From parent to population:

the influence of the social environment on individual fitness

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A thesis submitted for the degree of Doctor of Philosophy

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March 2017

Abstract

In this thesis, I investigated how the social environment – the phenotypes of all an individual's social partners – influences metrics related to individual fitness. Using data from a population of cooperatively breeding Seychelles warblers *Acrocephalus sechellensis*, I explored relationships between various fitness-related parameters (body condition, telomere length, reproductive success and survival) and components of the social environment during both development and adulthood. I first investigated whether the presence of a nestmate influenced nestling physiological costs. While nestmate presence during development was costly, this cost was lower for relatively strong competitors. Intriguingly, strong competitors who survived to adulthood outperformed those raised alone in the nest. I further investigated early-life competition in the context of communal breeding, where two females sometimes raise their offspring in a joint-nest. Relatedness between competitors, which should dictate the degree of conflict over parental resources, had no effect on the cost of competition, most likely because conflict was resolved through increased parental provisioning in communal nests. Next, I examined how mate choice affects reproductive success by testing for offspring inbreeding depression. Individuals who reproduced with a relative produced offspring with shorter telomeres, but this was mainly evident in low-quality years. I also explored the influence of adult social partners on fitness-linked metrics, both within social groups and between neighbouring groups. Within groups, dominants appeared to benefit from larger group sizes, while subordinate females had better condition in small groups. At the population level, I found that male territory owners gained mass and lost telomeres when their male neighbours were either relatives or familiar individuals. The benefit of having related or familiar neighbours was greatest in high-density areas of the population. My research demonstrates the importance of considering ecological context, such as resource availability and intrinsic individual properties, when attempting to understand the link between socially-mediated behaviour and fitness.

Acknowledgments

Just as a multitude of social partners contribute to the expression of a phenotype, a multitude of social partners have contributed to the making of this thesis. I would like to thank my supervisor David Richardson for his guidance and for granting me the freedom to explore many more ideas than could ever have led to testable hypotheses. I am also indebted to the other members of my supervisory team, Lewis Spurgin and Matt Gage, whose doors were always open when I needed advice, academic or otherwise. Jan Komdeur, who unquestioningly accepted my adopting of him as an extra supervisor upon my move to Groningen, has been an abundant source of enthusiasm and insight during my time in the Netherlands.

My thanks also go to the Seychelles warbler team, past and present, for making this work possible. Particular thanks to current team members Terry, Hannah, Owen, Sjouke, Frank, Michela, Martijn, Sara and Dave, who contributed not only vast amounts of data, but also academic discussion and moral support. I feel that Megan, Frank and Sara also deserve a special mention for their unrelenting optimism and good humour when the 2015 El Niño turned our little bounty island into a storm-stricken swamp. All the fieldwork conducted in this project, submerged or otherwise, was only possible thanks to the continued support of Nature Seychelles and the Cousin Island team; Tom, Sam, April and Cheryl helped make every trip to that most magical of islands a wonderful experience.

I'd also like to thank everyone at UEA who helped make PhD life so rewarding. A great deal of gratitude goes to my wonderful lab teacher Ellie; through her incredible knowledge and patience, I arrived at my PhD carrying only a behavioural ecologist's field notebook, but left with the notebook in one hand and the beginnings of a molecular ecologist's toolkit in the other. Cat Morrison and Richard Davies provided brilliant statistical advice about siblings and neighbours respectively. Special thanks also go to Ellen, Sarah, Ellie and Dave; they coloured my PhD with mischief, madness and wonderful friendship.

I'm hugely grateful to my family for being a source of encouragement and laughter, be it over skype or over a pint of excellent west-country cider, throughout my PhD and in the various adventures that preceded it. Katherine and Rachel, who began the academic journey with me back in 2009, and Emma, the real expert in animal behaviour, have been amazingly supportive friends in the face of considerable geographical distance. To my partner Sjouke, who throughout the development of this thesis has also assumed the varying roles of friend, mentor, collaborator and fellow adventurer, I owe an enormous debt of gratitude.

Lastly, I feel it is only fitting to acknowledge the existence of many non-human social partners – in particular, one rather complex lagomorph who dutifully ate her way through drafts 1-99 of this thesis and reminded me that social behaviour is a phenomenon about which I still have much to learn.

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Author contributions

At the time of submission, two of the five data chapters presented in this thesis have been published in peer-reviewed journals and another is currently under review. The appendix, a conceptual comment on a recently published article, is also under review. I am the lead author on all manuscripts, published and unpublished, and have been responsible in all cases for the large majority of the work therein. Below, I provide details about the specific contribution I have made to each data chapter.

Chapter 2: Bebbington K, Kingma SA, Fairfield EA, Spurgin LG, Komdeur J, Richardson DS 2016. *Behavioral Ecology* doi: 10.1093/beheco/arw167*.*

- I developed the hypotheses, compiled the data, analysed the data and drafted the manuscript (80%)

Chapter 3: Bebbington K, Fairfield EA, Spurgin LG, Kingma SA, Dugdale H, Komdeur J, Richardson DS (under review, *Behavioral Ecology*)

- I developed the hypotheses, compiled the data, analysed the data and drafted the manuscript (80%)

Chapter 4: Bebbington K, Spurgin LG, Fairfield EA, Dugdale HL, Komdeur J, Burke T, Richardson DS (2016) *Molecular Ecology* 25: 2949-2960.

- I compiled the data, lead the analysis and drafted the manuscript (70%)

Chapter 5: Bebbington K, Spurgin LG, Fairfield EA, Komdeur J, Kingma SA, Richardson DS (in prep)

- I developed the hypotheses, compiled the data, analysed the data and drafted the manuscript (80%)

Chapter 6: Bebbington K, Kingma SA, Fairfield EA, Dugdale HL, Komdeur J, Spurgin LG, Richardson DS (under review, *Proceedings of the National Academy of Sciences USA*)

- I developed the hypotheses, compiled the data, analysed the data and drafted the manuscript (80%)

Appendix: Bebbington K, Kingma SA 2017. *Evolution Letters* doi: 10.1002/evl3.18.

- I conceived the idea and drafted the manuscript (65%)

Chapter 1

General introduction

“…harmony can arise from extreme competition, in the evolution of both sex and sociality.”

Mary Jane West-Eberhard, 1979.

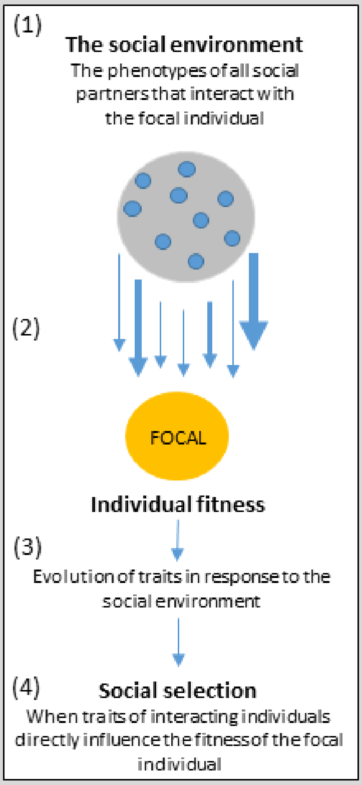
“Twee vogels” (Nr. 18) by MC Escher, 1938.

1.1 Social selection and evolution

No individual organism goes through its life entirely isolated from the influence of others. From the moment of fertilisation onwards, a suite of both direct and indirect social interactions with parents, family members, breeding partners and others in the population can greatly affect an individual's phenotype and ultimate fitness (West-Eberhard 1979). Individuals interact under a variety of circumstances including competition for resources, signalling of behavioural intentions and intrinsic quality, performance of altruistic acts and participation in mutualistic partnerships (Wolf et al. 1999 and references therein). Darwin (1859) recognised the importance of such social interactions for understanding individual fitness and evolution, and today the behavioural component of social interactions forms a cornerstone of behavioural ecology (Székely et al. 2010). In addition to broadly underpinning our understanding of animal behaviour, the form and function of social interactions are addressed in a now-extensive literature that aims to understand the power of social selection (Box 1.1) as an evolutionary process (Oh and Badyaev 2010).

Box 1.1 Social selection

Figure 1.1 (right) shows a schematic of the process of social selection.
(1) The phenotypes of all social partners (blue circles) – parents, siblings, members of the social group, neighbours – collectively form the social environment[1] (grey circle) of the focal individual (yellow circle). (2) Some components of the social environment may have more influence on individual fitness than others, as indicated by the thickness of the blue arrows. (3) Variation in fitness caused by the social environment leads to the evolution of traits in response to that environment[2]. (4) Social selection has the potential to act when these traits interact with the traits of other individuals ("interacting phenotypes")[3].



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2. Moore AJ, Brodie III ED, Wolf JB 1997. Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution* 51: 1352-1362.
3. McGlothlin JW, Moore AJ, Wolf JB, Brodie III ED 2010. Interacting phenotypes and the evolutionary process. III. Social evolution. *Evolution* 64: 2558-2574.

Whenever the social environment influences individual fitness, there is the potential for social selection to operate (Crook 1972; West-Eberhard 1979; McGlothlin et al. 2010; Box 1.1). In order to understand this process and how it promotes the evolution of complex behavioural phenomena such as cooperation and mutualistic partnerships, we must first understand the circumstances under which it can act. Those circumstances involve *the social environment and the nature of its influence on individual fitness* (McGlothlin et al. 2010).

Empirical work designed to measure the effect of the social environment on individual fitness has encompassed a broad range of topics including maternal effects (Mousseau and Fox 1998), mate choice (Westneat et al. 2000), intraspecific competition (West-Eberhard 1979) and cooperative breeding (Dickinson and Hatchwell 2004). However, despite the fact that selection can act differently on individuals at different stages of life (e.g. through antagonistic pleiotropy [Rose 1982]), there has been relatively little systematic effort to understand how the social environment might have varying influences on individual fitness during development compared to adulthood. Organisms interact with different conspecifics as they go through life (e.g. Hennessy et al. 2006; Hansen et al. 2008), meaning that different components of the social environment are likely to dominate at each stage of ontogeny, possibly also affecting individual phenotypes through different pathways. Below, I summarise the various components of the social environment that are likely to influence individuals at different life stages and discuss the mechanisms underlying their influence on individual fitness.

1.1.1 Social environments during development

Parental influence normally constitutes the most prominent component of the social environment during development (Mousseau and Fox 1998). Parents are responsible for determining the location and quality of the site where individuals develop, which can greatly influence both the early- and later-life fitness of the offspring (e.g. Shine and Harlow 1996; Mitchell et al. 2013). One of the best-studied components of post-natal parental care is the progressive provisioning of food to dependent offspring found in many bird, mammal, insect, amphibian and crustacean species (reviewed in Royle et al. 2012). As one of the primary mechanisms behind variation in individual development trajectories (Metcalfe and Monaghan 2001), early-life food availability can have a profound influence on factors such as immunological function (Saino and pape Møller 1997), physiological condition (Emlen et al. 1991) and growth trajectories (Stamps and Tanaka 1981), all of which have the capacity to affect offspring fitness. In some species parents can also influence offspring fitness through the transfer of skills and behaviours that are important for adult life. Parental influence on the development of traits such as song (Grant and Grant 1996), foraging skills (Müller and Cant 2010) and other social behaviours (reviewed in Thornton and Clutton-Brock 2011) have all been shown to affect offspring performance of that trait later in life, thus forming an important mechanism by which the parental social environment can influence individual fitness in more behaviourally complex taxa.

The other major component of the social environment during development is the presence of similar-aged individuals that compete for parental resources; a phenomenon broadly addressed under the umbrella term of sibling rivalry (Mock and Parker 1997). The presence and number of contemporaneous competitors (and arguably even the physical and behavioural traits of those competitors [Groothuis et al. 2005]) during an individual's development are largely a product of parental investment decisions and could therefore be viewed as a subset of the parental component of the social environment. However, co-existing competitors also act as agents of social influence in their own right; while their existence may be determined by parents, their evolutionary interests are rarely aligned with those of parents (Trivers 1974). I therefore treat competitors, both here and later in the thesis, as separate entities from parents that can exert their own influence on the fitness of an individual.

The implications of sibling rivalry for individual fitness are extensive and well-documented across a range of taxa including birds (Mock and Parker 1997), mammals (Hudson and Trillmich 2008) and plants (Shaanker et al. 1988). The most direct influence that sibling rivalry has on individual fitness is through obstruction of resource availability. Competitively stronger offspring have the capacity to deprive an individual of the parental resources required for successful growth and development, which can have severe implications for survival both during and after the developmental period (e.g. Magrath 1991; Mock et al. 2009). Besides directly affecting direct resource acquisition, sibling rivalry can also lead to the expression of competitive traits that become sub-optimal for fitness later in life. In species with altricial young, one such trait is flexible growth strategy. Selective investment in growth of certain aspects of morphology may increase the individual's competitive ability during development but come at a considerable cost later in life (Metcalfe and Monaghan 2001); for example in terms of reduced growth rate and fat deposition (Hale 1997) or lifespan (Birkhead et al. 1999). Sibling competition and the associated behavioural conflict can also induce the expression of certain behavioural syndromes such as abnormally low stress responses (Diaz-Real et al. 2016), although the extent to which variation in behavioural phenotypes influences fitness is not currently well known.

Clearly, early life social environments are important drivers of not only immediate fitness, but also fitness later in life. Capturing the short and long-term effects of early-life social environments not only requires high-resolution data and meaningful long-term metrics, but also relies on a detailed understanding of how different components of the early environment contribute to the emerging phenotype of an individual. However, studies that adopt such a holistic approach and incorporate sometimes conflicting influences of different social partners are still rare.

1.1.2 Social environments during adulthood

In adulthood, one of the most ubiquitous components of the social environment is the social and/or genetic mate. Regardless of whether that mate contributes gametes, parental care, or both, to a reproductive event, partner quality has strong implications for the reproductive tactics an individual employs, such as 1) overall investment in the reproductive attempt (e.g. Dixon et al. 1994; Harris and Uller 2009), 2) the degree of extra-pair copulations (e.g. Foerster et al. 2003) and 3) the reproductive success (Sandvik et al. 2000; Drickamer et al. 2003) and survival (Wedell 1996) of the individual. One prominent and well-studied mechanism by which an individual's choice of genetic mate can influence its reproductive success is inbreeding depression. Offspring whose parents are related to each other inherit a relatively high proportion of homozygous alleles and often under-perform in terms of various fitness components (Keller and Waller 2002). In species with biparental care, the social mate can also influence the cost of the reproductive attempt itself. For example, the degree of sexual conflict over care has the potential to influence not only physiological costs of reproduction (Houston et al. 2005) but also the fitness of any offspring produced (Lessells and McNamara 2012; Bebbington and Hatchwell 2016).

