may also change over time for each individual due to aging (Clobert *et al.* 1988; Lebreton *et al.* 1992). Survival may also vary due to density dependent processes; a key process often used to explain why populations normally fluctuate between certain boundaries (Murdoch 1994; Sæther *et al.* 2002). However, it has long been recognised that many natural populations will remain relatively constant when left undisturbed, and that density dependence will be impossible to detect, or confounded, in these circumstances (Murdoch 1994; Sæther 1997). Consequently experiments involving deliberate perturbations of population size, e.g. through translocations, are needed to test the causality of density dependence in natural populations. Although many studies have investigated the role of density dependence on life history traits (for reviews see :Sinclair 1989; Newton 1998; Both 2000), the detection of such processes is also dependent on the spatial scale at which such effects are investigated (e.g. Ray & Hastings 1996; Wilkin *et al.* 2006). Intra-specific competition for resources, one of the main density dependent processes (Newton 1992), will work mainly on a local scale in territorial species. The size of the territory and number of individuals living in it will, therefore, be the appropriate measure of density with which to investigate such effects (Both & Visser 2000). In contrast to non-cooperatively breeding species, where increased density may result in a negative effect on survival, in cooperatively breeding species a higher local density may be advantageous if individuals survive or reproduce better in larger cooperative groups (Woolfenden 1975; Brown 1987; Magrath 2001; Clutton-Brock 2002). Finally, the effects of density dependence may also be confounded if individuals of higher quality are able to obtain higher quality territories (Garant *et al.* 2005). Consequently territory quality, as well as local density, should be taken into account when analysing density dependent effects.

Environmental factors also play an important role in survival. In passerines, survival is often affected by food availability (Jansson *et al.* 1981; Newton 1998), which in turn can be affected by rainfall and temperature fluctuations. Moreover, such climatic factors can also influence survival directly. Variation in natal environment can influence the life-histories of complete cohorts (van der Jeugd & Larsson 1998; Lindström 1999; Reid *et al.* 2003). However, cohorts which are successful, because of factors such as food availability, may also be influenced by negative feedback effects through density dependence.

Most of what is known about survival comes from studies of temperate species. There is little knowledge about how factors such as environmental variation affect survival and population regulation in tropical species (Martin 1996). In tropical areas, environmental factors are generally considered to vary relatively little between seasons (Karr 1976; Martin & Karr 1986; Loiselle & Blake 1991, 1992). Life histories of species in these regions are typically characterised by small clutches, long developmental periods and extended parental care of juveniles associated with high juvenile and adult survival probabilities (Skutch 1949; Lack 1954; Martin 1996). Another important feature is that cooperatively breeding species are more prevalent in (sub)tropical areas than in temperate zones (Arnold & Owens 1999a).

Studies investigating the effect, on survival, of both density dependence and territory quality at an individual level are lacking, despite the fact that they would give a better understanding of the small scale processes of population regulation. Furthermore, we know of no study which has investigated the long-term effect of local density on survival in a cooperatively breeding species. Here we analyse the long-term dataset from a population of the Seychelles warbler (*Acrocephalus sechellensis*), a long lived tropical endemic confined to a few small islands in the Seychelles. In the 1960s, the entire world population of Seychelles warblers was restricted to just 26–29 individuals on Cousin Island. This population, which has since recovered and stabilized at around 320 adult individuals, has been studied since 1985 (Komdeur 1992; Richardson *et al.* 2003). The transfers of Seychelles warblers to the islands of Aride and Cousine allowed us to test the causality of density dependence. The Seychelles warbler is also a cooperatively breeding species, with group sizes varying from one to six birds per territory. As the population is a closed system, with no migration on or off the island (Komdeur *et al.* 2004), accurate, unbiased, survival probabilities can be calculated without the problems encountered by most other studies, where local survival estimates are confounded by dispersal away from the study site. By using capture-recapture models we investigate whether juvenile and adult survival is regulated by density dependence, both on a local and on a population level. This analysis should, for the first time, provide insight into the long-term effect of group living on survival. To determine if group size effects are caused by territory quality, we also investigate the effect of an index of territory quality per individual on survival. Finally, we test whether temporal variation in survival can be explained by environmental factors such as rainfall and temperature.

Methods

Study area and data collection

The data were collected as part of the long term study of the Seychelles warbler population on Cousin Island (04°20' S, 55°40' E). Except for in 1992, this population has been monitored every year since 1985. During the main breeding season,

each territory was checked for breeding activity at least once every two weeks by following the resident female for 30 minutes (Komdeur 1992). Nests were observed throughout the breeding cycle. Birds were either ringed as nestlings, or as fledglings while still resident in the natal territory and dependent on their parents (birds of known age and origin), or later when independent (birds of unknown age and origin). Birds were ringed with a unique combination of three UV-resistant colour rings and a British Trust for Ornithology ring. Blood samples (c. 15 µl) were collected by brachial venipuncture and diluted in 800 µl of 100% ethanol in a 2.0-ml screw-cap microfuge tube and stored at room temperature. Molecular sexing using the PCR method was used to determine the sex of each sampled individual since 1994 (Griffiths *et al.* 1998). As part of a conservation project, 29 warblers were translocated to each of the islands of Aride (1988) and Cousine (1990) (Komdeur 1994a). These translocations created an experimental reduction in density on Cousin.

To investigate whether environmental conditions play a role in survival, weather data were obtained from the Meteorological Services of Praslin Island, an island 2 km south of Cousin. Each year was divided into four periods of three months. Mean temperature (°C) and total rainfall (mm) were calculated for the main breeding season period. Furthermore, total rainfall was calculated for the pre-breeding season, for the period when fledglings were still dependent on their parents and for the period when the young were able to forage independently. As variation in temperature between years is largest in the breeding season, only the effect of breeding season temperature on survival was tested. As both very dry and very wet years might have strong effects on survival probabilities, the squared effects were also tested.

Seychelles warblers are insectivorous, taking 98% of their insect food from leaves (Komdeur 1991b). Therefore, the number of insect prey available, the territory size and foliage cover were used to calculate an index of territory quality following Komdeur (1992). Territory quality was calculated for each territory in 1987, 1990, 1996-1999 and 2003-2004. For the remaining years, territory quality for each territory was calculated as the average from the preceding and following period. Median territory quality did not increase during our study period ($r_6 = 0.04$, $N = 8$, $P = 0.93$). Repeating the analyses including only those years in which territory quality was measured, gave similar results (not shown).

For each bird, local density was calculated as; i) natal local density: group size during the individual's first year of life and, ii) lifetime local density: the average group size from the individual's second year on. Group size reflects local density as territory size does not proportionally increase with group size (territory size = $0.16 + e^{0.09} \times$ group size, $F = 16.6$, $d.f. = 429$, $P < 0.001$). For four years for which accurate territory sizes were available, the average territory size (in ha ± S.E.) per group size was as follows: group size = 1: 0.16 ± 0.04 ($N = 6$); group size 2: 0.22 ± 0.01 ($N = 229$); group size = 3: 0.23 ± 0.01 ($N = 142$); group size = 4: 0.25 ± 0.01 ($N = 43$); group size = 5: 0.27 ± 0.05 ($N = 8$); group size = 6: 0.27 ± 0.03 ($N = 3$). Natal territory quality was the territory quality during the individual's first year of life. Lifetime territory quality was calculated as the mean quality of all the

territories inhabited by an individual from the second year on. To disentangle territory quality effects from group size effects, natal food availability was calculated by dividing natal territory quality by the number of birds in the territory. Lifetime food availability was calculated as the mean quality (per individual) of the territories inhabited from the second year on. After performing these calculations natal food availability was still correlated with natal group size ($r = -0.18$, $N = 438$, $P < 0.001$); and lifetime food availability was still correlated with average lifetime local density: $r = -0.13$, $N = 736$, $P = 0.001$), though the variation in territory quality explained by group size was only 3.2 % and 1.7 % respectively.

Overall population size was estimated for each year, except for 1991–1994, as the number of birds older than three months of age alive in the breeding season, after correcting for the resighting rate. In 1991–1994 only a portion of the population was monitored (68% of territories). As the number of territories is relatively constant across years (average \pm S.E. 1986–1990, 1995–2004: $N = 112.3 \pm 1.2$), population size for these years was estimated by multiplying the number of birds observed by the proportion of territories monitored.

Survival analyses

To estimate survival, we constructed the capture-resighting histories of 991 marked individuals that were monitored between 1986 and 2004. Of these, 438 individuals were ringed as juveniles and were of known age. Another 553 individuals were ringed as adults and therefore only a minimum age could be assigned to these individuals. Two resighting periods were defined; the first from 1 July to 1 September during the main breeding season, the second from 1 January to 1 March. However, most birds were re-sighted within the first two weeks of each study period. Individuals were recorded as present if observed or caught at least once within this period or absent if not. No capture-resighting data were available for 1992 and data for January–March was only available in 1998, 1999 and 2003. If not available, dummy variables were created by including zeros in the encounter histories, with the survival parameter (Φ) set equal to 1 and the resighting parameter (recapture, P) set equal to 0. The re-sighting history files were used as input files in the program MARK (White & Burnham 1999).

To investigate survival and resighting probability, we employed an *a priori* approach in which a set of candidate models was created based on biological reasoning. Firstly, we investigated whether survival and resighting vary between age classes, cohorts or years using the data set of known age birds. As Seychelles warblers are dependent on their parents for at least three months, and stay in their natal territory until they are at least six months of age (Komdeur 1996a), survival and resighting rate may differ between one year and older birds. Consequently, we allowed survival and resighting rate to vary between years and between these age classes in the global model. Secondly, we included birds of unknown age to analyse the effects of sex, and group (ringed as juvenile or adult). The most parsimonious model resulting from this analysis was then used to investigate the effects of density, territory quality

and weather variables. The effect of sex on survival was analysed using individuals ringed between 1994 and 2004 for which blood samples were available for molecular sexing, thereby creating two groups of 417 males and 370 females. Population density and weather variables were tested by including the covariate as a linear constraint on survival probabilities. Local density, territory quality and food availability were included as individual covariates for each bird whereby lifetime local density and food availability were always tested as an interaction with age. The individual covariates were analysed by using the logit link function, with values of the individual covariates standardized to z-scores (by subtracting the mean and dividing by the standard deviation). Model notation was followed according to Lebreton *et al.* (1992).

Model selection and goodness of fit

Akaike's information criterion, corrected for the sample size (AIC_c), was used to select the most parsimonious model (Akaike 1973; Burnham & Anderson 2002). Additionally, we calculated the normalized Akaike weights to assess the relative likelihood of competing models. The goodness-of-fit of the global model was checked by using parametric bootstrap procedures. The variance inflation factor (\hat{c}) was calculated by dividing the model deviance by the bootstrapped deviance. Both the known age ($\hat{c} = 1.16$) and the unknown age ($\hat{c} = 1.29$) dataset fitted the data well with no strong evidence for overdispersion. Therefore, AIC_c values were adjusted to allow for the extent of overdispersion measured by \hat{c} , through quasi likelihood (QAIC_c). For one model to be selected above another ΔQAIC_c should > 2 (Burnham & Anderson 2002). To account for model selection uncertainty, we used model averaging techniques which weigh the impact of all models for a given parameter according to its AIC_c weight (Burnham & Anderson 2002).

Results

Effects of year, age and sex

The most parsimonious model for the known age data set showed that survival was lower in an individual's first year of life than older birds and varied between the years (Table 2.1, model 1). The resighting probability was lower for individuals in their first two years of life than for older birds (Table 2.1, model 1). After including the unknown age birds, the most parsimonious model showed that the resighting probability, but not the survival probability, of birds ringed as adults was higher than that of adult birds ringed as juveniles (Table 2.2, model 5 vs. 6 and model 1 vs. 2). Furthermore, the model best supported by the data showed that the resighting and survival probabilities varied between years and also differed between the age classes, with similar variation between years for both age classes (Table 2.2, model 1). This final accepted model was 3.7 times better supported by the data than the next most parsimonious model (Table 2.2, model 1 vs. model 2). Fig. 2.1 shows the

Table 2.1 Summary of model selection statistics of year and age effects in survival and resighting probabilities of the subset of Seychelles warblers of known age (N = 438). (t) = year, (a) = age, (.) = constant, (c) = cohort.

No	Model	No.Par.	Quasi deviance	ΔQAIC_c	QAIC _c weights
1	$\Phi_{(t+a1,2+)}P_{(a1-2,3+)}$	23	306.2	0.0	0.72
2	$\Phi_{(t+a1-2,3+)}P_{(a1-2,3+)}$	23	308.1	1.87	0.28
3	$\Phi_{(txa1-2,3+)}P_{(a1-2,3+)}$	40	284.1	13.4	0.00
4	$\Phi_{(txa1-2,3+)}P_{(a1-2,3,4+)}$	41	283.2	14.7	0.00
5	$\Phi_{(txa1-2,3+)}P_{(t+a1-2,3+)}$	58	247.3	15.4	0.00
6	$\Phi_{(txa1-2,3+)}P_{(a1,2+)}$	40	287.3	16.7	0.00
7	$\Phi_{(txa1-2,3+)}P_{(.)}$	37	298.4	21.4	0.00
8	$\Phi_{(txa1-2,3+)}P_{(t)}$	55	260.7	22.2	0.00
9	$\Phi_{(txa1-2,3+)}P_{(c)}$	54	267.0	26.4	0.00
10	$\Phi_{(t)}P_{(a1-2,3+)}$	22	337.8	29.5	0.00
11	$\Phi_{(txa1-2,3+)}P_{(txa1-2,3+)}$	70	236.5	30.9	0.00
12	$\Phi_{(c)}P_{(a1-2,3+)}$	20	376.0	63.6	0.00
13	$\Phi_{(a1,2+)}P_{(a1-2,3+)}$	4	413.5	68.5	0.00
14	$\Phi_{(a1-2,3+)}P_{(a1-2,3+)}$	4	416.1	71.1	0.00
15	$\Phi_{(a1-2,3,4+)}P_{(a1-2,3+)}$	5	414.7	71.7	0.00
16	$\Phi_{(.)}P_{(a1-2,3+)}$	3	450.6	103.5	0.00

Table 2.2 Summary of model selection statistics of year and group (ringed as adult) effects in survival and resighting probabilities of the Seychelles warbler (N = 991). (t) = year, (a) = age, and (g) = group (ringed as adult).

No	Model	No.Par.	Quasi deviance	ΔQAIC_c	QAIC _c weights
1	$\Phi_{(t+a1,2+)}P_{(t+g+a1-2,3+)}$	42	831.0	0.0	0.77
2	$\Phi_{(t+g+a1,2+)}P_{(t+g+a1-2,3+)}$	44	829.4	2.6	0.21
3	$\Phi_{(g+txa1,2+)}P_{(t+g+a1-2,3+)}$	60	801.3	7.3	0.02
4	$\Phi_{(txg+a1,2+)}P_{(t+g+a1-2,3+)}$	60	820.4	26.4	0.00
5	$\Phi_{(txg+txa1,2+)}P_{(t+g+a1-2,3+)}$	76	792.0	31.0	0.00
6	$\Phi_{(txg+txa1,2+)}P_{(t+a1-2,3+)}$	75	800.4	37.3	0.00
7	$\Phi_{(txg+txa1,2+)}P_{(g+txa1-2,3+)}$	94	763.0	39.6	0.00
8	$\Phi_{(txg+txa1,2+)}P_{(txg+a1-2,3+)}$	92	783.2	55.6	0.00
9	$\Phi_{(txg+txa1,2+)}P_{(txg+txa1-2,3+)}$	111	754.7	67.0	0.00

weighed survival estimates per year for both age classes. On average the annual survival probability for birds in their first year was 0.61 ± 0.09 (resighting probability = 0.87 ± 0.05) and 0.84 ± 0.04 (resighting probability = 0.92 ± 0.03) for adult birds. The final model was used to investigate whether survival probabilities varied between the sexes and/or were influenced by density and environmental factors.

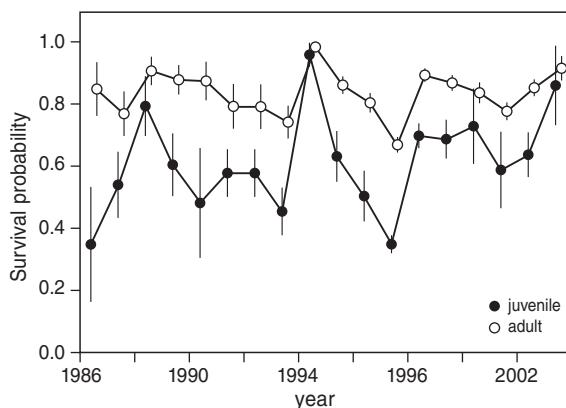


Figure 2.1 Maximum likelihood probabilities (\pm S.E.) of annual juvenile and adult survival for Seychelles warblers between 1986 and 2004. Estimates are derived by model averaging.

Survival and resighting probabilities did not differ between the sexes. A model with equal survival and resighting probabilities for both sexes was 2.2 times better supported by the data than the next most parsimonious model with different survival probabilities ($\Delta\text{QAIC}_c = 1.63$; $B \pm \text{S.E.} = 0.07 \pm 0.12$).

Effects of density

The translocations of 29 birds caused experimental reductions in density of 10% and 9% in 1988 and 1990 respectively. Although the population has been fairly stable since 1982 (Komdeur 1994a), the number of birds within the population does fluctuate a little between years (average: 308, range: 265–358, coefficient of variation = 10%). However, population size did not explain between year variation in survival for either juveniles or adults (Table 2.3a, model 4 and 5 vs. model 2). As the total number of birds in the population does not necessarily reflect local competition, we also investigated density on an individual scale. Individuals that lived in high density territories throughout their life had lower adult survival than individuals that lived in low density territories (Table 2.3a, model 1 vs. model 2; Fig. 2.2). This model was 32 times better supported by the data than the starting model. However, natal local density did not influence juvenile or adult survival (Table 2.3a, model 3 vs. model 2).

Effects of environmental factors

Lifetime territory quality did affect adult survival (Table 2.3b, model 8 vs. model 9), however the effect was negative. After correcting for group size, lifetime food availability did not affect survival (Table 2.3b, model 10 vs. model 9), indicating that the negative effect of lifetime territory quality was caused by group size effects. Natal territory quality and natal food availability did not influence juvenile or adult survival (Table 2.3b, model 11 and model 12 vs. model 9). However, the effect of life-

Table 2.3 Summary of model selection statistics of (A) density, (B) territory quality and (C) weather variables on survival probabilities of the Seychelles warbler. The resighting probability was similar for all models: $P_{(t+g+a1-2,3+)}$. (t) = year, (a) = age, and (g) = group (ringed as adult), (lds) = lifetime local density, (natalld) = natal local density, (popsize) = population size, (fa) = lifetime food availability, (nfa) = natal food availability, (tq) = lifetime territory quality, (ntq) = natal territory quality and (bs) = rainfall in breeding season.

No	Model	No.Par.	Quasi deviance	ΔQAIC_c	QAIC _c weights
(A)					
1	$\Phi_{(t+ldsxa1,2+)}$	45	3689.2	0.0	0.96
2	$\Phi_{(t+a1,2+)}$	42	3702.1	6.7	0.03
3	$\Phi_{(t+natallda1,2+)}$	43	3702.0	10.7	0.00
4	$\Phi_{(popsizexa1,2+)}$	25	3868.9	137.3	0.00
5	$\Phi_{(popsize+a1,2+)}$	24	3871.2	137.6	0.00
(B)					
6	$\Phi_{((t+a1,2+)tq)}$	61	3646.6	0.0	0.88
7	$\Phi_{((t+a1,2+)fa)}$	61	3657.4	4.7	0.09
8	$\Phi_{(t+tqxa1,2+)}$	44	3694.5	6.8	0.03
9	$\Phi_{(t+a1,2+)}$	42	3702.1	10.4	0.00
10	$\Phi_{(t+faxa1,2+)}$	44	3700.8	13.1	0.00
11	$\Phi_{(t+ntqa1,2+)}$	44	3702.0	14.3	0.00
12	$\Phi_{(t+nfaxa1,2+)}$	44	3701.5	15.9	0.00
(C)					
13	$\Phi_{(t+a1,2+)}$	42	3702.1	0.0	1.00
14	$\Phi_{((a1,2+)+bs+bs^2)}$	25	3859.6	128.0	0.00
15	$\Phi_{((a1,2+)+bs)}$	24	3863.0	129.2	0.00
16	$\Phi_{((a1,2+)\times bs)}$	27	3856.9	129.4	0.00

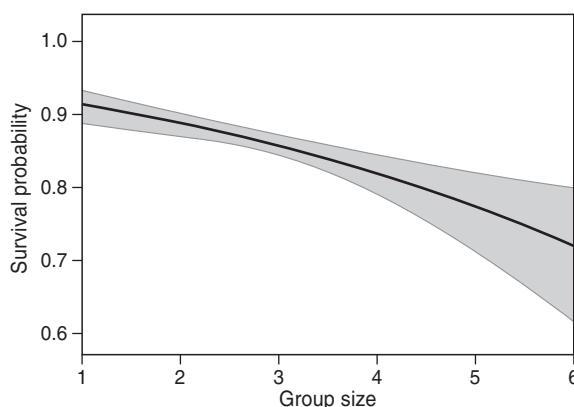


Figure 2.2 Annual adult survival probabilities of Seychelles warblers for an average year in relation to group size with 95% confidence interval.

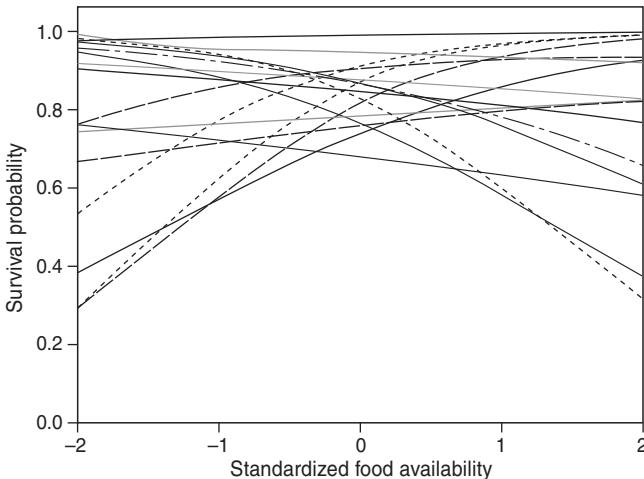


Figure 2.3 Annual adult survival probabilities of Seychelles warblers for an average group size, in relation to lifetime food availability, shown in standard deviations from the mean for each year between 1986 and 2004.

time territory quality and food availability did vary between years (Table 2.3b, model 6 and 7 vs. model 9; Fig. 2.3). In none of the years did territory quality or lifetime food availability have a significant effect on survival.

Weather variables did not covary with population size, nor did they change systematically across the study period (all: $-0.37 < r < 0.37$, $P > 0.15$, $N = 17$). Variation in temperature or rainfall during the breeding season did not explain variation in survival between years (Table 2.3c, shown for rainfall only: model 14 and 15 vs. model 13). Furthermore, the effect of rainfall in the breeding season did not affect age classes differently (Table 2.3c, model 16 vs. model 13). Similarly the pre-breeding season rainfall, the fledging period rainfall and the independence period rainfall did not affect either adult or juvenile survival ($\Delta\text{QAIC}_c > 119$, not shown). However, the two most extreme years, in relation to rainfall (breeding season rainfall: 1986: 60.3 mm and 1997: 1031.9 mm), also showed the lowest juvenile survival estimates (Fig. 2.1), indicating that extreme levels of rainfall did influence survival.

Discussion

Density dependent survival

Overall population density did not affect survival in our study. The experimental reduction of density caused by the translocations of warblers lies within the population's natural variation in density, which at only 10%, corresponds with the low levels of between year variation observed in other tropical species (Greenberg &

Gradwohl 1986). In addition, long lived species tend to have smaller annual population variability than short-lived species (Holyoak & Baillie 1996). Therefore it is not surprising that we do not find an effect of population density on survival. Local density, measured as territory group size, did affect survival; birds living at higher local density had lower survival than those at low local density. In cooperatively breeding species, positive effects of group size on reproduction or survival are widespread (Rood 1990; Allainé *et al.* 2000; Clutton-Brock *et al.* 2001; Magrath 2001; Conner *et al.* 2004; Khan & Walters 2004) but negative effects are rarely reported. Studies on non-cooperative, group living animals showed that the negative effects of increasing group size could be caused by larger groups attracting more predators (Begon *et al.* 1990) or increasing competition for food (Krause 1994; Bednekoff & Lima 2004). However, the species investigated all suffer from considerable predation pressure, which is not the case in the Seychelles warbler. Although eggs and small nestlings are predated upon by the endemic Wright's skinks (*Mabuya wright*), the Seychelles fody (*Foudia sechellarum*) and Seychelles Magpie Robin (*Copsychus sechellarum*), there is no predation on adult warblers (Komdeur 1994a). Competition for resources is, therefore, the most probable cause of the decreasing survival with increasing group size observed in the Seychelles warbler.

In the Seychelles warbler, the negative effect on survival of living at high local density appears to be compensated for by a gain in reproductive success (Richardson *et al.* 2002). Subordinates often gain direct fitness by co-breeding but, importantly, the dominants also gain from this through indirect kin benefits as the subordinates are normally closely related to them (Richardson *et al.* 2001). Removal experiments have shown that the presence of one or two helpers improves the reproductive success of a group, but that the presence of three or more helpers has a negative effect on reproductive success (Komdeur 1994b). This means that living in groups that are too large will not be profitable for Seychelles warblers, as both reproductive success and survival decreases for groups containing more than five birds. Future research taking effects of the complicated breeding system of the Seychelles warbler (Richardson *et al.* 2001) into account could give more insight in the effects of local and population density on reproduction.

Survival in tropical and island species

The yearly adult survival calculated for the Seychelles warbler was high (84%) in comparison with other European passerines, which usually have probabilities of < 50% (Sæther 1989; Peach *et al.* 2001). This result concurs with the idea that the life history strategies of tropical species are typified by high survival rates and small clutch sizes. The absence (or infrequency) of periods of severe food shortage and climatic severity in the tropics allows high survival rates. However, even in comparison with related African passerines, survival is remarkably high in the Seychelles warbler; for example, the Cape reed warbler (*Acrocephalus gracilirostris*) and African marsh warbler (*Acrocephalus baeticatus*) have annual survival probabilities of $55.7 \pm 8.7\%$ and $76.5 \pm 6.9\%$ respectively (Peach *et al.* 2001). However the survival probabilities

reported in these studies may be underestimated through the inclusion of individuals that are presumed dead when in reality they have migrated out of the study area. The inclusion of such migrants is not a problem in our study as the population is totally confined to Cousin Island (Komdeur *et al.* 2004). Another important feature of island species is the absence of predators. The fact that nothing that preys upon adult Seychelles warblers will also contribute to the high survival rates observed. In addition, the island biogeography theory suggest that island species will have less parasites and pathogens than their mainland relatives (MacArthur & Wilson 1967) resulting in them experiencing less adverse effects on survival. Preliminary results of parasite studies in the Seychelles warbler confirm this theory (D.S. Richardson pers. comm.).

Although lower than that of adults, the post-fledging survival of the Seychelles warbler was also relatively high (61%). This may be due to the prolonged parental care and delayed dispersal which juveniles undergo in this species as a response to habitat saturation and the birds lack of willingness, or ability, to emigrate from the island (Komdeur 1992; Komdeur *et al.* 2004). Fledged Seychelles warblers are often fed until three months of age which may enhance survival while developing foraging skills. Delayed dispersal has also been shown to promote juvenile survival in Siberian jays (*Perisoreus infaustus*, Ekman *et al.* 2000) and brown thornbills (*Acanthiza pusilla*, Green & Cockburn 2001).

Environmental effects on survival

In this study we found that, contrary to Komdeur (1992), higher food availability experienced during an individuals' life did not result in higher survival. In his study there was a positive relationship between territory quality and survival, however, mark-recapture analyses was not used, sample sizes were much smaller and no between-year variation was taken into account. In the present study, we found a significant interaction between year and lifetime food availability/territory quality, however this only showed that there was significant variation between years and that an index of territory quality, rather than the true territory quality, was measured. Although the repeatability of food availability during an individuals' life was 0.30 ± 0.02 (calculated according to Lessells & Boag 1987), there might be too much variation, caused by changes in group size and food availability between years, to find an effect on survival. On the other hand, individuals may be mitigating (or avoiding) the negative effects on survival of living on a low quality territory by postponing or reducing reproduction. Indeed, in the Seychelles warbler reproductive success is positively influenced by territory quality (Komdeur 1992).

Although we found temporal variation in survival, we did not find an effect of weather variables. In temperate regions, the weather is a very important factor that influences survival, either indirectly, via food availability (Newton 1998), or directly, e.g. via thermoregulation (Cuthill & Houston 1997). In our study, temperature did not affect survival, though this is not surprising as the variation in mean temperature between years is very limited (range mean temperature: 25.6-26.6 °C). That

rainfall did not affect survival estimates is surprising as rainfall is known to influence insect numbers in our study area (Komdeur 1996b). However, the two years showing extreme levels of rainfall, i.e. drought (1986) or flood (1997) also show the lowest survival probabilities. This might indicate that survival is influenced by weather conditions, but that our data set does not span enough years, to detect any effects. Alternatively, we might not be able to detect effects on post-fledging survival as mortality might have occurred before this stage while the chick was still in the nest.

Our study shows that in a territorial species, although density dependent effects might not be detectable at the population level they can be detected at the individual territory level - the scale at which individuals compete. These results will help provide a better understanding of the small scale processes involved the dynamics of a population in general, but in particular, in tropical species living in relatively stable environments.

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Top: Jeroen Reimerink extracting a warbler from a mist net.

Bottom left: Oscar Vedder checking a mist net for presence of birds.

Bottom right: Arno Kangeri colour-ringing a warbler



3

Chapter

Experimental evidence for density dependent reproduction in a cooperatively breeding passerine

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David S. Richardson, and Jan Komdeur

Abstract

Temporal variation in survival, fecundity and dispersal rates are associated with density dependent and density independent processes. Stable natural populations are expected to be regulated by density dependent factors. However, detecting this by investigating natural variation in density is difficult because density dependent and independent factors affecting population dynamics may covary. Therefore, experiments are needed to assess the density dependence of demographic rates. In this study, we investigate the effect of density on demographic rates of Seychelles warblers (*Acrocephalus sechellensis*). This species, endemic to a few islands in the Indian Ocean, went through a severe population bottleneck in the middle of the last century with only ca. 30 individuals left on one small island, but has since recovered. Our monitoring shows that since reaching the island's carrying capacity, population density has remained stable. However, we did neither detect density dependent reproduction nor survival on the basis of natural density variation during this stable period. For conservation reasons new populations have been established by transferring birds to nearby suitable islands. Using the change of numbers during the process of saturation as a natural experiment, we investigate whether we can detect regulation of numbers via density dependent survival and reproduction within these new populations. We found that reproduction, but not survival, decreased with increasing population density. Variation in mean reproduction between islands can be explained by food abundance, measured as insect density. Islands with the highest insect densities also had the highest bird densities and the largest breeding groups. Consequently, we suggest that the density dependent effect on reproduction is caused by competition for food. This study provides an example of how conservation management can be used to explore population regulation processes.

Introduction

Populations fluctuate between years as a result of temporal variation in survival, fecundity and dispersal rates. Such variation in demographic rates is often caused by interplay between density dependent and density independent processes. Analysis of these processes is crucial to population dynamics as density dependence is a key factor often used to explain why populations normally fluctuate between specific boundaries (Murdoch 1994, Sæther *et al.* 2002). In contrast, density independent factors, like climate, affect reproduction or survival irrespective of population size. The influence of both types of processes varies by environment and according to species' life history and they interact to affect population size and stability. Research on population regulation has focused on density dependent processes, and while many studies find negative relationships between density and reproduction (Kluyver 1951, Perrins 1965, Alatalo & Lundberg 1984, Arcese *et al.* 1992) or survival (Tinbergen *et al.* 1985, Francis *et al.* 1992, Lieske *et al.* 2000), not all studies do (van Balen & Potting 1990, Nur & Sydeman 1999, Loison *et al.* 2002, Festa-Bianchet *et al.* 2003). Furthermore even within species there can be considerable variation in the occurrence of density dependence (Both 2000). In contrast to negative effects of increasing densities, at very low densities an increase in population size can have a positive effect on population growth, known as the Allee effect (Allee 1951, for review see: Courchamp *et al.* 1999).

The existence of density dependent processes regulating populations is undisputed but whether they can be detected depends to a large extent on the relative scale and the co-variation of density dependent and independent factors that affect the fluctuation of populations. Factors which can confound the study of density dependence are, for example, incomplete knowledge of dispersal patterns or inter- and intra-annual variation in quality of territories or population composition. Although very stable natural populations are expected to be regulated strongly by density dependence, detecting this may be difficult because of the lack of variation in population density (Murdoch 1994, Sæther 1997). Consequently, the most fruitful way to assess the causality of density dependence on demographic rates has been to manipulate population density and study its effects. Many studies have done this indirectly by manipulating resources, e.g. by adding or removing nest boxes (Tompa 1967, Alatalo & Lundberg 1984, Török & Tóth 1988, Dhondt *et al.* 1992, Both 1998), or by supplementary feeding (Ewald & Rowher 1982, Davies and Lundberg 1984, Arcese & Smith 1988, Hoodless *et al.* 1999). Manipulating density directly is a better solution as changes in density are then less confounded by factors such as individual quality. However such experiments are rare especially on wild living vertebrates (but see: Both & Visser 2000). In the present study we both investigate density dependent patterns within a very stable population and utilize experimental populations to investigate the causality of density dependent processes.

Despite all the attention, the underlying mechanisms regulating populations remain largely unknown. Many studies assume that a crowding mechanism regu-

lates abundance (Sinclair 1989, Murdoch 1994). Crowding might result in a density dependent increase in agonistic interactions among conspecifics for limiting resources such as food (Newton 1998), territory space (McClerey & Perrins 1985), nest sites (Brawn & Balda 1988) or mating opportunities (Karvonen *et al.* 2000) and, consequently, to a decrease in demographic rates. All individuals within a population may be affected by density to the same extent, or alternatively this may differ between individuals. For example, at low population densities all individuals reside in high quality habitat, but with increasing density new individuals are forced to settle in lower quality habitat, resulting in a decline in average demographic rates (Kluyver & Tinbergen 1953, Brown 1969). In the present study we aim to investigate the underlying mechanisms regulating populations by analyzing whether the variance in reproduction increased as a result of lower quality habitat being occupied.

Translocations of individuals to suitable uninhabited islands are employed as successful tools to save species and have been a major component of conservation management (Bell & Merton 2002). We aim to utilize translocation populations as an experimental study of density dependence, as such populations start at low densities but undergo rapid changes in vital rates as the population grows (Armstrong & Ewen 2002, Nicoll *et al.* 2003, Armstrong *et al.* 2005). Although analyzing trends can have the disadvantage that there are natural changes through time, we can control for this by using data from a stable population.

We test the causality of density dependence by using data from translocations of the Seychelles warbler (*Acrocephalus sechellensis*) to three different islands done as part of ongoing conservation of this species. The Seychelles warbler is a facultative cooperative breeding species endemic to a few islands in the Indian Ocean. The species went through a severe bottleneck with only ca. 30 individuals left on Cousin Island (29 ha) between 1940 and 1968 (Crook 1960, Loustau-Lalanne 1968). This population, which has since recovered and stabilized at around 320 adult individuals, has been studied since 1986 (Komdeur 1992, Richardson *et al.* 2003). In order to save the species from extinction, new populations were established by transferring birds to the islands of Aride (1988) and Cousine (1990), which were subsequently monitored (Komdeur 1994a). As these three islands are located within an area of only a few km², a fourth population was established in 2004 on Denis Island, 60 km north of Cousin, to reduce the chance of extinction through natural disasters such as tropical storms or tsunami.

Here we investigate whether we can detect regulation of numbers via density dependent survival and reproduction. A previous study showed that both the natural variation in density and the experimental reduction in density caused by the removal of warblers for translocation was not associated with a change in either juvenile or adult survival probabilities on Cousin Island (Brouwer *et al.* 2006). We now investigate whether this is also true for reproduction. Furthermore, the change in numbers during population growth to saturation in the newly established populations will be used as a natural experiment in which to examine how survival and reproduction are influenced by density. In addition to studying the regulating mechanisms, we

will also investigate inter island differences in what limits population size by including food density measures. By studying both the limiting factors and density dependent patterns we hope to shed light on what the important density dependent processes are in these populations.

Methods

Study species

The Seychelles warbler is an insectivorous species that has long term pair bonds. Dominant pairs maintain territories all year round and although warblers can breed independently in their first year, a lack of suitable habitat in a saturated population drives them to become subordinate within their natal territory (Komdeur 1992). Both sexes of subordinates occasionally help with territory defense and the rearing of young (Komdeur 1994b, Richardson *et al.* 2002). Parentage analysis has shown that joint nesting occurs frequently with 44% of subordinate females producing offspring and 40% of offspring resulting from extra-group paternity (Richardson *et al.* 2001). Group size is defined as the number of independent birds resident in the territory, irrespective of whether they reproduce themselves. The main breeding peak is between July–September, with some breeding activity between January–March, although after translocation year-round breeding was observed (Komdeur 1996).

Study area and data collection

Data were collected on four different islands belonging to the Seychelles: Cousin (29 ha; 04°20' S, 55°40' E), Cousine (26 ha, of which 19 ha are natural habitat (Komdeur 1996); at 04°19' S, 55°39' E), Aride (68ha; at 04°13' S, 55°44' E) and Denis (144 ha of which 140 ha are natural habitat; at 03°48' S, 55°40' E). On Cousin, data were collected as part of the long term study of the Seychelles warbler population. Except for in 1992, this population has been monitored every year since 1986. From 1991 to 1994 only a part of the population was monitored (68% of territories). As the number of territories is relatively constant across years (average \pm S.E. 1986–1990, 1995–2006: $N = 112.3 \pm 1.2$), population size for these years was estimated by multiplying the number of birds observed by the proportion of territories monitored. During the main breeding season, each territory was checked for breeding activity at least once every two weeks by following the resident female for 30 minutes (Komdeur 1992). Territory borders were mapped based on observations of individual warblers and the outcome of disputes between groups. For conservation reasons, 29 warblers were translocated each to Aride in 1988 and Cousine in 1990 (Komdeur 1994a). The total Aride population was studied from establishment in September 1988 until November 1991. After 1991, research focused only on the most accessible plateau area (5.2 ha) of the island. Each year between 1993–2000 capture-recapture data was collected by mist netting, allowing us to estimate survival and resighting probabilities separately. From 1995–2000, except for 1997, each

territory was checked for the number of birds. In addition, during the main breeding season in 1995 and 1996, and all year round in 1999, the study area was completely monitored for reproduction. Furthermore, point sampling and line transect sampling were carried out across the whole island in 1997 and on the plateau in 2003 to estimate population size (Betts 1998). In 1988 the whole area which was covered by territories was estimated and in 1999 all territories sizes were measured separately. The population on Cousine Island was studied for two months after the founding of the population in July 1990 and then during the main breeding seasons in 1991 and 1995–1997. In 1994 and 1998 each territory was checked for the number of birds. Furthermore, during the main breeding seasons of 1994–1999 mark-recapture data was collected by mist netting and in 1994, 2002 and 2006 several visits during the main breeding season resulted in estimates of the number of territories and birds. In the years in which the populations were completely monitored population sizes were estimated as the number of independent birds (i.e. excluding juveniles fed by adults), after correcting for the resighting rate of that period (= number of counted individuals/resighting rate). As resighting rates were very high for these periods ($P > 0.9$), the estimates are very accurate. During the breeding seasons of 1990 and 1996, three insect counts (each a month apart) were performed to estimate food abundance on the three islands simultaneously. In addition insects were counted on Cousin throughout the study period. Insects were counted on the under side of 50 leaves of each of the three main tree species on at least 15 different locations per island, for more details see Komdeur (1992).

In 2004 a population of Seychelles warblers was established on Denis Island. This population was studied for two months after the translocation in June 2004, and then in January 2005 and in July-August 2005 and 2006. As birds within this population are attempting to breed year-round the estimated yearly reproduction will probably be underestimated, as some juveniles may have died before being recorded.

During each monitoring period as many birds as possible were caught, either as a nestling or after fledging using mist nets, and ringed with a unique combination of three ultra-violet-resistant colour rings and a British Trust for Ornithology metal ring. Body mass to the nearest 0.1 g and tarsus size to the nearest 0.1 mm were recorded.

Reproduction

To investigate whether reproduction on Cousin is related to natural variation in density and whether group size, territory size and territory quality are associated with reproduction, we analyzed reproduction of the years where both the main breeding season and the minor breeding season were completely monitored: 1986–1989, 1998–1999 and 2004–2005. For this analysis reproduction was defined as the total number of fledglings produced per territory in a year, with the total number of territories remaining relatively constant. Estimates of the available insect prey and foliage cover were used to calculate territory quality according to Komdeur (1992),

but without including territory size as a component of the calculation. Territory size and group size are correlated, therefore we investigated the effect of territory size separately by including the relative territory size per bird by dividing territory size by group size. Removal experiments have shown that the presence of one or two helpers improves the reproductive success of a group, but that the presence of three or more helpers negatively affects reproductive success (Komdeur 1994b). Therefore, group size was also included as a squared effect. Although the removal of birds from Cousin in 1988, 1990 and 2004 gave us the opportunity to investigate the effects of reduced density on reproduction, this analysis suffered from some difficulties. First, the transfer of birds in 1988 occurred after the breeding season, consequently effects of reduced density on reproduction could only be tested in 1989 at which time the population already returned to its original level. Second, after the transfer of 1990 the population was not intensively monitored for reproduction. Third, the transfer in 2004 was followed by an extreme drought (26.2 mm rain vs. on average 144.5 ± 22.9 ($N=18$) mm rain in June and July) which resulted in very few territories with nesting activity.

To investigate whether the change in numbers and, consequently, the increase in density of the newly established populations resulted in a change in reproduction we analyzed the per capita (per breeding season) reproduction on Aride, Cousine and Cousin in one model. For Aride and Cousine all breeding seasons in which reproduction data was collected were used, and the same seasons were selected for Cousin. In a cooperative breeding system reproduction can be defined in several ways and the method of analysis may illuminate different biological processes. We first investigated the per capita reproduction in relation to population density. However, with increasing density, group sizes will also increase and therefore any reduction in per capita reproduction might be a result of increasing group sizes. Consequently, we also investigate the fledgling production per territory in relation to population density.

Survival

To estimate survival, we constructed the capture-resighting histories of all marked individuals that were monitored on Aride, Cousine and Denis. On Aride, 539 birds were monitored between 1988 and 2000, of these 188 individuals were ringed as juveniles and were of known age. As only the plateau area was completely monitored dispersal may have caused survival to be underestimated. Consequently the survival probabilities for Aride represent local rather than true survival. On Cousine 183 birds were monitored between 1990 and 1998. Of these, 51 individuals were ringed as juveniles and were of known age. On Denis 93 birds were monitored between 2004 and 2006. Of these, 25 individuals were ringed as juveniles on Denis Island. The resighting period was defined as the main breeding season. The resighting history files were used as input files in the program MARK (White & Burnham 1999).

Data analyses

In the analyses of natural variation in reproduction on Cousin there are many repeats of the same territories between years which might be inter-correlated. Similarly different territories might be correlated within year due to between year differences. Consequently, we modeled year identity and territory identity as cross-classified random effects with reproduction (number of fledglings) of each territory nested within these random effects in MLwiN 2.02 (Rasbash *et al.* 2004). Reproduction was fitted using a Poisson response model with logit-link function and the 2nd order PQL estimation procedure. To investigate the effect of density on reproduction between populations, the average per territory reproduction was log transformed and then fitted as a normal response model with year defined as a random effect to account for systematic differences between years. Similarly, in the analyses of the effect of density on group size year was included as a random effect.

To investigate whether differences in population density also lead to differences in structural size measurements we analyzed body mass and tarsus size measurements from adult birds. As no data are available during the process of saturation we only investigate whether the difference in population densities between the populations had any effect. Tarsus measurements of translocated birds were not taken into account when measured in the new population. Although body mass might fluctuate over the season and day time, we do not think this could cause a bias in the data as similar catching strategies were performed in all populations. Body mass and tarsus size were fitted using a normal response model. As measurements from the same bird or of the same observer might be inter-correlated, bird identity and observer identity were included as cross-classified random effects with the measurements nested within these random effects.

To investigate whether insect densities differed between populations, monthly insect counts were fitted as a normal response model with month included as a random effect. All analyses mentioned above were performed in MLwiN 2.02 (Rasbash *et al.* 2004). Model selection was based on stepwise backwards elimination of the non-significant terms in the order of their significance assessed by the Wald statistic. The final model contained all significant explanatory terms. All eliminated terms were reintroduced to the final model to confirm their lack of contribution. Means are expressed with standard errors.

To investigate survival and resighting probabilities we employed an *a priori* approach in which a set of candidate models was created based on biological reasoning. Previous analyses showed that the survival of Seychelles warblers on Cousin varied between years and was lower in their first year of life (juvenile survival) than for older birds, with similar variation between the years for both age classes (Brouwer *et al.* 2006). Furthermore, there was no variation in survival nor in resighting probability between the sexes and no association with territory quality (Brouwer *et al.* 2006). Consequently, our global model allowed survival and resighting rate to vary between years. For Aride, birds ringed as juvenile were included as a group in the analysis and their survival probabilities were allowed to vary between the age

classes (first year and older). However, for Cousine just few juveniles were ringed over a long time-span and therefore these were only included in the analysis as an adult after their first year of life. Due to the small dataset for Denis, juvenile survival probabilities were not allowed to vary over time and their resighting probabilities were kept similar to those of adults. For the populations of Aride and Cousine we then used the most parsimonious model to test whether survival rates changed with increasing population density, whereby population density was included as a linear constraint on survival probabilities. Years in which no estimate of population density was available were extrapolated from nearest known numbers available. Akaike's information criterion corrected for sample size (AIC_c) was used to select the most parsimonious model, with better fitting models resulting in lower AIC_c values (Akaike 1973). We calculated the normalized Akaike weights to assess the relative likelihood of competing models. We checked the goodness-of-fit for the global model by using median \hat{c} procedure, which showed little evidence for overdispersion (Aride $\hat{c} = 1.16 \pm 0.03$; Cousine: $\hat{c} = 1.32 \pm 0.03$, Denis: $\hat{c} = 1.04 \pm 0.05$). Therefore, AIC_c values were adjusted to allow for the extent of overdispersion measured by \hat{c} , through quasi likelihood ($QAIC_c$). To account for model selection uncertainty, we used model averaging techniques which weigh the impact of all models for a given parameter according to its $QAIC_c$ weight (Burnham & Anderson 2002). The weighed averages were then used to investigate the relation between population density and survival between populations in a linear regression.

Results

Population dynamics

The population of Seychelles warblers on Cousin Island has been fairly stable since 1986 with only little natural fluctuation in the number of birds between years (average: 10.8 ± 0.2 birds/ha, C.V. = 0.10). The translocations of 29 birds in 1988 and 1990 caused experimental reductions in density of 10% and 9% respectively, within the 10% natural variation (Fig. 3.1). The translocation of 58 warblers in 2004 however, reduced the population density by 16%. In all cases the population recovered within one or two years to its original size, suggesting strong density dependence.

After the transfer in 1988 the birds on Aride experienced extremely low densities: 12 out of the 29 translocated birds established themselves on the plateau area (2.3 birds/ha). The population on the plateau increased tenfold to an asymptote of around 120 birds (25 birds/ha) in 1998, and was relatively stable thereafter (Fig. 3.1). A survey in 1997 indicated that the total population size was 1600 birds (23.5 birds/ha) (Betts 1998), a more than fifty-fold increase in density since the introduction. The population on Cousine Island showed a similar pattern of population growth. After the release of 29 birds (1.5 birds/ha) in 1990 the population grew to an asymptotic size of around 130 individuals by 1996 (6.8 birds/ha). Denis Island is the largest island and therefore had the lowest density of 0.41 birds/ha (58 birds)

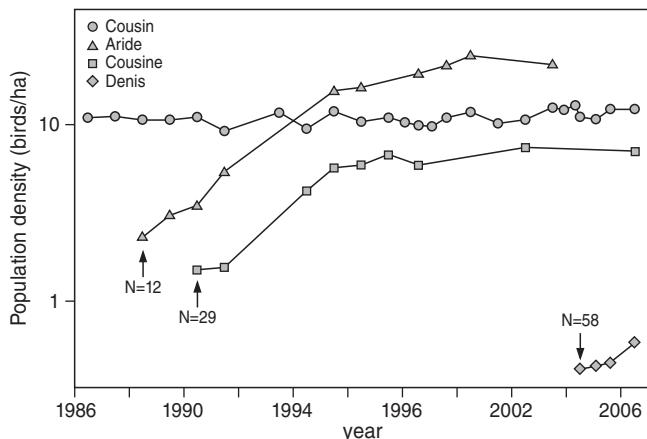


Figure 3.1 Annual fluctuations of population density (on log scale) of Seychelles warblers on the islands of Cousin, Aride (plateau), Cousine and Denis. Arrows with sample size indicate the introduction of birds to each island.

when first established in 2004 and numbers had only increased to 82 birds (0.5 birds/ha) by 2006.

During the process of population growth on Aride the number of territories increased as a result of birds expanding their range over the island however the size of the territories simultaneously decreased fivefold, from an average of 0.81 ha ($N = 6$) in 1988 to 0.16 ± 0.01 ha ($N = 34$) in 1999. Average territory size at saturation (1999) on Aride was significantly smaller than the average territory size on Cousin (0.23 ± 0.01 ha, $N = 107$; $U = 701.0$, $P < 0.001$).

As density increased so did group sizes: on Aride group size increased from 2.0 ± 0.3 ($N = 6$) in 1988 to 3.3 ± 0.2 ($N = 36$) birds per territory in 1999 and was significantly density dependent ($\chi^2_1 = 13.0$, $P < 0.001$). Similarly, group sizes on Cousine increased, from 1.6 ± 0.2 ($N = 11$) in 1991 to 2.7 ± 0.2 ($N = 41$) birds per territory in 1998, though this density dependent trend was not quite significant ($\chi^2_1 = 3.22$, $P = 0.07$). After saturation, territories on Aride contained the largest groups (3.3 ± 0.2), which were significantly larger than on Cousin (Cousin = 2.8 ± 0.1 , $N = 107$; $U = 1552$, $P = 0.04$) and Cousine ($U = 541.0$, $P = 0.02$). Group sizes on Cousine were not significantly smaller than on Cousin ($U = 2063.0$, $P = 0.41$). On Denis, cooperative breeding has not been observed in the two years since the translocation and all territories still consist of single birds or pairs.

The population growth rates (r) are shown as the slopes of the relation between log population densities and year (Fig. 3.1). Population growth rates clearly decrease with increasing population densities during the process of saturation. Population density alone did not account for all the observed variation between islands; at very low densities during the year after translocation the population growth rates on Cousine and Denis Island were similar ($r = 0.015$) but were lower

than on Aride ($r = 0.12$) (Fig. 3.1). However, over a number of years (1991–1995) the population growth rates were similar for Aride and Cousine, but the final population density on Aride was twice as high as that on Cousine, and was also higher than on Cousin, suggesting that the limiting factors differed in magnitude between islands.

Reproduction

On Cousin the average per capita reproduction was 0.27 ± 0.03 fledglings per year (range: 0.17–0.42). Reproduction varied between years, but the variation was unrelated to population density, the amount of rainfall during the breeding season or the total amount of rainfall in that year (Table 3.1). Furthermore, no correlation was found between territory quality and annual fledging production (Table 3.1). The available territory space per individual (= territory size/divided by groups size) was however, positively associated with reproduction, independent of group size (Table 3.1). In addition, reproduction was positively related to group size, but did not increase further after group size five (Table 3.1, Fig. 3.2).

The per capita reproduction was strongly negatively related to the population density on both Aride and Cousine: after translocation reproduction was initially high but declined as the population grew (Fig. 3.3A and 3.3B). The seasonality of reproduction was also affected by the translocation, on Aride year round breeding occurred when the population was at a low density. The per capita reproduction on Aride was on average 5.6 times higher in the first three years after translocation than on Cousin, and twice as high as in the first year on Cousine (Fig. 3.3A). At low density reproduction on Cousine was also higher than at saturation on Cousin (Fig. 3.3A). Analyzing the per capita reproduction per breeding season showed that with

Table 3.1 Results of hierarchical cross-classified model examining the annual reproductive output per territory (measured as no. fledglings/bird) of Seychelles warblers on Cousin Island between 1986–2006, ($N = 902$ territories). Density, total rainfall and breeding season rainfall are year variables and consequently modeled on the year variation, whereas the other variables are modeled at the level of the individual territories.

Parameter	B ± S.E.	χ^2	d.f.	P
Final model:				
Intercept	-2.91 ± 0.38		1	
Territory size/bird	3.24 ± 0.81	14.0	1	< 0.001
Group size	1.12 ± 0.20	27.4	1	< 0.001
Group size ²	-0.09 ± 0.03	11.7	1	< 0.001
Random effects: $\sigma_{\text{year}}^2 = 0.16 \pm 0.15$, $\sigma_{\text{territory}}^2 = 0.01 \pm 0.01$				
Rejected:				
Density	0.14 ± 0.20	0.50	1	0.48
Territory quality	0.002 ± 0.01	0.02	1	0.89
Total rainfall	0.001 ± 0.001	0.46	1	0.50
Breeding season rainfall	0.001 ± 0.001	0.10	1	0.75

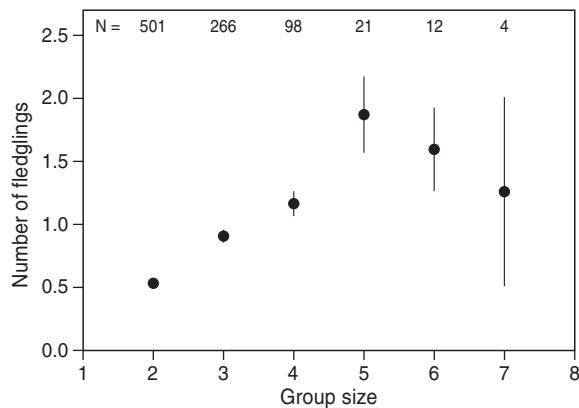


Figure 3.2 Mean annual reproduction (number of fledglings) per territory in relation to group size on Cousin Island. Values are corrected for territory size and between year and territory variation according to the final model in Table 3.1. Numbers on top indicate the total sample size.

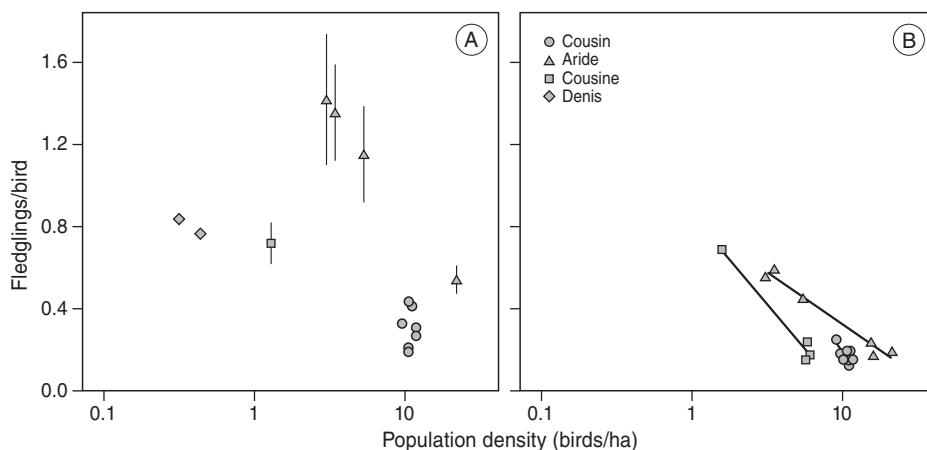


Figure 3.3 The per capita reproduction in relation to population density for Seychelles warblers of the islands of Cousin, Aride, Cousine and Denis per A) year and B) main breeding season (July-September). Values per breeding season are corrected for between year variation. Lines are the regression lines according to the model shown in Table 3.2A.

increasing density reproduction decreased significantly on Aride and Cousine (Table 3.2A). The strength of the decrease in reproduction with increasing density was stronger on Cousine than on Aride (Table 3.2A). After the populations reached their stable size, per capita reproduction was very similar among the islands.

Although group size also increased with increasing density, and could therefore have caused the decrease in per capita reproduction, this was not the case. Analyzing the reproduction at the territory level gives similar results; with increasing density reproduction significantly decreases both on Aride and Cousine (Table 3.2B).

Table 3.2 Relation between population density and the average: A) per capita and B) per territory reproduction per breeding season for Seychelles warblers on the Islands of Cousin, Aride and Cousine. Estimates of effect sizes are $\times 10$ on log scale.

Parameter	B ± S.E.	χ^2	d.f.	P
A) Final model:				
Intercept	-2.47 ± 1.04		1	
Density	-0.26 ± 0.07	12.3	1	< 0.001
Population*		1.02	2	0.31
(β_{Cousin})	3.37 ± 6.23)			
(β_{Cousine})	1.69 ± 1.73)			
Density x population†		6.67	2	0.04
(β_{Cousin})	-0.62 ± 0.58)			
(β_{Cousine})	-0.84 ± 0.33)			
Random effect:	$\sigma_{\text{year}}^2 = 2.68 \pm 1.44$			
B) Final model:				
Intercept	2.24 ± 1.18		1	
Density	-0.25 ± 0.09	8.07	1	0.004
Population*		0.29	2	0.87
(β_{Cousin})	2.77 ± 7.48)			
(β_{Cousine})	1.04 ± 2.21)			
Density x population†		5.60	2	0.06
(β_{Cousin})	-0.62 ± 0.70)			
(β_{Cousine})	-0.10 ± 0.04)			
Random effect:	$\sigma_{\text{year}}^2 = 0.14 \pm 0.11$			

* reference category is Aride population and is given by the intercept

† reference category is Aride population and is given by the density main effect

To investigate whether the variance in reproduction per territory increased as a result of lower quality habitat being occupied, the variance in reproduction at low density was compared with that at high density. However, with increasing density the variance in reproduction on Aride and Cousine did not change (Levene's test, Aride: $F_{5,32} = 0.86$, $P = 0.36$; Cousine: $F_{8,42} = 0.62$, $P = 0.44$).

Survival

The most parsimonious model for the Aride data set showed that survival and resighting probabilities were lower in an individual's first year than later in life (Table 3.3A, model 1). Furthermore resighting, but not survival, varied between years in a similar way for both age classes (Table 3.3A, model 1). On average the annual survival probability was 0.68 ± 0.05 (resighting probability = 0.56 ± 0.14) for juveniles and 0.77 ± 0.02 (resighting probability = 0.79 ± 0.07) for adults. The most parsimonious model for the Cousine data set showed that survival, but not resighting probabilities, varied between years (Table 3.3B, model 1). The average annual adult survival probability was 0.83 ± 0.07 (resighting: 0.95 ± 0.02).

Table 3.3 Summary of model selection statistics of year, age and density effects on survival and resighting probabilities in the Seychelles warbler for the populations on; A) Aride and B) Cousine Island. (t) = year, (a) = age, (.) = constant, (density) = population density.

No	Model	No.Par.	Quasi deviance	ΔQAIC_c	QAIC_c weights
(A)					
1	$\Phi_{(a1,2+)} P_{(t+a1,2+)}$	13	352.7	0.0	0.53
2	$\Phi_{(\text{density}+a1,2+)} P_{(t+a1,2+)}$	14	352.6	2.0	0.19
3	$\Phi_{(\text{density}x a1,2+)} P_{(t+a1,2+)}$	15	350.8	2.3	0.17
4	$\Phi_{(t+a1,2+)} P_{(t+a1,2+)}$	23	336.1	4.4	0.06
5	$\Phi_{(t)} P_{(t+a1,2+)}$	22	340.3	6.5	0.02
6	$\Phi_{(txa1,2+)} P_{(t+a1,2+)}$	33	317.1	7.0	0.02
7	$\Phi_{(txa1,2+)} P_{(txa1,2+)}$	41	301.4	9.1	0.01
8	$\Phi_{(txa1,2+)} P_{(txa1-2,3+)}$	40	304.9	10.3	0.00
9	$\Phi_{(txa1,2+)} P_{(t)}$	32	323.7	11.5	0.00
10	$\Phi_{(txa1,2+)} P_{(a1,2+)}$	25	418.0	90.6	0.00
(B)					
1	$\Phi_{(t)} P_{(.)}$	7	42.27	0.0	0.95
2	$\Phi_{(t)} P_{(t)}$	11	39.86	6.1	0.05
3	$\Phi_{(\text{density})} P_{(.)}$	3	62.79	12.3	0.00
4	$\Phi_{(.)} P_{(.)}$	2	66.88	14.3	0.00

Models were ranked according to their QAIC_c value, with the best supported model on top. ΔQAIC_c being the difference between the QAIC_c of the best supported model and the model considered.

Increasing density during the process of saturation did not result in decreasing survival rates for adults on Aride and Cousine or for juveniles on Aride (Table 3.3A, models 2 and 3; Table 3.3B, model 3). Furthermore, when comparing between island variation there is no indication that higher density results in lower survival probabilities ($r = 0.14$, $P = 0.27$; Fig. 3.4).

Food as cause of density dependence?