Aside from breeding partners, other members of the immediate family or social group can have a considerable effect on individual fitness in adulthood. This group-level component of the social environment has, by definition, been best-studied in social species where non-reproductive interactions with other conspecifics are frequent. The nature and frequency of interactions with other group members influences the degree of cooperation between social partners, which in turn has important implications for reproductive success and survival (reviewed in Silk 2007). Social hierarchies, almost ubiquitous in group-living species, have a large effect on individual fitness through a variety of pathways. An individual's social rank can determine whether it experiences restriction (or monopolisation) of reproductive opportunities (e.g. Nelson-Flower et al. 2011; Harrison et al. 2013), its foraging success (e.g. Monaghan and Metcalfe 1985), or the level of aggression it experiences from other group members (Creel 2001). In addition, the nature of relationships and degree of aggression within an individual's social group can influence dispersal decisions and consequent fitness (Ekman et al. 2002; Aguillon and Duckworth 2015).

Even in species that do not live in exclusive social groups, conspecifics still interact and influence each other's fitness through a variety of pathways. One promising emerging area of study in this respect is that of social networks – spatial descriptions of all interacting individuals in the population and the relationships between them (Skyrms and Pemantle 2000; Wey et al. 2008). An individual's position in a social network can influence its mating success (Oh and Badyaev 2010), level of physiological stress (DeVries et al. 2003), or likelihood of receiving cooperative help from neighbours (van Dijk et al. 2014). More broadly, the majority of interactions between conspecifics outside of social groups involve competition over resources (West-Eberhard 1979) such as food (Gobalek et al. 2012) or reproductive opportunities (Harris 2010). One notable example is spatial territoriality, where individuals defend their exclusive use of an area of habitat and the resources it contains from surrounding conspecifics. The influence of conspecifics on both the relative size of spatial territories (Adams 2003) and the cost of maintaining them (Eason and Switzer 2004) are considerable. Perhaps less well-understood is how the influence of the population-level component of the social environment translates into differences in individual fitness. While some evidence suggests that the identity of neighbours and their relationship with an individual can affect its reproductive success (reviewed in Hatchwell 2010), the physiological and mechanistic processes behind such patterns remain unclear.

1.2 The social environment and context-dependent fitness

In their paper, McGlothlin et al. (2010) advocate the definition of social selection outlined in Box 1.1, claiming that this definition allows us to measure *"the effect of social-partner or group traits on the fitness of a focal individual, while controlling for the effect of that individual's own traits"*. However, the effect of the focal individual's traits, and that of traits in the broader social and physical environment, are arguably some of the most important factors to consider when aiming to understand how the social environment affects individual fitness. In other words, the ecological context of a social interaction and the intrinsic properties of the individuals involved are hugely important because they have the potential to dampen, or even completely reverse, directional effects of the social environment. For example, a component of the social environment that reduces individual fitness under unfavourable ecological circumstances or for individuals of poor quality may have little or no effect in more favourable conditions or for high-quality individuals because in the latter two cases, the individual has the necessary resources to buffer any associated fitness costs. Below, I outline some of the mechanisms that can drive context-dependent fitness responses to the social environment, which can largely be assigned to one of two categories.

1.2.1 The physical environment

It is important to consider the physical environment – such as climatic conditions, resource availability or structural properties of the landscape – when considering influences of the social environment on fitness because it has the potential to influence how well an individual is able to buffer negative effects, or capitalise on positive effects, of its social partners (Keller et al. 2002; Barbraud and Weimerskirch 2005). Resource availability often constitutes an important part of the physical environment in this respect, as it dictates how much individuals are willing or able to invest in mediating social influences. For example, population-wide levels of food availability can affect competitive interactions and determine the strength of selection for certain behavioural phenotypes (Dingemanse et al. 2004). In cooperatively breeding birds, resource availability often affects the degree to which a helper-at-the-nest increases the reproductive success of the breeders – depending on the ecology of the individual species, helpers can have greater influence when resources are scarce (Komdeur 1994) or plentiful (Koenig et al. 2011).

Resource availability is almost always linked to broader climatic conditions – certain weather patterns produce a greater abundance of food and other important resources (Stenseth et al. 2002). However, other specific aspects of the climate such as temperature or rainfall can also have important influences on the relationship between the social environment and individual fitness. For example, in communally-roosting bird species, roosting position is strongly contested among group members and can have an important role on the benefits of group-thermoregulation (Hatchwell et al. 2009). However, since thermoregulatory costs are typically dependent on ambient temperature (du Plessis et al. 1994; Hatchwell et al. 2009), the benefits and costs of different roosting positions are presumably also temperature-dependent. Other aspects of climatic favourability may also influence the way in which individuals interact: for example, the quantity of parental care received during the developmental period can be influenced by rainfall (Öberg et al. 2014). One particularly fascinating study in superb fairy wrens *Malurus cyaneus* showed that the timing of male moult and the availability of helpers-at-the-nest both vary according to patterns of rainfall, in turn producing temporal variation in extra-pair paternity and thus the strength of sexual selection (Cockburn et al. 2008). This example of an interaction between the physical environment and the social environment demonstrates how social selection (in this case, sexual selection [Lyon and Montgomerie 2012]) is best understood in the light of context-dependent relationships and a detailed knowledge of the system in question. In Chapters 2-4 of this thesis, I therefore explicitly consider how social environmental influences vary according to the ecological context an individual experiences, thus shedding light on how selection landscapes might change with climatic, temporal or physical conditions.

1.2.2 Intrinsic state

Intrinsic qualities or characteristics of the individual can have a profound influence on the degree to which its social environment affects its fitness. Some characteristics, such as sex, are (in most species) to a large part irreversible components of the individual's phenotype that interact with the social environment in a similar way across life. For example, intense male-male competition may enhance the effect of factors such as inbreeding (Meagher et al. 2000) or costs of mate-guarding (Ancona et al. 2010) on male fitness, while simultaneously having no effect on a female's fitness (although the inverse can also be true [Le Galliard et al. 2005]). Yet other qualities of the individual are more flexible, varying according to the physical environment or the life stage of the individual. For example, competitive ability, which is largely a product of strength and aggression (Petrie 1988; Arnott and Elwood 2009), may vary with resource availability. Intrinsic competitive ability has the potential to mitigate or amplify the effect of the social environment depending on the relative competitiveness of an individual compared to its social partners. One well-studied example is that of sibling rivalry: the fitness costs imposed by the presence of competing offspring during development are known to vary considerably according to an individual's place in the competitive hierarchy of the family (Forbes and Glassey 2000; Wahaj and Holekamp 2006; Nettle et al. 2015). Another flexible component of intrinsic state is age; especially in long-lived species, the effect of the social environment on individual fitness may be very different for individuals that are early or late in their reproductive lifespan. For example, white-browned scrub wren females are known to benefit from helpers-at-the-nest most in their first year of breeding (Macgrath 2001), possibly because helpers help mitigate inefficiency caused by young females' lack of experience. In cooperative breeders more generally, it also seems plausible that the presence of helpers-at-the-nest might be more influential on the breeder's fitness during very late life when reproduction is more costly (e.g. Descamps et al. 2009). The interaction between individual characteristics, whether fixed or flexible, and the social environment is likely to be a key avenue of further research. In Chapters 2, 3 and 5 of this thesis, I consider relative competitive ability as one such factor that might influence the outcome and long-term consequences of social environmental interactions.

Another important factor that may mediate social environmental influences is the relationship between an individual and its social partners. Since Hamilton's (1964) seminal work on the evolution of sociality through kin selection, relatedness between social partners has been invoked as a mediator of social behaviour in a wide variety of contexts, including cooperative breeding (Clutton-Brock 2002), parent-offspring conflict (Trivers 1974) and maintenance of public goods (van Dijk et al. 2014). Broadly speaking, kinship between social partners has the potential to affect how an individual's social environment impacts its fitness because the sharing of genes between social partners is predicted to make their interactions more cooperative (Hamilton 1964). For example, nestlings of species with frequent extra-pair paternity, and hence relatively low relatedness between competing offspring, grow faster than nestlings of species where competitors are more related (Royle et al. 1999), suggesting that non-kin are in greater conflict over parental resources. In adult life, kinship with conspecifics in an individual's immediate surroundings have been shown to increase reproductive success (Mappes et al. 1995; Lee et al. 2009) and adult growth rates (Brown and Brown 1993; Gerlach et al. 2007), although the exact mechanisms behind these patterns are not yet clear. However, it is important to note that social interaction with kin can also have negative consequences because competition between kin for limited resources can reduce inclusive fitness (West et al. 2002). The degree to which costs of kin competition could outweigh benefits of living and cooperating with relatives has been questioned; ecological factors such as dispersal patterns and population-level processes are likely to limit the number of biologically plausible scenarios in which kin competition becomes a problem (Platt and Bever 2009). Clearly, more empirical work is needed to understand the effect of kinship in different social interactions – Chapters 3 and 6 of this thesis aim to address this problem.

Variation in social environmental effects can also arise through forms of social bond other than kinship. For instance, the degree of conflict between breeding partners, who are by definition almost always non-kin, can reduce over time as a result of negotiation of parental care (Lessells and McNamara 2012). This is an example of how social partners vary in their influence depending on how established, and hence cooperative, the social bond is. Generally, established relationships such as those between long-term breeding partners, but also between neighbouring territory owners (Grabowska-Zhang et al. 2012), can generate more positive influences of the social environment on individual fitness. It seems reasonable to assume that the nature of relationships between social partners is an important mediator of the effect of social environments on individual fitness that should be explicitly included in studies of social evolution more generally. In Chapter 6 of this thesis, I provide one example of how a non-genetic relationship between social partners can influence the costs of behaviours linked to reproduction.

1.3 Metrics linked to individual fitness

In studies of wild populations, the measurement of a given genotype's fitness (i.e. its expected abundance in the population at some point in the future [Hamilton 1964; Dawkins 1982]) is problematic (Jakob et al. 1996). Researchers therefore tend to measure factors linked to fitness – specifically, metrics of individual reproductive success including lifespan, fecundity, mating success or survival of offspring (Clutton-Brock 1988). These components rely on the assumption that individuals who produce fewer offspring during a specified period or across their lifetimes, or who die at a younger age and hence have fewer opportunities to produce offspring, contribute less genetic material to future generations and hence have lower fitness. While logical in terms of their relationship with fitness, both reproductive and survival measures can also be complicated by environmental or ecological influences, along with stochastic processes, such that meaningful measures of individual fitness through these fitness metrics can be difficult to obtain (Miller and Coltman 2014). In addition to measuring survival and reproduction directly, researchers therefore commonly measure individuals' physiological state. Such measurements assume that a "snapshot" view of an individual's current physiological state provides insight into factors like resource availability, competitive ability, current allostatic load and ability to cope with stress (Jakob et al. 1996), all of which are expected to correlate with its reproductive success, longevity and ultimate fitness. Such physiological measures also provide a currency with which to measure the "cost" of activities that improve fitness such as growth, acquisition of food and reproduction (Zera and Harshman 2001). In order to understand the influence of the social environment on individual fitness, it is important to investigate how a suite of fitness-associated metrics, both direct (survival and reproduction) and physiological, vary with social influences. Below, I outline two of the key metrics of physiological state that are used to predict physiological state in wild populations.

1.3.1 Body condition

Various forms of body condition have been used to measure individuals' energetic state and provide a potential index that correlates with fitness (Mitchell et al. 1976; Greggor et al. 2017). The rationale behind using body condition as a measure of physiological state is that individuals carrying more metabolisable materials (i.e. proteins and fats) have more resources available to allocate towards activities that improve fitness (Schulte-Hostedde et al. 2005) such as regulation of immune defences (Møller and Petrie 2001; Gleeson et al. 2005) or gamete production (Pilz et al. 2003; O'Dea et al. 2014). Body condition indices aim to control for structural body size differences between individuals in order to focus only on differences in nutritional state (Jakob et al. 1996). Body condition does appear to correlate with reproduction and survival in a range of species (e.g. Dobson 1992; Shine et al. 2001; Bowers et al. 2014), but the link between current body condition and fitness is far from ubiquitous and may be confounded by costs of carrying extra mass in certain situations (e.g. Lima 1986), non-linearity (Barnett et al. 2015), or the fact that it is highly subject to temporal fluctuation (e.g. Chapter 5 of this thesis). Thus, body condition as an indication of fitness should preferably be used either only in cases where it is known to affect fitness, or in combination with other measures of physiological state that can corroborate any observed patterns.

In terms of its potential to reflect influences of the social environment on an individual's physiological state, body condition is perhaps best suited to reflect socially-induced stressors at the time of sampling. Immediate social influences on body condition are probably largely linked to an individual's current access to food (e.g. according to parental investment or the presence of competing group members or neighbours). However, body condition may also reflect socially-induced changes in an individual's hormonal state – for example, through social stress caused by dominance-related aggression or intimidation (Creel 2001; Creel et al. 2013).

1.3.2 Telomeres

Over the last decade, telomeres have emerged as a promising biomarker of individual physiological state. Telomeres are regions of non-coding DNA and associated protein structures found at the end of all eukaryotic chromosomes that provide chromosome stability and protection to coding DNA during cell replication (reviewed in Blackburn 1992). During each cycle of cell replication, a small amount of genetic material is lost in what is known as the "end-replication problem" – the protective telomere structure therefore becomes progressively shorter in each replication but in doing so provides a buffer against the loss of important coding DNA (Levy et al. 1992). In addition to this cell-replication shortening, telomeres also shorten when exposed to reactive oxygen species (von Zglinicki 2002). These DNA-damaging molecules are a natural by-product of metabolism and other processes such as immune responses, but can accumulate to cause oxidative stress, an imbalance in favour of oxidants over detoxifying anti-oxidant molecules (Finkel and Holbrook 2000). Because a critically-short telomere would no longer be able to perform its protective function for the chromosome, eukaryotes also express the enzyme telomerase, which restores telomeres that have been damaged. However, telomerase expression is limited to certain tissues, typically those in the germ line (Forsyth et al. 2002), leading to a general trend of telomere shortening throughout the life of a cell, until a critical point at which the cell senesces or dies (Hornsby 2002).