To assess whether food plays an important role in both the island differences in reproduction and the density dependence within islands, we investigate whether there are differences in body mass and tarsus length of the birds on the different islands. Both body mass and tarsus length differed significantly among islands, with Aride having the largest (but similar to Cousin) and heaviest birds, and Cousine having the smallest birds (Fig. 3.5). That these differences are due to effects of population density seems unlikely because on the island of highest density (Aride) the birds are biggest and heaviest. As no size measurements are available during the process of population saturation on Aride and Cousine we cannot test whether an increase of density within islands leads to lower body mass or structural size.

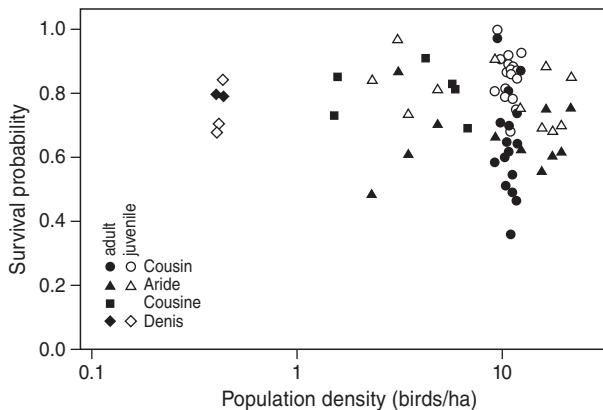


Figure 3.4 Annual adult and juvenile survival probabilities of Seychelles warblers in relation to population density for each of the four populations. Estimates of survival are derived by model averaging (see Table 3.3).

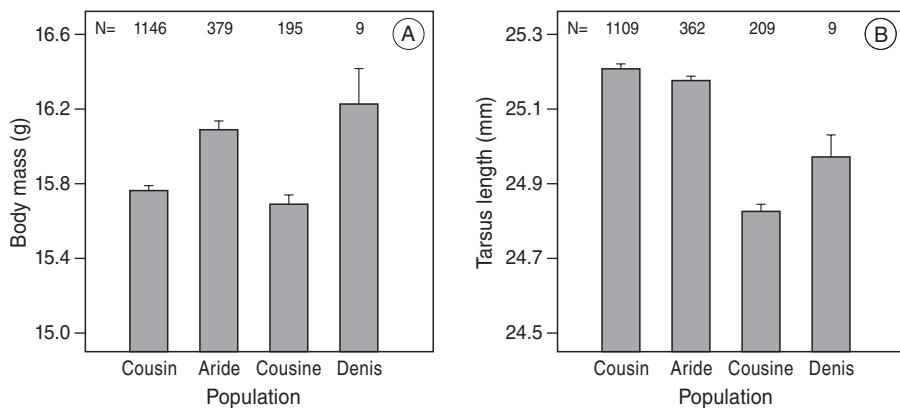


Figure 3.5 A) Body mass and B) tarsus length measurements of adult Seychelles warblers for the populations of Cousin, Aride, Cousine and Denis, corrected for observer (bodymass: $\sigma^2 = 0.08 \pm 0.04$; tarsus: $\sigma^2 = 0.07 \pm 0.03$) and between individual (bodymass: $\sigma^2 = 0.71 \pm 0.06$; tarsus: $\sigma^2 = 1.06 \pm 0.06$) variation. Numbers on top indicate total sample sizes.

Food abundance, measured as insect density, might explain variation in body mass between populations better than density. In 1990, food abundance on Aride (233.7 ± 44.7 insects, $N = 3$) was on average 3.1 times higher than on Cousin (74.7 ± 10.4 insects, $N = 3$) ($\chi^2_1 = 17.7$, $P < 0.001$). On Cousine (124.0 ± 35.2 insects, $N = 3$) food abundance was on average 1.7 times higher, but this was not significantly different ($\chi^2_1 = 1.7$, $P = 0.19$). By 1996, after both islands had reached saturation, insect availability was only 1.8 times higher on Aride (231.7 ± 54.7 insects) than Cousin (127.0 ± 26.7 insects), and 0.7 times lower on Cousine (86.0 ± 14.7

insects) in comparison to Cousin (Aride: $\chi^2_1 = 16.4$, $P < 0.001$; Cousine: $\chi^2_1 = 2.5$, $P = 0.11$). Over the years insect densities on Cousin remained constant ($F_{1,9} = 0.62$, $P = 0.45$). Therefore the island with least food (Cousine) has the lowest asymptotic density and the smallest birds, while the island with the highest food availability (Aride) has the highest density, the highest reproduction at low density, the largest groups per territory and the biggest birds.

Discussion

Density dependence

After translocation to new islands, the Seychelles warbler populations were initially far below carrying capacity but grew rapidly to reach an asymptotic level, which differed among islands. This experiment provided good evidence that reproduction was strongly negatively related to population density. Asymptotic population density was higher on the island with higher food abundance and individual birds were structurally bigger there. Furthermore, the strength of the decline with population density was weaker for the island with high food abundance and initial reproduction was higher there, although this could be a result of between year variation. These results indicate that regulation of numbers takes place via density dependent reproduction, probably caused primarily by competition for food.

We found no evidence that survival was important in regulating population density, which concurs with the results of a previous study of the effect of reduced density, caused by removal of birds, on survival probabilities (Brouwer *et al.* 2006). As our results are based on the establishment of multiple new populations, and not on fluctuations caused by changing environmental conditions or the manipulation of resources, the decline in reproduction must have been caused by increasing density itself. Although density dependent reproduction is a commonly reported phenomenon, density dependent survival is more difficult to detect because of the difficulties in distinguishing between survival and dispersal in most systems. However, studies that have investigated survival often show that juvenile, but not adult, survival probabilities might be regulated in a density dependent way (Clutton-Brock *et al.* 1987, Dhondt *et al.* 1990, Arcese *et al.* 1992, Armstrong and Ewen 2002, Nicoll *et al.* 2003, Armstrong *et al.* 2005). In our study, juvenile survival may have been biased by dispersal as our estimates were based on the plateau area of Aride only. Estimates based on the whole Aride population suggests that juvenile survival was higher (average 82%) during the first three years after translocation, however even at high densities high (even underestimated) estimates are not uncommon (e.g. 1996: 0.75 ± 0.08). In the comparison with other bird species in which density dependence was observed, it should be noted that these were all on temperate species with lower annual survival rates and often larger broods. In contrast, our species has a typical tropical life history, with small brood sizes, long lasting parental care and high juvenile survival rates, and juveniles may thus be less affected by competition.

We did not detect an association between the relatively low levels of natural and experimental fluctuations in a saturated population and mean reproduction. However, the rapid recovery of the population back to saturation after each translocation of significant proportions of the population is, in itself, direct evidence for density dependence.

Food as cause of density dependence?

Food availability has been shown to affect reproduction and survival in many species (for reviews see: Martin 1987, Boutin 1990, Newton 1998). However, in most studies relationships between population size and food availability can always be confounded by selection on specific high quality individuals settling in areas with high food availability, rather than a direct effect of food availability. In isolated populations we showed that insect availability was associated with both asymptotic population density, reproduction at low density and the structural size of individuals. Another indirect line of argument to suggest that food played a major role in competition is found in the reduction of reproduction with a large number of subordinates in the territory, and negative effect of territory space per individual on reproduction. Although insect densities on Cousin remained constant since 1986, it is unclear whether insect densities on Cousine decreased as a result of the introduction of warblers on the island. Although birds in the population with lowest food abundance (Cousine) were smallest, they had similar body weight to birds in the original Cousin population and must therefore have been relatively heavier. As structural size is determined during the early growth period it seems plausible to suggest that the availability of food during the nestling period may be responsible for limiting their growth. Structural size also has been shown to be strongly heritable in different bird populations (Merilä *et al.* 2001), and therefore the possibility that natural selection may have played a role cannot yet be excluded. An alternative explanation is that birds are selected to be relatively large at high density because at high competition it is more advantageous to be larger (Both *et al.* 1999). We do not have the data to show that selection for large size is indeed stronger when density increases, but it may be the case that under high densities a larger proportion of resources is allocated to chick quality rather than quantity (Mesterton-Gibbons & Hardy 2004). The reason why insect availability differs between the islands in our study remains unknown. It could be a consequence of different vegetation composition, renewal rates as a consequence of differences in the food webs or differences in micro-climate, or even different levels of depletion by the predators. Unfortunately, we do not yet have the data to investigate this further, nor to investigate whether the introduction of the birds on islands had any significant effect on the food availability.

Komdeur (1992) found that territory quality was associated with reproduction, but our analysis on reproduction within the saturated population failed to find the same effect. However, this difference can be explained by the inclusion of territory size in the earlier studies' calculation of territory quality. We now show that territory size was positively associated with reproduction, independent of group size.

Although the between island comparison shows that insect availability is very important and therefore that a relation between reproduction and territory quality within the saturated population is expected, our measure of territory quality might not be accurate enough to detect this. Insect availability was counted at 15 sampling points across the island (Komdeur 1992) and not in each territory separately. Furthermore, our measurement of territory quality includes estimates of foliage cover which might not directly or proportionally translate into increased insect availability. Consequently, our results show that an index rather than true territory quality was measured.

Cooperative breeding

Offspring hatched in the newly established populations did not delay dispersal to stay as a subordinate in the territory until one and two years after translocation on Cousine and Aride respectively. As the population densities on these islands further increased so did group sizes, and eventually group sizes on the island with highest food availability (Aride) becoming significantly larger than in all other populations. Instead of establishing new territories, more subordinates stayed in the natal territory, because the costs of defending a territory are possibly too high at such high densities. Alternatively, as territory size is also associated with reproduction, such territories might be too small for successful reproduction and not outweigh the benefits of staying and gaining indirect fitness benefits, especially as territory sizes were already significantly smaller than in the Cousin population.

Increased population density and, consequently, group size resulted in a decrease in per capita reproduction. However, not only did fewer individuals start to reproduce independently but also, reproduction decreased at the territory level. This was not a consequence of lower quality habitat being occupied resulting in lower average reproduction as the variance in reproduction per territory at high and low density did not change. We suggest that with increased density crowding results in increased competition for food, which in turn leads to a decrease in both the number of individuals able to reproduce and the number of offspring produced per bird.

Conclusion

We found that reproduction, but not survival, decreased with increasing population density. Variation in mean reproduction between islands could be explained by food abundance, measured as insect availability. Islands with the highest insect availability also had the highest bird densities. Hence, we suggest that the density dependent reproduction observed is caused primarily by competition for food, however experiments (e.g. supplementary feeding) are needed to confirm this. We show that next to monitoring numbers, data on behaviour and reproduction can add important information about the mechanism regulating populations during the process of saturation. The mechanism by which density dependence occurs determines to a large extent the outcome of evolutionary life history models (Mylius & Diekmann 1995), quantitative models of cooperative breeding (Pen & Weissing 2000), but also popula-

tion viability analyses, which provide a quantitative assessment of the probability of a population to decline to extinction (Beissinger & Westphal 1998). Consequently, our results can contribute to this field, especially because our results are based on a tropical species, the area where biodiversity threats are especially prominent nowadays.

Acknowledgements

The Department of Environment and Seychelles Bureau of Standards gave permission for field-work and sampling. We would like to thank the owners and staff of Aride, Cousine and Denis Island for allowing us to work on their islands. Furthermore we would like to thank Camille Hoareau for his great help on Denis Island. We thank all the volunteers and students who have assisted with data collection since 1986, Michael Betts and Janet Hunter-Bowler for providing data collected on Aride and Alberto Castillo Solis and Arno Kangeri for help with data entry. This work was supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO, 84-519) allocated to JK.

Box A

Translocation of Seychelles warblers to Denis Island: another conservation success story?

Lyanne Brouwer, Jeroen J. Reimerink, Cas Eikenaar, Rachel Bristol, Jan Komdeur and David S. Richardson

Introduction

The establishment of new populations through the translocation of individuals to new suitable habitat is an important tool for conservation management. For several species, e.g. the Seychelles magpie robin (*Copsychus sechellarum*) and the Mauritius kestrel (*Falco punctatus*), the establishment or reintroduction of new populations has been crucial to the species continued existence (Bell & Merton 2002). The Seychelles warbler (*Acrocephalus sechellensis*) is one of eight endangered bird species endemic to Seychelles. Until 1988 the Seychelles warbler population was confined to Cousin Island (29 ha). Because of the vulnerability of a single population, and the fact that there is no dispersal between islands (Komdeur *et al.* 2004), birds were translocated to the islands of Aride (1988, 68 ha) and Cousine (1990, 26 ha) (Komdeur 1994a). In 2004 the warbler was listed as ‘vulnerable’ on the IUCN red list (BirdLife International 2004), on the basis of its tiny range: a total of only 120-ha over three islands containing an estimated population of approximately 2000 birds. As these island populations were at carrying capacity, a new translocation was planned to improve the conservation status of the Seychelles warbler. After extensive survey and habitat restoration work by Nature Seychelles, Denis Island (144 ha at 03°48' S, 55°40' E) was chosen as the island on which to try to establish a fourth population. Although Denis Island lies outside of the known historical range of the Seychelles warbler, it was considered to be suitable for a viable population for three reasons. First, the eradication of rats and cats provided a predator-free island. Second, partial removal of dense coconut-strands and planting of native trees had extended the remnant native forests. Consequently, Denis Island holds 30 ha of forest dominated by native broad-leaved species such as *Terminalia* and *Pisonia*, which provide good feeding opportunities for Seychelles warblers (Komdeur 1991a). Third, Denis Island is relatively distant from the three already existing populations, thus making the species less vulnerable to local catastrophic events, such as hurricanes and diseases. In this Box, we examine demographic rates in the first two years after translocation to investigate whether the translocation of Seychelles warblers to Denis Island has, so far, been successful.

Methods

Translocation

The translocation was undertaken in conjunction with Nature Seychelles. Birds were caught on Cousin Island and transported to Denis Island by helicopter approximately one month before the anticipated start of the breeding season on Cousin. The timing was chosen to coincide with the optimal condition of the birds on Cousin and to avoid disturbance of breeding birds. 58 birds were translocated of which 25 were females and 33 males. To be able to investigate MHC dependent mate choice translocation of complete groups and pairs was avoided and a surplus of males after

the first release was aimed for allowing ample female mate choice (Richardson *et al.* unpublished data). On the mornings of 29th and 30th May 2004 a total of 21 males and 14 females were moved. After two weeks of monitoring on Denis Island we were confident the translocated birds were doing well and another 12 males and 11 females were translocated. In total 50 birds were captured from 07:30h to 11:00h and translocated the same morning. The other 8 individuals were captured on the evening prior to translocation and kept overnight. Birds were kept individually in small cardboard boxes which contained food (termite eggs), water and a perch. On Denis the birds were released in the centre of the island, where the vegetation is richest. The vegetation at the release site was sprayed with water to provide the birds with drinking opportunities. During the third translocation birds were weighed before release. The population was subsequently studied for two months immediately after the translocation, and additionally in January 2005 and in July – August 2005 and 2006.

Data collection

During each fieldwork period the whole of Denis was intensively searched. Birds were located by listening for their song, sometimes stimulated by playback song and whistling, or the distinctive 'snapping' of the bill during foraging. Individual birds, identified by their colour rings, were followed for a minimum of 30 minutes in order to determine breeding status, territory boundaries (recorded using GPS) and the composition of their breeding group. Thereafter each territory was checked for breeding activity at least once every two weeks by following the resident female for 30 minutes (Komdeur 1992). If accessible, nestlings were ringed in the nest, otherwise young birds were caught after fledging using mist nets. Birds were ringed with a unique combination of three UV-resistant colour rings and a metal British Trust for Ornithology ring. Blood samples (ca. 15 µl) were collected by brachial venipuncture and then diluted in 800 µl of 100% ethanol in a 2.0 ml screw-cap microfuge tube and stored at room temperature. Molecular sexing using the PCR method was used to determine the sex of each individual (Griffiths *et al.* 1998). Sex-ratio was expressed as the proportion of males in the sampled population. Reproductive success was defined as the average number of fledglings produced per year per bird. Each monitoring period monthly insect counts were performed to estimate food abundance (Komdeur 1992).

Data analyses

To estimate the yearly survival probabilities of Seychelles warblers on Denis island, we analyzed the encounter histories of 93 marked individuals using program MARK (White & Burnham 1999). Of these marked birds, 25 individuals were ringed as juveniles on Denis Island. The resighting period was defined as each fieldwork period. Our global model (Table A.1, model 5) allowed adult survival and resighting to vary between periods. As survival and resighting probabilities for the last period are confounded, we subsequently simplified the model by including similar resighting

Table A.1 Summary of model selection statistics of year, age and sex effects on survival and resighting probabilities of juvenile (j) and adult (a) Seychelles warblers on Denis island (N = 93). (a1,a2+) = two age classes, first year and older, (s) = sex, (t) = time, (p) = period.

No	Model	No.Par.	Deviance	ΔQAIC_c	QAIC _c weights
1	$\Phi^j_{(a1,a2+(s))}\Phi^a(s) P(p)$	5	28.77	0.0	0.41
2	$\Phi^j = \Phi^a(s) P(p)$	4	30.95	0.1	0.40
3	$\Phi^j_{(a1(s),a2+(s))}\Phi^a(s) P(p)$	6	28.77	2.2	0.14
4	$\Phi^j_{(a1,a2+(s))}\Phi^a(tx(s)) P(p)$	9	25.22	5.2	0.03
5	$\Phi^j_{(a1,a2+(s))}\Phi^a(tx(s)) P(t)$	10	25.22	7.4	0.01
6	$\Phi^j_{(a1(s),a2+(s))}\Phi^a(tx(s)) P(p)$	10	25.22	7.4	0.01
7	$\Phi^j_{(a1,a2+(s))}\Phi^a(tx(s)) P(.)$	8	33.13	10.9	0.00
8	$\Phi^j_{(a1,a2+(s))}\Phi^a(tx(s)) P(tx(s))$	13	24.46	13.6	0.00

probabilities for July 2005 and July 2006. Although there is no possibility to test whether this assumption was correct, resighting was close to 1 in July 2005 and resighting effort was similar during both periods. Birds ringed as juvenile were included as a group in the analysis to estimate juvenile (first year) survival, however as only five fledglings were ringed in 2004 and none in January 2005, juvenile survival probabilities were not allowed to vary over time and juvenile resighting probabilities were kept similar to those of adults. Akaike's information criterion corrected for small sample size (AIC_c) was used to select the most parsimonious model (Akaike 1973). Additionally, we calculated the normalized Akaike weights to assess the relative likelihood of competing models. We checked the goodness-of-fit for the global model by using median \hat{c} procedure, which showed no evidence for overdispersion ($\hat{c} = 1.04 \pm 0.05$). To account for model selection uncertainty, we used model averaging techniques which weigh the impact of all models for a given parameter according to its AIC_c weight (Burnham & Anderson 2002).

Results

Population establishment

The first pairing of warblers was observed just five hours after release on Denis Island and two of the newly formed pairs started nest building three days after the translocation. In 2004, a total of 23 territories were located, of which 15 pairs attempted breeding in the first two months after translocation. All but one bird was re-sighted during this period and birds that did not settle as a breeder during this initial two month period were seen as floaters on the island or established a territory on their own. Population size, number of territories and number of pairs all

increased after establishment of the population, reaching a total of 99 birds in 2006 (Figure A.1A). However, the number of females did not increase as fast as the number of males and, consequently, the number of pairs did not increase as fast as the number of territories, with 14 territories occupied by single males in 2006 (Figure A.1A).

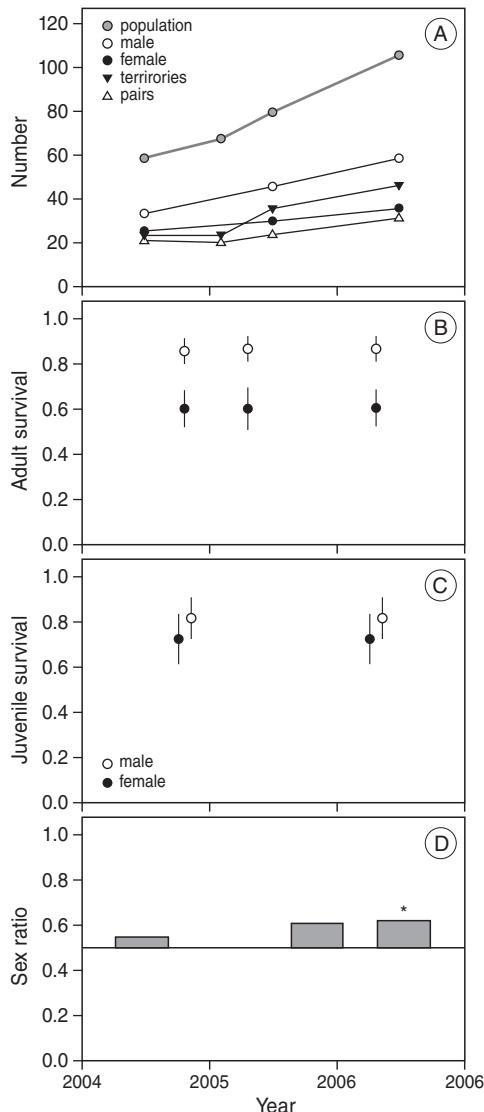


Figure A.1 Details of population dynamics of Seychelles warblers after introduction on Denis Island (June 2004–August 2006) with A). total population size, number of males, females, pairs and territories, B). annual adult survival (\pm S.E.), C). annual juvenile survival (\pm S.E.), and D). population sex-ratio. Survival probabilities were based on model averaging. * indicate significance at $\alpha = 0.05$.

Reproduction

In January 2005 no birds were caught, however at the end of August 2005 and 2006 all but 4 and 6 respectively recently fledged young were ringed and sampled. In July 2005 a total of 33 unringed birds were recorded on Denis, while in July 2006, 40 new birds were recorded. These figures lead to an estimated average annual reproduction rate of 0.58 and 0.63 fledglings per adult bird in 2005 and 2006 respectively. In contrast to the saturated Cousin population, no cooperative breeding was observed on Denis.

Survival

The most parsimonious models show that adult survival differed between the sexes and that this did not vary between years, with females having lower survival probabilities than males (Table A.1, model 1 and 2; Figure A.1B). Although female juvenile survival was slightly lower than male juvenile survival, including different juvenile survival probabilities for both sexes was not supported by the data (Table A.1, model 3 vs. model 1; Figure A.1C). Juvenile survival was high and a model including different survival probabilities for juveniles and adults was not supported by the data (Table A.1, model 1 vs. model 2). Resighting probabilities did not vary between the sexes (Table A.1, model 8 vs. model 5), but the variation between years could be reduced to two periods with lower resighting in January 2005 (0.72 ± 0.06) than in July 2005 and 2006 (0.95 ± 0.03) (Table A.1, model 4 vs. model 5), and this dichotomy was better supported by the data than constant resighting probabilities (Table A.1, model 4 vs. model 7).

Although the population was slightly male biased at establishment, this was not statistically significantly different from unity (binomial test, $N = 58$, $P = 0.35$). However, as a result of higher female mortality, the population sex ratio tended to be male biased in 2005 (binomial test, $N = 74$, $P = 0.08$) and was significantly male biased in 2006 (binomial test, $N = 93$, $P = 0.02$; Figure A.1d). Breeding birds did not respond to the biased population sex-ratio by producing more female offspring; the sex-ratio of the newly produced birds was not significantly different from unity (sex-ratio = 0.54; binomial test, $N = 70$, $P = 0.55$). However, biased mortality could already have taken place in this sample before the surviving fledglings were caught. However, the direction of the trend remained opposite to expectation even when we confined the analysis to birds sampled as nestling or fledgling (sex-ratio = 0.61, $N = 18$).

Food availability

Comparing insect densities on tree species which occurred on both islands revealed that insect densities were similar between the islands (paired t-test, $t = 0.34$, d.f. = 14, $P = 0.74$). However, the most common tree species on Denis (*Terminalia catappa*) does not occur on Cousin and insect densities on this tree species are significantly higher than the insect densities on one of the most common tree species on Cousin ($U = 1194$, $n_1 = 42$, $n_2 = 98$, $P < 0.001$).

Discussion

The strong growth of the Denis Island population over the two years since establishment means that this translocation can be considered a success so far. Nonetheless, the relatively high mortality of females compared to males and to the average annual survival on Cousin (84%) does give reason for some caution. On Denis birds were breeding year-round and the rate of reproduction was much higher than in the original population on Cousin (0.60 vs. on average 0.30 fledglings/bird). The higher mortality in females may therefore be a cost of reproduction. However, translocations to Aride and Cousine also resulted in increased reproduction but higher mortality was not observed (Brouwer *et al.* 2007b). Nevertheless, the estimates of reproduction on Denis are underestimated as only the surviving birds are counted. Furthermore, when assuming that only paired birds produced offspring the estimates get much higher (\approx 0.8 fledgling/bird). Future studies that include parentage analyses are needed to determine whether female mortality is linked to the production of more offspring. Male Seychelles warblers also put a lot of effort in reproduction by guarding of the nest and feeding of young, yet they do not suffer from high mortality. However, food availability does not seem a limiting factor as insect densities were similar to those on Cousin, and birds on Denis were even significantly heavier than on Cousin (Brouwer *et al.* 2007b). Furthermore, both the densities of conspecifics and predators (some main predator species even absent) are much lower than on Cousin. This might indicate that the costs for males are not as high as for females which lay and incubate the eggs, even several times a year. Alternatively, the higher mortality for females might be associated with their incubation behaviour itself, parasites associated with the nest could in this way be able to infest the females. Preliminary results of gastro-intestine parasite research however, shows that no parasites are present in faecal samples collected from birds on Denis (K. Hutchings, pers. comm.).

Although the total population size increased quickly, the effective population size is increasing at much lower rate as the number of females is a limiting factor. Further research is needed to asses whether the reduced female survival occurs in the coming years and whether females hatched on Denis will also suffer from low adult survival. Although the results from the first two years of monitoring of this fourth population of Seychelles warblers look promising, some worries persist regarding the biased population sex-ratio. Consequently we cannot yet state that this translocation has been another conservation success story.

Acknowledgements

We would like to thank Nature Seychelles for organizing the translocation and the Mason family for allowing us to work on Denis Island. Furthermore, we would like to thank Camille Hoareau for his hospitality and help during the field work.



Species interacting with Seychelles warblers. Top: the Seychelles magpie robin and Seychelles fody predate on warbler-eggs; the hermit crab and Wright's skink scavenge dead warblers and hamper ring-recoveries of dead warblers. Bottom: the introduced ship rat –probably the greatest threat to warblers– is present in most of the Seychelles but absent from all warbler islands.



4

Chapter

Long-term effects of rearing conditions on survival and recruitment in a cooperative breeder

Lyanne Brouwer, David S. Richardson and Jan Komdeur

Abstract

Conditions during the rearing period can have far reaching consequences for the survival and reproduction of offspring later in life. Such conditions can vary due to parental or environmental effects. In this study, we used multi-state capture-recapture models to investigate the effect of conditions during the rearing period on the survival and recruitment probabilities in a closed population of the cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*). We investigated whether territory quality, group size, the number of helpers, non-helpers and nest mates in the rearing territory were associated with offspring survival and recruitment. To distinguish rearing conditions from non-causal effects resulting from quality of the parents or territory, we used a nestling cross-foster experiment to investigate the effect of natal versus rearing territory conditions. We found that the number of helpers in the rearing territory, but not in the natal territory, was positively associated with survival of offspring in their first year, and also later in life. This was not caused by the number of subordinates in the territory, as the number of non-helping birds was not associated with survival. Neither the quality, group size, nor the number of nest mates in the rearing territory affected juvenile or adult survival. Furthermore, rearing conditions were not associated with an individuals' subsequent probability of acquiring a breeding position. Our results show that helpers not only increase offspring survival in the short-term, but also increase subsequent adult survival and, therefore, have important fitness consequences later in life.

Introduction

Variation in fitness is already shaped by the conditions an individual experiences during the rearing period. Conditions during the rearing period can vary due to differences in the quality of the environment and/or of the parents. It is obvious that variation in rearing conditions will immediately affect offspring fitness. Originally, however, it was thought that this variation would not affect long-term fitness as the environmental stochasticity that individuals experience would accumulate during their lifetime and because selection gradients on fitness become weaker over the course of life (Charlesworth 1980; Caswell 2001). However the importance of the long-term consequences of rearing conditions has recently been emphasized (Lindström 1999) and several studies have now shown that such conditions can have important fitness consequences later in life (Magrath 1991; Sedinger *et al.* 1995; Green & Cockburn 2001; Cam *et al.* 2003; van de Pol *et al.* 2006).

Variation in rearing conditions due to environmental effects can differ between individuals (e.g. due to variation in territory quality) or can affect entire cohorts (e.g. due to bad weather in a specific year: (van der Jeugd & Larsson 1998; Reid *et al.* 2003). Parental effects can affect the offspring directly via their genes, but parental quality can also affect offspring as a result of the parents' reproductive decisions, e.g. the trade-off between quantity and quality of offspring (McNamara & Houston 1992; McNamara & Houston 1996; Morris 1998) or because of the quality of their parental care, e.g. feeding effort (Hakkarainen & Korpimäki 1994). Parental quality can also contribute to how the environment affects early development (Lindström 1999). Furthermore, environmental conditions can be manipulated by parents, for example by adjusting parental care or by laying eggs of different quality. Egg quality can differ because of differences in size or composition and this can affect offspring growth, survival or immune function (Hipfner 2000; Eising *et al.* 2001; Saino *et al.* 2003).

The long-term effects of rearing conditions have rarely been investigated in cooperatively breeding species. In such species some individuals delay dispersal and often help to rear kin (e.g. Brown 1987). The presence of helpers adds an important component to the rearing conditions as the presence of helpers has been shown to positively affect offspring survival and weight (Brown 1987; Emlen 1991). However, whether the presence of these helpers can have long-term fitness consequences for offspring has received little attention (but see: Hatchwell *et al.* 2004). Furthermore, experiments are needed to test this as the decision to help might depend on the quality of the territory or parents, resulting in non-causal correlations between the presence of helpers and offspring fitness.

The main difficulty in interpreting how variation in rearing conditions affects long-term fitness is the inability to distinguish rearing effects from the parental/territory quality effects. Cross-foster experiments, in which nestlings are swapped between nests, are able to resolve this problem by separating the rearing from the parental/territory effects. Although many studies have shown effects of rearing

conditions on survival and recruitment, effects of rearing conditions are also typically related to dispersal (Verhulst *et al.* 1997). Therefore results might be confounded by the unobserved effects of dispersal outside the study population (Ims & Hjermann 2001; Bennets *et al.* 2001). Ideally, the effects of rearing conditions on survival and recruitment should be studied in a closed population with no immigration and emigration.