For evolutionary ecologists, the justification for measuring telomeres as an indication of physiological state are twofold. First, telomeres provide a record of the past oxidative stress that an individual has experienced. Since oxidative stress arises when an individual lacks the resources to upregulate antioxidant defences and buffer effects of heightened metabolism, infection and other sources of physiological stress, telomere length (or the loss of telomeres over time) can be used as a measurable index of any form of “life-stress” that a researcher is interested in. Recently, a plethora of studies have used telomere dynamics to demonstrate variation in physiological state associated with a suite of molecular, physiological and behavioural factors including inbreeding (Chapter 4 of this thesis, Bebbington et al. 2016), parasite infection (Asghar et al. 2015), reproduction (Bauch et al. 2013) and even seasonal migration (Schultner et al. 2014). Second, telomeres appear to be not only linked to oxidative stress, but also to survival prospects. The predictive function of telomeres in determining individual lifespan has been reported in humans (Bakaysa et al. 2007), birds (Haussmann et al. 2005; Bize et al. 2009; Barrett et al. 2013) and mammals (Vera et al. 2012). Whether this pattern is causally driven by the relationship between critically short telomeres and cell senescence, or whether both short telomeres and somatic failure are caused by a third-party factor, is unclear. Current evidence points to the latter (Simons 2015), although a recent study has demonstrated the ability to rejuvenate senescent mouse cells and apparently reverse whole-organism ageing (Baar et al. 2017). However, this distinction is not directly relevant in the use of telomeres as a biomarker for somatic costs – the predictive power of telomere dynamics is useful for examining variation in individual costs, regardless of the mechanism.

The ability of telomeres to reflect the accumulation of stress-related damage over a period of time provides researchers of the social environment with two key benefits. First, telomere shortening over a given time period can be used to judge the influence of the social environment during that period on an individual’s internal state. Second, telomere length measured at any point in time can be used to link past social environments (e.g. social influences during development) not only with current physiological state, but also with future survival prospects. In this sense, telomeres provide a more complete way of measuring social environmental influences on fitness than other physiological metrics such as body condition, because they give an indication of the long-term damage and ultimate survival costs that the social environment can induce. Social influences on telomeres are likely to include all socially-induced factors that can affect oxidative stress, such as suboptimal growth regimes forced on developing individuals by the presence of competitors, or metabolic costs of signalling to or physically fighting with competitors in adulthood.

1.4 Aims and outline of the thesis

The overall aims of this thesis are twofold. First, I aim to identify the influence of different components of the social environment on metrics linked to individual fitness at various life history stages. This will provide us with a basic but important understanding of when social interactions between individuals can be expected to drive selection for traits important in social contexts (West-Eberhard 1979). A simultaneous second aim is to explore whether the influence of the social environment on individual physiological state is context-dependent. Without investigating how intrinsic state and the physical environment influence the effects of social interactions, we cannot hope to understand between-individual variation in fitness. I address the aims of the thesis in five research chapters and an appendix that collectively span a range of life-history stages and levels of social organisation (Fig. 1.2).

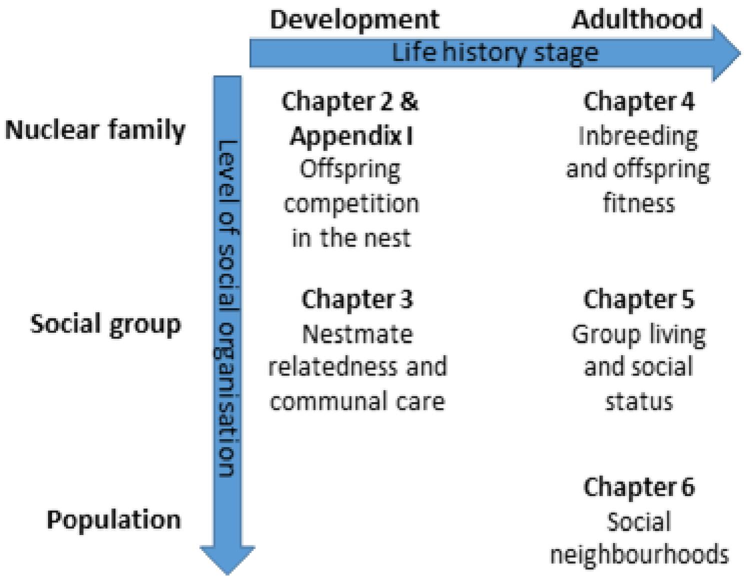

Figure 1.2 Overview of components of the social environment investigated in the thesis in relation to life history stage and level of social organisation.

Very few components of the social environment have lacked research attention – indeed, sibling rivalry and genetic mate choice are some of the oldest topics in evolutionary biology (Lack 1947; Robertson 1952). However, even well-studied phenomena can reveal important new insights when considered in a different framework (Travis 2006). One poignant example is that of sexual selection, an enormous field in its own right that, under the umbrella of social selection, can be more simply interpreted as a particular manifestation of the same set of principles that also apply to non-sexual phenomena such as parent-offspring conflict, sibling rivalry and social competition (Lyon and Montgomerie 2012). The idea of unifying social interactions in a single concept, while simultaneously considering context-dependent fitness effects, is in principle a good one, but is often extremely difficult to achieve empirically. Such a holistic approach to testing relationships between social environments and fitness requires unique data, incorporating lifelong sampling, behavioural observations, genetics and environmental conditions. There are an increasing number of model systems that fit this requirement, and such systems are often the source of the most comprehensive studies (Clutton-Brock and Sheldon 2010). The long-term study of Seychelles warblers *Acrocephalus sechellensis* on Cousin Island, Seychelles, is particularly well-fitted to holistic investigation of social environments and individual fitness. In Box 1.2 I describe the ecology of this species and the nature of the long-term study that has been conducted in this population over the last 30 years.

1.4.1 Thesis outline

In Chapter 2, I investigate how the presence of another dependent offspring during an individual's development influences physiological state, both in early life and in adulthood, while considering the influence of competitive ability on nestling interactions. In Chapter 3, I explore how relatedness between competitors in the nest and the presence of alloparental carers in the breeding group influence the physiological costs of nestling competition for resources, and discuss mechanisms by which cooperation between non-kin can evolve. In Chapter 4, I test whether an individual's choice of genetic mate influences its reproductive success through inbreeding depression, and investigate how the consequences of this manifest across life. In Chapter 5, I test the hypothesis that costs of group living vary with social status and explore sex-related differences in physiological costs of dominance and subordination. In Chapter 6, I explore how costs of territory maintenance vary according to the relationship between an individual and its immediate neighbours, with whom it interacts at territory borders. In the Appendix, I discuss and challenge the interpretation of a recently-published comparative analysis that explored the effect of social environmental factors on variation in the honesty of offspring begging signals. Finally, in Chapter 7 I provide a synthesis of the research reported in the thesis and discuss broad implications for our understanding of variation in individual fitness in the context of interactions with social partners.

Box 1.2 The Seychelles warbler *Acrocephalus sechellensis*

Study population

Cousin Island (Fig. 1.3) hosts a stable population of ca. 320 Seychelles warblers[1]. The population has been monitored every year since 1997 as part of an ongoing long-term research project[2]. Each breeding season, a complete census of all individuals is conducted and all nesting attempts are located and monitored until fledging or failure. Almost all birds in the population are ringed with a British Trust for Ornithology ring and a unique combination of 3 colour rings (Fig. 1.4a). Birds are also opportunistically re-captured repeatedly throughout their life, which provides longitudinal sampling of physiological and molecular measurements.

Figure 1.3. Map of Cousin Island (4°20'S, 55°40'E, marked in capitals) in relation to other granitic islands in the Seychelles Inner Islands archipelago and the broader location of the Seychelles in the Indian Ocean (inlet).

Population structure

Cousin supports ca. 110 Seychelles warbler territories, which cover all suitable habitat on the island. Territories are defended year-round against conspecific intruders by a breeding pair[3]. Foraging for insect prey occurs exclusively within the territory, and the availability of insects varies both spatially across the island and temporally between seasons [1].

Social system

Due to a combination of habitat saturation and benefits of philopatry[4], many territories contain not only a breeding pair but also subordinate individuals, which can either be retained offspring of the breeding pair or immigrants from another group[3] (Fig 1.4c).

Breeding system

Approximately 70% of nesting attempts involve only a breeding male and female (biparental breeding). However, subordinate individuals can become helpers-at-the-nest by either incubating the egg or feeding the offspring (cooperative breeding). Around 44% of female subordinates also lay their own egg in the nest of the breeding pair and help to raise both offspring alongside the dominant breeders (communal breeding)[5] (Fig 1.4b).

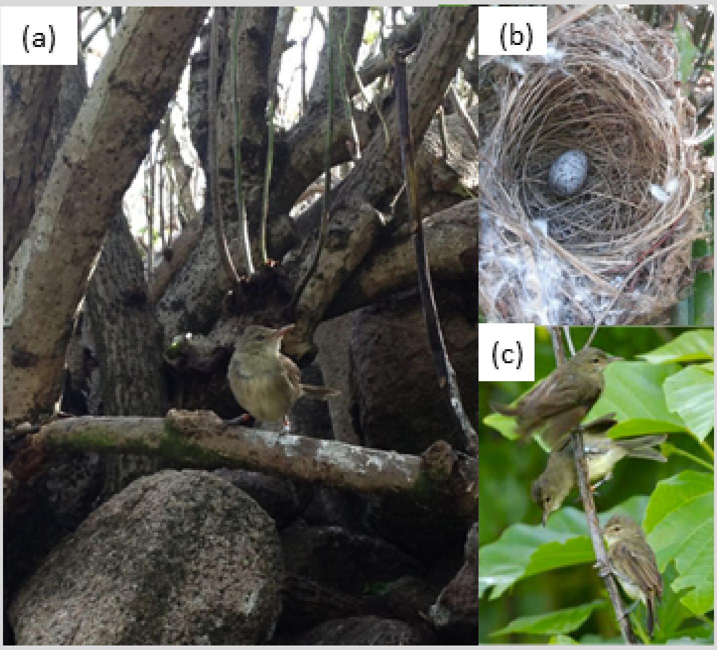

Figure 1.4. The Seychelles warbler: (a) a colour-ringed adult; (b) a completed single-egg clutch, laid in an open cup nest; (c) a social group consisting of a dominant male, a dominant female and a subordinate. The subordinate may assist the breeding pair in raising the offspring.

Benefits of the system for studying social environments

The Seychelles warbler population on Cousin provides an ideal long-term study system in which to test how different social environments affect individuals. We monitor individuals throughout their entire lives from the moment they hatch and, since individuals virtually never leave the island[5], we know that any individuals that disappear from the population have died. Repeat blood sampling throughout life allows us to not only measure telomere length, but also to determine individual genotypes and the genetic relatedness between individuals. Combined with complete individual-level monitoring of the population, we can therefore accurately identify the social and genetic relationships between an individual and all its social partners.

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Chapter 2

Consequences of sibling rivalry vary across life in a passerine bird

A version of this manuscript is published in *Behavioral Ecology* doi: 10.1093/beheco/arw167

Seychelles warbler nestlings are either raised alone in the nest or with a single competitor. Photos by K Bebbington.

2.1 Abstract

Many studies have assessed the costs of sibling rivalry in systems where offspring always have competitors, but conclusions about sibling rivalry in these species are restricted to interpreting the cost of changes in the relative level of competition and are often complicated by the expression of potentially costly rivalry related traits. Additionally, the majority of studies focus on early-life sibling rivalry, but the costs of competition can also affect later-life performance. We test a suite of hypothesised immediate (early-life body mass, telomere length, and survival) and delayed (adult reproductive potential and lifespan) costs of sibling rivalry for offspring of differing competitive ability in Seychelles warblers, where most offspring are raised singly and hence competitor success can be compared to a competition-free scenario. Compared to those raised alone, all competing nestlings had lower body mass and weaker competitors experienced reduced survival. However, the stronger competitors appeared to have longer adult breeding tenures and lifespan than those raised alone. We propose that comparisons with competition-free groups, as well as detailed fitness measures across entire lifetimes, are needed to understand the evolution of sibling rivalry and thus individual reproductive strategy in wild systems.

2.2 Introduction

When coexisting offspring are raised in a joint "nursery" such as in the multiple-offspring broods or litters of many vertebrates (Mock and Parker 1997), conflict between offspring for limited parental resources results in sibling rivalry (Trivers 1974; Parker et al. 2002a). Such sibling rivalry is expected to incur costs according to the degree to which the competitors' evolutionary interests are aligned; ultimately, this depends on the direct fitness benefit of acquiring resources and the indirect fitness cost of denying them to siblings (Parker 1989).

Many studies have aimed to determine the costs of sibling rivalry for offspring (reviewed in Shaanker et al. 1988; Hudson and Trillmich 2008). When the relationship between per-capita parental investment and number of competing offspring is less than 1, offspring experience a reduction in parental resources. For each offspring, the extent of this resource-based cost depends on its relative competitive ability and the number of competitors. Although parents may have some capacity to increase overall provisioning to larger numbers of young (Hegner and Wingfield 1987), evidence for decreasing per-capita investment with increasing brood size is widespread (Mock and Forbes 1995). Reduced food intake in early life may impair a suite of physiological components (e.g. growth rates: Stamps and Tanaka 1981, body size and mass: Emlen et al. 1991, immunocompetence: Saino et al. 1997), which can in turn reduce survival to adulthood (Magrath 1991; Christe et al. 1998; Mock et al. 2009). Hence, by consuming a portion of available resources, coexisting offspring inflict a resource-based cost on each other, which may or may not be symmetrical across the brood (see below). A second type of sibling rivalry cost concerns the behavioural adaptations that evolve as a consequence of sibling rivalry, which can be elaborate and diverse across species—ranging from nonphysical behavioural contests to obligate siblicide (Mock and Parker 1997). Sibling rivalry may be costly in terms of the production, maintenance, and expression of such traits (Godfray 1995). For example, behavioural (begging and jostling for optimal position) and physiological (growth strategies and morphological signals) adaptations to competition are found in a broad range of taxa (Manser and Avey 2000; Kilner 2001; Smiseth and Moore 2002). The energetic costs of maintaining rivalry traits, independent of parental resource depletion, may be an important component of sibling rivalry. Such traits are expected to be costly (MacNair and Parker 1979) and there is some empirical evidence for energetic costs to avian nestling begging (Kilner 2001; Neuenschwander et al. 2003). However, the magnitude of these costs appears generally limited (Smiseth and Parker 2008; reviewed in Chappell and Bachman 2002) and perhaps context-dependent (e.g. based on environmental conditions; Leech and Leonard 1996).