Here we present data from a closed population of the Seychelles warbler (*Acrocephalus sechellensis*), a species endemic to a few small islands in the Indian Ocean. Nestlings are fed in the nest for 18–20 days prior to fledging, and then up to three months before reaching independence (Komdeur 1991a). Although warblers can breed independently in their first year, a lack of suitable habitat drives them to become subordinate within their natal territory (Komdeur 1992). Using multistate mark-recapture analyses on 20 years of data, we investigate the long-term effect of conditions during the rearing period upon future juvenile and adult survival and the probability of being recruited into a breeding position. Conditions during the rearing period are defined by territory quality, group size, the number of helpers and non-helpers and the number of nest mates in the rearing territory. Previous analyses have shown that natal territory quality and group size do not affect survival, but whether they could be associated with recruitment probabilities is unknown (Brouwer *et al.* 2006). Here we use data from a cross-fostering experiment of nestlings, performed as part of an earlier study (Richardson *et al.* 2004) to distinguish rearing from parental/territory effects, by investigating the effects of the conditions of the original territories of the cross-fostered offspring as well. When effects of rearing conditions on survival or recruitment are the result of non-causal relationships caused by higher quality parents or territories, the conditions of the original territories of the cross-fostered offspring should show this effect as well.

Methods

Study area and data collection

Data were collected from 1986 to 2006 as part of the long term study of the Seychelles warbler population on Cousin Island (04°20' S, 55°40' E). During the main breeding season (July to September), and in some years during the minor breeding peak (January to March), each territory was checked for the presence of birds and breeding activity (Komdeur 1992; Brouwer *et al.* 2007a). Most Seychelles warblers produce one clutch per season (Komdeur 1996b) and this normally consists of just one egg, but about 20% of nests contain two or three eggs (Richardson *et al.* 2001). Parentage analyses have shown that egg dumping does not occur, but joint nesting occurs frequently with 44% of subordinate females producing offspring and 40% of offspring resulting from extra-group paternity (Richardson *et al.* 2001). As Seychelles warblers stay in their natal territory until they are at least six months of age (Komdeur 1996a), rearing conditions were defined as the conditions during the

breeding season the bird hatched. Nestlings were individually colour-ringed and blood-sampled at approximately 12 days old. If a nest could not be reached, the nestling was caught within the natal territory after fledging but while still dependent upon its parents using mist nets. Molecular sexing using the PCR method (Griffiths *et al.* 1998) was used to determine the sex of each individual sampled since 1993. Before that birds were sexed based on observations and biometry (Komdeur 1991a). Seychelles warblers are insectivorous, taking 98% of their insect food from leaves. Therefore, the number of insect prey available, the territory size and foliage cover were used to calculate an index of territory quality following Komdeur (1992). Territory quality was calculated for each territory in 1987, 1990, 1996-1999 and 2003-2006. For the remaining years, territory quality for each territory was calculated as the average from the preceding and following period (Brouwer *et al.* 2006).

During each season we assigned the status of all birds in the population. The 'primary' male and female was defined as the dominant, pair-bonded male and female in the territory. All other birds, older than eight months, resident in the territory were defined as 'subordinate' (Richardson *et al.* 2002) which could either be a 'helper' or a 'non-helper'. Nests were observed throughout the breeding cycle and, when subordinates were present in the territory, ninety minute focal watches during the incubation and the nestling feeding stage were performed to asses whether a subordinate was helping or not. A subordinate was defined as helper when it contributed to the rearing of the offspring, through incubation (females only) and/or the feeding of young (both sexes). When a juvenile was found in the fledgling stage and no observations had been conducted on the nest to determine the status of subordinate birds in the territory, the subordinate(s) was assumed to have been helping at the nest if it had been observed helping in the previous and/or subsequent breeding events. Co-breeders might have wrongly been assigned as helper, however, our main interest lies in how additional birds affect the rearing of young.

Survival and recruitment

To investigate the effect of rearing conditions on survival and recruitment, we analysed survival, resighting and transition probabilities between the states of, 'juvenile', 'subordinate' and 'primary' in one combined model, using multistate mark-recapture models based on resightings (e.g. Nichols & Kendal 1995). The survival and transition probabilities for all states were estimated according to Fig. 4.1. We constructed the capture-resighting histories of 1018 marked birds that were monitored between 1986 and 2006. Of these, 499 were ringed as nestling or fledgling (known age and territory). Offspring are considered as juveniles until they are at least 8 months old, and, therefore, can be considered as juveniles in two consecutive periods in years where both breeding peaks are monitored. To include this in the multistate model the juvenile state was divided into two states: 'fledgling' and 'juvenile'. Juvenile birds could subsequently become 'subordinates' recruit to a 'primary' (breeding) position or die (Fig. 4.1). We tested whether survival in the different

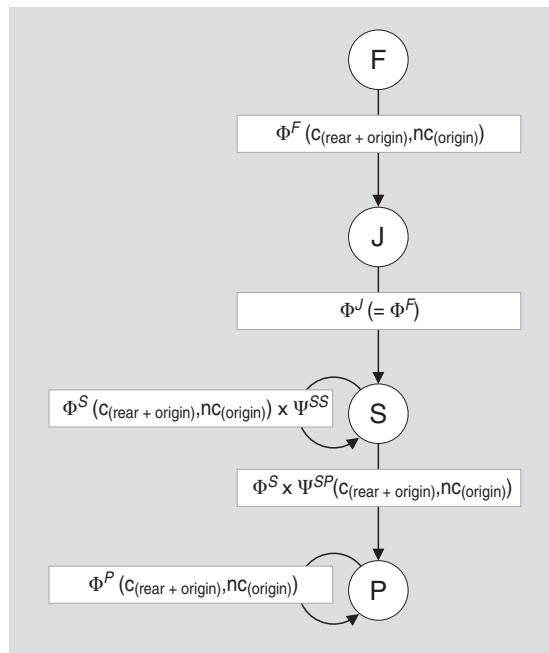


Figure 4.1 Schematic overview of the life cycle of the Seychelles warbler, with the four main life stages: fledgling (F), juvenile (J), subordinate (S) and primary (P) and the survival (Φ) and transition (ψ) parameters as estimated in the multistate capture-recapture model as a function of covariates of the rearing (rear) and original (origin) territory. (c) = cross-fostered, (nc) = non-cross-fostered.

states, and recruitment to a breeding position, were affected by the conditions experienced during the rearing period; i.e. territory quality and group size. Furthermore, for the cohorts 1997–1999 and 2002–2005, additional data on rearing conditions were available; i.e. the number of nest mates, helpers and non-helpers in the rearing territory. Birds ringed as adult (unknown age and origin) entered the data set as either ‘subordinate’ or ‘primary’ birds and were included to improve the estimation of the survival, resighting and recruitment probabilities. The transition probabilities from fledgling to juvenile, and from juvenile to subordinate, were fixed to one as all fledglings and juveniles either survive and move to the next state, or die (Fig. 4.1). Twenty-nine out of 1047 birds in our dataset lost their ‘primary’ status and became ‘subordinate’ again. Although this is an interesting phenomenon (see: Richardson *et al.* 2007), we did not include these birds in our sample as we are primarily interested in recruitment here. Consequently, the transition from ‘primary’ to ‘subordinate’ was constrained to zero (Fig. 4.1). In a few cases (100 out of 5567 resightings) the status of a bird was unknown; these cases were treated as not being resighted.

To disentangle the effects of rearing conditions and parental/territory quality

effects on long-term fitness, 74 same age (\pm 2-day difference) nestlings were cross-fostered between 0 and 6 days of age in the years 1997–1999. Nests with two or three nestlings ($N = 11$) were reduced to one by increasing the brood size of another nest. The cross-fostered offspring were included as a group in the analyses, thereby allowing us to test whether rearing conditions, and/or the conditions of the original territory, affect survival or recruitment.

Data analyses

Two resighting periods were defined; the first from 1 July to 1 September during the main breeding season, the second from 1 January to 1 March. Individuals were recorded as present if observed at least once within a period. Although the resighting periods were not instant, most birds were re-sighted within the first two weeks of each study period. No capture-resighting data were available for 1992, and data for January-March was only available in 1998, 1999, 2004 and 2005. If not available, dummy variables were created by including zeros in the encounter histories, with the survival parameter (Φ) set to 1 and the resighting parameter (recapture, p) and transition parameter (ψ) set to 0. The encounter histories were used as input files in the program MARK (White & Burnham 1999).

Construction of our global model was based on the findings of a previous study (Brouwer *et al.* 2006). Consequently, our global model (Table 4.1, model 6) allowed survival to vary between years and states, with different survival probabilities for individuals in their first year of life (fledgling and juvenile state) than for older birds (subordinate and primary state). To simplify and avoid the overparameterisation of our model we assumed time independent resighting rates but allowed resighting rates to vary between states. Our global model allowed transition (recruitment) probabilities to vary over time and between the sexes. In addition, three groups were

Table 4.1 Summary of model selection statistics examining survival, resighting and transition probabilities for fledgling (F), juvenile (J), subordinate (S) and primary (P) Seychelles warblers from 1986 to 2006 ($N = 1018$). (t) = time, (s) = sex, (.) = constant.

No Model	No.Par.	QDeviance	ΔQAIC_c	QAIC_c weights
1 $\Phi^{FJ}(t) \neq \Phi^S = \Phi^P(t) p^{FJ}(.) = p^S(.) \neq p^P(.) \psi^{SP}(t)$	67	10879.8	0.0	0.72
2 $\Phi^{FJ}(t) \neq \Phi^S = \Phi^P(t) p^{FJ}(.) = p^S(.) \neq p^P(.) \psi^{SP}(t+s)$	68	10879.8	2.1	0.26
3 $\Phi^{FJ}(t) \neq \Phi^S = \Phi^P(t) p^{FJ}(.) = p^S(.) \neq p^P(.) \psi^{SP}(.)$	48	10926.1	7.3	0.02
4 $\Phi^{FJ}(t) \neq \Phi^S = \Phi^P(t) p^{FJ}(.) = p^S(.) \neq p^P(.) \psi^{SP}(txs)$	84	10861.6	17.0	0.00
5 $\Phi^{FJ}(t) \neq \Phi^S(t) \neq \Phi^P(t) p^{FJ}(.) = p^S(.) \neq p^P(.) \psi^{SP}(txs)$	106	10844.9	46.3	0.00
6 $\Phi^{FJ}(t) \neq \Phi^S(t) \neq \Phi^P(t) p^{FJ}(.) \neq p^S(.) \neq p^P(.) \psi^{SP}(txs)$	107	10844.5	47.9	0.00

Models were ranked according to their QAIC_c value, with the best supported model on top. ΔQAIC_c being the difference between the minimum QAIC_c of the best supported model and the model considered.

created in the analyses, one group for birds of known age and origin, one group for (known age and origin) cross-fostered offspring, and one group for the birds of unknown age and origin. Conditions of the rearing territory were included for both the cross-fostered birds and for birds of known age and origin, whereas the pre-foster conditions (of the original territory) were included for the cross-fostered birds only. Akaike's information criterion corrected for the sample size (AIC_c) was used to select the most parsimonious model with better fitting models resulting in lower AIC_c values (Akaike 1973; Burnham & Anderson 2002). We calculated the normalized Akaike weights to assess the relative likelihood of competing models. We checked the goodness-of-fit of our global model by using the median \hat{c} procedure, which showed some evidence for overdispersion (median $\hat{c} = 1.51 \pm 0.02$). Consequently, AIC_c values were adjusted to allow for the extent of overdispersion measured by \hat{c} , through quasi likelihood (QAIC_c).

We employed an *a priori* approach in which a small set of candidate models was created based on previous knowledge and hypotheses of interest. We first investigated whether the probability of resighting or survival depended on state, we expected resighting probability to be highest for primary birds, as they stay in the same territory after settling, in contrast to subordinates which make forays around the island in search of a vacancy (Eikenaar *et al.* 2007). We investigated whether the recruitment rate varied over time and whether it differed between the sexes. We then investigated whether survival and recruitment were associated with conditions during the rearing period for both the cross-fostered birds and the birds of known age and origin. To do this we first investigated the effect of each covariate on juvenile survival. Where we found evidence for an association with juvenile survival, we investigated whether there was an association with adult survival as well. Finally, we investigated whether we can distinguish rearing conditions from parental/territory quality effects by including conditions of the original territory for cross-fostered offspring only.

The individual covariates were analysed by using the logit link function with values of the individual covariates standardized to Z-scores (by subtracting the mean and dividing by the standard deviation). Model notation was followed according to Lebreton *et al.* (1992).

Results

Natural variation in rearing conditions

During the whole study period on average 39% of the territories had one or more subordinates living in it and the focal nest watches showed that on average 49% of subordinates helped with incubation or feeding of the nestling in the territory. Although group size varied from 2 to 7 birds, the maximum number of helpers observed per territory was two. High quality territories did not have more helping subordinates than low quality territories ($\chi^2_1 = 0.87, P = 0.35$).

Effect of rearing conditions on survival and recruitment

We investigated whether survival, recruitment and resighting probabilities differed between individuals in the different states. Annual resighting probabilities were similarly high for first year birds as subordinates (0.83 ± 0.02) (Table 4.1, model 5 vs. 6), but even higher for primary birds (0.97 ± 0.01). Survival probabilities were similar for subordinates and primaries (Table 4.1, model 4 vs. model 5). There was no evidence for differential recruitment between the sexes (Table 4.1, model 1 vs. model 2), and this did not change between years (Table 4.1, model 2 vs. model 4). A model with equal recruitment probabilities for both sexes was 2.8 times better supported by the data than a model which included sex (Table 4.1, model 1 vs. model 2). However, overall recruitment rate did vary between years (average \pm S.E.: 0.60 ± 0.04 ; Table 4.1, model 1 vs. model 3). Consequently model 1 (Table 4.1) was used as a starting model to investigate the effects of conditions during the rearing period on survival and recruitment.

Although there was just little evidence that the number of helpers in the rearing territory was associated with juvenile survival (Table 4.2, model 2), it was positively associated with survival later in the subordinate and primary state (Table 4.2, model 1; Fig. 4.2). Including the number of helpers in the rearing territory as a covariate

Table 4.2 Summary of model selection statistics examining conditions during the rearing period on survival and recruitment probabilities of fledgling (F), juvenile (J), subordinate (S) and primary (P) Seychelles warblers. Covariates of the rearing territory: (h) = number of helpers, (nh) = number of non-helpers, (tq) = territory quality, (n) = number of nest mates, (gs) = group size, (t) = time. Resighting probabilities were similar for all models: $p^{FJ}_{(.)} = p^S_{(.)} \neq p^P_{(.)}$

No	Model	No.Par.	QDeviance	ΔQAIC_c	QAIC_c weights
1	$\Phi^{FJ}_{(t+h)} \neq \Phi^S = \Phi^P_{(t+h)} \psi^{SP}_{(t)}$	68	10875.5	0.0	0.32
2	$\Phi^{FJ}_{(t+h)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10877.4	2.0	0.12
3	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	67	10879.8	2.3	0.10
4	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+bs)}$	68	10878.2	2.8	0.08
5	$\Phi^{FJ}_{(t+nh)} \neq \Phi^S = \Phi^P_{(t+nh)} \psi^{SP}_{(t)}$	68	10878.9	3.4	0.06
6	$\Phi^{FJ}_{(t+nh)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.0	3.5	0.05
7	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+gs)}$	68	10879.5	4.1	0.04
8	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+h)}$	68	10879.6	4.2	0.04
9	$\Phi^{FJ}_{(t+gs)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.6	4.2	0.04
10	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+tq)}$	68	10879.7	4.2	0.04
11	$\Phi^{FJ}_{(t+n)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.8	4.3	0.04
12	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+nh)}$	68	10879.8	4.3	0.04
13	$\Phi^{FJ}_{(t+tq)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.8	4.4	0.04
14	$\Phi^{FJ}_{(txh)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	72	10874.2	7.0	0.01

Models were ranked according to their QAIC_c value, with the best supported model on top. ΔQAIC_c being the difference between the minimum QAIC_c of the best supported model and the model considered.

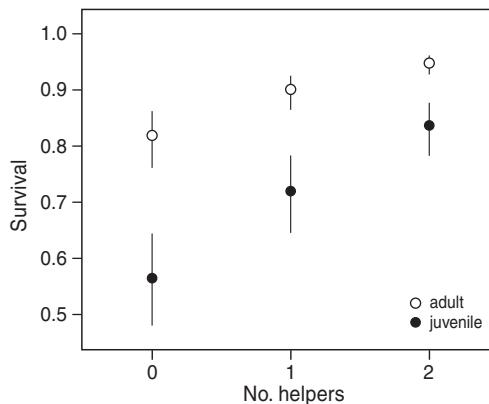


Figure 4.2 Annual adult and juvenile survival probabilities (with 95% CI) of Seychelles warblers for an average year, in relation to the number of helpers in the rearing territory.

on survival was 3 times better supported by the data than a model without this effect (Table 4.2, model 1 vs. model 3). The higher survival for birds from territories with helpers was caused by the number of helpers *per se* as the number of non-helping subordinates was not associated with survival (Table 4.2, models 5 and 6 vs. model 3). Birds from territories with helpers did not, however, have a higher probability of recruiting to the primary state (Table 4.2, model 7 vs. model 3). There was no evidence that any of the other factors experienced in the rearing period were associated with survival (Table 4.2, models 9, 11, 13, and 14).

The chance of recruitment to the primary state was not associated with either the quality of the territory on which offspring were reared (Table 4.2, model 10 vs. model 3), nor on the size of the group or brood in which they were reared (Table 4.2, model 4, 7 and 12 vs. model 3).

Rearing vs. quality effects

The association between the number of helpers in the rearing territory and offspring survival was not a result from a non-causal relationship caused by higher quality parents/territories; for the cross-fostered offspring there was no evidence that number of helpers of the original territory was associated with either survival or recruitment probabilities (Table 4.3 model 2, 3 and 6 vs. model 4). Furthermore, none of the other factors, i.e. group size, brood size, territory quality or the number of non-helpers were associated with survival or recruitment (Table 4.3, model 5, 7-13). This was not caused by a lack of power as there was a positive association between the number of helpers in the rearing territory and both juvenile and adult survival for the cross-fostered offspring (Table 4.3, model 1). Although QAICc increased by only 1.6, this model was 2.3 times better supported by the data than a model without this effect (Table 4.3, model 1 vs. model 4).

Table 4.3 Summary of model selection statistics examining the pre-foster conditions on survival and recruitment probabilities of cross-fostered Seychelles warblers ($N = 74$). Covariates from original territories: (h) = number of helpers, (nh) = number of non-helpers, (tq) = territory quality, (n) = number of nest mates, (gs) = group size, (t) = time and (fosh) = number of helpers on rearing territory. Resighting probabilities were kept constant: $p^{FJ}(.) = p^S(.) \neq p^P(.)$

No	Model	No.Par.	QDeviance	ΔQAIC_c	QAIC_c weights
1	$\Phi^{FJ}_{(t+fosh)} \neq \Phi^S = \Phi^P_{(t+fosh)} \psi^{SP}_{(t)}$	68	10876.2	0.0	0.21
2	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+h)}$	68	10877.3	1.1	0.12
3	$\Phi^{FJ}_{(t+h)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10877.7	1.5	0.10
4	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	67	10879.8	1.6	0.09
5	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+n)}$	68	10877.9	1.7	0.09
6	$\Phi^{FJ}_{(t+h)} \neq \Phi^S = \Phi^P_{(t+h)} \psi^{SP}_{(t)}$	68	10878.3	2.2	0.07
7	$\Phi^{FJ}_{(t+n)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10878.3	2.2	0.07
8	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+gs)}$	68	10878.6	2.5	0.06
9	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+tq)}$	68	10879.2	3.1	0.05
10	$\Phi^{FJ}_{(t+nh)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.5	3.4	0.04
11	$\Phi^{FJ}_{(t+tq)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.6	3.4	0.04
12	$\Phi^{FJ}_{(t+gs)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t)}$	68	10879.7	3.5	0.04
13	$\Phi^{FJ}_{(t)} \neq \Phi^S = \Phi^P_{(t)} \psi^{SP}_{(t+nh)}$	68	10879.8	3.6	0.03

Models were ranked according to their QAIC_c value, with the best supported model on top. ΔQAIC_c being the difference between the minimum QAIC_c of the best supported model and the model considered.

Discussion

Our study shows that helpers have long-term effects on offspring fitness; the number of helpers in the rearing territory affected not only the juvenile survival, but also the later adult survival of the offspring helped. This was entirely due to the number of helpers, and not to the overall number of birds, in the territory as the number of non-helping subordinates was not associated with offspring survival. Evidence that helpers can increase reproductive success by increasing juvenile survival has previously been shown by removal experiments (Brown *et al.* 1982; Mumme 1992; Shreeves & Field 2002; Brouwer *et al.* 2005). However, that helpers can affect offspring fitness even in the long-term has rarely been shown. As far as we know only one study, on the cooperatively breeding long-tailed tit (*Aegithalos caudatus*), has shown that the number helpers has a positive effect on the recruitment of helped offspring (Hatchwell *et al.* 2004). It is possible that the decision to help depends on, and/or is positively associated with the quality of the parents or territory a subordinate lives in, and therefore that offspring of these high quality parents/territories have high survival independent of the actual helping. However, we showed that it was not the number of helpers in the original territory, but the number of helpers in

the rearing territory that was associated with the survival of cross-fostered offspring. Consequently, increased survival is not a consequence of higher quality but due to helping *per se*. This is confirmed by a helper removal experiment which showed that helping increased reproductive success by increasing nestling survival (Komdeur 1994b).

Neither the number of nest mates or the territory quality experienced during the rearing period were associated with survival at any stage of an individuals' life, nor with the probability of recruitment. A previous study on the Seychelles warbler has already shown that although there was variation in juvenile survival between seasons, this did not affect a cohorts' adult survival probability (Brouwer *et al.* 2006). The long period in which the Seychelles warbler offspring are dependent on their parents might counterbalance any negative effects experienced during early life. Alternatively, selective disappearance (mortality) of the low quality individuals may result in similar adult survival probabilities for cohorts which experienced adverse conditions as for other cohorts.

Many studies have found that parental quality affects offspring fitness (e.g. Amundsen & Stokland 1990; Bolton 1991; Meathrel *et al.* 1993; Risch & Rohwer 2000). Parents can affect their offspring directly via genetic (Merilä 1996; Sheldon *et al.* 1997) or maternal effects (Eising *et al.* 2001), but also by feeding or care (Clutton-Brock 1991). Our results, which show that the number of helpers affects offspring survival, suggest that there is a direct effect of the additional care, probably as a result of the extra provisioning gained by nestlings. Furthermore, a previous analysis found evidence for maternal effects, as maternal heterozygosity at microsatellite loci was positively associated with offspring survival (Brouwer *et al.* 2007a). It is possible that parental effects are, therefore, a more important source of variation in quality than the effects of the environment and territory. The Seychelles warbler lives in a relatively stable tropical environment and birds time their reproduction to periods with high food availability and choose whether to lay one or two eggs (Komdeur 1996b). With such a strategy, adverse conditions might be avoided.

None of the rearing conditions was associated with recruitment to a primary position. This is not surprising as a previous study showed that proximity to a vacancy is the most important factor determining whether a subordinate will acquire a territory and not an individual's size or mass (Eikenaar *et al.* unpublished data). This might indicate that recruitment to a breeding position is, unless an individual does not survive, mainly a chance process.

Primary birds did not have lower survival probabilities than subordinates, which could suggest there is little cost associated with reproduction. However, the assessment of status was based on observations only. A previous study showed that 44% of subordinate females are co-breeders each year (Richardson *et al.* 2001); therefore they may also suffer any cost of reproduction. Furthermore, subordinates may have been investing considerable effort in helping. Unfortunately, we could not differentiate helper and non-helper survival as the minor breeding peak was not monitored in all years. In general, many studies fail to show any cost of reproduction (Reznick

et al. 2000) this may be caused by problems with the analytical methods used, or in how accurate the assessment was of whether individuals were reproducing or not. Finally, it might be that heterogeneity in quality between individuals allows high quality individuals to reproduce without bearing the cost of reduced survival (Cam & Monnat 2000).

Our results show that helpers not only increase offspring survival in the short-term, but also increase their subsequent adult survival and, therefore, have important fitness consequences later in life. On the other hand, the other investigated conditions during the rearing period appear to have little long term fitness consequences. Although studies on temperate species might show greater effects of early conditions, in tropical species with less variation in the environmental conditions, parental effects and decisions might be more important.

Acknowledgements

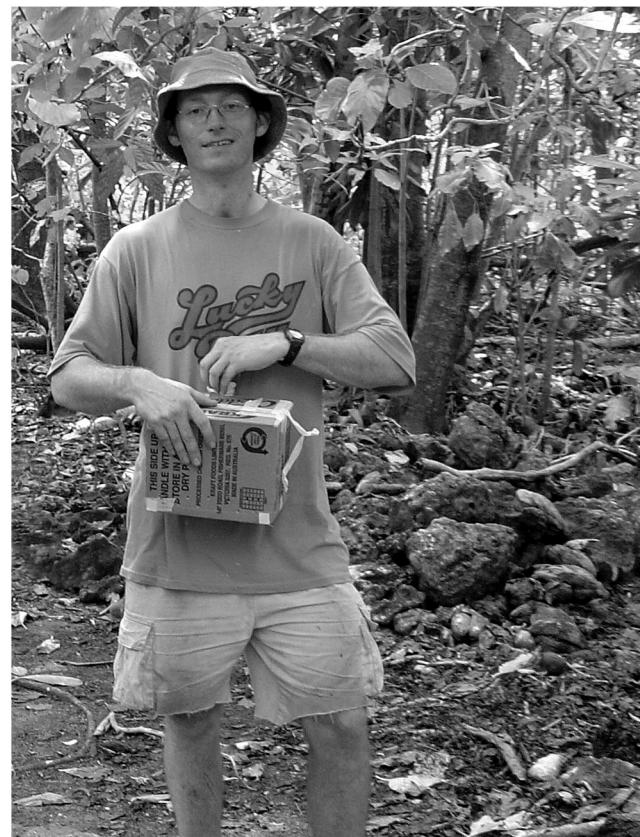
Nature Seychelles kindly allowed us to work on Cousin Island. The Department of Environment and Seychelles Bureau of Standards gave permission for fieldwork and sampling. We thank all the people who assisted with data collection since 1986. Martijn van de Pol provided helpful comments on the manuscript. This work was supported by Netherlands Foundation for the Advancement of Tropical Research (WOTRO, 84-519) awarded to JK and by Marie Curie and NERC postdoctoral fellowships awarded to DSR.



Top: research house on Cousin Island.

Bottom left: Lyanne Brouwer clearing a path through dense vegetation.

Bottom right: Cas Eikelaar puts a warbler into a box for translocation to Denis Island.



5

Chapter

Do primary males physiologically suppress subordinate males? An experiment in a cooperatively breeding passerine

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Abstract

Knowing why certain individuals within groups refrain from reproduction is fundamental to our understanding of the evolution of cooperative breeding. Although many studies have focused on the role of life history and ecological factors, the proximate mechanisms underlying this behaviour have received considerably less attention. We determined the natural variation in plasma testosterone (T) level and cloacal protuberance (CP) size - an indication of sperm storage - in successfully reproducing primary male and in apparently sexually inactive subordinate male Seychelles warblers (*Acrocephalus sechellensis*). Primary males had significantly higher T levels during the female's fertile period and significantly larger CPs throughout the breeding season than subordinate males. Furthermore, subordinate males had lower residual body mass and higher buffy coat values, which is a measure of immunocompetence, indicating that subordinate males of this species are physiologically suppressed or of lower quality. To test if the lower T levels of subordinates were caused by their social status per se, we experimentally promoted subordinates to primary males, through permanent removal of primary males from their territories. The experimentally promoted males increased their CP sizes significantly, suggesting suppression by primary males. However, once social suppression was removed their T levels were still lower than those of primary males, indicating that subordinate males are either of low quality or that full sexual maturation takes a substantial time period.

Introduction

In cooperative breeding species, individuals refrain from reproduction and often help to rear kin instead of reproducing themselves (e.g. Brown 1987). This intriguing phenomenon has been the subject of many studies in which numerous adaptive hypotheses have been investigated, e.g. kin-selection (Komdeur and Hatchwell 1999; Griffin and West 2003), pay to stay (Balshine-Earn *et al.* 1998), group augmentation (Clutton-Brock 2002) and the role of ecological (Arnold and Owens 1999; Hatchwell and Komdeur 2000) and life history factors (Arnold and Owens 1998). To gain insight into the proximate mechanisms that contribute to delayed breeding and helping, an increasing number of studies have investigated hormonal blood levels of breeders and non-breeders in cooperatively breeding species (Schoech *et al.* 1991; Vleck *et al.* 1991; Wingfield *et al.* 1991; Creel *et al.* 1992; Poiani and Fletcher 1994; 1996; Roberts *et al.* 1996; Brown and Vleck 1998; Peters *et al.* 2000; Khan *et al.* 2001). Gonadal steroid hormones such as testosterone (T) play a key role in the stimulation of male reproductive behaviour (for review see: Nelson 2000). In male birds, elevated T levels during the breeding season can favour the development of secondary sexual characteristics (Kimball and Ligon 1999) and the expression of behaviours involved in mate attraction (de Ridder *et al.* 2000), territory defence (Moore 1984; Wingfield 1994) and mate guarding (Saino and Møller 1995), needed to gain reproductive success. In most cooperatively breeding species subordinate males appear to be sexually inactive (Koenig and Mumme 1987; Poiani and Fletcher 1994) and higher T levels for sexually active male breeders compared to reproductively inactive subordinates and/or helpers have been reported in several studies (Schoech *et al.* 1991, 1996; Poiani and Fletcher 1994). Several hypotheses have been proposed to explain this sexual inactivity. Subordinate males could be (1) reproductively immature (delayed maturation, Brown 1987), (2) in poor body condition or physically unable to produce high T levels due to physical suppression by the primary male (physiological suppression, Wingfield *et al.* 1991; Wingfield and Farner 1993; Schoech *et al.* 1997), (3) not receiving appropriate stimulation from females (lack of stimuli, Poiani and Fletcher 1994; Schoech *et al.* 1996), or (4) being prevented from reproducing by social interference (behavioral suppression, Mumme *et al.* 1983; Emlen and Wrege 1986; Mays *et al.* 1991). Alternatively, subordinates could facultatively delay investment in T, to avoid aggression from the primary male (Grant 1990; Schoech *et al.* 1991), to reduce the costs of increased levels of T (Ketterson *et al.* 1991), or to avoid inbreeding (Poiani and Fletcher 1994). Experiments are needed to test these hypotheses and to disentangle whether the relation between T levels and status is really due to status per se rather than age, sexual immaturity or lack of breeding opportunities of the subordinate males.

The cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*) is a year round territorial species that has long term pair bonds and is endemic to a few islands in the Seychelles. Although warblers can breed independently in their first year, lack of unoccupied habitat on most islands drives many young individuals to

become subordinates within a territory (Komdeur 1992). Both sexes of subordinates occasionally help with territory defence and the rearing of young (Komdeur 1994; Richardson *et al.* 2002). However, male subordinates never gain paternity outside their own territory and only seldom within (Richardson *et al.* 2001). In this study we will investigate, both descriptively and experimentally, differences in T levels and sperm storage capacity between primary and subordinate male Seychelles warblers. We will assess differences in sperm storage capacity by measuring the cloacal protuberance (CP), an anatomical structure resulting from the enlargement of the seminal glomerus (the site of sperm storage, Lake 1981), that is known to accurately reflect sperm storage and copulation frequency (Nakamura 1990; Birkhead *et al.* 1991). Furthermore, we will investigate body condition of primary and subordinate males using three indices; haematocrit, 'buffy coat' and the residuals of body mass and size. Haematocrit is the volume of packed red blood cells as a percent of total blood volume and reflects the extent and efficiency of oxygen uptake and transfer to tissues. Buffy coat is the volume of leucocytes as a percent of total blood volume. Leucocytes form the basis of the immune system and their main function is the protection against various pathogenic antigens. Elevated leucocyte number is symptomatic of stress syndrome and inflammatory processes (Ots *et al.* 1998).

Here we study a tropical bird species. In contrast to temperate species, most bird species in the tropics are territorial and mated all year round and therefore do not need to establish a territory and attract a mate each year. Consequently, T levels often remain basal throughout the year but can be elevated in response to a challenge (Wikelski *et al.* 1999; Hau *et al.* 2000). However, it remains unclear if the challenge hypothesis applies for tropical species in general (Wingfield *et al.* 1991; Moore *et al.* 2004). Our study species breeds seasonally, despite being territorial and mated all year round. Furthermore, in cooperatively breeding species the primary males have to be able to compete with subordinate males. Consequently, we expect that our males should be able to increase T in response to a challenge.

The specific aims of this study were (1) to investigate the seasonal variation in plasma T levels of subordinate and primary males before and during the breeding season. Previous work in the Seychelles warbler has shown that both T levels and CP are elevated during the nest building stage, but sufficient data of subordinates were lacking (van de Crommenacker *et al.* 2004). (2) To investigate experimentally if subordinates are able to elevate their T levels and sperm storage capacity after they become a breeder. Other studies manipulated T levels of subordinate males directly through T implants and looked at the effect on reproductive or parental behaviour (Peters *et al.* 2002; de la Cruz *et al.* 2003). The disadvantage of such studies is that T levels may exceed levels seen under natural variation. In the present study we were able to experimentally change the status of subordinate males and subsequently record their change in T levels and sperm storage capacity. This was done by removing primary males, thereby creating vacancies for subordinate males to become primary males. (3) To investigate if subordinate males are in poorer body condition than primary males and whether their condition changes after removal of

the dominant male, by determining haematocrit, 'buffy coat' and the residuals of body mass and size.

Methods

Study population

We monitored the entire population of Seychelles warblers on the island of Cousin (29 ha) from June-September in 2002, 2003 and 2004. As this population has been monitored since 1986, we could assign sex, age and status to the majority of the population (> 98%). The 'primary' male and female were defined as the dominant, pair-bonded male and female in the territory. All other birds, older than six months, resident in the territory were defined as 'subordinate' (Richardson *et al.* 2002). Each territory was checked for nesting activity at least once every two weeks by following the primary female for 15 minutes (Komdeur 1992). Once a nest was located it was monitored throughout the breeding season. The reproductive season was divided into the following stages: pre-nesting; nest building (circa 20 days from the start of building until egg laying); incubating (circa 18 days from egg laying until hatching) and offspring provisioning stage (circa 30 days from hatching).

Bird capture and sampling

Birds were caught using mist nets. No challenge was used during the first 15 min session of catching, but when this failed to catch the bird an audible challenge was used at the net during the second 15 minutes. The challenge consisted of a continuous playback of unfamiliar Seychelles warbler song. This procedure was repeated with 15 min intervals of silence until up to a maximum of four challenges in a catching session. Birds were defined as 'not challenged' when it was caught within the first 15 min of catching. Nets were checked at least every 15 minutes. When a bird was caught the maximum time the bird had spent in the net (delay time in min), the number of challenges and the time lapse between the start of the first challenge and capture (challenge time in min) were recorded. Blood samples (ca. 70 ml) were taken by brachial venipuncture using heparanized capillary tubes immediately after catching. These were then centrifuged at 8,000 r.p.m. for 8 min. Haematocrit and buffy coat were determined by measuring the height of the red blood cell and leucocyte layers with a digital calliper (± 0.01 mm) and the use of a 10x magnifying glass (Gustafsson *et al.* 1994; Ots *et al.* 1998) allowing the calculation of the volume of the plasma collected. The plasma was diluted in 1 ml of 100% ethanol in a screw-cap microfuge tube and stored at room temperature. The volume of the CP was calculated by measuring the width (w) and height (h) from the anterior end of the CP with a calliper (± 0.1 mm). As the CP resembles a barrel shape from its anterior to posterior end, the volume of the protuberance was calculated as $h \times r^2$ where r represents $w/2$ (Mulder and Cockburn 1993). T levels were determined for 65 different primary and 31 different subordinate males CP was measured for 61 primary and 31 subordinate males.

Testosterone analyses

T levels (nanograms per milliliter of plasma) were analyzed using radioimmunoassay (RIA). Samples from the 2002 season were extracted using Extrelute columns (van de Crommenacker *et al.* 2004). In 2003 and 2004 the samples in ethanol were analysed with a commercial Spectria Testosterone RIA kit (Orion diagnostica). The diluted plasma was centrifuged at 14000 r.p.m. for 2 min and the supernatant was poured off. The tubes in which the plasma had been stored were washed again with ethanol which was again centrifuged and the supernatant was poured off. The combined samples were then used in the assay after drying under a stream of nitrogen and dissolving the sample in phosphate buffered saline. This procedure leads to an almost 100% recovery. The antibody has a very low cross-reactivity with antigens other than T (< 3%). Due to very small blood samples we could not run the RIA in duplicate. We performed two assays. Inter assay variation was 10.1% and 13.8% for two samples run in seven similar assays.

Experimental promotion of status

For conservation purposes 58 warblers (16% of the population) were translocated from Cousin to Denis island prior to the breeding season of 2004 (Brouwer 2007). The birds were caught all over Cousin Island and randomly selected with respect to age and status. In this way breeding vacancies were created, which were subsequently occupied by 15 subordinate males without prior breeding experience. These subordinate males started nest building on average 32.2 ± 6.1 days after their promotion. Of these experimentally promoted males, we were able to sample a random set of six individuals during the nest building stage, the period in which we expected differences between subordinate and primary males to be largest (see results below). These males are hereafter referred to as 'experimental' males.

Data analyses

The values for T level and CP size were log-transformed to normalize the data. To investigate the seasonal variation in T values and CP sizes the data of 2002 – 2004 were combined. To correct for the different extraction methods, T values were standardized to z – scores by subtracting the mean and dividing by the standard deviation. The mean T value was based on the overlapping period between the data sets. By correcting for the use of different extraction methods we can not test for differences between 2002 and the two other years. However, the fact that there were no differences between 2003 and 2004 ($\chi^2_1 = 0.28, P = 0.60$) and that this is a tropical species with little environmental variability (Komdeur 1996) means we can reasonably assume that there is no between year variation. To investigate the effect of experimental promotion on T levels and CP sizes only data from 2003 and 2004 were used.

Some individuals were sampled in several years and can therefore not be considered as independent data points. To take this non-independence of the data into

account multilevel mixed-modelling procedure was used in MLwiN 2.1 (Rasbash *et al.* 2004) with individual identity included as a random effect. T levels and CP sizes were fitted in a multivariate response model allowing us to estimate the covariation between T and CPs. We included social status, reproductive stage, number and duration of challenges, the presence of male subordinates and delay to sampling in the model, as well as several interactions specified in the results section. Furthermore, CP measures were corrected for between observer variation. To investigate if subordinate males are in poorer body condition than primary males, haematocrit, 'buffy coat' and the residuals of body mass and tarsus size were fitted using a normal response model. Model selection was based on stepwise backward elimination of the non-significant terms in the order of their significance assessed by its Wald statistic. The final model contained the constant and all significant explanatory terms. All eliminated terms were reintroduced to the final model to confirm their lack of contribution. Means are expressed with standard errors.

Results

Natural variation

Fig. 5.1A shows the natural variation in T levels for primary and subordinate males during the different reproductive stages for 2003 and 2004 combined. Primary males had low levels of T during the pre-nesting stage, increased T levels during the nest building stage, and decreased their T levels during incubation and offspring provisioning stage to similar low T levels as during the pre-nesting stage. T levels of subordinate males remained at low levels throughout the breeding stages. To investigate the effects of social status and nest stage on T levels, data from 2002 to 2004 were combined (Fig. 5.1B) and the results of the full and final model are shown in Table 5.1A. T levels varied significantly with nest stage, but this differed between primary and subordinate status (Table 5.1A). Examining this in more detail revealed that primary males significantly increased their T levels during nest building ($\chi^2_1 = 34.7, P < 0.001$) and significantly decreased them thereafter ($\chi^2_1 = 9.30, P = 0.002$) but their T levels during the incubation stage were still higher than during the pre-nesting stage ($\chi^2_1 = 4.78, P = 0.03$). With the reference category of status set to primary male and of nest stage set to nest building, the status effect gives the difference between primary and subordinate T levels during the nest building stage and this shows that primary males had significantly higher T levels than subordinate males during the nest building stage (Table 5.1A). Furthermore, primary males also had significantly higher T levels during the incubation stage ($\chi^2_1 = 5.35, P = 0.02$). In addition, within the same territory, subordinate males always had lower T levels than the primary male in cases where they were caught within two days of each other (paired t-test: $t = 4.25, n = 5, P = 0.013$). Primary males were significantly older than subordinate males (mean age in years; primary males: $5.3 \pm 0.4, n = 79$, versus subordinate males: $1.9 \pm 0.2, n = 35; t = 8.20, P < 0.001$). However when

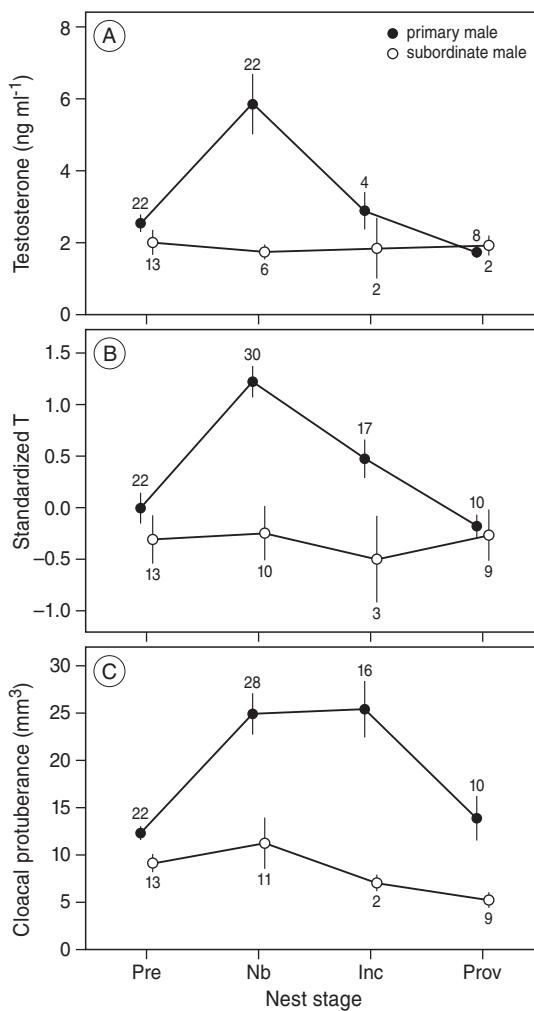


Figure 5.1 The relation between breeding stage and (A) plasma T levels for 2003 – 2004, (B) standardized plasma T levels for all years combined and (C) CP sizes, for primary and subordinate male Seychelles warblers. Numbers indicate sample sizes. Pre = pre-nesting; Nb = nest building; Inc = incubating; Prov = offspring provisioning stage.

tested during the nest building stage, older primary males did not have higher T levels than younger primary males (Spearman $r = 0.13$, $n = 30$, $P = 0.48$). This removes age as a potential explanation of the difference between primary and subordinate males.

The mean time of delay till sampling was 4.8 ± 0.5 min (range 0.5–15 min). Longer delays resulted in lower T levels (Table 5.1A). Fifty four birds were caught without the use of a challenge and 60 with the use of one or more challenges. The number of challenges used and challenge time did not affect T levels (Table 5.1A).

Table 5.1 Model summaries examining effects on (A) standardized T levels ($N = 114$) and (B) cloacal protuberance ($N = 110$) in primary and subordinate Seychelles warblers. Summaries derived from a multivariate normal response mixed-modelling procedure. Final models shown in bold. Estimates of effect sizes (B) for cloacal protuberance are given on the log scale.

Parameter	B ± S.E.	χ^2	d.f.	P
A) Testosterone				
Intercept	0.96 ± 0.16		1	
Status*	-1.52 ± 0.27	31.2	1	<0.001
Nest stage⁺		45.7	3	<0.001
Pre-nesting	-1.22 ± 0.21			
Incubating	0.70 ± 0.23			
Provisioning	-1.40 ± 0.28			
Nest stage⁺ × status*		11.2	3	0.01
Pre-nesting × status	-1.08 ± 0.37			
Incubating × status	0.44 ± 0.54			
Provisioning × status	1.20 ± 0.44			
Delay	-0.04 ± 0.01	5.95	1	0.01
No. Male subordinates	-0.02 ± 0.13	0.02	1	0.88
No. Male subordinates × status	0.33 ± 0.33	1.02	1	0.31
No. Challenges	-0.03 ± 0.07	0.16	1	0.69
Challenge time	-0.01 ± 0.02	0.10	1	0.75
Random effect	$\sigma_{\text{individual}}^2 = 0.06 \pm 0.12$			
B) Cloacal protuberance				
Intercept	1.08 ± 0.06		1	
Status*	-0.37 ± 0.07	25.5	1	<0.001
Nest stage⁺		34.0	3	<0.001
Pre-nesting	0.30 ± 0.06			
Incubating	0.12 ± 0.07			
Provisioning	-0.10 ± 0.08			
Nest stage⁺ × status*		12.3	3	0.006
Pre-nesting × status	0.24 ± 0.10			
Incubating × status	-0.28 ± 0.17			
Provisioning × status	-0.01 ± 0.12			
Observer		47.6	4	<0.001
Random effect	$\sigma_{\text{individual}}^2 < 0.001 \pm 0.001$			

* reference category is primary male

+reference category is pre-nesting stage

However, subordinate males might not be able to increase their T levels at all and primary males could only increase their T levels when they have not reached high levels yet. Consequently, the relation between the number of challenges and challenge time were tested for primary males during the different nest stages, but this also yielded no significant results (number of challenges, all: Spearman $-0.17 < r < -0.20$, $P > 0.43$; challenge time, all: Spearman $-0.16 < r < 0.35$, $P > 0.45$). In

addition, the presence of subordinate males in the territory did not cause primary males to have higher T levels (Table 5.1A). A test for the primary males during the nest building only confirmed this ($t = 0.88, n_1 = 21, n_2 = 9, P = 0.39$).

CP sizes showed a similar pattern as T levels (Fig. 5.1C). CPs of primary males increased during the nest building stage, and they remained large during the incubation stage. Throughout the breeding cycle primary males had on average 2.8 times larger CPs than subordinate males. Analyses show that CPs varied significantly with nest stage, but this differed between primary and subordinate status (Table 5.1B). Examining this in more detail revealed that CPs of primary males significantly increased after the pre-nesting stage ($\chi^2_1 = 22.2, P < 0.001$) and were still significantly larger during the incubation stage ($\chi^2_1 = 30.1, P < 0.001$). CPs of primary males were significantly larger than CPs of subordinate males during the nest building stage (Table 5.1B), but also during the incubation ($\chi^2_1 = 17.2, P < 0.001$) and provisioning stage ($\chi^2_1 = 16.1, P < 0.001$). This was the case both with and without correcting for observer effect (not shown). CP sizes of primary males during the nest building and incubation stage did not increase with age (Spearman $r = 0.20, n =$

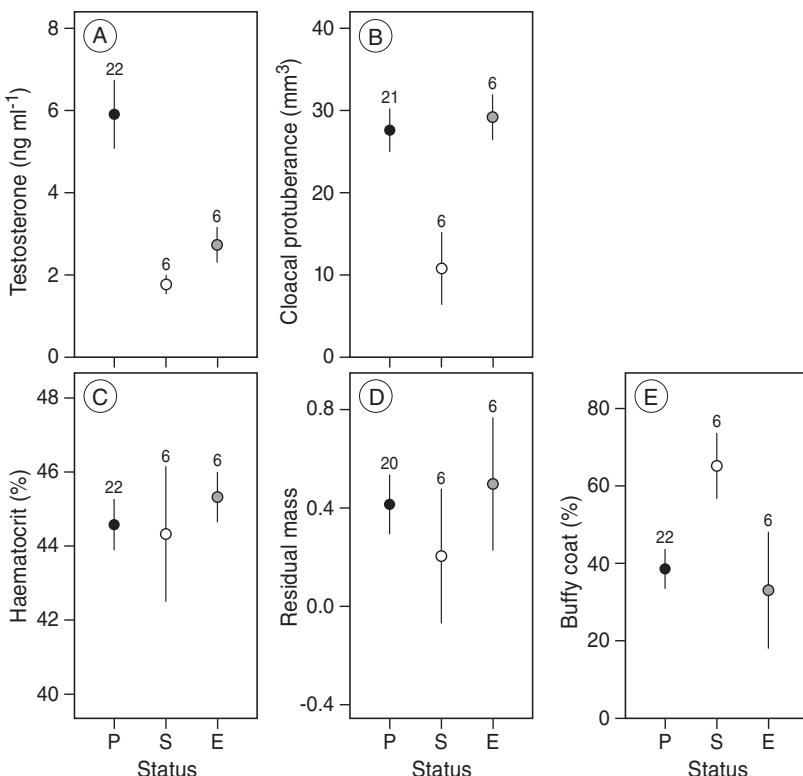


Figure 5.2 (A) Plasma T levels, (B) CP sizes, (C) haematocrit, (D) residual mass and (E) buffy coat for primary (P), subordinate (S) and experimentally promoted (E) male Seychelles warblers during the nest building stage. Numbers indicate sample sizes.

46, $P = 0.18$). T levels were not correlated with CP sizes as the covariance between T levels and CPs was not significant (covariance = 0.019 ± 0.014 , $\chi^2_1 = 1.77$, $P = 0.18$).

Experimental promotion of status

The experimental promotion of subordinate males revealed that these males were physically not able to elevate their T levels to similar levels as normal primary males in the first few weeks after filling a breeder vacancy (Fig. 5.2A). T levels varied significantly with male status (Table 5.2A) and although the experimental males had significantly higher T levels during the nest building stage than subordinate males ($\chi^2_1 = 3.85$, $P = 0.05$) the increase was just little and their T levels were significantly lower than those of the primary males ($\chi^2_1 = 4.55$, $P = 0.03$; Fig. 5.2A). Three of four promoted experimental males caught as subordinates in the previous year increased their T levels after promotion, one already had a high T level and decreased it. However, before promotion these experimental males did not differ significantly in their T levels from subordinates which did not naturally promote to the primary status (mean \pm S.E. = 2.9 ± 1.1 ng/ml ($n = 4$) versus 2.0 ± 0.4 ng/ml ($n = 4$). $t = -0.35$, $P = 0.74$).

In contrast to the results of T, experimental males increased their CPs after promotion (Fig. 5.2B). CPs varied significantly with status (Table 5.2B) with CPs of experimental males being significantly larger than those of subordinate males ($\chi^2_1 = 8.27$, $P = 0.004$), but similar to those of primary males ($\chi^2_1 = 0.38$, $P = 0.54$). CPs

Table 5.2 Model summaries examining effects on (A) T levels ($n = 34$) and (B) cloacal protuberance ($n = 33$) in primary, subordinate and experimentally promoted Seychelles warblers during the nest building stage. Summaries derived from a normal response modelling procedure. Final model shown in bold. Estimates of effect sizes (B) are given on log scale.

Parameter	B \pm S.E.	χ^2	d.f.	P
A) Testosterone				
Intercept	0.57 ± 0.12		1	
Status*		27.2	2	<0.001
Primary	0.23 ± 0.11			
Subordinate	-0.26 ± 0.13			
Delay	-0.014 ± 0.007	4.93	1	0.03
B) Cloacal protuberance				
Intercept	1.23 ± 0.10		1	
Status*		9.40	2	0.009
Primary	-0.04 ± 0.09			
Subordinate	-0.35 ± 0.11			
Observer		17.6	2	<0.001

* reference category is experimental male

of the four promoted experimental males previously caught as subordinates did not differ from other non promoted subordinates (mean \pm S.E. = 10.9 ± 3.8 ($n = 4$) versus 10.1 ± 2.0 ($n = 4$); $t = 0.11$, $P = 0.92$).

Body condition

Haematocrit did not differ between primary and subordinate males and did not vary over the breeding season (Fig. 5.3A, Table 5.3A). However, subordinate males had significantly higher buffy coat values than primary males, and this did not change

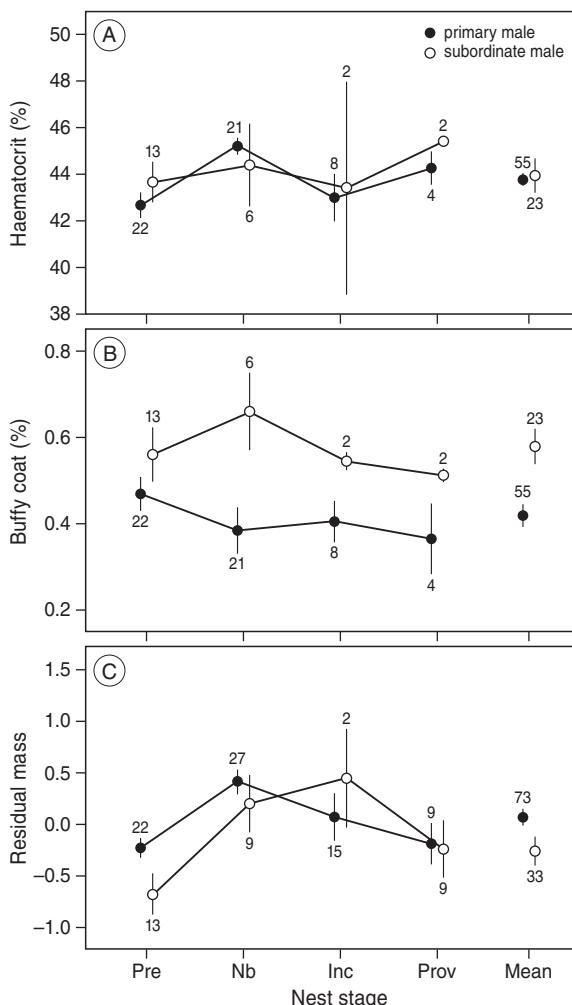


Figure 5.3 (A) Haematocrit values, (B) buffy coat values and (C) residual body mass of primary and subordinate Seychelles warblers during the different nest stages and the mean values. Numbers indicate sample sizes. Pre = pre-nesting; Nb = nest building; Inc = incubating; Prov = nestling provisioning stage.

Table 5.3 Model summaries examining effects on (A) haematocrit ($n = 78$) and (B) buffy coat ($n = 78$) and (C) residual body mass ($n = 106$) in primary and subordinate Seychelles warblers. Summaries derived from a normal response mixed-modelling procedure. Significant p-values are shown in bold.

Parameter	d.f.	A) Haematocrit			B) Buffy coat			C) Residual body mass		
		B ± S.E.	χ^2	P	B ± S.E.	χ^2	P	B ± S.E.	χ^2	P
Intercept	1	43.6 ± 0.38			0.44 ± 0.04			-0.33 ± 0.13		
Status*	1	-0.05 ± 0.06	0.56	0.45	0.16 ± 0.05	10.5	0.001	-0.32 ± 0.15	4.33	0.04
Nest stage ⁺	3	0.08 ± 0.06	1.41	0.70	-0.04 ± 0.05	1.19	0.76	0.71 ± 0.14	26.5	<0.001
Nestbuilding		-0.01 ± 0.08			-0.04 ± 0.07			0.64 ± 0.18		
Incubating		0.11 ± 0.11			-0.08 ± 0.09			0.29 ± 0.18		
Provisioning		0.04 ± 0.12	0.97	0.32	0.18 ± 0.11	2.65	0.45		2.94	0.40
Nest stage+ × Status*	3	0.03 ± 0.24			0.05 ± 0.17			0.21 ± 0.31		
Nestbuilding × status		0.15 ± 0.25			0.05 ± 0.18			0.72 ± 0.56		
Incubating × status		$\sigma^2 = 0.09 \pm 0.02$			$\sigma^2 < 0.001 \pm 0.001$			0.48 ± 0.36		
Provisioning × status								$\sigma^2 = 0.33 \pm 0.09$		
Random effect										

* reference category is primary male

+ reference category is pre-nesting stage

during the season (Fig. 5.3B, Table 5.3B). Residual body mass differed between primary and subordinate males, with primary males having significantly higher residual body mass during the pre-nesting stage (Fig. 5.3C, Table 5.3C). Furthermore, residual body mass varied with nest stage for both primary and subordinate males (Table 5.3C) with a significant increase from the pre-nesting to the nest building stage ($\chi^2_1 = 24.0$, $P < 0.001$), which remained high at the incubation stage ($\chi^2_1 = 12.0$, $P < 0.001$) but decreased to pre-nesting stage values during the offspring provisioning stage ($\chi^2_1 = 2.47$, $P = 0.12$) (Fig. 5.3C, Table 5.3C). The differences between primary and subordinate males were not an effect of age, older primary males did not have lower buffy coat values or higher residual body mass than younger primary males (buffy coat: Spearman $r = 0.21$, $n = 56$, $P = 0.12$; residual mass: Spearman $r = 0.09$, $n = 77$, $P = 0.46$).

Experimental males did not differ in haematocrit nor in residual body mass from either primary or subordinate males (haematocrit: $\chi^2_1 = 0.32$, $P = 0.85$; residual body mass: $\chi^2_1 = 0.47$, $P = 0.79$; Fig. 5.2C and 5.2D). Interestingly, their buffy coat values were similar to those of primary males ($\chi^2_1 = 0.25$, $P = 0.62$) and were significantly lower than those of subordinate males ($\chi^2_1 = 6.70$, $P = 0.009$; Fig. 5.2E).

Discussion

Influence of status on T levels and CPs

We studied blood plasma levels of testosterone and indicators for body condition of subordinate and primary males in a cooperative breeding avian species, both descriptively and experimentally, to test several proximate hypotheses for why subordinate males do not gain paternity. Primary male Seychelles warblers had significantly higher T levels during the nest building and incubation stage than subordinate males. Subordinate males never gain paternity outside their resident territory and only rarely within their territory (Richardson *et al.* 2001), suggesting that elevated T levels are needed to be capable to reproduce. This is consistent with other studies in cooperative breeding systems that report differences in T levels between sexually active male breeders and reproductively inactive subordinates and/or helpers: e.g. Florida scrub-jay (*Aphelocoma coerulescens*, Schoech *et al.* 1991; Schoech *et al.* 1996); white-browed sparrow weaver (*Plocepasser mahali*, Wingfield *et al.* 1991) and bell miner (*Manorina melanophrys*, Poiani and Fletcher 1994). Interestingly, in cooperatively breeding species where subordinate males are sexually active, T levels did not differ between subordinate and primary males: Harris hawk (*Parabuteo unicinctus*, Mays *et al.* 1991) and Australian magpie (*Gymnorhina tibicen*, Schmidt *et al.* 1991). However, there is one exception, in the red-cockaded woodpecker (*Picoides borealis*) subordinate males are sexually inactive, but do produce similar T levels compared to reproducing primary males (Khan *et al.* 2001). The authors suggest that these subordinate males are behaviourally suppressed by active exclusion from the nesting sites.

The lower residual body mass and higher buffy coat values for subordinate males suggest that subordinates males are physiologically suppressed or of lower quality than primary males. The finding that the experimentally promoted subordinates quickly reached similar levels of residual mass and buffy coat as primary males, suggest that not individual quality but suppression by the dominant male explains their lower condition when subordinate. It is intriguing that these males were not able to raise their T levels to that of normal primary males. Since these promoted males had normal interactions with their females that also started nest building, lack of female stimulation can not explain their low T levels. Also, since variation in T levels of primary males are not related to the presence of subordinate males, the low T levels in the experimental males are unlikely to be due to the lack of competition with subordinates. The lack of a substantial increase in T levels in the promoted males is interesting since convincing evidence exists that in many taxa males can quickly elevate T levels in response to social stimulation (Oliveira 2004). It indicates that suppression by dominant males may down regulate the sensitivity of the Hypothalamus-pituitary-gonadal axis to the social context.

Despite the lack of a substantial increase in T production in the experimental males, their CPs were similar to those of normal primary males. Furthermore, the mates of the experimental males did show nest building behaviour and laid eggs. This might indicate that high T levels are not necessary for reproduction. Alternatively, eggs from these females could have been fertilised by males from other territories, which usually already occurs in 40% of territories (Richardson *et al.* 2001). Unfortunately, low sample size due to high egg predation does not allow us to test whether offspring was fathered by the experimental males.

Although subordinate males that filled a breeding vacancy could potentially differ from subordinate males that did not, we do not have clear evidence that this is the case. First, distance to a vacancy is the most important factor determining whether a subordinate will acquire a territory (Eikenaar *et al.* unpublished data). Second, the first subordinate observed in a territory always became primary male of that territory and no subsequent interactions between the first and other subordinates were observed in these territories (Eikenaar *et al.* unpublished data).

Temporal variation

The Seychelles warbler is territorial and paired with a mate year round and therefore does not need to re-establish a territory or attract a mate before the breeding season. However, T levels of primary males peaked during the nest building stage and decreased after egg laying in the same way as monogamous species of the temperate zone in which males participate in parental care (Wingfield and Farner 1993; Beletsky *et al.* 1995). It has been hypothesized that in species which defend their territory year-round (as in most tropical species) it would be beneficial to keep plasma T levels at a basal level to avoid the potential detrimental effects of elevated T levels (Wingfield *et al.* 2001) and to have low-amplitude cycles with slight elevations during the breeding season (Dittami and Gwinner 1990; Levin and Wingfield

1992; Wikelski *et al.* 1999; Hau *et al.* 2000; but see: Goymann *et al.* 2001; Stutchbury and Morton 2001). However, in the Seychelles warbler, the increase during the nest building stage was substantial (more than two fold increase). As there is no need for competition over territories and mates during this time this suggests that in this species T levels are not influenced by aggression. On the other hand, as the risk of cuckoldry is very high in this species (40%, Richardson *et al.* 2001), increase of T may facilitate mate defence since the frequency of male intrusion is high (Komdeur *et al.* 1999). CPs showed similar patterns as T except that the period of increased CPs extended into the incubation stage. Although T levels will initially result in an enlargement of the CP by production of sperm, additional T does not seem to influence CPs. In contrast to T levels, CP sizes increased after experimental promotion to similar size as those of primary males. Apparently the increase of CP requires a relative low elevation of T levels.

Challenge

In male Seychelles warblers a challenge (conspecific song) did not increase T levels (van de Crommenacker *et al.* 2004; this study), while in other species this resulted in increased T levels within minutes (Wingfield and Wada 1989; Wingfield and Soma 2002). According to the ‘challenge hypothesis’ (Wingfield *et al.* 1990) agonistic interactions rapidly elevate T levels and thereby create a positive feedback system in which subsequent aggressive behaviour could be promoted. An explanation for the lack of response in our study might be that during the nest building period, when the response was expected to be highest, the T levels of primary males had already reached their maximum. A lack of response during the other periods might be explained by insensitivity to a challenge outside the period of sexual activity. Our findings suggest that in Seychelles warblers, like in other tropical bird species, sensitivity in the T response to aggressive challenges is low (Wingfield & Lewis 1993, McDonald *et al.* 2001, Moore *et al.* 2004). The presence of subordinates did not result in higher T levels for primary males, indicating that primary males are not challenged by the presence of subordinate males. This could be a mechanism to avoid social disruption of the group.

Conclusion

Our data suggest that subordinate males are physiologically suppressed. Whether this is caused by active suppression by the primary male or a facultative delay of reproduction, remains unknown. It could well be that subordinates, because of their poorer body condition, decide not to invest in T to reduce physiological costs. The higher T levels of primary males indicate that these are needed to reproduce and may play a role both in the regulation of sexual behaviour and defence against extra pair copulations. The latter may explain seasonality in T levels despite the stable social system.

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Top: abandoned coconut plantation is not the preferred habitat of the Seychelles warbler, but areas with native tree species is (bottom).



Chapter 6

Heterozygosity-fitness correlations in a bottlenecked island species: a case study on the Seychelles warbler

Lyanne Brouwer, Jan Komdeur and David S. Richardson

Abstract

We used capture-mark-recapture models to investigate the effects of both individual and parental heterozygosity, measured at microsatellite loci, on the survival of Seychelles warblers (*Acrocephalus sechellensis*). This is an endemic island species which went through a severe population bottleneck in the middle of the last century. We found that an individual's survival was not correlated with multilocus heterozygosity, or with heterozygosity at any specific locus. However, maternal, but not paternal, multilocus heterozygosity was positively associated with offspring survival, but only in years with low survival probabilities. A nestling cross-fostering experiment showed that this was a direct maternal effect as there was an effect of the genetic mother's, but not of the social mother's, heterozygosity. Heterozygosity-fitness correlations at microsatellite markers were generally assumed to reflect genome-wide effects. Although this might be true in partially inbred populations, such correlations may also arise as a result of local effects with specific markers being closely linked to genes which determine fitness. However, heterozygosity at the individual loci was not correlated and therefore does not seem to reflect genome-wide heterozygosity. This suggests that even in a small bottlenecked population heterozygosity-fitness correlations may not be caused by genome-wide effects. Support for the local effects hypothesis was also equivocal; although three specific loci were associated with offspring survival, including all single locus heterozygosities as independent predictors for the variation in survival was not supported by the data. Furthermore, in contrast to the local effects hypothesis, the loci which contributed most to the heterozygosity-survival relationship were not more polymorphic than other loci. This study highlights the difficulties in distinguishing between the two hypotheses.

Introduction

Inbreeding between closely related individuals will result in decreased heterozygosity in offspring which may lead to reduced fitness (Hartl & Clark 1997). Several studies have shown direct relationships between heterozygosity measured across multiple microsatellite loci and components of fitness, e.g. birth weight (Coltman *et al.* 1998), survival (Bean *et al.* 2004), recruitment (Hansson *et al.* 2001), reproductive success (Slate *et al.* 2000; Amos *et al.* 2001) and sperm quality (Gage *et al.* 2006). Variation in multilocus heterozygosity has also recently been shown to be associated with variation in sexually selected fitness traits, such as territory size (Seddon *et al.* 2004), plumage ornamentation (Foerster *et al.* 2003) and song repertoire size (Marshall *et al.* 2003). However it is still difficult to assess the generality and importance of heterozygosity-fitness correlations in natural populations as null results are likely to be under-represented in the literature (Coltman & Slate 2003), (but see: Whitlock 1993; Duarte *et al.* 2003; Overall *et al.* 2005). Furthermore, the underlying mechanisms which are normally responsible for the heterozygosity-fitness correlations observed in natural populations are still unresolved.

Heterozygosity-fitness correlations can arise in different ways (reviewed by Hansson & Westerberg 2002). First, heterozygous individuals may have higher fitness as a result of the scored loci which themselves influence fitness in an overdominant manner, the 'direct effect' hypothesis (Mitton 1997; David 1998). However, this does not easily explain correlations between fitness and heterozygosity at non-coding markers, such as microsatellite loci, as these are considered to be selectively neutral (Queller *et al.* 1993). Second, heterozygosity across multiple microsatellite markers could reflect genome-wide heterozygosity, the 'general effect' hypothesis (e.g. Lynch & Walsh 1998). This idea was initially favoured by studies investigating heterozygosity-fitness correlations (Coulson *et al.* 1998; Coltman *et al.* 1998; Coulson *et al.* 1999; Slate *et al.* 2000). However heterozygosity at microsatellite markers may only reflect genome-wide heterozygosity in partially inbred populations, and when very large numbers of markers are analysed, which is often not the case (Slate & Pemberton 2002). Third, heterozygosity-fitness correlations can be explained by the 'local effect' hypothesis, in which the heterozygote advantage is a result of a genetic association between a neutral marker and a locus under selection (Lynch & Walsh 1998; David 1998). This requires linkage disequilibria - the non-random associations of alleles at different loci - which is expected in small or recently bottlenecked populations (Hartl & Clark 1997; Lynch & Walsh 1998). Until recently, few studies had considered the local effect hypothesis as an appropriate explanation for heterozygosity-fitness correlations, as linkage disequilibria were only considered to occur over a limited area of the genome, i.e. a few kilobases (kb). However, recent empirical studies have shown that high levels of linkage disequilibrium may occur in natural populations (Reich *et al.* 2001; McRae *et al.* 2002; Dawson *et al.* 2002; Sinervo & Clobert 2003). Consequently, linkage disequilibrium might be an important factor causing heterozygosity-fitness correlations in natural populations.

Indeed, in several studies some loci seem to contribute more than others to (associated measures of) heterozygosity-fitness correlations (Merilä *et al.* 2003; Bean *et al.* 2004; Hansson *et al.* 2004; Acevedo-Whitehouse *et al.* 2006).

It is also possible that heterozygosity may not only affect the fitness of the individual but also of its offspring. Offspring fitness could be affected directly by the parents via somatic effects or hormones (direct parental effects) (e.g. Eising *et al.* 2001), or indirectly through the environment created by parents, e.g. via provisioning or parental care (environmental parental effects) (e.g. Hakkarainen & Korpimäki 1994). Several studies have shown effects of inbreeding on egg hatchability (van Noordwijk & Scharloo 1981; Bensch *et al.* 1994; Keller 1998; Westemeier *et al.* 1998; Kruuk *et al.* 2002; Hansson 2004) and/or offspring fledging success (van Noordwijk & Scharloo 1981; Kruuk *et al.* 2002; Jamieson *et al.* 2003). Although evidence that parental heterozygosity-offspring fitness correlations occur is currently lacking.

Here we investigate heterozygosity-fitness correlations in a population of the Seychelles warbler (*Acrocephalus sechellensis*), an endemic species restricted to a few islands in the Indian Ocean. Genome-wide inbreeding effects could be important in this species as the last remaining population on Cousin island went through a severe bottleneck. Only c. 30 individuals survived between 1920 and 1968 due to habitat deterioration as result of the planting of coconuts (*Cocos nucifera*) in the 1910s (Crook 1960; Loustau-Lalanne 1968). Since then the Cousin Island population has expanded and has been stable since 1982 with c. 320 individuals (Komdeur 1992). Importantly, these conditions could also generate considerable linkage disequilibrium (Nei, 1975; Reich *et al.* 2001). In this population, close inbreeding - resulting in decreased offspring multilocus heterozygosity - has been shown to occur for over 5% of offspring (Richardson *et al.* 2004). An earlier study has suggested that low multilocus heterozygosity of the genetic mother is linked to reduced first year survival of the offspring (Richardson *et al.* 2004). However this study did not investigate the effect of multilocus versus loci specific heterozygosity nor did it investigate the effects of heterozygosity on adult survival. Furthermore the survival analysis did not use mark-recapture analysis which incorporates resighting probabilities and is a more robust technique than logistic regression (Lebreton *et al.* 1992).

In the present study we investigate the effect of both the individual's own heterozygosity, and that of its parents - measured across 14 microsatellite loci - upon future survival using capture-mark-recapture analyses. First, we investigate the role of parental multilocus heterozygosity on offspring survival after testing for individual multilocus heterozygosity. Second, we use data from a cross-fostering experiment to disentangle direct parental and environmental parental effects by testing the effect of both the social and genetic parents' multilocus heterozygosity on offspring survival probabilities. Third, as close inbreeding occurs in this population we investigate whether heterozygosity is correlated across loci and therefore could reflect general effects. Fourth, we investigate whether all single locus heterozygosities explain variation in survival better than multilocus heterozygosity, which would indicate

local effects. This is, to our knowledge, the first time that capture-mark-recapture analyses have been used to investigate heterozygosity fitness correlations.

Methods

Study area and data collection

Data were collected from *Acrocephalus sechellensis* offspring which hatched between 1997 and 1999 and which were subsequently monitored up to 2004 as part of the long-term study of the Seychelles warbler population on Cousin island (04°20' S, 55°40' E). During the main breeding season (July to September), and the minor breeding peak (January to March) each territory was checked for breeding activity at least once every two weeks by following the resident female for 30 minutes (Komdeur 1992). Nests were observed throughout the breeding cycle. Most Seychelles warblers produce one clutch per season (Komdeur 1996b) and this normally consists of just one egg, but about 20% of nests contain two or three eggs (Richardson *et al.* 2001). Nestlings were ringed with a unique combination of three ultraviolet light-resistant colour rings and a British Trust for Ornithology ring at approximately 12 days of age. Blood samples (c. 15 µl) were collected by brachial venipuncture and then diluted in 800 µL of 100% ethanol in a 2.0-mL screw-cap microfuge tube and stored at room temperature. If a nest could not be reached, the nestling was caught with mistnets after fledging but while still dependent upon its parents within the natal territory. We attempted to sample all offspring produced in each breeding season. Although a large proportion of nests could not be reached, it is hard to imagine that birds nesting higher also have a different heterozygosity as there is large variation in selection of the height of the nest sites within individuals (personal observation). Offspring that were found dead in the egg ($n = 8$) or nest ($n = 10$) were included in the survival analysis. These early deaths, and an additional seven eggs and 16 nestlings sampled in 1995 and 1996, were used to investigate whether there was any relation between multilocus heterozygosity and early mortality. Molecular sexing using the PCR method was used to determine the sex of each individual (Griffiths *et al.* 1998).

Genotypes were identified for all individuals in the population using 14 polymorphic microsatellite markers previously isolated in the Seychelles warbler (Richardson *et al.* 2000). Multilocus heterozygosity was calculated, for offspring and their parents, as the number of heterozygous loci divided by the number of loci examined. Parentage was assigned with > 80% confidence (Richardson *et al.* 2001) for 181 offspring sampled between 1997 and 1999.. This is the same dataset as used in Richardson *et al.* (2004), except that we did not exclude the 1997 cohort in which mortality was high due to severe weather, but included year effects in our model (see below). These offspring were used to investigate whether the offspring's own multilocus heterozygosity or the multilocus heterozygosity of the genetic parents was associated with their juvenile and adult survival probabilities (Table 6.1). The

Table 6.1 Design of data sets used for testing the effects of individual, paternal and maternal heterozygosity on Seychelles warbler survival probabilities. G, genetic parent; F, foster parent.

Offspring	Design		Effect of heterozygosity		
	Father	Mother	Individual and direct parental	Environmental paternal	Environmental maternal
Within-group	G = F	G = F	+	-	-
Extra-group	G ≠ F	G = F	+	+	-
Cross-fostered	G ≠ F	G ≠ F	+	+	+

degree of linkage disequilibrium was estimated in Arlequin 2.0 (Schneider *et al.* 2001). Gametic phase was unknown and pairwise tests for linkage disequilibrium were therefore performed using a likelihood ratio test whose empirical distribution was obtained by a permutation procedure (Slatkin & Excoffier 1996). In this test the haplotype frequencies were estimated using the expectation-maximization (EM) algorithm to obtain the likelihood of the data not assuming linkage equilibrium. The EM algorithm has a very high ability of inferring correct haplotypes when sample sizes are large (> 100 individuals) as is the case in this investigation (Excoffier & Slatkin 1995).

To disentangle direct parental and environmental parental effects on survival probabilities, same age (\pm 2-day difference) nestlings between 0 and 6 days of age were cross-fostered between nests on different territories. 45 cases of the noncross-fostered offspring had extra-group paternity (EGP, sired by a male outside the group, Richardson *et al.* 2001). Consequently environmental parental effects - i.e. the multi-locus heterozygosity of the social mother ($n = 58$) and the multilocus heterozygosity of the social father (cross-foster + EGP, $n = 103$) - as well as direct parental effects on offspring survival probabilities could be analysed (Table 6.1).

Data analyses

Two resighting periods were defined; the first from 1 July to 1 September, the second from 1 January to 1 March, however most birds were re-sighted within the first two weeks of each fieldwork period. Individuals were recorded as present if observed at least once within this period, or absent if not. Data for January-March were not available for 2000–2002, for these years dummy variables were created by including zero's in the encounter history, with the survival parameter (Φ) set equal to 1 and the resighting parameter (recapture, ρ) set equal to 0. The resighting history files were used as input files in the program MARK (White & Burnham 1999). Akaike's information criterion corrected for sample size (AIC_c) was used to select the most parsimonious model, with better fitting models resulting in lower AIC_c values (Akaike 1973; Lebreton *et al.* 1992; Burnham & Anderson 2002). For one model to be selected above another, the AIC_c should decrease with at least 2 (Burnham &

Anderson 2002). Additionally, we report the normalized Akaike weights to assess the relative likelihood of competing models.

To investigate survival and resighting probabilities we employed an a priori approach in which a set of candidate models was created based on biological reasoning. Previous analyses showed that the survival of Seychelles warblers was lower in their first year of life (juvenile survival) than for older birds ('juvenile survival', Brouwer *et al.* 2006). Furthermore, survival varied between years, with similar variation between the years for both age classes. Resighting probabilities varied across the age classes, with a lower resighting probability for individuals in their first two years of life and higher probabilities for older birds (Brouwer *et al.* 2006). There was no variation in survival nor in resighting probability between the sexes, and neither territory quality (measured according to Komdeur (1992)), the number of nest mates or the number of non-helping subordinates in the natal territory were associated with survival probabilities (Brouwer *et al.* 2006, L. Brouwer, D.S. Richardson & J. Komdeur unpublished data). However, the number of helpers in the natal territory had a positive effect on offspring survival (L. Brouwer, D.S. Richardson & J. Komdeur unpublished data) and the local density, measured as the average group size that an individual lived in, had a negative effect on survival (Brouwer *et al.* 2006). However, the number of helpers and the average group size an individual lived in were not correlated with individual or parental multilocus heterozygosity (all: $-0.09 < r < 0.09$, $P > 0.24$). Consequently, we started our analyses on a global model with time dependent juvenile and adult survival and two age classes in the resighting probability (Table 6.2A, model 12). We checked the goodness-of-fit of this model by using parametric bootstrap procedures implemented in the program MARK, which suggested that our global model fitted the data well (Bootstrap goodness-of-fit, $n = 1000$, $P = 0.18$). The global model was then used to investigate the effects of individual and parental heterozygosity on survival. These effects were also tested in an interaction with age and year, with the main effects always included in the model when testing for an interaction. A model including heterozygosities at all 14 loci was then compared to the models with multilocus heterozygosity to investigate whether such a model would fit the data better and consequently whether correlations arise as a result of local effects. Including individual multilocus heterozygosity with parental heterozygosity in the same model did not change the results, but for clarity we present each effect separately.

Heterozygosity was included as an individual covariate in the analyses. As direct parental and environmental parental effects can only be differentiated using the cross-fostered/EGP offspring, two groups were created in which the covariates were only included for the group for which this data was available (see section 'study area and data collection'). In this way the complete data set could still be used to estimate the common survival and resighting parameters. The individual covariates were analysed by using the logit link function, with values of the individual covariates standardized to Z-scores (by subtraction of the mean and division by the standard deviation). Model notation was followed according to Lebreton *et al.* (1992).

Results

Early mortality

There was no indication that individual multilocus heterozygosity influenced mortality in the early stages of life. First, the multilocus heterozygosity of eggs that did hatch did not differ from that of eggs which did not hatch (hatched vs. non-hatched: mean \pm S.E. = 0.50 ± 0.01 , $n = 173$ vs. 0.56 ± 0.05 , $n = 15$, Mann-Whitney: $U = 1207.0$, $P = 0.58$). Second, the multilocus heterozygosity of fledglings did not differ from that of nestlings which did not fledge (fledged vs. non-fledged: mean \pm S.E. = 0.49 ± 0.01 , $n = 163$ vs. 0.52 ± 0.03 , $n = 26$, Mann-Whitney: $U = 1847.0$, $P = 0.29$).

Juvenile and adult survival

Our initial model structure (Table 6.2A, model 12) showed a clear division in survival between seasons. Juvenile survival in the first two seasons (average: 0.30 ± 0.06) was lower than in the remaining seasons (average: 0.68 ± 0.05), however adult survival was relatively constant (0.88 ± 0.02). Creating a model with constant adult survival and two groups of seasons for juvenile survival was better supported by the data than the fully time dependent model (Table 6.2A, model 2 vs. model 12). Resighting probability was lower in the first two years of life (0.92 ± 0.02) and higher for older birds (0.96 ± 0.01). Model 2 (Table 6.2A) was used as a starting model to test for effects of parental and individual heterozygosity on survival.

Individual multilocus heterozygosity was not associated with survival; a model including individual multilocus heterozygosity was not supported by the data (Table 6.2A, model 7 vs. model 2). Furthermore, individual multilocus heterozygosity did not affect the age classes differently (Table 6.2A, model 10 vs. model 2). Even under adverse conditions, an association between juvenile survival and multilocus heterozygosity did not become apparent; a model including an interaction between multilocus heterozygosity and season quality did not provide a better alternative to the starting model (Table 6.2A, model 9 vs. model 2). The survival of individuals with extremely high or low multilocus heterozygosity did not differ from individuals with intermediate multilocus heterozygosity; including multilocus heterozygosity as a squared effect was not supported by the data (Table 6.2A, model 6 vs. model 2).

No direct effect of paternal multilocus heterozygosity on offspring survival was found; including multilocus heterozygosity of the genetic father was not supported by the data (Table 6.2A, model 8 vs. model 2). Furthermore, multilocus heterozygosity of the genetic father did not affect the age classes differently (Table 2a, model 11 vs. model 2), nor was it associated with survival in low quality seasons (Table 6.2A, model 3 vs. model 2). Multilocus heterozygosity of the genetic mother was not directly associated with offspring survival (Table 6.2A, model 5 vs. model 2), nor did it affect the age classes differently (Table 6.2A, model 4 vs. model 2), but it was positively associated with juvenile survival in low quality seasons (Table 6.2A, model 1 vs. model 2; Fig. 6.1). This model was almost eight times better supported by the data than was the model without these effects (Table 6.2A, model 1 vs. model 2).

Table 6.2 Summary of model selection statistics for the effects of: (A) the individual's and genetic parents' multilocus heterozygosity, and (B) the social parents' multilocus heterozygosity on survival probabilities of the Seychelles warbler. Survival was dependent on the following (individual) covariates: (t), year; (a1,2+), two age classes: first year and older; (sq), season quality; (hz), individual heterozygosity; (gm), heterozygosity of genetic mother; (gf), heterozygosity of genetic father; (sm), heterozygosity of social mother; and (sf), heterozygosity of social father. Estimates of effect sizes (B , main effect (upper) and interaction (lower)) of individual covariates are given on the logit scale.

No	Model	No. Par.	Deviance	ΔAIC_c	Deviance	AIC_c weights	$B \pm S.E.$
(A)							
1	$\Phi_{(sqxgm+a1,2+)}$	8	749.8	0.0	749.8	0.46	-0.03 ± 0.12 0.90 ± 0.35
2	$\Phi_{(sq+a1,2+)}$	6	758.0	4.0	758.0	0.06	
3	$\Phi_{(sqxgf+a1,2+)}$	8	753.9	4.1	753.9	0.06	-0.12 ± 0.12 0.66 ± 0.34
4	$\Phi_{(sq+gm_a1,2+)}$	8	754.0	4.1	754.0	0.06	0.32 ± 0.17 -0.42 ± 0.17
5	$\Phi_{(sq+gm+a1,2+)}$	7	757.2	5.3	757.2	0.03	0.10 ± 0.11
6	$\Phi_{(sq+hz2+a1,2+)}$	7	757.6	5.7	757.6	0.03	-0.08 ± 0.12
7	$\Phi_{(sq+hz+a1,2+)}$	7	757.8	5.9	757.8	0.02	-0.04 ± 0.10
8	$\Phi_{(sq+gf+a1,2+)}$	7	757.9	6.0	757.9	0.02	-0.03 ± 0.11
9	$\Phi_{(sqxhz+a1,2+)}$	8	756.7	6.8	756.7	0.02	-0.10 ± 0.12 0.27 ± 0.26
10	$\Phi_{(sq+hzxa1,2+)}$	8	757.7	7.9	757.7	0.01	-0.01 ± 0.15 -0.06 ± 0.21
11	$\Phi_{(sq+gfa1,2+)}$	8	757.9	8.0	757.9	0.01	-0.01 ± 0.17 -0.04 ± 0.23
12	$\Phi_{(t+a1,2+)}$	14	754.0	16.6	754.0	0.00	
(B)							
13	$\Phi_{(sq+sm+a1,2+)}$	7	755.1	3.2	755.1	0.09	-0.35 ± 0.22
14	$\Phi_{(sqxsm+a1,2+)}$	8	754.5	4.6	754.5	0.05	-0.41 ± 0.23 0.59 ± 0.75
15	$\Phi_{(sq+smxa1,2+)}$	8	755.1	5.3	755.1	0.03	-0.34 ± 0.30 -0.04 ± 0.44
16	$\Phi_{(sq+sf+a1,2+)}$	7	757.8	5.9	757.8	0.02	-0.07 ± 0.13
17	$\Phi_{(sqxsf+a1,2+)}$	8	756.8	7.0	756.8	0.01	-0.001 ± 0.15 -0.32 ± 0.34
18	$\Phi_{(sq+sfxa1,2+)}$	8	757.7	7.8	757.7	0.01	-0.12 ± 0.18 0.10 ± 0.27

The resighting probability was similar for all models with a lower resighting probability for individuals in their first two years of life and a higher probability for older birds. Models were ranked according to their AIC_c value, with the best supported model on top. ΔAIC_c being the difference between the AIC_c of the best supported model and the model considered.

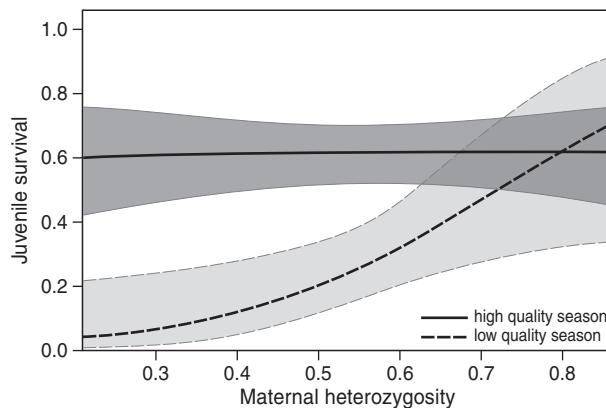


Figure 6.1 The relation between juvenile survival and maternal multilocus heterozygosity in low and high quality seasons with the 95% confidence interval based on model 1 (Table 6.2A).

In order to resolve between direct and environmental parental effects on offspring survival, the heterozygosity of the social parents was also investigated. However, multilocus heterozygosity of the social parents was not associated with offspring survival (Table 6.2B, models 13 and 16 vs. model 2) and there were no effects of the social parents' multilocus heterozygosity in an interaction with season quality or age (Table 6.2B, models 14, 15, 17 and 18 vs. model 2).

General vs. local effects

For multilocus heterozygosity to reflect genome-wide heterozygosity, heterozygosity at the individual loci should be correlated. We estimated heterozygosity twice for each individual, once using seven randomly sampled markers and then again based on the heterozygosity of the remaining seven markers (Balloux *et al.* 2004). A regression on both estimates indicates that heterozygosity at the individual loci was not correlated as $r^2 < 1\%$. In addition, we performed an analysis based on the sum of covariances in heterozygosity between every pair of loci (for details see: Slate *et al.* 2004). This yielded $\sigma = 0.26$. To determine the statistical significance, individual heterozygosity was randomised across individuals at each locus and the sum of covariances was recalculated. Repeating this 10.000 times also showed that heterozygosity at the individual loci was not correlated, as our test statistic was not significantly different from random ($P = 0.38$). Similarly, such correlations were not significant in the mothers of the individuals born in the low quality years ($N = 49$, $r^2 < 1\%$).

Analyses of linkage disequilibrium showed that 24 out of 91 pairs of loci were in significant linkage disequilibrium, which was much higher than would be expected by chance (4.6 cases at $\alpha = 0.05$). After sequential Bonferroni correction, still 8 pairs of loci showed significant linkage disequilibrium (Ase: 48-37, 9-37, 48-13, 42-13, 37-13, 10-35, 48-6, 37-6, 13-6), indicating that local effects could be important in this study.

To investigate whether the effect of mother's multilocus heterozygosity on offspring survival in low quality seasons was caused by local effects at specific loci, we included all single locus heterozygosities as independent predictors in one model. However this model was not better supported by the data than the model including multilocus heterozygosity, not surprisingly as the number of parameters increased with 26 (Table 6.3, model 3 vs. model 1). Investigating the estimates of the effect sizes of maternal heterozygosity at each locus on offspring survival showed that locus *Ase48* was associated with juvenile offspring survival in low quality seasons and loci *Ase35* and *Ase9* were associated with offspring survival in all years, with a positive effect of *Ase35* and a negative effect of *Ase9* on offspring survival (Table 6.4, Fig. 6.2). These effects remained when these loci where tested separately (not shown). Furthermore, loci *Ase56*, *Ase6* and *Ase37* show similar effects to locus *Ase48*, but locus *Ase37* in opposite direction (Table 6.4). When testing these loci separately, the effects of loci *Ase56* and *Ase6* remain similar, but their confidence intervals overlap with zero (not shown). However, the effect of locus *Ase37* disappears and was apparently caused by its strong linkage disequilibrium.

To test whether the single loci that were more associated with offspring survival were also more polymorphic than the other loci, we analysed the strength of the heterozygosity-survival correlation for each locus measured by its AIC_c value in relation to the expected heterozygosity and the number of alleles at each locus. However, the loci which were more associated with offspring survival were not more polymorphic than the other loci (expected heterozygosity: $r_s = -0.29$, $n = 14$, $P = 0.32$; no. of alleles: $r_s = -0.10$, $n = 14$, $P = 0.74$).

As correlations among pairs of loci might have masked an effect of multilocus heterozygosity on individual survival we then went back and tested the effect of heterozygosity at each locus in one model. However, a model including heterozygosity

Table 6.3 Summary of model selection statistics for the effect of heterozygosity at maternal loci and an individuals own loci on survival probabilities of the Seychelles warbler. Survival was dependent on the following (individual) covariates: (sq), season quality; (a1,2+), two age classes: first year and older; (gm), multilocus heterozygosity of genetic mother; (gmloci), heterozygosity at each locus of genetic mother; and (loci), heterozygosity at each locus.

No.	Model	No. Par.	Deviance	ΔAIC _c	AIC _c weights
1	$\Phi_{(sq\times gm+a1,2+)}$	8	749.8	0.0	0.88
2	$\Phi_{(sq+a1,2+)}$	6	758.0	4.1	0.11
3	$\Phi_{(sq\times gmloci+a1,2+)}$	34	703.1	9.2	0.01
4	$\Phi_{(sq\times loci+a1,2+)}$	34	722.5	17.7	0.00

The resighting probability was similar for all models with a lower resighting probability for individuals in their first two years of life and a higher probability for older birds. Models were ranked according to their AIC_c value, with the best supported model on top. ΔAIC_c being the difference between the AIC_c of the best supported model and the model considered.

Table 6.4 Locus names with their allele sizes, observed (HO) and expected (HE) heterozygosity and estimate of effect size (B) of maternal single-locus heterozygosity on offspring survival as a main effect (upper) and in interaction with season quality (lower) (on logit scale), based on model 3 (Table 6.3).

Locus	Allele sizes (bp)	HO	HE	B ± S.E.
Ase48	272, (278 + 279), 284	0.56	0.65	0.02 ± 0.16 2.33 ± 1.02
Ase9	(130 + 131), 133, 138	0.42	0.44	-0.32 ± 0.13 0.04 ± 0.49
Ase10	122, 130, 143	0.48	0.50	-0.16 ± 0.15 -0.39 ± 0.75
Ase42	249, 253	0.30	0.28	-0.10 ± 0.13 0.01 ± 0.47
Ase37	237, 241, 247	0.42	0.49	0.22 ± 0.15 -2.57 ± 1.23
Ase18	184, 188, 196	0.47	0.49	-0.09 ± 0.15 -0.05 ± 0.51
Ase58	283, 293, 299, 305, 310	0.73	0.72	-0.08 ± 0.15 1.12 ± 0.70
Ase25	173, 197, 202, 206, 209, 213	0.64	0.73	-0.04 ± 0.16 1.62 ± 0.89
Ase27	184, 209, 213, 217, 222, 225, 230	0.64	0.67	-0.20 ± 0.13 0.34 ± 0.48
Ase13	140, 147, 154	0.49	0.53	0.06 ± 0.15 0.32 ± 0.55
Ase35	230, 232, 234	0.54	0.60	0.30 ± 0.14 0.48 ± 0.50
Ase4	106, 108	0.45	0.42	0.09 ± 0.14 -0.53 ± 0.58
Ase56	299, 301, 305	0.31	0.39	0.01 ± 0.14 1.55 ± 0.67
Ase6	117, 119, 125, 129	0.69	0.69	-0.08 ± 0.14 1.31 ± 0.50

Alleles in brackets were lumped due to difficulties in resolving alleles with only one base pair difference (see Richardson *et al.* 2001).

at each locus was not supported by the data (Table 6.3, model 4 vs. model 2). Although two loci seem to be associated with survival in low quality seasons, locus Ase48 ($B \pm S.E. = 1.00 \pm 0.48$) and Ase13 ($B \pm S.E. = -1.79 \pm 0.71$), their effects are in opposite direction and disappear when testing them separately, apparently caused by their strong linkage disequilibrium.