A third, less studied consequence of sibling rivalry is the potential for delayed costs in terms of later-life performance. If competition in early life causes suboptimal phenotypic development, it is possible that individuals become more susceptible to early mortality either through premature ageing (Nettle et al. 2015) or reduced ability to acquire resources (Merilä and Svensson 1997). Poor early life development may also affect an individual's ability to compete for reproduction (Verhulst et al. 1997) and this may be exacerbated if competing offspring influence the later-life reproductive potential of rivals after independence (Ekman et al. 2002; West et al. 2002; Tarwater 2012). However, very few studies have tested for such delayed costs, presumably due to the difficulty of monitoring individuals across their lifespan.

If competitive ability varies within the brood, sibling rivalry costs may be asymmetric. Competitive asymmetry typically arises through age or size differences (Mock and Forbes 1995) resulting from asynchronous birth (Drummond et al. 1986; Bonisoli-Alquati et al. 2011) or differences in growth induced by prenatal allocation of maternal resources (Einum and Fleming 1999; Royle et al. 2001). Competitor hierarchies and asymmetric competitive ability can have pronounced effects on the within-brood distribution of costs (Parker et al. 2002b), and empirical studies often suggest that the strongest competitors in a brood suffer no net cost of sibling rivalry (Cook et al. 2000; Sykes et al. 2007; Roulin and Dreiss 2012). Due to the difficulty of determining rivalry costs for the most competitive individuals (see below), the validity of this latter argument remains unclear.

Despite extensive research into sibling rivalry, there remain multiple key avenues for future research. Perhaps most importantly, many studies to date have considered broods that contain multiple offspring, where sibling rivalry will always be expected (e.g. Smale et al. 1995; Michaud and Leonard 2000, but see Emms and Verbeek 1991; Drummond et al. 2011; López-Jiménez et al. 2015).Within a brood, each individual is prenatally provisioned to deal with an expected level of competition (e.g. Harper 1986) in terms of developing the necessary morphological and behavioural platforms to express postnatal competitive traits. For individual offspring, the cost of experimentally varying the level of competition (e.g. by brood-size manipulations) will depend on the level of competition the offspring is equipped to encounter, because changing the postnatal level of competition cannot reverse the costs (or benefits) of such prenatal provisioning by parents. Thus, although previous studies have facilitated our understanding of variation in sibling rivalry, they may over or underestimate the true costs of competition, which might be better resolved by comparing competing individuals to noncompeting individuals.

Importantly, a naturally occurring competition-free comparison group would best enable us to determine whether even the strongest competitors in a brood suffer rivalry costs.

In addition to the rarity of studies comparing competing and noncompeting individuals, few studies have considered competition beyond the earliest stage of dependence (but see Arroyo et al. 2002; Ekman et al. 2002; Drummond et al. 2011; Tarwater 2012). In particular, extended sibling rivalry may play an important role in social species with delayed offspring dispersal (Mock and Parker 1997); ignoring this may limit our understanding of the ultimate fitness consequences of sibling rivalry. Additionally, sibling rivalry in early life may produce delayed or ongoing costs after offspring have dispersed and no longer interact, which could affect downstream lifespan or reproductive performance (Spear and Nur 1994). Our knowledge about delayed sibling rivalry costs in wild systems is limited to a few studies in seabirds (Drummond et al. 2011; Müller et al. 2011; Carmona-Isunza et al. 2013)—information from a broader array of taxa is needed to infer when and how early-life rivalry has lifelong effects (Drummond et al. 2011).

The Seychelles warbler *Acrocephalus sechellensis* provides a useful system in which to improve our understanding of the lifelong costs of sibling rivalry, taking into account both prenatal priming and delayed rivalry costs outlined above. This insectivorous passerine, which is endemic to the Seychelles (Safford and Hawkins 2013), has been intensively studied on Cousin Island and provides a highly tractable system in which to explore some of the gaps in our current understanding of sibling rivalry. Modal brood size on the island is 1 but a small proportion of nests (13%) contain 2 nestlings (Komdeur 1994; Richardson et al. 2001). Since the majority of offspring therefore never experience competition from a coexisting nestmate and selection driving the evolution or “priming” of traits designed to manipulate competitive ability is likely to be relatively weak, we can effectively test the effect of sibling rivalry against a competition-free comparison group. Moreover, following the ca. 17-day nestling period, the Seychelles warbler has an extensive period of postfledging care (3 months, Komdeur 1991) and prolonged parent–offspring association of up to several years can occur due to habitat saturation and dispersal constraints (Komdeur 1992; Eikenaar et al. 2007), meaning that sibling rivalry can persist long after offspring become independent. Importantly, the availability of accurate reproductive and survival data allows us to test for delayed rivalry costs in terms of lifelong reproductive potential and longevity. It is evident that there are many possible mediators and outcomes of sibling rivalry, which may have a profound influence on the evolution of reproductive strategy, resolution of evolutionary conflicts, and population dynamics. With these in mind, we test a suite of hypothesised costs of sibling rivalry (Table 2.1) across individuals' entire lifetimes and determine whether these costs are greater for the weaker of 2 competitors (asymmetric costs, Table 2.1). First, we test whether nestlings with a competitor experience different resource availability levels to those raised alone. We then test for differences in immediate physiological condition as a function of rivalry in terms of early-life body mass (reflecting an individual's energetic state; Schulte-Hostedde et al. 2005, Gil et al. 2008) and telomere length (an established predictor of intrinsic condition and survival across many taxa including the Seychelles warbler; Barrett and Richardson 2011; Barrett et al. 2013). We also test for an immediate survival cost to rivalry in terms of survival to adulthood. Among offspring that survived to adulthood, we test the hypothesis that individuals who were raised with a competitor suffer reduced reproductive potential (in terms of breeding position acquisition, age at first reproduction, and breeding tenure, Table 2.1) and lifespan. This investigation of multiple components and consequences of sibling rivalry will enable us to disentangle the costs of competition *per se* and allows us to detect consequences of early-life sibling rivalry at every stage of an individual's lifespan.

Table 2.1 Framework for testing hypothesised immediate and delayed costs of sibling rivalry via a suite of predictions.

Fitness component	**Hypothesis**	**Prediction**	**Prediction met in Seychelles warblers?**	**Evidence**
Early life intrinsic condition and survival	*Resource availability*	Nestlings with a competitor receive less food	**Yes** – per capita provisioning rate is lower in nests with two nestlings	Fig. 2.1
	Physiological condition	a) Competing offspring have lower body mass	**Yes** – in nestlings, both A- and B- offspring have lower mass than their single counterparts	Table 2.2, Fig. 2.2A
		b) Competing offspring have lower telomere length	**No** –A- and B- offspring have equal telomere length to their single counterparts	Table 2.2
	Survival	Competing offspring are less likely to survive to adulthood	**Yes** – B-offspring have lower survival than single offspring	Fig. 2.2A, Fig 2.2B
	Asymmetric cost	Physiological and recruitment costs are greater for weaker competitors	**Partially** – body mass costs apply to both competitors, survival costs only to B-offspring	Fig. 2.2
Adult reproductive potential and lifespan	*Reproductive potential*	a) Competing offspring are less likely to become breeders	**No** - A- or B-offspring are equally as likely to become breeders as their single counterparts	Table 2.3, Fig. 2.3A
		b) Competing offspring are slower to gain a breeding position	**No** - A- or B-offspring first breed at the same age as their single counterparts	Table 2.3, Fig. 2.3B
		c) Competing offspring have shorter breeding tenures	**No** – A-offspring have longer breeding tenures than their single counterparts	Table 2.3, Fig. 2.3C
	Lifespan	Competing offspring have lower lifespans	**No –** A-offspring have longer lifespans than their single counterparts	Fig 2.3, Fig. 2.3D
	Asymmetric cost	Reproductive potential and lifespan costs are greater for weaker competitors	**No –** B-offspring have similar reproductive potential and lifespan to their single counterparts	Table 2.3, Fig. 2.3

2.3 Material and methods

2.3.1 Study system and field data

Data were collected in the Seychelles warbler population on Cousin Island between 1995 and 2014. Across this period of intensive study, nearly all birds on the island received a unique British Trust for Ornithology ring and a combination of colour rings for individual identification (Richardson et al. 2001; Hammers et al. 2013). Each year during the main breeding season (June–September) and in some years during the minor breeding season (January–March [Komdeur 1996]), a census of the entire population was followed by intense monitoring of all nesting attempts on the island. These censuses, combined with negligible off-island dispersal (Komdeur et al. 2004), yield a >90% re-sighting probability (Brouwer et al. 2006) so death dates can be accurately inferred from the time of disappearance from the population. Each season, the majority of first-year birds were caught and ringed either as nestlings (ca. day 10 of the nestling period, during a small window of development within which nestlings are big enough to fit with rings but small enough not to present a risk of force-fledging), dependent fledglings, or independent subordinates in their natal territory. Age at catch was determined by eye colour (Komdeur 1991); in this study we only use data from birds caught when <1 year of age and distinguish between dependent (fledglings observed begging, <3 months, grey eyes) and independent (3–11 months, brown eyes) individuals. To determine physiological condition, body mass (to 0.1 g) and tarsus length (to 0.1 mm) were recorded and a small blood sample (ca. 25 µl) was taken via brachial venipuncture and stored in absolute ethanol.

Seychelles warblers defend year-round territories occupied by a breeding pair and 0–5 independent subordinates (Komdeur 1992). The identity of the breeding pair in each territory was determined from behavioural interactions during censuses (Richardson et al. 2003). Nesting attempts were located by following the breeding female for signs of nesting activity. If the nest was accessible (by hand or using a pole and mirror), the clutch and/or brood size was recorded. All nests were followed until failure or fledging (hatching and fledgling success are 46% and 80%, respectively [Komdeur 1994]). In a small proportion of nests, partial brood loss may mean that one nestling died before the brood size was recorded. To minimize error in our brood size classification, we therefore only classified nestlings as “single” if they were alone in the nest on or before day 12 of the nestling period. However, we were able to record the clutch and hatching brood size for 41% of nestlings and the remaining 59% were, on average, classified earlier than day 12 (mean ± SE = 8 ± 4 days). Thus, although some “single” nestlings may therefore have had a nestmate that died prior to the classification, the proportion is likely to be small (we were only aware of 3 partially fledged nests in our nestling dataset). Furthermore, the direction of any error will be in the opposite direction to the hypotheses in Table 2.1, thus making our assignment conservative. To determine survival to adulthood for all sampled nestlings, fledglings, and independent offspring, we recorded the presence of each individual in the population in the year following birth and all surviving individuals were subsequently followed for their entire lives as part of continued seasonal monitoring to determine adult reproduction and lifespan (Table 2.1).

In order to test for asymmetric costs (Table 2.1), we calculated each nestling's body condition as the residuals of a regression of mass on tarsus length, controlling for the time of day and month in which sampling took place, separately for males and females. Where 2 nestlings from the same brood were sampled, we used body condition to determine each offspring's size rank and assigned them as either the A-offspring (higher condition) or B-offspring (lower condition). Ranking competitors in this way reduces the variance in condition in each group compared to that of single offspring; in order to make a more meaningful comparison with our competition-free comparison group, we therefore also assigned each single nestling either as a "high-quality" or "low-quality" single offspring according to whether its body condition fell above or below the mean condition of all single offspring. A-offspring and B-offspring could then be compared to similarly classified single counterparts rather than to all single offspring.

The Seychelles warbler has obligate biparental care (Komdeur 1992) and subordinates can become helpers-at-the-nest by incubating or provisioning nestlings—the latter increases total provisioning rate to the brood (Komdeur 1994, Richardson et al. 2002). For 86 nests, food provisioning watches of approximately 1 hour (mean duration ± SD = 64.3 ± 13.2) were conducted on days 10–11 of the nestling period (mean age ± SD = 10.7 ± 5.1) to quantify overall nest provisioning rate (the number of provisioning events per hour) and to determine which (if any) subordinates helped in provisioning. Watches were focused around this stage of the nestling period to coincide with approximate asymptote of provisioning rate. For a small subset of nests (n = 20), a provisioning watch was also conducted on day 3 of the nestling period. We used this subset of nests to determine the repeatability of our provisioning rate measures (see section 2.3.3). We tested the resource availability hypothesis (Table 2.1) by calculating per-capita provisioning rate as the total provisioning rate divided by brood size. Observations of nestling provisioning provide evidence that food partitioning is equal between nestlings (Supplementary Table S2.4, see section 2.5 for details).

There is pronounced spatial and temporal variation in habitat quality on Cousin (Brouwer et al. 2006). During each season, the quality of every territory was calculated as a function of foliage density, insect abundance, and territory size following Komdeur (1992) and Brouwer et al. (2006). In this study, we define territory quality as the natural log of this measure and per-capita territory quality as territory quality divided by the number of independent birds (>3 months) present in the territory that season, following Brouwer et al. (2006). Insect availability across the island also varies annually, so for each season we calculated food availability as the mean number of insects counted across the whole island during each breeding season following Brouwer et al. (2006).

2.3.2 Molecular methods

DNA for molecular sexing and telomere measurement was extracted using a DNeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions with modification of overnight lysis at 37 °C and a final DNA elution volume of 80 µL. We determined the sex of all offspring using the PCR method developed by Griffiths et al. (1998).

We used quantitative PCR (qPCR) to obtain relative telomere length (henceforth telomere length) measurements as described for the Seychelles warbler in full detail elsewhere (Barrett et al. 2013; Bebbington et al. 2016, Chapter 4 of this thesis). Briefly, we ran each DNA sample in duplicate and used LinRegPCR 2014.2 to correct baseline fluorescence, determine the window-of-linearity for each amplicon, and calculate individual well efficiencies. Threshold values (Nq) were set in the centre of the window-of-linearity per amplicon for all samples. We corrected for variation across plates using a golden sample interplate calibrator and then calculated telomere length for each sample as the amount of telomere DNA relative to that of a constantly expressed reference gene (GAPDH) that was simultaneously amplified on the same plate, following equation 1 in Pfaffl (2001).