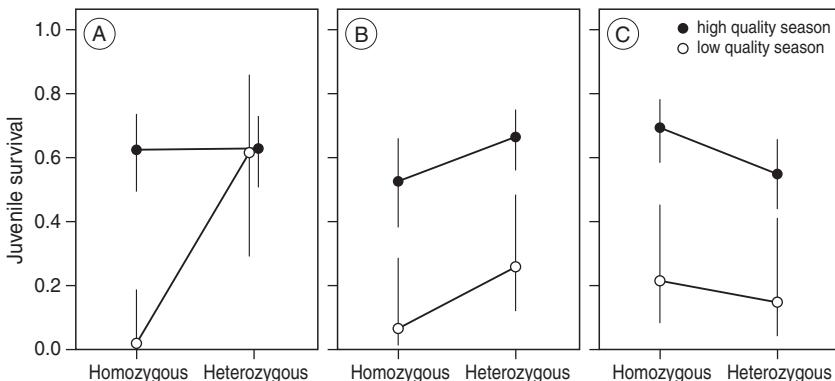


Figure 6.2 The relation between juvenile survival and maternal homozygosity and heterozygosity at; A) locus Ase48 B) locus Ase35 and C) locus Ase9 in low and high quality seasons with the 95% confidence interval based on model 3 (Table 6.3).

Discussion

General vs. local effects

Despite the fact that a previous study showed that offspring heterozygosity decreased with increasing parental relatedness, i.e. with inbreeding (Richardson *et al.* 2004), we found that neither multilocus or single locus heterozygosity was associated with an individual's own survival. However, the correlation between offspring heterozygosity and parental relatedness might be circular as parentage analyses and parental relatedness were based on genotypic similarity at the same microsatellite loci. The lack of heterozygosity-survival correlations is not surprising given that the heterozygosities of the microsatellite loci were not correlated either. Although there seemed to be high levels of linkage disequilibrium in the Seychelles warbler, none of the specific loci appeared to be linked to genes that were associated with an individual's own survival. High levels of linkage disequilibrium might have reduced the degree of information of the microsatellite loci, as they are not independent. Alternatively, the lack of an association between single loci heterozygosity of an individual and its survival might be explained by the fact that any linked loci are not overdominant or that the variation in fitness is only revealed under adverse conditions. In our study, survival in two seasons was very low, but even under these conditions individuals with lower heterozygosity did not, themselves, have decreased survival. The similar levels of heterozygosity in eggs that did or did not hatch, and nestlings that did or did not fledge, indicates that heterozygosity linked mortality did not take place at an earlier stage of life. Another possibility is that any recessive deleterious alleles that had an effect directly upon individual survival have been purged. Although recent work suggests that purging will not substantially reduce inbreeding depression for most field populations (Keller & Waller 2002), the popula-

tion we studied does meet the conditions where purging is likely to be most effective; the population went through a severe bottleneck (26 – 29 individuals) which occurred for several generations. As purging is likely to be most effective on alleles subject to strong selection (Hedrick 1994), the bottleneck in our population might have lead to purging of the deleterious alleles affecting an individuals own survival directly, but not those alleles affecting offspring survival.

Many studies have investigated heterozygosity-fitness correlations (for review see Hansson & Westerberg 2002), however only a few have considered the local effect hypothesis as an appropriate explanation. Some studies have shown that one or a few loci contribute more to a heterozygosity-fitness correlation than others (Merilä *et al.* 2003; Bean *et al.* 2004; Hansson *et al.* 2004; Acevedo-Whitehouse *et al.* 2006). However, this is expected by chance even under the general effect hypothesis. Comparing a model with multilocus heterozygosity to a model including heterozygosity at each locus would be the best way to distinguish between the hypotheses (David *et al.* 1997). However, due to the increase in the number of parameters the power of such analysis will decrease with an increasing number of loci tested. Ideally, corrections for genome-wide inbreeding effects should be made, as for example in the great reed warbler (*Acrocephalus arundinaceus*) study by Hansson *et al.* (2004) where full siblings with different heterozygosity values at microsatellite loci are compared. In that study the heterozygosity-survival correlation was especially pronounced at four loci. However, as complete pedigree data is often not available it is possible that local effects, rather than general effects, may explain the heterozygosity-fitness correlations observed in many other studies (see also: Hansson & Westerberg 2002; Balloux *et al.* 2004). Part of the reason for this may be that the procedure used to select microsatellites for use in molecular studies on populations is biased. For instance, for studies that include parentage analyses (as in this present study) only the most polymorphic loci are selected for use. The higher variance at these loci might be a result of the longer coalescence time between the alleles, but could also be an artefact of either non-stepwise mutation processes or high mutation rates, resulting in stronger linkage disequilibrium (Slate & Pemberton 2002). Hansson *et al.* (2004) showed that the more polymorphic loci were related to survival. This is not the case in our study. However, with only 14 microsatellites and the low variance in the number of alleles (Richardson *et al.* 2000) our power to detect such a relationship was low.

Parental heterozygosity

We found evidence that the multilocus heterozygosity of the genetic mothers was positively associated with juvenile survival of their offspring, however only in low quality seasons. The local effects hypothesis was not supported as maternal multilocus heterozygosity explained the variation in survival better than including the single locus heterozygosities. However, we found that the maternal heterozygosity survival correlation in low quality seasons was mainly due to locus *Ase48*, but loci *Ase56* and *Ase6* show similar effects. Loci *Ase9* and *Ase35* were also associated with

offspring survival in all seasons but to a much lesser degree. Surprisingly, higher heterozygosity at locus Ase9 resulted in lower offspring survival. Why this should be the case is unknown, but one explanation might be that a rare deleterious allele causes higher mortality and that selection cannot act on this allele because it is fixed or linked to another locus.

Heterozygosity of both the genetic and social father was not associated with offspring survival. This is in contrast to the earlier study by Richardson *et al.* (2004), where heterozygosity of the social father was positively associated with offspring survival. However, in that study the direct parental and environmental parental effects were not completely separated as heterozygosity of the social parents was analysed on both cross-fostered and noncross-fostered offspring. Furthermore, year and age effects were not accurately corrected for as only cohort effects were taken into account. Nevertheless, the study of Richardson *et al.* (2004) did show the negative trend between maternal heterozygosity and juvenile survival.

Maternal effects

Our cross-foster experiment shows that there is only an association between offspring survival and heterozygosity of the genetic mother, and not of the foster mother. Consequently, the genes involved in the heterozygosity-survival association must affect fitness through direct maternal effects and not via maternal feeding or care. It is known that maternal effects may profoundly influence the survival of offspring (Mousseau & Fox 1998; Wolf *et al.* 1998) and that they can occur through differential investment in eggs, e.g. differences in egg size (Potti 1999), hormone levels (Eising *et al.* 2001) or antibodies (Heeb *et al.* 1998). It is possible that, in the Seychelles warbler, these fitness genes are related to one or more of the above factors and that being heterozygous at these loci provides an advantage. Unfortunately we do not have any data on egg size or hormone composition of this data set. Two other studies, one on the song sparrow (*Melospiza melodia*, Keller 1998) and the other on the takahe (Keller 1998; Jamieson *et al.* 2003) have shown that the mother's, but not the father's, level of inbreeding affects offspring fitness. However in these studies reduced offspring fitness may have been due to decreased heterozygosity in the offspring itself and not to the mother's level of inbreeding *per se*. To our knowledge the present study is the first to show a direct relationship between maternal heterozygosity and offspring fitness in a natural population, although only under adverse conditions.

Seasonal selection events

Maternal heterozygosity is only associated with juvenile offspring survival in seasons with low survival probabilities. During the almost 20 years during which the population of Seychelles warblers on Cousin has been studied such bad years (with below 50% juvenile survival) have occurred only twice, in 1997-1998 and in 1986 (Brouwer *et al.* 2006). These two years were characterised by extreme weather conditions, specifically by either very high (1997) or very low (1986) levels of rainfall

(Brouwer *et al.* 2006). With an average lifespan of four years after surviving their first year (average survival probability = 84%, Brouwer *et al.* 2006), many birds will never face such a selection event. Furthermore, even during such events selection is not acting directly on the mother, but indirectly on her offspring, consequently the mother will face more opportunities to reproduce (in high quality seasons) and pass on her genes. Consequently, selection against any deleterious alleles exposed through homozygosity must be relatively weak, and this may explain how these alleles have persisted in the small bottlenecked population of the Seychelles warbler. Our results support the idea that genetic effects should not be considered independently of environmental effects (Bijlsma *et al.* 1999), as has been shown through a number of other studies on wild populations. For example, in the inbred population of song sparrows on Mandarte island, outbred individuals survived better than inbred individuals under environmental stress (Keller *et al.* 1994). In Darwin's finches (*Geospizinae*) oscillating selection acts on morphological characters depending on the food supply (Gibbs & Grant 1987). While in the inbred takahe (*Porphyrion hochstetteri*) newly established island populations had a higher level of reproductive failure than the population living in the natural habitat (Jamieson *et al.* 2003).

The identity of associated genes

Several of the microsatellite markers isolated from the Seychelles warbler (Richardson *et al.* 2000) were also used in a study of the great reed warbler (*Acrocephalus arundinaceus*, Hansson *et al.* 2004). Intriguingly, one locus (*Ase58*), used in both studies had a positive significant effect on survival in great reed warblers, but not in the Seychelles warbler. As the environment these species live in is completely different, it might be that the gene linked to *Ase58* is of lesser importance in the Seychelles warbler than in the great reed warbler. Alternatively, as this locus appeared to have a direct effect on the survival of great reed warblers any deleterious alleles at this locus may have been purged in the Seychelles warbler. Whether the loci *Ase48*, *Ase9* and *Ase35* associated with offspring survival in the Seychelles warbler are also associated with offspring survival in the great reed warbler has not yet been investigated.

A predicted passerine genome map of microsatellites shows that one of the loci associated to the maternal effect in the Seychelles warbler (locus *Ase9*) is located on chromosome 3, while another one (*Ase48*) is located on chromosome 5 of the chicken genome (Dawson *et al.* 2006). Unfortunately, the three *Ase* loci associated with survival in the great reed warbler study were not successfully mapped. Future research taking more loci into account and comparing which loci affect fitness across a range of populations of the same or closely related species should make it possible to detect the location and even the function of the genes causing the heterozygosity-fitness correlation and by this get a better understanding of the underlying processes involved. Furthermore, complete pedigrees of populations will help us to investigate how known levels of *f* relate to heterozygosity and/or fitness.

Conclusion

Our study shows that heterozygosity-fitness correlations can occur in bottlenecked populations, although such correlations might be very limited, in our case only heterozygosity of the mother was associated with offspring survival in low quality seasons. Our results support the idea that even in a small inbred population heterozygosity measured at microsatellites might not reflect genome-wide heterozygosity or, therefore, individual inbreeding. However, support for local effects caused by linkage disequilibrium between specific neutral markers and fitness genes was also equivocal, highlighting the difficulty in distinguishing between the two hypotheses.

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7

Chapter

Summary and general discussion



The faunas of tropical islands are particularly rich in endemic species, and constitute a disproportionately large proportion of global biodiversity (Collar & Stuart 1985; Stattersfield & Capper 2000). In the last few centuries island faunas have become among the most threatened in the world, mainly because of anthropogenic effects such as human disturbance and the introduction of predators or competitor species (Stattersfield & Capper 2000). In contrast to species from temperate regions, we have little knowledge of the processes that regulate populations of tropical bird species, despite the fact that this knowledge is crucial to their conservation. In this thesis I studied population regulation in small isolated populations of the cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*). As this is a vulnerable species, direct manipulation of density through permanent removal of birds was not possible. However, translocations carried out as a part of a conservation project gave me the possibility to study density dependence of reproduction and survival in newly established populations. In addition I investigated the proximate mechanisms involved in helping behaviour and the long-term fitness consequences of group living. In this final chapter I will summarize some of the major findings of my research, discuss these in a general framework and explore their relevance to conservation.

Tropical environment

The life history strategies of tropical species are typified by high survival rates and small clutch sizes. In chapter 2 we showed that in the Seychelles warbler annual adult survival was high (84%) compared to European passerines, which usually have probabilities of < 50% (Sæther 1989; Peach *et al.* 2001). One of the reasons for this may be that the absence (or infrequency) of periods of severe food shortage and/or climatic severity in the tropics allows for high rates of survival. Our findings support this hypothesis as although we found temporal variation in survival, we did not detect an effect of weather variables (chapter 2). In temperate regions, the weather is a very important factor that influences survival, either indirectly, via food availability (Newton 1998), or directly, e.g. via thermoregulation (Cuthill & Houston 1997). We found no evidence that temperature was associated with survival in Seychelles warblers, though this was not surprising as the variation in mean temperature between years is extremely limited (range mean temperature: 25.6–26.6 °C). That rainfall did not affect survival (chapter 2) or reproduction (chapter 3) is surprising, as rainfall is known to influence insect numbers in our study area (Komdeur 1996b). However, the two years with extreme levels of rainfall, i.e. drought (1986) or flood (1997) also coincided with the lowest survival probabilities (Figure 2.1). This might indicate that survival is influenced by extreme weather conditions, but that our data set does not span enough years, to detect any significant effect. Alternatively, we may not be able to detect effects on post-fledging survival as mortality might have occurred before the fledging stage. Although the initiation of the breeding season was related to increasing rainfall (Komdeur 1996b), average annual

reproduction was not affected by the amount of rainfall (chapter 3). The ability of the Seychelles warbler to time their reproduction to periods with high food availability and choose whether to lay one or two eggs (Komdeur 1996b) might allow it to avoid adverse conditions. Moreover, with a life expectancy (period after which 50% of population is still alive) of 4 years after surviving their first year, many birds will never face the kind of severe weather conditions, and consequently selection event, which occurred less than once a decade. However, such sporadic events can have major ramifications for the evolution of a species, like for example, in the inbred population of song sparrows (*Melospiza melodia*) on Mandarte island, where outbred individuals survived better than inbred individuals under environmental stress (Keller *et al.* 1994). Or in Darwin's finches (*Geospizinae*) where oscillating selection acts on morphological characters depending on the food supply (Gibbs & Grant 1987). So if selection is normally weak in the Seychelles warbler, the strong selection events every decade might be have important consequences for the species' evolution.

Population regulation

In a closed population, the population size is determined by both density dependent and density independent processes affecting reproduction and survival. We investigated whether survival was density dependent in a saturated population of Seychelles warblers, but did not detect any evidence for this (chapter 2). Furthermore, we were unable to detect density dependent survival or reproduction after the removal of birds from the saturated population. However, the fact that within a year of the removal the population had increased back to the carrying capacity observed before the removal is, in itself, direct evidence for density dependence (Figure 3.1). Although many studies have shown associations between vital rates and density, the causal effect of density can only be shown by the use of experiments. As part of a conservation program, Seychelles warblers were translocated to a number of new islands. After translocation the newly established populations quickly expanded in size as a result of rapid rates of reproduction, but after a few years population growth rates started to decline (Figure 3.1). In chapter 3, we show that this decline in growth rate was a consequence of a decrease in reproduction, but not in survival, indicating that these populations are regulated by density dependent reproduction. Such density dependent reproduction has been observed in many other studies, but only few of these are experimental (Kluyver 1951; Kluyver 1971; Dhondt *et al.* 1992; Both 1998). Furthermore, these few experimental studies were normally performed by manipulating resources and very rarely by experimentally manipulated density itself (e.g. Armstrong *et al.* 2005) as done in the Seychelles warbler. Density dependent survival is hard to prove because of the difficulty in distinguishing between survival and dispersal in most study systems. Some experimental studies, which could control for dispersal, did find that survival decreased with

increasing densities (Armstrong & Ewen 2002; Nicoll *et al.* 2003; Armstrong *et al.* 2005). However, in the Seychelles warbler system, where dispersal was virtually impossible, we have no indication that density dependent survival occurred at the population level, either within or between populations (chapter 2 and Figure 3.4).

The detection of density dependence may also depend on the spatial scale at which such effects are investigated (e.g. Ray & Hastings 1996; Wilkin *et al.* 2006). In territorial species, especially year-round territorial species such as the Seychelles warbler, competition for resources will work mainly on a local scale, i.e. the territory (Both & Visser 2000). Consequently, we investigated the effect of local density, measured as group size during an individual's life, on survival in the saturated Cousin population. Individuals living in larger groups had lower survival probabilities than birds living in smaller groups (Figure 2.2). Therefore, although density-dependent survival was not detectable at the population level it was detected at the territory level - the scale at which individuals compete.

We showed that monitoring populations before and after translocation events can provide useful experiments and uncover interesting results which cannot be detected in saturated populations. Monitoring reproduction and well as survival can also add important information about which mechanisms regulate populations during the process of saturation. As such mechanisms can have consequences for the success of translocations, investigating and understanding them may also be important for conservation.

Food availability as a density dependent mechanism?

The newly established populations on Aride and Cousine grew rapidly to asymptotic levels, but these levels differed among all the islands, with higher population densities, larger groups and larger birds on islands with higher insect densities (chapter 3). Hence, we suggest that the density dependent reproduction was caused by competition for food (chapter 3).

At the territory level, increasing density (larger group sizes) was associated with lower survival (chapter 2). Studies on non-cooperative, group living animals have shown that the negative effects of increasing group size can be caused by larger groups attracting more predators (Begon *et al.* 1990), or because of increased competition for food (Krause 1994; Bednekoff & Lima 2004). As adult Seychelles warblers do not suffer from any predation pressure, competition for food is the most likely cause of the reduction in survival with increasing group size observed in the Seychelles warbler. Although the effects of both population and local density on survival are consistent with the idea that competition for food is causes the density dependent effects, manipulation of food itself, e.g. by supplementary feeding, is required to confirm this. In practice such an experiment might be hard to perform as removing food is too risky as this might have severe effects on the warbler and other (endangered) species. The disadvantage of adding food in a territory is that this will

also attract more competitors or potential egg predators (Wright's skinks (*Mabuya wright*)), Seychelles fodie (*Foudia sechellarum*) and Seychelles Magpie Robin (*Copsychus sechellarum*), therefore this should be done in such a way that only warblers can profit from it.

Rearing conditions

It is obvious that variation in rearing conditions will have immediate effects on offspring fitness, however they may also have important long-term fitness consequences (Lindström 1999). Rearing conditions can only be distinguished from parental/territory effects by the cross-fostering of nestlings. In the Seychelles warbler variation in rearing conditions is besides parental quality, also caused by variation in the number of nest mates, group size and territory quality of the rearing territory. However, none of these factors were associated with either juvenile or adult survival or recruitment (chapter 4). Furthermore, although there was variation in juvenile survival between seasons, this did not affect a cohorts' adult survival probability (chapter 2). The long period over which Seychelles warbler offspring are dependent on their parents might counterbalance any negative effects experienced during early life. Alternatively, selective disappearance (mortality) of low quality individuals may result in cohorts which experienced adverse conditions having similar adult survival probabilities as other cohorts.

The long period of offspring dependence, the stable environment and the ability of parents to synchronize reproduction with high food availability may all mean that parental effects are more important than environmental variation in rearing conditions in the Seychelles warbler. An indication that maternal effects play a role in determining offspring fitness comes from chapter 6; here we showed that maternal, but not paternal heterozygosity was positively associated with offspring survival, but only in seasons with low survival (Figure 6.1).

Territory quality

Although Komdeur (1992) found that territory quality was associated with reproduction and survival, we did not find this in our analysis. There was no association between territory quality and survival (chapter 2) or reproduction within the saturated populations (chapter 3). Furthermore, the quality of the rearing territory did not affect offspring recruitment (chapter 4). The former association between territory quality and reproduction (Komdeur 1992) is due to the inclusion of territory size in his calculation of territory quality. We now showed that territory size, and not the quality measure, was positively associated with reproduction, independent of group size (chapter 3). That previous studies found that survival was associated with territory quality (Komdeur 1992) while this study did not, can probably be explained by

the fact that the earlier studies were less thorough; mark-recapture analyses were not used, sample sizes were much smaller and no between-year variation was taken into account.

Although the comparisons in densities between islands shows that insect availability is very important and, therefore, that a relation between reproduction and territory quality within the saturated Cousin population is expected, our measure of territory quality might not be accurate enough to detect this. Insect availability was only counted at 15 sampling points across the island (Komdeur 1992) and not in each territory separately. Furthermore, our measurement of territory quality includes estimates of foliage cover which might not directly, or proportionally, translate into increased insect availability. This indicates that an index, rather than the true territory quality, was measured.

Cooperative breeding

In cooperatively breeding species, positive effects of group size on reproduction or survival are widespread (Rood 1990; Allainé *et al.* 2000; Clutton-Brock *et al.* 2001; Magrath 2001; Conner *et al.* 2004; Khan & Walters 2004; Hatchwell *et al.* 2004), but negative effects are rarely reported. Removal of Seychelles warbler helpers has shown that the presence of one or two helpers improved the reproductive success of a group, but that the presence of three or more helpers had a negative effect (Komdeur 1994b). We showed that birds that had lived in larger groups during their life had lower survival probabilities than birds in smaller groups (Figure 2.2). These results indicate that living in a group of five or more birds in total will not be profitable for Seychelles warblers in the Cousin population, as both reproductive success and survival decreased for such groups. In smaller groups, however, the presence of subordinates which help does increase both the juvenile and adult survival of the helped offspring (Figure 4.3). This effect is caused by the presence of active helpers *per se* and not by the presence of non-helping subordinates, indicating that helpers do really help. Finally, we ruled out the possibility that this effect was due to a non-causal relationship between the presence of helpers and parental/territory quality, as cross-fostering showed that only the number of helpers in the rearing, but not in the original territory, affected offspring survival.

A fundamental drawback of most studies on cooperatively breeding species relates to the poverty of accurate cost-benefit analyses of group living. Although costs in cooperative breeding seems obvious as individuals refrain from reproduction and risk mortality while waiting for breeding opportunities, helping itself can also be costly (e.g. Taborsky 1984; Heinsohn & Cockburn 1994). Furthermore, cooperative breeding can occur in many forms and helping behaviour varies both within and between species and is therefore not a discrete strategy with two levels (disperse or stay and help), but rather a continuous variable (Heinsohn 2004). The shape of the costs and benefits curves will differ between species as a result of variation in life

histories and social system, but might also differ within species as a result of environmental variation, for example, for Seychelles warblers on Aride (with higher insect densities) the optimal group size is probably larger than for the Cousin population. Benefits gained by increased reproduction will depend on the relatedness between group members and offspring, but will also differ between group members (i.e. primary and subordinate). Cost-benefit analyses of cooperative breeding will only get more complex with our findings that helping can also have long-term fitness effects for the offspring helped, and that also living in groups itself can have costs. However, hopefully this can contribute to our understanding of the large variation in helping behaviour both within and between species.

Hormones and behaviour

A lack of unoccupied habitat seems to provide a clear explanation as to why Seychelles warblers delay dispersal and become subordinate within a territory (Komdeur 1992). However, this cannot explain why, despite the high levels of extra-pair paternity seen in this species, male subordinates never gain paternity outside their own territory and only seldom within (Richardson *et al.* 2001). However, birds are able to breed independently in their first year of life, as this occurs in the new translocated population (pers. obs.; Komdeur 1992; Komdeur 1996a). To gain insight into the proximate mechanisms that contribute to delayed breeding and, subsequently, to helping, we investigated circulating hormone levels in male breeders and non-breeders. We investigated blood testosterone levels as this is a key hormone in the stimulation of male reproductive behaviour (for review see: Nelson 2000). Primary males had significantly higher testosterone levels during the nest building stage, and significantly larger cloacal protuberances (an indication of sperm storage) throughout the entire breeding season, than did subordinate males (chapter 5). Experimental promotion of subordinates to primary status (through the permanent removal of primary males), showed that promoted males increased their cloacal protuberance, but not their testosterone, to levels equal to those seen in normal primary males (Figure 5.2a). Lower body mass and measure of immunocompetence for subordinate males suggest that they are in poor body condition, possibly caused by physical suppression by the primary male (physiological suppression, Wingfield *et al.* 1991; Wingfield & Farner 1993; Schoech *et al.* 1997). It appears that more time is needed after promotion for previously subordinate males to be able to increase their testosterone to the levels usually seen in primary males. Repeating the experiment and subsequently sampling promoted subordinates at different time intervals might show what time frame is needed for the males to produce the typical primary male testosterone levels.

Small populations

Due to anthropogenic influences which have resulted in fragmentation and loss of habitat, many populations have decreased in size and become more scattered. As a result many species worldwide are now threatened with extinction (Gaston *et al.* 2003). The population dynamics of island populations are highly relevant to the conservation of bird species, as a large proportion of endangered birds occur on islands (Bell & Merton 2002) and most bird species extinctions have occurred on islands (Caughley & Gunn 1996). Small and/or isolated populations are inherently more vulnerable to external environmental perturbations, but also to chance fluctuations in local survival and fecundity, for example caused by insufficient numbers of one sex to sustain productivity. Although we do know that severe weather can affect survival in the Seychelles warblers, the limited fluctuations in numbers in the saturated Cousin population (Figure 3.1) show that the population has a strong ability to quickly respond to perturbations. The warblers are long-lived, territorial year round and time their reproduction to coincide with high food availability (Komdeur 1996b), and therefore appear to be very flexible to response to severe events. Furthermore, their cooperative breeding system might act as a buffer for periods of high mortality; subordinates quickly occupy breeding vacancies when these become available (Eikenaar *et al.* unpublished data) and by doing so prevent the overall breeding population being affected. In general, cooperative breeding might be a very successful strategy to buffer effects of environmental or demographic stochasticity. This was also shown in individual-based simulation models of the Red-cockaded woodpecker (*Picoides borealis*) (Walters *et al.* 2002), and by the presence of a non-breeding class in wolves (*Canis lupus*) (Vucetich & Creel 1999).

Inbreeding may also become an important factor in the survival of small populations as the chances of mating with relatives increases as numbers decrease. Inbreeding between closely related individuals will result in decreased heterozygosity in offspring and might lead to the expression of recessive deleterious alleles or the loss of the heterozygote advantage. This could result in decreased fitness, known as inbreeding depression (Hartl & Clark 1997). Many studies have shown direct relationships between heterozygosity measured across multiple microsatellite loci and components of fitness (Coltman *et al.* 1998; Slate *et al.* 2000; Hansson *et al.* 2001; Amos *et al.* 2001; Bean *et al.* 2004). However it is unclear whether these correlations indicate inbreeding effects as heterozygosity at microsatellite markers may not reflect genome-wide heterozygosity, except in partially inbred populations and/or when very large numbers of markers are analyzed (Slate & Pemberton 2002). Heterozygosity-fitness correlations can also be explained by the 'local effect' hypothesis, in which the heterozygote advantage is a result of a genetic association between a neutral marker and a locus under selection (Lynch & Walsh 1998; David 1998). In the Cousin population, close inbreeding - resulting in decreased offspring multilocus heterozygosity - has been shown to occur for over 5% of Seychelles warbler offspring (Richardson *et al.* 2004). However, in chapter 6 we showed that

inbreeding depression was very weak; multilocus heterozygosity of an individual was not associated with its own survival, but multilocus heterozygosity of the mother was associated with offspring survival, however only in low quality seasons (Figure 6.1). Furthermore, heterozygosity at the individual loci was not correlated and therefore does not seem to reflect genome-wide heterozygosity.

Our results support the idea that even in small inbred populations, heterozygosity measured at microsatellites might not reflect genome-wide heterozygosity or, therefore, individual inbreeding. The absence of a heterozygosity-survival association could be caused by the fact that any recessive deleterious alleles that had an effect directly upon individual survival have been purged. As purging is likely to be most effective on alleles subject to strong selection (Hedrick 1994), the bottleneck in our population might have lead to purging of the deleterious alleles affecting individual survival directly, but not those alleles affecting offspring survival. Although inbreeding depression effects are weak at the individual level, at the population level this might be different. As the Cousine and Aride population originated from only 29 individuals, alleles that are rare in one population might be very common in another and also the variation in the number of alleles could differ. Investigating heterozygosity-fitness correlations between populations might show that there are differences between populations and that inbreeding depression could be stronger at the population level.

Effective population size

In this study we used the total population size (N), the number of adult individuals in the population, to investigate the dynamics and regulation of populations. However, the genetically effective population size (N_e) - the number of individuals that actually breed and contribute to future generations - is a more meaningful measure of population size for threatened and small populations. Effective population size is defined as the size of an ideal population (i.e. one that experiences no mutation, selection and migration and in which mating is random) that would undergo genetic drift at the same rate as N (Wright 1931). If only a few individuals are responsible for the majority of offspring, for example through gaining extra-pair fertilizations, the effective population size will be smaller than expected under social monogamy (Waite & Parker 1997). N_e / N can be calculated as follows (Nunney & Elam 1994; Parker & Waite 1997).

$$N_e / N = \frac{4r(1-r)T}{r[A_f(1+I_{Af}) + I_{bf}] + (1-r)[A_m(1+I_{Am}) + I_{bm}]}$$

Where r is the proportion of adult males in the population; A_i is the mean adult lifespan of sex i ; T = generation time (age of first reproduction + $A - 1$); I_{ai} stan-

dardized variance (variance/mean²) in adult lifespan of sex i (= annual survival probability in case of age independent survival); and I_{bi} standardized variance in reproductive success of sex i. As precise estimation of N_e is rather complex (e.g. Araki *et al.* 2007), I calculate rough estimates for a Seychelles warbler population at low and high population density here. At low density where no cooperative breeding occurs almost all individuals participated in reproduction. Thus $r = 0.5$, $T = 6.25$, $A_i = 6.25$ for both sexes (chapter 2), $I_{ai} = 0.84$, $I_{bf} = 0.26$ (based on Aride) and assuming that $I_{bf} = I_{bm}$, $N_e / N = 0.53$. However, with increasing density, group size increased and cooperative breeding occurred (chapter 3), thus the proportion of birds participating in reproduction decreased and the variance in reproduction increased, $I_{bf} = 2.47$ (based on data Aride). Consequently N_e / N would decrease to 0.44. However, the mean age of first reproduction increased from 1 to 3.5 (based on 60% annual recruitment rate, chapter 4) and therefore the generation time increased, $T = 8.75$. This results in $N_e / N = 0.62$ (Figure 7.1)

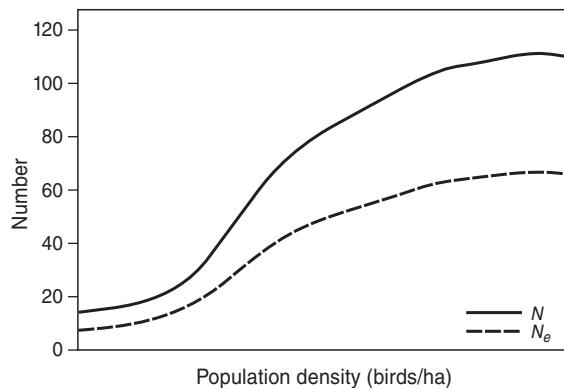


Figure 7.1 Population density in relation population size (N) and effective population size (N_e).