2.3.3 Statistical analyses

We examined the costs of sibling rivalry using a total of 349 nestling and juvenile Seychelles warblers. Unless stated otherwise, all analyses were conducted using a mixed modelling procedure in the lme4 (Bates et al. 2015) package in R (R Core Team 2015). All models included year of birth to account for variation in island density, climate and resources between years. In models using data from two individuals from the same nest we also included nest identity to account for non-independence between nestmates. We removed variables for which P > 0.05 from the final reported models. Stepwise elimination of nonsignificant variables can increase the likelihood of type I error (Mundry and Nunn 2009), but can be appropriate in cases of specific hypothesis testing with a small number of variables (Bolker et al. 2009), as is the case in this study. We minimize the potential for type I error by reintroducing all excluded variables back into the minimum model before considering them nonsignificant ($P > 0.05$ in all combinations). We report estimates from the final model including only significant terms and fixed effects; we obtained estimates for nonsignificant terms by reintroducing these terms individually to the final minimum adequate model.

To test for differences in resource availability, we first tested for inherent differences in the physical and social environment between nests containing 1 and 2 nestlings. We modelled brood size as a binomial response and tested for relationships with territory quality, food availability, and group size. In our investigation of variation in per-capita provisioning rate, we first determined how well per-capita provisioning rate reflects general resource availability at a given nest. Using the 20 nests for which a day 3 provisioning watch was also performed, we built a linear model with day 10 provisioning rate as the response variable and tested the strength of relationship with day 3 provisioning rate. Using each nest as a single data point, we then examined whether per-capita provisioning rate on day 10 (response variable) was related to brood size. We included 1) brood size, 2) helper presence (only 9 [5%] nests had >1 helper), 3) nest age in days, 4) observation time (early: 0630– 1100; midday: 1100–1500; late: 1500–1800 hours), because provisioning rate may vary across the day (e.g. Knapton 1984), 5) territory quality, and 6) food availability, as provisioning rate may depend on resource availability or foraging time (e.g. Tremblay et al. 2005). These latter 2 measures are correlated ($R^2 = 0.17$), but not strongly enough to cause collinearity in our analysis (VIF = 1.08). We also tested whether helper presence, territory quality, and food availability interacted with brood size.

We examined physiological condition separately in nestlings and juveniles by testing the relationship between size rank and 2 Gaussian response variables: body mass and telomere length. In nestlings, we created separate models for high-quality (A-offspring and high-quality single offspring) and low-quality (B-offspring and low-quality single offspring) categories. In juveniles, we compared all A-, B-, and single offspring together to maximize power under limited sample sizes.

We tested whether body mass was related to competitor presence and size rank. We included time (classified as above) and month of capture, the interaction between tarsus length and sex (to account for sex-specific scaling of mass and tarsus), territory quality, and food availability (which may affect offspring body mass through maternal effects [Richardson et al. 2004, Russell et al. 2007] or provisioning rate to offspring [Schroeder et al. 2012]) as additional predictors. For nestlings, we also included helper presence to account for varying food acquisition and for juveniles we included sampling age (dependent or independent) and used the per-capita measure of territory quality to account for group-size mediated postfledging competition (Brouwer et al. 2006; Ridley and Raihani 2007). To investigate telomere length, we used the same additional predictors as for body mass. For nestlings, we also added tarsus length to control for variation in growth rates between nestlings. In all models, we tested for interactions between competitor presence or size rank and food availability and territory quality; and in nestlings, we also tested the interaction with helper presence.

To analyse survival to adulthood of nestlings and juveniles, we used a generalized linear mixed model with a binomial error structure and survival to adulthood as a binary response. In nestlings, we performed the quality-based comparisons described above: A-offspring versus higher-quality single offspring and B-offspring versus lower-quality single offspring. In juveniles, we compared all A-, B-, and single offspring. We did not include food availability or territory quality based on a prior study reporting no effect of these variables on juvenile survival (Brouwer et al. 2006).

Among individuals that survived to adulthood, we compared the reproductive potential and lifespan of A- and B-offspring with that of their single counterparts as described above. Some individuals in our dataset (n = 19) were selected at random to be translocated to different islands as part of a planned expansion of the species' range (Richardson et al. 2006; Wright et al. 2014)—any of these individuals that did not yet hold a breeding position when translocated were excluded from our analyses of breeding position acquisition and age at first reproduction and all translocated individuals were excluded from analyses of breeding tenure and lifespan. Acquisition of a breeding position was modelled as a binomial response in a standard generalized linear model, excluding 3 individuals who were still alive at the time of analysis but had not yet gained a breeding position (2 single offspring and 1 B-offspring). We investigated age at first reproduction, breeding tenure, and life span using cox proportional hazards survival analyses in the "survival" package (Therneau 2015) in R. Because some individuals were still alive at the time of analysis, our data were left-censored: each individual was classified as either dead or alive in the model. The assumption of proportional hazards were met in all models (Cox 1972). We report the hazard coefficient, or "risk", of becoming a breeder (age at first reproduction), ceasing to be a breeder (breeding tenure), and dying (life span) for individuals who had a competitor compared to those raised alone, separately for high- and low-quality offspring. We included sex and natal group size (number of independent birds in the territory) as additional predictors in all models to account for potential sex differences in breeding performance and group-size–mediated differences in reproductive opportunities. We also tested the interactions between these 2 predictors and competitor presence.

2.4 Results

Our nestling dataset contained 161 (71%) single nestlings and 66 (29%) nestlings with a nestmate. For simplicity, we report model estimates for size rank and any additional predictors of early-life sibling rivalry costs for which $P < 0.25$. Model estimates for all other nonsignificant additional predictors and nonsignificant interaction terms are available in Supplementary Tables S2.1-S2.3.

2.4.1 Resource availability in nestlings

Brood size was not significantly related to territory quality ($\beta \pm SE = -0.30 \pm 0.21$, $P = 0.15$) or food availability ($\beta \pm SE = 0.01 \pm 0.01$, $P = 0.51$), but did increase with group size ($\beta \pm SE = 0.36 \pm 0.14$, $P = 0.01$).

Among nests where 2 provisioning watches were conducted, the per-capita provisioning rates of the 2 watches were significantly positively correlated ($\beta \pm SE = 0.55 \pm 0.14$, $P < 0.01$) with an R^2 of 0.45 (Supplementary Fig. S2.1). This repeatability suggests that our day 10 measures of per-capita provisioning rate reflect general resource availability at a given nest. Across all nests for which we had day 10 provisioning data ($n = 86$), nestlings with a nestmate each received less food than those raised alone (Fig. 2.1) as found in a previous study (Komdeur 1994). Per-capita provisioning rate varied throughout the day (β ± SE vs. early: midday 1.01 ± 1.74, $P = 0.56$; late 4.21 ± 1.71, $P = 0.02$). There was a nonsignificant tendency for per-capita provisioning rate to increase with helper presence ($\beta \pm SE = 2.46 \pm 1.48$, $P = 0.10$) but neither food availability nor territory quality affected per-capita provisioning rate and there were no significant interactions between brood size and any other variables (Supplementary Table S2.1).

2.4.2 Physiological condition

In nestlings, the body mass of both A- and B-offspring was lower than that of their single counterparts (Fig. 2.2A, Table 2.2). Territory quality, food availability, and helper presence had no effect on nestling mass and were not significant in interactions with size rank (Supplementary Table S2.2). Nestling telomere length did not vary with competitor presence (Table 2.2) but declined with increasing tarsus length in low-quality individuals, likely as a function of increasing nestling age (Table 2.2). Food availability, territory quality, and helper presence had no effect on nestling telomere length and did not significantly interact with competitor presence (Supplementary Table S2.2). Juvenile body mass was not related to nestling size rank (Fig. 2.2B, Table 2.2) but the sample size for B-offspring was very low. None of the additional predictors were related to juvenile body mass (Supplementary Table S2.2), nor were present in interactions (Supplementary Table S2.2). Juvenile telomere length was not related to size rank (Table 2.2) nor to any additional predictors (Supplementary Table S2.2) and there was no interaction between size rank and any other predictor on juvenile telomere length (Supplementary Table S2.2).

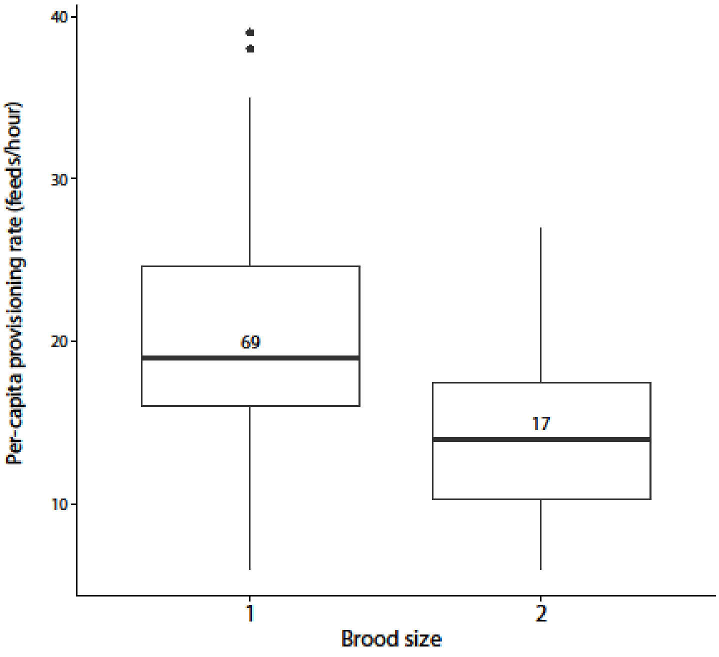

Figure 2.1 Boxplot showing median (horizontal line) per-capita provisioning rate to nestlings with and without a competitor. Numbers on each box denote sample sizes per group. Nestlings with a competitor received significantly less food than those raised alone (β ± SE = -5.76 ± 1.79, P = 0.002).

2.4.3 Survival cost

In nestlings, there was not a significant difference between the survival of A-offspring and their single counterparts (β ± SE = −0.47 ± 0.47, P = 0.32, Fig. 2.2C) but B-offspring were significantly less likely to survive to adulthood than low-quality single offspring (β ± SE = −1.00 ± 0.50, P = 0.04 Fig. 2.2C). A similar pattern occurred in juveniles: A-offspring were equally likely to survive as single offspring (Fig. 2.2D), but B-offspring were less likely to survive than single offspring (β ± SE = −2.80 ± 1.09, P = 0.01, Fig. 2.2D). B-offspring tended to have lower survival than A-offspring, but not significantly so (β ± SE = −2.20 ± 1.33, P = 0.10, Fig. 2.2D).

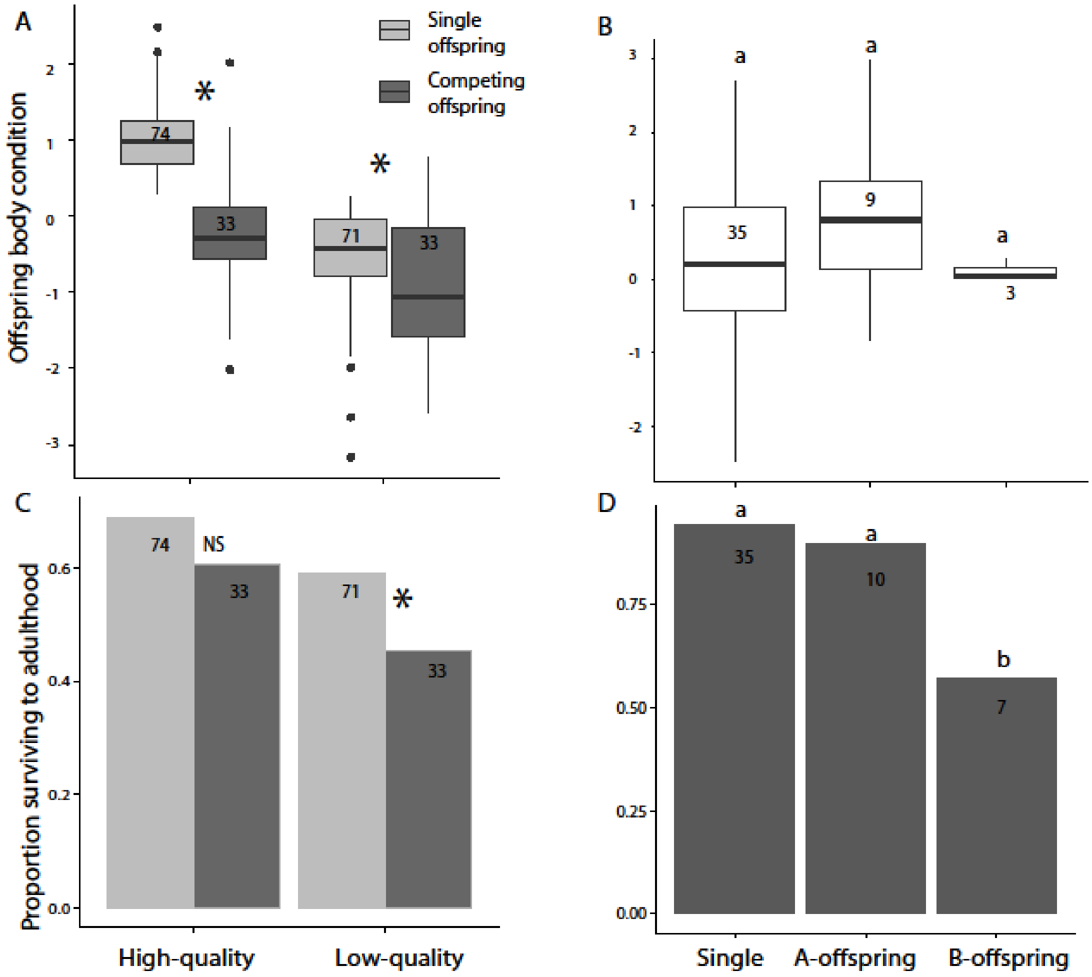

Figure 2.2 Early life body condition and recruitment costs of sibling rivalry. A) Nestling body condition; B) Juvenile body condition; C) Nestling survival to adulthood; D) Juvenile survival to adulthood. In nestlings, high-quality refers to A-offspring and single offspring with greater than average body condition, and low-quality refers to B-offspring and single offspring with lower than average body condition (see Section 2.3.1). * = significant relationships, NS = non-significant relationships. In juveniles, A- and B-offspring are compared with all single offspring). Different letters between groups denote significant differences. Throughout, numbers denote sample sizes per group, boxplots display median values per group and bar plots display mean values per group.

Table 2.2 Predictors of nestling and juvenile body mass and telomere length in Seychelles warblers. Significant terms are in bold.

Physiological measure	Comparison	Predictor	Estimate ± SE	*P* - value
Nestling body mass (*n* = 211)	High-quality	**Competitor presence**	**-1.23 ± 0.14**	**<0.01**
		Catch time	**Mid 0.31 ± 0.15**	**0.04**
		(versus morning)	**Late 0.44 ± 0.17**	**0.01**
		Tarsus length * Sex	**0.20 ± 0.07**	**<0.01**
	Low-quality	**Competitor presence**	**-0.52 ± 0.18**	**<0.01**
		Catch time	Midday 0.29 ± 0.18	0.11
		(versus early)	Late 0.27 ± 0.23	0.24
		Catch month	**0.17 ± 0.06**	**<0.01**
		Tarsus length * Sex	0.13 ± 0.09	0.18
Nestling telomere length (*n* = 172)	High-quality	Tarsus length	-0.03 ± 0.02	0.12
		Competitor presence	-0.05 ± 0.09	0.60
	Low-quality	**Tarsus length**	**-0.06 ± 0.03**	**0.02**
		Competitor presence	-0.08 ± 0.10	0.43
Juvenile body mass (*n* = 46)	All offspring	Age (vs independent)	-1.07 ± 0.58	0.07
		Size rank	A- offspring 0.24 ± 0.48	0.62
			B- offspring -0.16 ± 0.57	0.78
Juvenile telomere length (*n* = 44)	All offspring	Size rank	A- offspring -0.10 ± 0.08	0.21
			B- offspring 0.13 ± 0.10	0.22

2.4.4. Reproductive potential and lifespan

Among individuals that survived to adulthood, neither competitor presence (Fig. 2.3A) nor group size influenced the likelihood of achieving a breeding position either for high-quality or low-quality offspring (Table 2.3), although males in the high-quality category were slightly more likely to become breeders (P = 0.08). Competitor presence (Fig. 2.3B), natal group size, and sex were also unrelated to age at first reproduction in both high- and low-quality offspring (Table 2.3). A-offspring had longer breeding tenures than their singleton counterparts, as indicated by a lower hazard ratio (Table 2.3), but the breeding tenure of B-offspring did not differ from low-quality single offspring (Table 2.3, Fig. 2.3C). Among both low- and high quality offspring, individuals from larger natal groups had lower breeding tenures, as indicated by a higher hazard ratio (Table 2.3). A-offspring also had longer lifespans than their single counterparts, whereas the lifespan of B-offspring and low-quality single offspring did not differ (Table 2.3, Fig. 2.3D). In both high-and low-quality categories, individuals from larger natal groups had lower life spans, as indicated by a positive hazard ratio (Table 2.3). There were no interactions between competition and either sex or group size for any of the 3 reproductive components or lifespan for either high- or low-quality offspring (Supplementary Table S2.3).

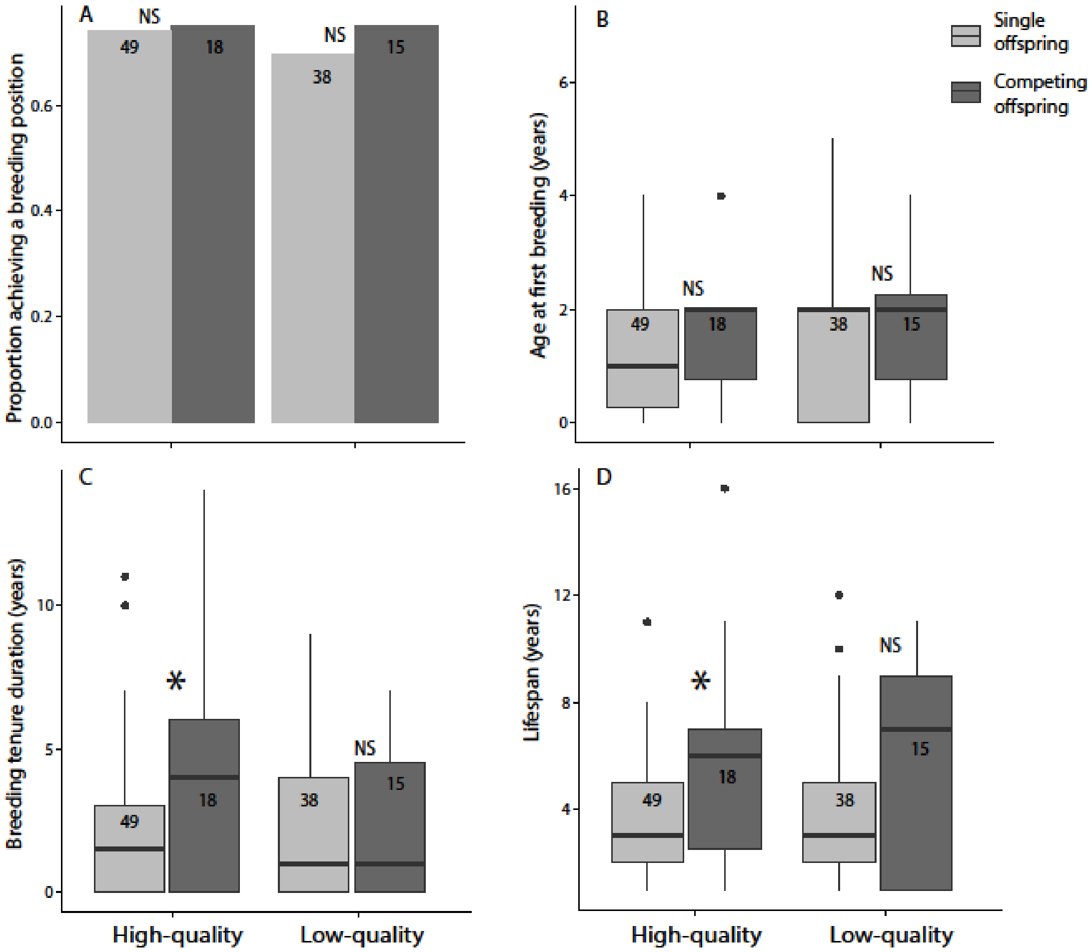

Figure 2.3 The relationship between competitor presence and A) proportion of individuals acquiring a breeding position, B) age at which the breeding position was attained, C) length of the breeding tenure and D) adult lifespan among individuals surviving to adulthood. High- and low-quality groups are defined as for figure 2 A (see Methods). * = significant relationships, NS = nonsignificant relationships and numbers denote sample sizes per group. Throughout, numbers denote sample sizes per group, boxplots display median values per group and bar plots display mean values per group.

Table 2.3 Predictors of reproductive potential and lifespan among Seychelles warbler offspring that survived to adulthood. The analysis of whether individuals achieved breeding status was performed with a logistic regression: all other models were based on survival analyses. Hazard ratio describes the risk of the event (becoming a breeder, ceasing to be a breeder or dying) for an individual raised with a competitor relative to an individual raised alone, such that values below zero indicate less risk to competing individuals. Significant terms are in bold.

Reproductive component	Comparison	Predictor	Coefficient ± SE	*P*	Hazard ratio
Achieved breeding status (*n* = 104)	High-quality	Competing offspring	0.52 ± 0.73	0.48	
		Group size	-0.27 ± 0.38	0.47	
		Sex (male)	1.23 ± 0.71	0.08	
	Low-quality	Competing offspring	-0.32 ± 0.73	0.67	
		Group size	-0.49 ± 0.31	0.12	
		Sex (male)	-0.68 ± 0.71	0.34	
Age at first reproduction (*n* = 102)	High-quality	Competitor presence	-0.22 ± 0.29	0.44	0.80
		Group size	0.10 ± 0.18	0.56	1.11
		Sex (male)	0.04 ± 0.27	0.87	1.04
	Low-quality	Competitor presence	-0.52 ± 0.36	0.15	0.59
		Group size	0.22 ± 0.13	0.08	1.26
		Sex (male)	0.48 ± 0.32	0.13	1.61
Breeding tenure (*n* = 100)	**High-quality**	**Competitor presence**	**-0.82 ± 0.37**	**0.03**	**0.44**
		Group size	**0.65 ± 0.21**	**<0.01**	**1.92**
		Sex (male)	-0.37 ± 0.32	0.25	0.69
	Low-quality	Competitor presence	-0.37 ± 0.39	0.34	0.69
		Group size	**0.47 ± 0.16**	**<0.01**	**1.60**
		Sex	0.24 ± 0.34	0.49	1.28
Lifespan (*n* = 100)	**High-quality**	**Competitor presence**	**-0.76 ± 0.36**	**0.04**	0.47
		Group size	**0.58 ± 0.21**	**<0.01**	**1.78**
		Sex	-0.12 ± 0.27	0.67	0.89
	Low-quality	Competitor presence	-0.49 ± 0.40	0.21	0.61
		Group size	**0.43 ± 0.15**	**<0.01**	**1.53**
		Sex	0.43 ± 0.35	0.22	1.54

2.5 Discussion

In this study, we tested a suite of hypothesised mediators and costs of sibling rivalry (Table 2.1). We found evidence for decreasing resource availability as a function of increased brood size, which translated into reduced physiological condition in both A- and B-nestlings when compared to competition-free, single nestlings of the same quality category. However, the survival cost imposed by having a competitor was asymmetric within broods: in nestlings, only B-offspring had lower survival than their single counterparts, and in juveniles, B-offspring were less likely to survive than single offspring. Among individuals who survived to adulthood, the relationship between sibling rivalry and adult reproductive potential and lifespan was positive for A-offspring, who outperformed their single counterparts in terms of breeding tenure and lifespan, and neutral for B-offspring, who performed equally well as their single counterparts in all tested aspects of adult success. We discuss these results in detail below.

2.5.1 Universal immediate costs: resource availability and physiological condition

Individuals in larger broods may suffer from resource depletion as a function of the number or strength of competitors (Forbes et al. 1997; Kitaysky et al. 2001), which can lead to reduced body condition (Emlen et al. 1991) and recruitment rates (Schwagmeyer and Mock 2008). In our dataset, we found no evidence that brood size was linked to territory quality or food availability, suggesting that resource depletion as a function of increased brood size is not mitigated by increased overall resource availability. We also found that nestlings with a competitor received substantially less food than those raised alone. This suggests that the reduced body mass found in competing nestlings is, at least partly, the result of reduced food intake; but without quantifying nestling begging behaviour, we cannot rule out additional energetic costs of behavioural competition. However, evidence for energetic costs of begging is limited (e.g. McCarty 1996; Chappell and Bachman 2002) and we suspect that such costs are low in the Seychelles warbler. Intrabrood scramble competition (Stamps et al. 1978; MacNair and Parker 1979) should occur whenever parents allocate non-divisible resources among nestlings (Royle et al. 1999), but anecdotal observations by the authors suggest that Seychelles warbler parents usually bring multiple small insects to the nest in a given trip and divide them equally between the nestlings (*pers. obs.*). Preliminary evidence collected earlier in the Seychelles warbler long-term study also shows that provisioning rate to each nestling appears approximately equal (Supplementary Table S2.4); although we acknowledge that we do not have sufficient data for a formal statistical analysis, taken together this anecdotal evidence is compatible with the hypothesis that resource-based rivalry costs should be relatively equal between the 2 competitors. The fact that A-offspring have lower nestling body mass than the highest-quality single offspring (Fig 2.2A) suggests that A-offspring do indeed suffer a cost associated with the presence of the B-offspring, but whether or not the relative extent of this cost is greater for B-offspring is difficult to determine. Differences in juvenile body mass and telomere length between A- and B-offspring would have allowed us to better determine whether physiological condition does indeed differ between competitors, but we found no differences in telomere length according to size rank. This lack of any effect may be due to the low power of our tests involving telomere measures, given the number of individuals involved (*n* = 172 nestlings and 44 juveniles). It may also be because telomeres lack the resolution to reflect differences in condition at the scale at which it was considered here. It would be interesting to test for differences in other physiological characteristics, such as immune function, between A- and B-offspring to determine whether either, or both, competitors suffer with respect to physiological condition more generally.

2.5.2 Asymmetric immediate costs: survival to adulthood

Although physiological condition was reduced among nestling competitors regardless of size rank, only B-offspring had lower nestling survival to adulthood than their single counterparts. In juveniles, B-offspring also experienced lower survival than all single offspring (Table 2.2) and tended to have lower survival than A-offspring, although this last result was not significant (P = 0.10).Together these results suggests that the physiological costs of sibling rivalry in early life have a disproportionately large impact on the survival of weaker competitors. If we apply the brood reduction (where weak offspring only survive in favourable circumstances [O'Connor 1978]) and egg insurance (where extra offspring are produced to mitigate the potential loss of a more valuable "core" offspring [Mock and Forbes 1995]) hypotheses to the Seychelles warbler system, we would predict that second eggs constitute a bet-hedging strategy by parents to optimize their reproductive output. We believe this to be unlikely for several reasons. First, B-offspring fledge as often as those raised alone (in all but three of the nests in the nestling analysis, the entire brood fledged) and we found no interaction between food availability and competitive ability on offspring condition (Supplementary Table 2.2). Second, approximately half of all nests containing 2 nestlings are the result of communal breeding of 2 females (Richardson et al. 2001) and it seems unlikely that this breeding strategy would remain stable if 1 female was restricted to laying an insurance egg (e.g. Clutton-Brock 1998, Chapter 3 of this thesis). Third, environmental predictability is very high in this system (Komdeur and Pels 2005) and so selection for "parental optimism" (Mock and Forbes 1995) in relation to brood size is likely to be weak. We therefore suggest that variation in brood size in this species is likely to reflect variation in parental perception of the likelihood of success of the whole brood.

2.5.3 Asymmetric delayed costs: adult reproductive potential and life span

Although our results clearly support the physiological condition and survival hypotheses of sibling rivalry in early life, we found limited support for the reproductive potential hypothesis. In contrast to our predictions, A-offspring who survived to adulthood had longer breeding tenures than high-quality single offspring and also lived longer than their single counterparts. Additionally, B-offspring had equal breeding tenure and survival to their single counterparts, so do not seem to be suffering any later-life costs to sibling rivalry if they survive to adulthood. A lack of later-life cost for B-offspring has also been shown in blue-footed boobies *Sula nebouxii*, where B-offspring suffer neither reduced survival nor reduced immunocompetence in adulthood (Drummond et al. 2011; Carmona-Isunza et al. 2013). These results suggest that, provided they reach adulthood, B-offspring are able to buffer any negative effects of early-life stress (Drummond et al. 2003).