These are only rough estimates, in which I assumed that $I_{bf} = I_{bm}$, however extra-pair paternity will increase I_{bm} and co-breeding will decrease I_{bf} . Furthermore, measured over multiple years I_{bi} will even increase more. Although I expected N_e to decrease with increasing population density, the increase in generation time counter-balances the effect of the increased variance in reproduction. This again shows that dynamics of cooperatively breeding species can be very different from monogamous species.

Future prospective

The mechanism by which density dependence occurs determines to a large extent the outcome of evolutionary life history models (Mylius & Diekmann 1995), quanti-

tative models of cooperative breeding (Pen & Weissing 2000), but also population viability analyses, which provide a quantitative assessment of the probability of a population to decline to extinction (Beissinger & Westphal 1998). These analyses have become an important tool in the management of threatened species and our results can contribute to this field, especially as they are from a tropical species, a group for which there is, as yet, little information despite the fact that the majority of endangered species occur in the tropics.

Our results show that ecology cannot be considered independently from behaviour. During the process of population saturation not only did density start to affect reproduction, but also other factors, such as the breeding system and thus the behaviour of individuals, which changed as cooperative breeding developed. Although we now have a clearer idea of the regulation processes at the population level, to describe the processes at the individual level is more complicated. The Seychelles warblers' complex breeding system, including extra-pair paternity, co-breeders and helpers, means that an estimate of the average reproduction per bird is not a good indicator of individual fitness. A pedigree currently being developed based on molecular parentage analyses should help us to get a better understanding of the processes and decisions which occur at the individual level, i.e. investment strategies, mate choice and dispersal. It is likely that individuals follow different strategies to maximize fitness, e.g. disperse and try to breed independently, stay and try to gain direct fitness benefits by co-breeding, or specialize in gaining EPP. Although some analysis of this has been done on a small data set (Richardson *et al.* 2002), long-term data might give insights into the strategies followed by individuals and their actual life time reproductive success and will allow accurate cost-benefit calculations of group living. Furthermore, genetic analyses for the newly established Denis population might give new insights into the processes occurring at low population densities, and will allow a more accurate calculation of effective population size. In addition, inbreeding can be measured directly and thus its effect on fitness.

Although knowledge about natural patterns and variation in populations is very useful, experiments are needed to assess the causality of correlations. The disadvantage of working with a 'vulnerable' species is that performing experiments always brings a certain risk for the population. Our study, however, provides an example of how conservation management can be used to experimentally explore population regulation and behavioural processes. With the expected increase of the warbler population the potential for experiments will also increase.

If the establishment of a fifth population and a total population size of $> 5,000$ birds can be reached, the Seychelles warbler will be considered for downgrading from 'vulnerable' to 'near -threatened' (Richardson 2001). The suitability of any island for a new Seychelles warbler population depends on a number of factors. However as we show that reproduction depends on insect availability, which in turn, depends on the occurrence of native broad leaved species, including *Terminalia*, *Pisonia*, and *Morinda*, these habitat requirements must be seen as a key factor. The threat of rising sea levels and the increased occurrence of severe weather also mean

that any new populations should, preferably, be established on islands with higher altitudes. However, the most important factor for a successful establishment is, undoubtedly, the absence of introduced predators (rats and cats) which limits the numbers of suitable islands drastically. As the Seychelles warbler is a long-lived species with low reproductive rate, the population growth rate will largely be affected by adult survival. Changes in adult survival caused, for example by predation, will therefore have large consequences for the viability of the population. Although the previous translocations to Aride and Cousine were successful, this does not guarantee that all newly established populations will be successful (see Box A) and the continued monitoring of not only numbers, but also individual behaviour is required on the new islands. However, the rapid increase of numbers after translocations and the flexibility of the species to respond to perturbations suggest that the risk of extinction is low and that there is a bright future for this remarkable species.

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Nederlandse samenvatting

Coöperatief broeden en dichtheids regulering van kleine eiland populaties Seychellen zangers

Een achtste van alle vogel soorten, een kwart van alle zoogdieren en een derde van alle amfibieën wordt momenteel bedreigd met uitsterven. De bedreiging is vooral groot in de tropen waar de biodiversiteit het grootst is en waar de fragmentatie en vernietiging van habitat in een onophoudelijke tred voortduurt. Op tropische eilanden komen verhoudingsgewijs veel endemische soorten voor, dit zijn soorten die elders niet voorkomen. In de laatste paar eeuwen zijn deze eiland fauna's sterk bedreigd geraakt door menselijke invloeden zoals verstoring en de introductie van predatoren. In tegenstelling tot soorten uit de gematigde zone hebben we weinig kennis van de soorten uit de tropen, terwijl juist in de tropen deze kennis zo hard nodig is. In dit proefschrift heb ik onderzocht hoe kleine geïsoleerde populaties van de coöperatief broedende Seychellen zangers worden gereguleerd.

Populatie regulatie

De groei van populaties wordt op een bepaalde manier gelimiteerd en dus gereguleerd, anders zou de wereld binnen de kortste keren te klein zijn. De regulatie van aantallen gaat via overleving, reproductie en de uitwisseling van individuen tussen populaties (dispersie). Dichtheidsonafhankelijke factoren, zoals klimaat of het type omgeving, hebben effect op deze processen, maar dit effect is onafhankelijk van de grootte van de populatie. Wanneer een populatie door dichtheid gereguleerd wordt betekent dit gewoonlijk dat de overleving en reproductie afnemen als de populatie groter wordt. Het begrijpen van hoe en in welke mate dichtheidsafhankelijke en onafhankelijke processen de aantallen in populaties beïnvloeden, zeker in kleine populaties, is belangrijk voor het begrip populatie regulatie. Verder is dit ook belangrijk voor de ontwikkeling van modellen en theorieën over hoe bedreigde populaties beheerd en beschermd kunnen worden, omdat de manier waarop regulatie plaats vindt grote verschillen in uitkomst van theoretische modellen geeft en natuurlijk ook heel verschillende beheer strategieën met zich meebrengt.

Voor het bestuderen van de demografie en verandering in aantallen in populaties zijn lange termijn data series nodig met een zo volledig mogelijke kennis van de levensgeschiedenissen van individuen. Hoewel dispersie een interessant en belangrijk fenomeen is, bijvoorbeeld omdat het de genetische variatie van een populatie in stand kan houden, is het ook erg lastig omdat individuen die zich buiten de populatie vestigen niet meer waargenomen kunnen worden. Dispersie is vaak dus een groot probleem bij het verzamelen van complete levensgeschiedenissen van individuen. Dit probleem wordt vermeden bij het bestuderen van zogenoamde gesloten populaties, zoals eiland populaties met individuen die zich niet tussen de eilanden verplaatsen.

Hoewel stabiele populaties zeer sterk dichtheidsafhankelijk gereguleerd kunnen zijn, is het toch vaak moeilijk om dit vast te stellen omdat dichtheid vaak meeveranderd met dichtheidsonafhankelijke processen. Als het klimaat bijvoorbeeld erg gunstig is en voor een toename in aantallen zorgt door verhoogde overlevingskansen, is er dus geen negatieve relatie tussen populatie dichtheid en overleving zoals je op

basis van dichtheidsafhankelijkheid zou verwachten. Om te onderzoeken of dichtheid effect heeft op overleving of reproductie is het noodzakelijk dat er experimenten worden uitgevoerd waarbij de aantallen kunstmatig veranderd worden, maar tegelijkertijd de dichtheidsonafhankelijke factoren gelijk blijven.

Kleine populaties

Populatie regulatie speelt een belangrijke rol in kleine populaties. In zulke populaties kunnen toevallige veranderingen in overleving of reproductie grote gevolgen hebben. Verder is het zo dat als een populatie erg klein wordt een toename in grootte juist positieve gevolgen kan hebben op de demografie, omdat bijvoorbeeld de kans om een partner te vinden groter wordt, dit wordt ook wel omgekeerde dichtheidsafhankelijkheid genoemd. Een ander belangrijke eigenschap van kleine populaties is dat de kans op inteelt toeneemt en dit vaak negatieve gevolgen heeft voor de nakomelingen en dus uiteindelijk voor de populatie zelf.

Translocaties, het verplaatsen van delen van populaties naar nieuwe gebieden, kunnen gebruikt worden als een manier om bedreigde soorten te beschermen. Zulke (her) introducties verschaffen een mooie gelegenheid om dichtheidsafhankelijkheid te bestuderen aangezien zulke populaties normaal met een klein aantal individuen beginnen. Terwijl de populatie groeit en de dichtheid dus toeneemt kunnen de eventuele veranderingen in overleving en reproductie bestudeerd worden. Het interessante is dat bij toenemende aantallen ook het gedrag van individuen veranderd. Met een toenemende dichtheid neemt ook het aantal beschikbare broedplaatsen af en hierdoor stellen individuen hun dispersie naar een broedplek uit en blijven in plaats daarvan bij hun ouders op het territorium. Soms gaan deze individuen zelfs helpen met het grootbrengen van halfbroers- en zussen, dit wordt coöperatief broeden genoemd.

Coöperatief broeden

Coöperatief broedende soorten worden gekenmerkt door de aanwezigheid van niet broedende individuen, vaak nakomelingen, die in het territorium van hun ouders blijven in plaats van zelf te gaan broeden. Vaak, maar niet altijd, helpen deze nakomelingen met het grootbrengen van nieuwe jongen en daarom worden ze 'helpers' genoemd. Wij geven de voorkeur aan de term 'subdominant' voor zulke individuen, omdat ze zoals gezegd dus niet altijd helpen. Aan de ene kant kan dichtheid dus de ontwikkeling van coöperatief broeden stimuleren; doordat nakomelingen nergens meer heen kunnen blijven ze 'thuis'. Aan de andere kant is het zo dat coöperatief broeden ook effect kan hebben op de dichtheid; een hogere dichtheid kan bijvoorbeeld voordelig zijn wanneer individuen beter overleven of reproduceren in grote coöperatieve groepen.

Vele hypotheses zijn bedacht om dit schijnbaar tegennatuurlijke gedrag, dat voorkomt in zowel zoogdieren, vogels, vissen en insecten, te kunnen verklaren. Zo zou het kunnen zijn dat door ruimtegebrek een individu geen andere keuze heeft dan het grootbrengen van verwante jongen, wat een betere optie is dan niets doen. Ook zou het kunnen dat bijvoorbeeld door hoge predatiedruk het beter is om in groepen te leven, of omdat een individu in de veilige omgeving van zijn familie wil blijven ‘huur’ moeten betalen, in de vorm van hulp bij het grootbrengen van de nakomelingen. Anderzijds zou het echter ook zo kunnen zijn dat subdominanten gewoon niet kunnen reproduceren, bijvoorbeeld omdat hun hormoonspiegels te laag zijn. Wij hebben testosteron levels gemeten in kleine bloedsamples van mannelijke Seychellen zangers (*Acrocephalus sechellensis*). Dit hormoon speelt een sleutelrol in de stimulatie van reproductief gedrag bij mannen. Het bestuderen van zowel adaptieve (waarom is dat zo) als mechanistische (hoe werkt het) verklaringen kan onze kennis in het begrijpen van dit soort gedrag vergroten.

Een lange termijn studie aan de Seychellen zanger

In dit proefschrift heb ik zowel het coöperatief broedgedrag als populatie regulatie in de Seychellen zanger bestudeerd. Dit is een zangvogel die alleen voorkomt op vier kleine eilandjes in de Indische oceaan. De Seychellen zanger is een facultatief coöperatief broedende vogel, hoewel ze dus coöperatief gedrag vertonen, zijn er ook vogels die in paartjes leven en is coöperatief gedrag niet noodzakelijk voor succesvolle reproductie of overleving. In 1968 bestond de hele wereld populatie nog maar uit 30 individuen op het kleine eiland Cousin (29 ha). Hoewel de soort voorheen tenminste op drie eilanden moet hebben geleefd, hebben de aanplant van kokospalm plantages en de introductie van katten en ratten er voor gezorgd dat de soort bijna uitgesorven was. Met de aankoop van het eiland Cousin in 1968 door BirdLife International werd een programma gestart voor het herstel van het eiland en de Seychellen zanger populatie. Door het kappen van palmbomen en aanplanten van originele vegetatie herstelde de zanger populatie snel tot een stabiel aantal van ongeveer 320 individuen in 1982. Sinds 1985 wordt de populatie intensief bestudeerd, eerst door Jan Komdeur en sinds 1997 ook door David Richardson en AIO’s. Vanwege de kwestbaarheid van een kleine populatie werd besloten om nieuwe populaties te stichten. In 1988 en 1990 werden beide kerken 29 zangers getransporteerd naar de nabij gelegen eilanden Aride (68 ha) en Cousine (26 ha). Echter doordat deze drie eilanden op zeer kleine afstand van elkaar liggen is het gevaar bij een natuurramp zoals een tsunami erg groot. Daarom werd in 2004 een nieuwe translocatie uitgevoerd van 58 zangers naar het op 60 km ten noorden van Cousin gelegen Denis eiland (144 ha). Mijn onderzoek zich richtte hierbij op het bestuderen van deze nieuwe populatie. De Seychellen zanger komt nu op vier eilanden voor met een totale populatie grootte van meer dan 2000 vogels.

De Seychellen zanger leeft in groepen van 2 tot 7 vogels per territorium. Dezan-

gers broeden in het droge seizoen tussen juli en september, met soms nog broedactiviteit in het regenseizoen van januari tot maart, en leggen per keer meestal maar één ei. De uitgevlogen jongen worden nog tot een leeftijd van 3 maanden oud gevoerd. De ontwikkeling van genetische analyses heeft laten zien dat het broedsysteem zelfs nog ingewikkelder is dan coöperatief broeden alleen. 44% Van de vrouwelijke subdominanten legt zelf een ei bij de primaire vrouw in het nest, verder is 40% van alle jongen niet van de primaire man in het territorium, maar van een primaire man van een ander territorium. Omdat er vrijwel geen dispersie is tussen de eilanden kunnen we nauwkeurig de overleving bepalen.

In deze studie heb ik de populatie dynamica van alle vier de populaties Seychellen zangers bestudeerd. Ik heb de translocaties als experiment gebruikt om dichtheidsafhankelijkheid te bestuderen aan de hand van de groei van de nieuwe populaties. Verder heb ik de lange termijn effecten van coöperatief broeden op overleving en de proximate factoren die coöperatief broeden kunnen veroorzaken onderzocht.

De belangrijkste resultaten

De Seychellen zanger heeft een hoge overlevingskans, gemiddeld overleeft 84% van alle volwassen individuen tot het volgende jaar, voor uitgevlogen jongen is dit gemiddeld 61%. De oudst levende vogel gedurende dit onderzoek was zelfs 18 jaar. Hoewel er geen directe relatie was tussen weersfactoren en overleving, was het wel zo dat de twee jaren met de laagste overleving ook de jaren met extreem weer waren: droogte (1986) en overstroming (1997). De aantallen van de Cousin populatie zijn echter al meer dan 20 jaar erg stabiel en de kleine aantalsveranderingen door de jaren heen hebben geen meetbaar effect op de overleving. De mogelijkheid van de Seychellen zanger om de reproductie te plannen in periodes met hoog voedselaanbod en te kiezen voor het leggen van één of twee eieren maakt de soort flexibel in het ontwijken van moeilijke omstandigheden. Het blijkt echter dat individuen die in grotere groepen leven een lagere overlevingskans hebben. Dit kan worden veroorzaakt doordat competitie in territoriale soorten, zoals de Seychellen zanger, voornamelijk op een locaal niveau plaats vind, het territorium dus. Dit is zeer interessant want hoewel grotere groepen in coöperatieve soorten meestal een positief effect op overleving of reproductie hebben, blijken er dus ook negatieve effecten van groepsleven te zijn.

De nieuw gestichte populaties op Aride en Cousine namen snel in grootte toe, tot ze na een aantal jaren een stabiel aantal bereikten. De afname in groei werd veroorzaakt door een afname in reproductie en niet in een afname van overleving. Dit betekent dus dat deze populaties gereguleerd worden door dichtheidsafhankelijke reproductie. Echter, de uiteindelijke dichthesen in Seychellen zangers verschillen enorm tussen de eilanden, met hogere dichthesen en grotere vogels op de eilanden met hogere insecten dichthesen. Dit duidt erop dat het mechanisme achter deze dichtheidsregulatie competitie om voedsel is. Insecten dichtheid is dus een belangrij-

ke factor waar rekening mee moeten worden gehouden bij het vastsellen van de geschiktheid van een eiland als toekomstige nieuw habitat voor de Seychellen zanger.

Hoewel het duidelijk is dat bepaalde omstandigheden in een territorium, zoals bijvoorbeeld de hoeveelheid voedsel, direct effect kunnen hebben op de groei en overleving van jongen, kunnen bepaalde factoren gedurende de opgroeiperiode ook nog effect hebben veel later in het leven. Onze resultaten laten zien dat jonge Seychellen zangers die tijdens hun opgroeiperiode naast hun ouders ook werden gevoerd door subdominanten, een hogere overlevingskans hebben, zelfs als ze al volwassen zijn. Dit werd niet veroorzaakt doordat deze jongen van hogere kwaliteit ouders zijn en de subdominanten in deze territoria dus om die reden kozen om te helpen. Een experiment waarbij kuikens tussen nesten werden verwisseld, laat zien dat het aantal helpers in het oorspronkelijke territorium geen effect had op de overleving van een jong, wat wel de verwachting is als het effect niet door het helpen zelf maar door een kwaliteitsverschil zou zijn veroorzaakt.

Uit onze resultaten naar de hormoonhuishouding bij primaire en subdominante mannelijke zangers blijkt dat primaire mannen significant hogere testosterone levels hebben gedurende de nestbouwfase. Testosteron is dus noodzakelijk voor succesvolle reproductie, maar waarschijnlijk speelt het ook een belangrijke rol bij de verdediging van de partner tegen buitenechtelijke copulaties. Het was echter onduidelijk waardoor er een verschil tussen primaire en subdominanten was. Komt dit door een verschil in kwaliteit of zijn ze nog niet oud genoeg? Door subdominanten de mogelijkheid te geven om te promoveren tot primaire man (door het verwijderen van primaire mannen tijdens de translocatie) hebben we laten zien dat deze subdominanten hun testosterone levels binnen korte tijd kunnen verhogen, maar in deze periode nog niet het niveau van normale primaire mannen bereiken. Gegevens aan lichaamsgewicht en een maat voor immuunrespons laten zien dat de subdominante mannen in slechtere conditie zijn. Dit is zeer interessant want hoewel er nooit agressie tussen primaire en subdominante mannen binnen het territorium wordt waargenomen, lijkt het er dus op dat subdominanten op de een of andere manier fysiologisch onderdrukt worden door de aanwezigheid van de primaire man. Het zou echter nog steeds zo kunnen zijn dat het gewoon lagere kwaliteit individuen zijn. Echter, het feit dat ze binnen een korte tijdsperiode na promotie wel in goede conditie zijn wijst erop dat het hier niet om een kwaliteitsverschil gaat, maar dat de aanwezigheid van de primaire man op de een of andere manier verhindert dat subdominanten hun testosterone spiegels verhogen. Dit zou een mechanisme kunnen zijn om te voorkomen dat de sociale orde binnen een groep verstoord wordt.

Om inzicht te krijgen of er negatieve effecten van in te eten zijn werd onderzocht of individuen met lagere heterozygotiteit, gemeten aan neutrale genetische merkers, ook slechter overleefden. Een organisme is heterozygoot voor een bepaalde eigenschap als het twee verschillende vormen (allelen) van een gen heeft. Nakomelingen van gerelateerde individuen zullen minder heterozygoot zijn en dus minder genetische variatie hebben. Ook wordt de kans vergroot dat twee nadelige recessieve allelen bij elkaar komen, wat een negatief effect op overleving of reproductie kan hebben. Het blijkt

echter dat minder heterozygote individuen niet slechter overleven. De heterozygositeit van vrouwelijke Seychellen zangers was echter wel gecorreleerd met de overlevingskans van haar nakomelingen, maar alleen in slechte jaren. Het feit dat er geen correlatie tussen heterozygositeit en overleving van een individu zelf was kan zijn veroorzaakt doordat nadelige recessieve allelen uitgeselecteerd zijn gedurende de periode waaring de populatie zeer klein was. Aangezien zo'n proces het effectiefst is op allelen die onder sterke selectie staan kan het zijn dat de allelen die een individu's overleving direct, maar niet degenen die alleen de overleving van nakomelingen beïnvloedt (en dan alleen onder slechte omstandigheden) uitgeselecteerd zijn. Dit zou kunnen betekenen dat het gevaar van inteelt op de overleving dus niet zo groot is. We kunnen dit echter pas met zekerheid zeggen als we de relatie tussen overleving en heterozygositeit gemeten aan niet neutrale merkers onderzocht hebben. Dit zijn merkers verbonden aan een genencomplex waar wel selectie op plaats vindt, zoals bijvoorbeeld het gencomplex dat een rol speelt bij de immuunrespons.

Conclusies

Kennis over de manier waarop populaties gereguleerd worden kan onder andere worden gebruikt in wiskundige modellen die voorspelling doen over de kans dat een soort binnen bepaalde tijd uit zal sterven. Onze resultaten kunnen bij dragen aan deze voorspellingen, zeker omdat onze studie gebaseerd is op een tropische soort, het gebied waar de meeste bedreigde soorten voor komen en waarvoor deze modellen bedoeld zijn.

We hebben laten zien dat gedrag en ecologie niet afzonderlijk beschouwd moeten worden; verandering in aantallen resulteerde ook in verandering in broedsysteem en dus gedrag van individuen. Hoewel we nu een beter beeld hebben van de regulatie processen op populatie niveau is het nog moeilijk om meer te kunnen zeggen over de processen op individu niveau. Het gecompliceerde broedsysteem leidt ertoe dat het gemiddelde aantal jongen geproduceerd per vogel per jaar geen nauwkeurige fitness maat is. Het zou namelijk kunnen zijn dat individuen een bepaalde strategie kiezen, bijvoorbeeld zo snel mogelijk zelf een broedpositie bemachtigen, juist in het ouderlijk territorium blijven en daar proberen zelf eieren te leggen of specialiseren in het verkrijgen van buitenechtelijke jongen. Een stamboom van alle Seychellen zangers in een populatie en berekingen aan de totale hoeveelheid nakomelingen geproduceerd gedurende een individu's leven kan hier meer duidelijkheid over geven.

De toekomst

Met de geplande vorming van een 5e populatie en een uiteindelijke totale populatie grootte van meer dan 5000 vogels zal de status van de Seychellen zanger op de rode

lijst verbeteren. De geschiktheid van een eiland voor translocatie hangt van verschillende factoren af, maar zoals wij in onze studie hebben laten zien wordt een groot deel van het succes van een populatie bepaald door de hoeveelheid aanwezig voedsel en dit hangt weer af van de aanwezigheid van bepaalde vegetatie. Het risico van zeespiegelstijging en de toename van extreem weer betekent ook dat nieuwe populaties het best op hoger gelegen eilanden kunnen worden gevestigd. Echter het meest belangrijke is ongetwijfeld de afwezigheid van predatoren zoals ratten en katten, wat het aantal geschikte eilanden drastisch verminderd. Hoewel de vorige translocaties succesvol waren betekent dit niet dat dit voor toekomstige translocaties ook geldt en het bestuderen van zowel aantallen als individueel gedrag is noodzakelijk in nieuwe populaties. Echter, de snelle toename in aantallen na translocatie, de flexibiliteit van de soort om te reageren op verstoringen en de gelimiteerde fluctuaties in de omgeving betekenen dat het risico op uitsterven klein is en dat deze opmerkelijke soort een bloeiende toekomst tegemoet kan zien.

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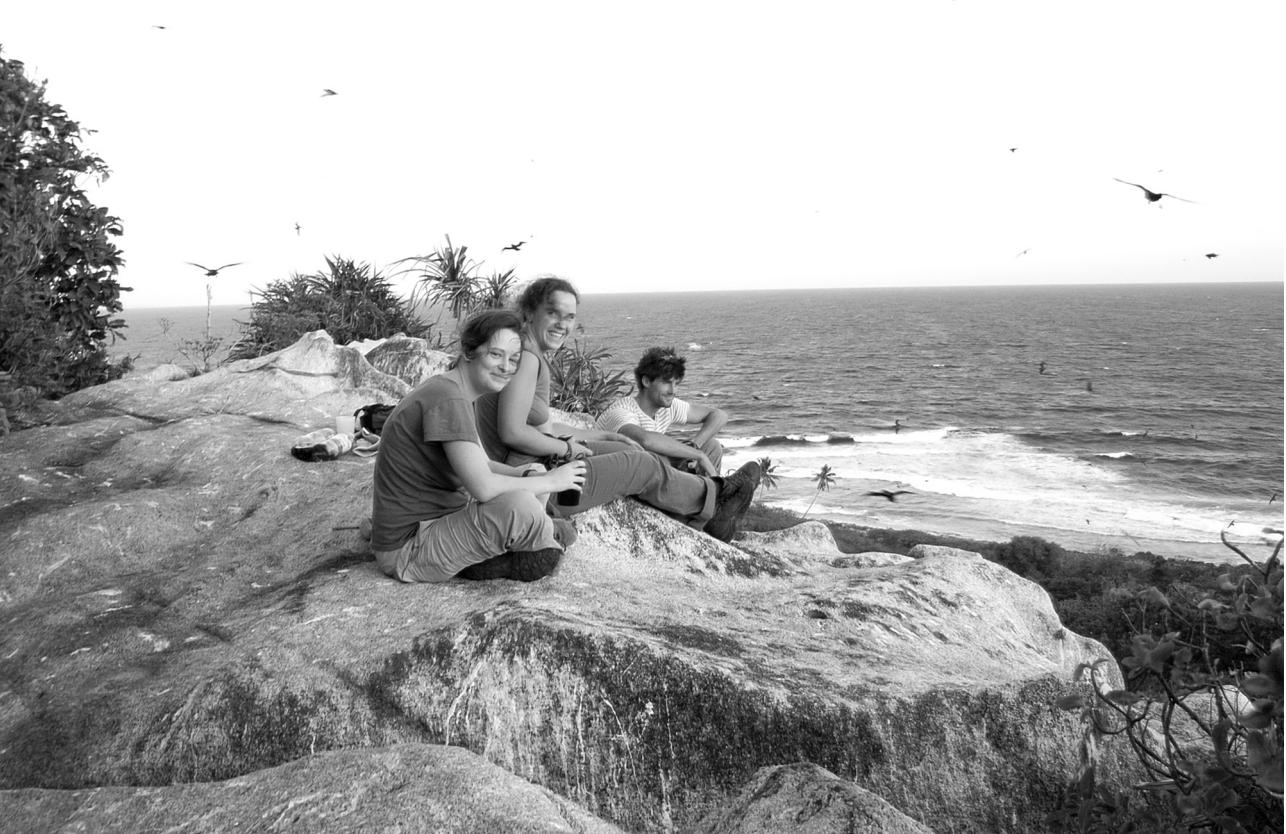
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Top: warbler researchers Kim Hutchings, Janske van de Crommenacker and Jeroen Reimerink enjoying the sunset at the viewpoint of Cousin Island. Bottom: Martijn van de Pol and Andrés López-Sepulcre wondering how a giant tortoise will manage to get to its feet again.



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Machteld en Martine, we kennen elkaar al vanaf de eerste dag op het biologisch centrum en ik vind het erg tof dat we elkaars paranifmen zijn, Martine zet hem op volgende week! Super bedankt voor alle leuke tijden eerst met z'n drieën en later met Arjen, Martijn en Martijn. Hoewel we nu wat verder van elkaar af wonen, hoop ik niet dat dat ten koste gaat van onze vriendschap. Martijn S., het was altijd erg gezellig om zomaar even wat te kletsen en je stond altijd klaar om me bij wat dan ook te helpen. Roos, even sporten na het werk zit er voorlopig niet in, maar misschien binnenkort wintersporten in Noorwegen?

Volleyballen was altijd een welkomme afleiding na een dag werken en hoewel mijn teamgenoten het wel apart vonden als ik weer maandenlang wegging, was er gelukkig altijd veel begrip. Denise en Ineke, hoewel met elkaar afspreken nu wat meer planning vergt dan tijdens onze studietijd in Groningen, hoop ik toch dat dit nog af en toe gaat lukken!

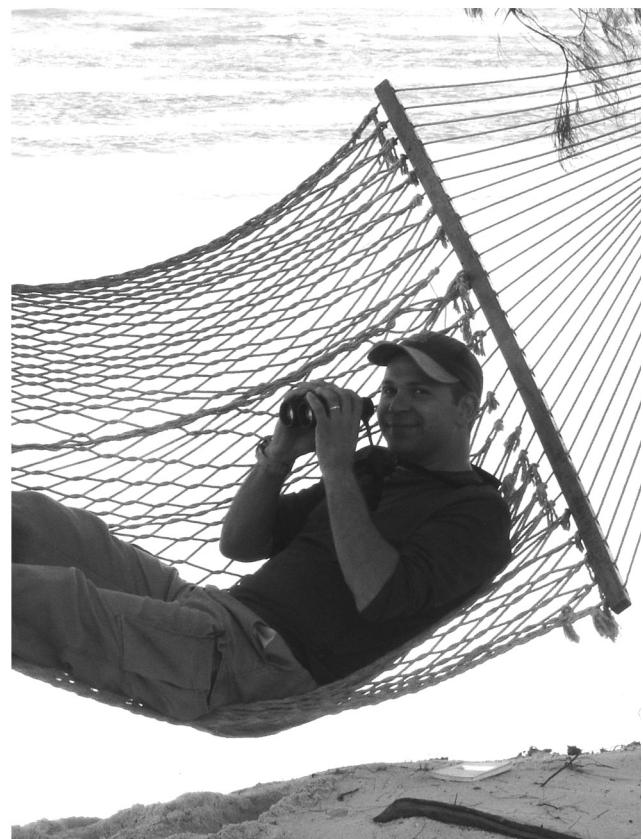
Mijn familie en schoonfamilie wil ik bedanken voor alle steun en het altijd welkom voelen. Opa Witte leerde me al heel vroeg van alles over het gedrag van honden, paarden en vogels en ik hoop dat hij trots geweest zou zijn. Ik ben mijn moeder enorm dankbaar dat ze me alle mogelijkheden en vrijheid heeft gegeven om te doen wat ik wilde. Maar het allerbelangrijkste is Martijn, ook al was het soms moeilijk als ik weer eens drie maanden weg was, jij hebt me altijd enorm gesteund en ik kijk erg uit naar alle avonturen die ons nog te wachten staan.



Top: wardens of Nature Seychelles in front of the boathouse on Cousin Island.

Bottom left: Camille Hoareau measuring a green turtle on Denis Island.

Bottom right: David Richardson looking for warblers from his hammock.



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