However, the positive effect of sibling rivalry on A-offspring adult performance is perhaps more perplexing. As Seychelles warblers typically occupy a breeding position until death (Hammers et al. 2015), breeding tenure and lifespan are inherently linked and we suggest that the positive effect of rivalry on A-offspring adult performance could arise through 3 non-mutually exclusive mechanisms. First, A-offspring may outperform single offspring because broods of 2 are only produced under highly favourable circumstances. Our results show that this is not the case in terms of territory quality or food availability, but it is possible that A-offspring are sired by better-quality parents and thus inherit that quality. However, because nestling body mass of A-offspring is lower than that of higher-quality single offspring, this seems an unlikely explanation. Second, it is possible that A-offspring who survive to adulthood are of higher quality or competitive ability due to some selective filter on poor-quality individuals, which leads to biases either in death rates or in tendency for individuals to gain a breeding position (as oppose to remaining as a subordinate in a territory). Finally, A-offspring may become better competitors through exposure to competition early in life and are therefore better able to obtain a higher-quality breeding position, where the costs of obtaining food and producing offspring are relatively low. Once in the breeding territory, low costs could result in greater somatic maintenance and hence lifespan. Empirical evidence, although rare, suggests that such early-life influence on behavioural phenotype can occur: in yellow-legged gull chicks *Larus michahellis*, last-hatched nestlings produce very different behavioural responses to first-hatched nestlings (Diaz-Real et al. 2016) and in Nazca boobies *Sula granti*, nestlings that experience more adult aggression tend to be more aggressive later in life (Müller et al. 2011). Due to the correlation inherent to individual resource availability and intrinsic condition, it is difficult to distinguish between these 2 latter alternatives. However, given that A-offspring do not out-perform single offspring during the first year of life, it at least seems likely that any observed “benefits” of competition for A-offspring arise after independence, either as a result of selective mortality or competitive traits that are not expressed until adulthood. We suggest that investigating behavioural and social competence as a function of early-life competition would be a highly interesting avenue for further study.

2.5.4 Sibling rivalry costs and competition-free comparisons

Parents can optimize the level of sibling rivalry to maximize their own fitness by creating asymmetric competitive hierarchies. These can arise through asynchronous hatching of eggs (Ricklefs 1993) or preferential allocation of pre or postnatal resources to specific offspring (Slagsvold 1997; Groothuis et al. 2005). Many studies of sibling rivalry have shown that costs are often much greater for weaker siblings as a result of these hierarchies (e.g. Mock and Ploger 1987; Forbes and Glassey 2000; Smiseth et al. 2007). However, studies often fail to determine the costs of competition *per se*, as many systems do not provide the opportunity to compare competing and noncompeting offspring. The costs for dominant siblings may therefore be masked by the level of rivalry expected in the population and the costs for weaker offspring underestimated. Our comparison between nestlings that were raised with and without competition did not involve experimental manipulations, hence we are unable to rule out all potential parental or environmental factors that might differ between these 2 groups. Nonetheless, our results suggest that comparison between competing and noncompeting offspring, experimentally assigned where possible, can provide important insights and enhance our understanding of sibling rivalry costs. For example, if the current study had compared 2-chick nests with nests containing 3 chicks (as are found on other isolated islands in the Seychelles warbler’s range [Komdeur et al. 1995]), we may have concluded that the physiological costs of sibling rivalry only affected second- or third-order nestlings. It was only through comparison with single offspring and specifically single offspring of a similar quality category, that we were able to detect an absolute cost of competition. Similarly, by removing single offspring from our analysis of juvenile recruitment, we may have concluded that there was no recruitment cost to rivalry, whereas actually B-offspring suffered relative to single offspring. These results add further support to the hypothesis of asymmetric costs of competition within broods, but also suggest a need to consider more global costs and benefits within families in order to understand the multiple drivers and mediators of sibling rivalry and reproductive strategy. However, it is important to note that the correlational nature of the current study limits our ability to control for variation in parental quality, which may influence the degree to which offspring raised with and without rivalry differ. Given that per-capita provisioning rate is lower in broods of 2, it seems reasonable to assume that nestlings raised with a competitor experience some kind of resource limitation regardless of any differences in parental quality; nonetheless, studies that are able to experimentally separate the effects of parental quality and sibling rivalry are required to more comprehensively explore the extend of sibling rivalry costs.

2.6 Conclusions

In this study, we used a comprehensive framework of hypothesised costs to understand the manifestation and extent of sibling rivalry in wild systems. Although our results provide strong evidence for both asymmetrical and universal costs of sibling rivalry, we also found that stronger competitors that did overcome the early-life costs of rivalry had a longer breeding tenure and lifespan than single offspring. We suggest that comparisons of individuals raised with and without sibling competition, combined with detailed monitoring of individuals throughout life, will be instrumental in future studies of sibling rivalry, evolution of parental investment, and individual reproductive strategies in wild systems.

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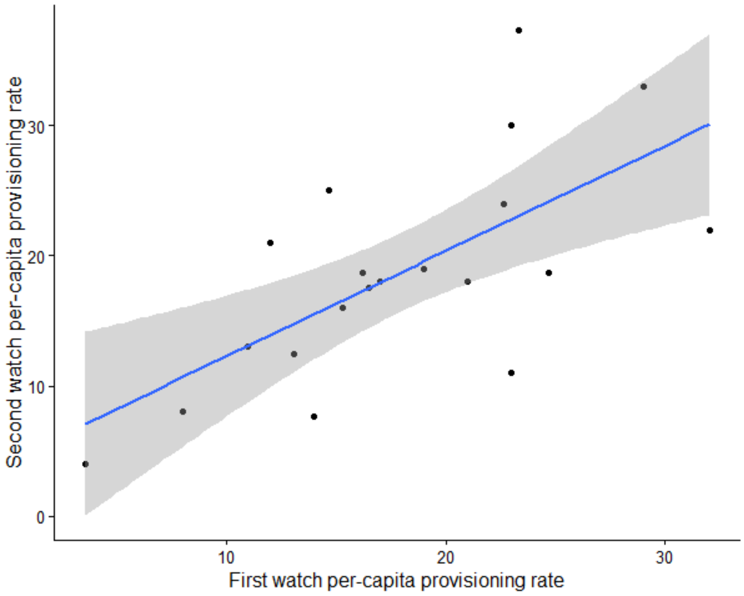
2.8 Supplementary information

Supplementary Table S2.1 Predictors of per-capita provisioning rate in Seychelles warbler nests (n = 86). Significant terms are in bold and estimates of main effects are reported from a linear model without interactions.

Predictor	Estimate ± SE	CI		*P*
		Lower	Upper	
Brood size	**-5.76 ± 1.79**	**-9.21**	**-2.30**	**0.002**
Observation time (vs early)	Mid 1.01 ± 1.74	-2.37	4.33	0.56
	Late 4.21 ± 1.71	**0.90**	**7.51**	**0.02**
Helper presence	2.46 ± 1.48	-0.38	5.31	0.10
Annual food availability	0.06 ± 0.05	-0.03	0.16	0.25
Nest age	0.19 ± 0.18	-0.15	0.53	0.30
Territory quality	0.67 ± 1.09	-1.44	2.72	0.54
Brood size * food availability	0.18 ± 0.11	-0.03	0.39	0.11
Brood size * territory quality	1.97 ± 2.41	-2.82	6.33	0.42
Brood size * helper presence	-1.16 ± 3.72	-8.27	5.96	0.76



Supplementary Figure S2.1 Relationship between observed per-capita provisioning rate recorded in two consecutive provisioning watches at a nest. $R^2 = 0.45$, $P < 0.01$. Points represent raw data, lines represent fitted values (linear regression) and shading represents credible intervals.

Supplementary Table S2.2 Model estimates for nonsignificant terms and interactions between competitive status and other explanatory variables of nestling and juvenile body condition and relative telomere length (RTL).

Physiological measure	Comparison	Predictor	Estimate ± SE	*P*
Nestling body mass (*n* = 211)	High-quality	Annual food availability	<-0.01 ± <0.01	0.49
		Helper presence	-0.05 ± 0.15	0.73
		Territory quality	<0.01 ± 0.10	0.95
		Catch month	0.06 ± 0.07	0.37
		Competitor presence * helper presence	0.18 ± 0.30	0.55
		Competitor presence * food availability	<-0.01 ± 0.01	0.68
		Competitor presence * territory quality	-0.05 ± 0.20	0.81
	Low-quality	Annual food availability	<-0.01 ± <0.01	0.44
		Helper presence	-0.14 ± 0.19	0.47
		Territory quality	0.03 ± 0.13	0.81
		Competitor presence * helper presence	-0.35 ± 0.24	0.14
		Competitor presence * food availability	0.01 0.01	0.44
		Competitor presence * territory quality	0.18 ± 0.27	0.51
Nestling RTL (*n* = 172)	High-quality	Helper presence	-0.09 ± 0.08	0.27
		Annual food availability	<-0.01 ± <0.01	0.40
		Territory quality	0.02 0.07	0.60
		Competitor presence * territory quality	<-0.01 ± 0.13	0.99
		Competitor presence * helper presence	<0.01 ± 0.17	0.99
		Competitor presence * food availability	<-0.01 ± <0.01	0.55
	Low-quality	Annual food availability	<-0.01 ± <0.01	0.72
		Territory quality	-0.02 ± 0.07	0.76
		Helper presence	-0.03 ± 0.10	0.77
		Competitor presence * helper presence	-0.20 ± 0.20	0.31
		Competitor presence * food availability	<-0.01 ± <0.01	0.38
		Competitor presence * territory quality	-0.04 ± 0.15	0.78
Juvenile body mass (*n* = 46)	All offspring	Annual food availability	0.03 ± 0.03	0.38
		Per-capita territory quality	<-0.01 ± 0.25	0.98
		Catch time (versus morning)	Mid -0.08 ± 0.41	0.85
		Catch month	0.04 ± 0.07	0.59
		Tarsus length * sex	-0.52 ± 0.31	0.31
		Competitor presence * food availability	A-offspring 0.02	0.87
		Competitor presence * territory quality	A-offspring -	0.09
Juvenile RTL (*n* = 44)	All offspring	Annual food availability	<0.01 ± <0.01	0.25
		Per-capita territory quality	0.05 ± 0.06	0.40
		Age (vs independent)	0.02 ± 0.10	0.85
		Competitor presence * food availability	A-offspring 0.02	0.31
		Competitor presence * territory quality	A-offspring	0.97

Supplementary Table S2.3 Model estimates for nonsignificant interactions between predictors of reproductive performance and competitive status among individuals that survived to adulthood.

Reproductive component	Comparison	Predictor	Estimate ± SE	*P*	Hazard ratio
Achieved breeding status (n = 104)	High-quality	Competitor presence * group size	-1.35 ± 1.02	0.18	
		Competitor presence * sex	-3.32 ± 1.85	0.06	
	Low-quality	Competitor presence * group size	-0.22 ± 0.72	0.76	
		Competitor presence * sex	-0.07 ± 1.58	0.97	
Age at first reproduction (n = 102)	High-quality	Competitor presence * group size	-0.14 ± 0.39	0.72	0.87
		Competitor presence * sex	0.21 ± 0.58	0.72	1.23
	Low-quality	Competitor presence * group size	0.27 ± 0.29	0.38	1.29
		Competitor presence * sex	0.97 ± 0.87	0.27	2.63
Breeding tenure (n = 100)	High-quality	Competitor presence * group size	-0.04 ± 0.40	0.92	0.96
		Competitor presence * sex	-0.14 ± 0.72	0.85	0.87
	Low-quality	Competitor presence * group size	0.37 ± 0.36	0.30	1.44
		Competitor presence * sex	0.85 ± 0.83	0.31	2.33
Lifespan	High-quality	Competitor presence * group size	-0.11 ± 0.36	0.76	0.90
		Competitor presence * sex	-0.05 ± 0.60	0.93	0.60
	Low-quality	Competitor presence * group size	0.16 ± 0.30	0.60	1.17
		Competitor presence * sex	0.86 ± 0.77	0.26	2.36

Supplementary Table S2.4 Division of parental provisioning between nestlings in two-chick nests, observed between 1987 and 1990. Provisioning watches were performed for one hour on ca. day 10 of the nestling period. The provisioning rate to each nestling was calculated as the number of times that that individual received food. The least-fed offspring was classified as Nestling 1.

Nest number	Nest provisioning rate (feeds/hour)	Nestling 1		Nestling 2	
		Provisioning rate	Proportion of feeds	Provisioning rate	Proportion of feeds
1	15.0	6.3	0.42	8.7	0.58
2	22.0	9.0	0.41	13.0	0.59
3	22.8	8.9	0.39	13.9	0.61
4	18.0	9.0	0.50	9.0	0.50
5	33.9	16.2	0.48	17.7	0.52
6	16.1	7.2	0.45	8.9	0.55
Average			**0.44**		**0.56**

Chapter 3

Joint-care can outweigh costs of non-kin competition in communal breeders

A version of this manuscript is under review at *Behavioral Ecology*

Seychelles warbler nestmates that were raised together despite being unrelated. Photo by K Bebbington.

3.1 Abstract

Competition between offspring can greatly influence offspring fitness and parental investment decisions, especially in communal breeders where unrelated competitors have less incentive to concede resources. Given the potential for escalated conflict, it remains unclear what mechanisms facilitate the evolution of communal breeding among unrelated females. Resolving this question requires simultaneous consideration of offspring in non-communal and communal nurseries, but such comparisons are missing. In the Seychelles warbler *Acrocephalus sechellensis,* we compare nestling pairs from communal nests (two mothers) and non-communal nests (one mother) with singleton nestlings. Our results indicate that increased provisioning rate can act as a mechanism to mitigate the costs of offspring rivalry among non-kin. Increased provisioning in communal broods, as a consequence of having two female parents, mitigates any elevated costs of offspring rivalry among non-kin: per-capita provisioning and survival was equal in communal broods and singletons, but lower in non-communal broods. Individual offspring costs were also more divergent in non-communal broods, likely because resource limitation exacerbates differences in competitive ability between nestlings. It is typically assumed that offspring rivalry among non-kin will be more costly because offspring are not driven by kin selection to concede resources to their competitors. Our findings are correlational and require further corroboration, but may help explain the evolutionary maintenance of communal breeding by providing a mechanism by which communal breeders can avoid these costs.

3.2 Introduction

When parents provide simultaneous care to more than one offspring, limitations on parental resources are expected to result in competition between offspring for those resources (Mock and Parker 1997). Such offspring rivalry can greatly affect offspring fitness, either through direct disruption of resource acquisition or through investment in the development and maintenance of competitive traits (reviewed in Hudson and Trillmich 2008). As a consequence, offspring rivalry may influence parental decisions regarding the optimal level of investment for a given reproductive attempt (Trivers 1974; Parker et al. 2002).

In communally breeding species (also referred to as plural breeding in mammals [Jennions and MacDonald 1994] or joint-nesting in birds [Vehrencamp and Quinn 2004]), the offspring of multiple parents are reared in a joint nursery. While communal breeding may have thermoregulatory, safety and energetic advantages in certain circumstances (reviewed in Vehrencamp and Quinn 2004), there are potential reproductive conflicts that must be overcome when offspring are reared in communal nurseries. As in singularly breeding species with multiple offspring, a communally-breeding parent can expect a reduction in the fitness of each of its offspring as a function of increasing brood/litter size but, unlike in non-communal breeders, does not enjoy the reproductive benefit of having produced a greater number of its own offspring (Hodge et al. 2009). Additionally, the presence of additional, non-descendent offspring in the nursery may facilitate disease transmission (Saino et al. 1997) to the focal parent's offspring, potentially further lowering the reproductive success of that parent. The extent to which offspring should compete with nursery-mates is partially determined by the benefit of acquiring resources and the cost of denying them to a related competitor (Parker 1989; Godfray 1995). Consequently, the lower within-brood relatedness inherent to communal nurseries (e.g. Williams 2004) provides a "battleground" for escalating offspring rivalry (Shen et al. 2010), potentially further increasing the cost of offspring competition for communally-breeding parents. However, explicit tests of the degree of offspring rivalry as a function of nest-mate relatedness, either in singular breeders or communal breeders, are largely missing.

There are two mediators of offspring rivalry that may play important roles in the evolutionary stability of mixed-relatedness nurseries in communally breeding species. Firstly, offspring rivalry arises as a result of limited parental resources (Mock and Parker 1997), but the increased number of caregivers in communal nurseries may increase per-capita resource availability to offspring so that costly competition is reduced (Shen et al. 2010); this may be particularly effective in systems where the ratio of carers to offspring is relatively high. Second, if parents have sufficient resources, they may attempt to mitigate the costs of competition for their own offspring by increasing prenatal investment to favour offspring growth and competitive ability, such as by producing heavier offspring (Hodge et al. 2009) or increasing prenatal provisioning of certain hormones (Schwabl 1996; Cariello et al. 2006). Thus, the extent of heightened offspring rivalry costs in communal nurseries depends on the balance between the negative effects of lower within-nursery relatedness and the positive effects of increased resource availability and prenatal provisioning.

In order to better understand the interplay between within-nursery relatedness, resource availability and offspring rivalry, we explored the costs of offspring rivalry in communal and non-communal nurseries in a facultative communally-breeding passerine bird, the Seychelles warbler *Acrocephalus sechellensis*. In this species, 87% of nests contain a single nestling (singleton broods) (Komdeur 1994) but some nests contain two nestlings, which can either both be laid by the same female (non-communal broods) or each be laid by a different female in the same social group (communal broods) (Richardson et al. 2001). Brood parasitism and egg-dumping are both entirely absent in this species (Richardson et al. 2001). By comparing nestlings raised with a competitor and singletons raised alone in the nest, we recently found that competition from a nestmate incurs body condition costs for all competitors and survival costs for the smaller of two nestlings (Bebbington et al. 2016a; Chapter 2 of this thesis). Given the inherent reproductive cost to raising two nestlings together, it is not clear how communal breeding remains stable in this system, nor indeed whether the costs of offspring rivalry vary between non-communal and communal broods. Unlike many other communally breeding species, where infanticide is common (e.g. Trail et al. 1981; Macedo et al. 2001; Vehrencamp and Quinn 2004), communal Seychelles warbler nurseries are relatively peaceful; egg-rejection does not occur (Komdeur et al. 2005) and neither infanticide nor siblicide have ever been observed or suspected (*pers. obs.*). Previous work has shown that additional female parents in communal broods are on average not more related to the breeding pair than females who do not participate in the communal nest (Richardson et al. 2002). This result indicates that the parental costs of communal breeding are not mediated by preferentially sharing reproduction with a more related group member. Since females are probably “aware” if their offspring will be competing with a less related nestmate (Cariello et al. 2006), they may be selected to produce a highly competitive offspring phenotype in order to mitigate the costs of offspring rivalry (Hodge et al. 2009). Importantly, unlike in many communally breeding animals, brood size is identical in communal and non-communal Seychelles warbler broods, providing an ideal situation to test the absolute costs of offspring rivalry without the confounding effect of variation in the number of nestling competitors.

In this study we use singleton nestling broods as a naturally-available comparison group to test for costs of offspring rivalry separately in non-communal and communal Seychelles warbler broods. Specifically we test whether 1) non-communal and communal broods differ from singleton nests in terms of per-capita resource availability to nestlings (including spatial, temporal and nest-level variation in food availability), 2) nestling pairs in non-communal and communal broods differ in terms of relatedness, brood size asymmetry and total brood mass, and 3) nestlings in non-communal and communal nests suffer differential costs of offspring rivalry as measured through reduced body mass, telomere length (both these metrics are known to reflect condition and survival in this species: Richardson et al. 2004; Barrett et al. 2013; Chapter 4 of this thesis; Bebbington et al. 2016b) and survival compared to singleton broods, and according to the relative competitive ability of each offspring.

3.3 Materials and methods

3.3.1 Data collection

We sampled 247 nestlings from 203 nests, using long-term data from the Seychelles warbler database (Version 0.56.1) between 1995 and 2014 from the population of Seychelles warblers on Cousin Island, Seychelles (04°20'S, 55°40'E). During all major (June-September) and some minor (December-March) breeding seasons, the entire population was censused and breeding adults were caught with mist nets. All birds were given a unique combination of colour rings for visual identification and ca. 25 µl of blood was taken for sex determination, genotyping and telomere analyses (see below). During each breeding season, all ca. 115 territories on the island were monitored for nesting activity. For all nests within reach, we sampled each nestling at between 10-14 days old, taking a small (15µl) blood sample and measuring mass and tarsus length to the nearest 0.1g and 0.1mm respectively. The time of day and month of catch were noted, since temporal variation in temperature and food provisioning may affect nestling mass. Where more than one nestling was sampled in a nest (n = 42 nests), we assigned each as either the "A-offspring" (higher mass) or "B-offspring" (lower mass) as described in Chapter 2 of this thesis and Bebbington et al. (2016a). Each nest was then monitored until fledging or failure. Yearly censusing, combined with extremely low off-island dispersal (0.1%; Komdeur et al. 2004) and a high re-sighting probability (ca. 92%, Brouwer et al. 2006) means that individuals who were no longer seen could safely be assumed to be dead, yielding highly accurate estimates of survival to adulthood (Brouwer et al. 2006; Barrett et al. 2013).

For 88 nests (43%) we performed provisioning watches of at least one hour (mean duration ± SD = 64.3 ± 13.2 minutes) immediately before sampling the nestlings. From these data we determined the number of caregivers provisioning the nestlings, which can vary from two to five, depending on the presence of provisioning subordinates (Komdeur 1994). Communal broods are always provisioned by at least three caregivers (the extra female parent always provisions [Richardson et al. 2003]), but the number of caregivers in singleton and non-communal broods is variable. Using the provisioning watches, we also determined variation in resource availability in terms of per-capita provisioning rate (total provisioning rate per hour divided by brood size). Previous work has shown that provisioning rates observed at the same nest across the nestling period are moderately correlated (r = 0.45), suggesting that our observation regime is sufficient to produce a representative measure of provisioning rate at a given nest (Chapter 2 of this thesis; Bebbington et al. 2016a).

There is also spatial and temporal variation in resource availability within the population, which we measured each year by calculating territory quality (foliage density, insect abundance and territory size) and food availability across the whole island (mean number of insects counted per territory in a given year) as described in Komdeur (1992) and Brouwer et al. (2006). Both of these measures were logged to provide a normal distribution.

3.3.2 Molecular methods

DNA for sexing, telomere measurement and relatedness assignment was extracted using a DNeasy blood and tissue kit (Qiagen). Nestling sex was determined as described in Griffiths et al. (1998). We used quantitative PCR to obtain a relative measure of nestling telomere length (henceforth telomere length) as described in detail elsewhere (Barrett et al. 2013; Bebbington et al. 2016b; Chapter 4 of this thesis).

Parent-offspring and nestmate-nestmate relatedness was calculated using a panel of 30 microsatellite loci previously developed for the Seychelles warbler (Richardson et al. 2001; Spurgin et al. 2014). To distinguish between communal and non-communal broods, we first assigned all two-nestling broods in territories with only one adult female present as non-communal (egg-dumping does not occur in this species [Richardson et al. 2001, Hadfield et al. 2006]). In territories with more than one resident female, we included all females as candidate mothers for each nestling and assigned maternity using maximum-likelihood estimation in MASTERBAYES 2.52 (Hadfield et al. 2006) with Wang's (2004) genotyping error model, following the MbG_Wang method of Patrick et al. (2012). Genotyping errors were set to 0.0005 – for full details see Chapter 4 of this thesis and Bebbington et al. (2016b). Any nests where each nestling was assigned to a different female were considered “communal” (*n* = 8) and those where both nestlings had the same mother were “non-communal” (*n* = 34). Relatedness (Queller and Goodnight’s R) between nestling dyads was calculated using Genalex 6 (Peakall and Smouse 2006).

3.3.3 Statistical methods

Unless otherwise stated, all analyses were conducted in R Studio (version 0.99.486, R Core Team 2015). We constructed generalized linear mixed models using the “lme4” package (Bates et al. 2015). Because we used multiple approaches and response variables to test our hypotheses, each of our analyses included different responses and predictor variables, not all of which were available for all individuals in the dataset. Sample sizes therefore vary between analyses; specific sample sizes for each analysis are therefore provided in Tables 3.1-3.2 and Figures 3.1-3.4. We checked for collinearity by calculating variance inflation factors for all our variables. *P* values were calculated using the Satterthwaite approximation in the R package lmerTest (Kuznetsova et al. 2015). In order to determine whether costs of offspring rivalry vary in non-communal and communal nests when compared to nestlings raised alone, we report effects of nest type with reference to singleton broods. However, we also calculated parameter estimates for multi-level factors by altering the reference level; these contrasts are reported in the figures and in Supplementary Table S3.3. In order to maximize available degrees of freedom, we removed any predictors for which $P > 0.1$ to produce a minimal model. The reported parameter estimates for these non-significant terms were obtained by reintroducing them individually into the minimal model.

3.3.3.1 Resource availability

We first tested whether resource availability was different between singleton and non-communal broods or between singleton and communal broods. We modelled per-capita provisioning rate as a Gaussian response and included nest type (singleton, non-communal, or communal, where each nest constituted a single data point and singletons were the reference group), observation time (early: 0630-1100; midday: 1100-1500; late: 1500-1800 hours) to account for variation in provisioning rates across the day and nest age (days since egg laying) as predictors. We included year of observation as a random effect to account for between-year differences. A second random effect of breeding pair identity nested in territory identity was included to account for repeat sampling of nests belonging to the same pair and territory across years.

To investigate differences in territory quality and island-wide food availability between nest types, we ran two separate logistic regressions: the first binary response was whether the nest was singleton or non-communal, the second whether the nest was singleton or communal. We used log measures of territory quality and island-wide food availability as predictors in both regressions and included a random effect of breeding pair nested in territory identity to account for sampling of nests from the same parents or territory across the study period.

3.3.3.2 Brood-level differences

Next we investigated brood-level differences between non-communal and communal nests. We first tested whether nestlings from non-communal broods were indeed more related than those in communal broods (since different rates of extra-pair paternity might influence the degree of relatedness difference between nest types) using pairwise nestmate relatedness. We also tested whether brood size asymmetry (as the proportion difference in mass between the A- and B-offspring) and total brood mass differed between non-communal and communal broods. Nestling relatedness was modelled as a Gaussian response, with nest type (non-communal or communal) as the single predictor. Brood size asymmetry (log-transformed) and total brood mass were modelled as Gaussian responses and we included nest age (days since egg-laying) and nest type as predictors. Territory identity was included as a random effect to account for repeat sampling of territories across the study period.

3.3.3.3 Costs of offspring rivalry

We then tested whether offspring rivalry in non-communal and communal broods infers costs in terms of reduced body mass, telomere length and survival to adulthood compared to singleton broods. We constructed mixed models that included nest identity (to account for common nest origin), year of sampling (to account for between-year environmental differences) and breeding pair nested in territory identity (to account for similarity in parental and rearing environments). In all models we included nest type (singleton, non-communal or communal, where singletons were the reference group) as a predictor. To investigate body mass (Gaussian response) we included tarsus length and its interaction with sex (to account for sex-specific mass-size scaling), along with time and month of sampling and nest age, as additional predictors. To investigate telomere length (Gaussian response) we included sex, nest age and tarsus length to account for potential differences in growth rate costs. To investigate survival to adulthood (binary response), we again included tarsus length and nest age. For all three response variables we also included territory quality and island-wide food availability as additional predictors and tested for an interaction between these variables and nest type on offspring rivalry costs.

3.3.3.4 Differential influences of competitive ability and resource availability

Lastly, we extended our analyses to investigate whether competitive ability and resource availability affected offspring rivalry costs differently for non-communal and communal broods. To do this we created separate models for body mass, telomere length and survival to adulthood, all of which included the random effects described above for the previous analyses (apart from breeding pair, which was unique for all nests in this analysis), along with any predictors that were significant in our initial analyses of offspring rivalry costs (see Table 3.2). Parameter estimates for these additional predictors were highly similar to those reported for the initial analyses and so are not reported here.

First, since the costs of offspring rivalry differ for the strongest and weakest of two competitors (Bebbington et al. 2016a; Chapter 2 of this thesis), we tested for two interaction effects. To determine whether asymmetry in costs varies between nest types, we tested the interaction between nest type (non-communal or communal) and size rank (A- or B-offspring), with the prediction that B-offspring may suffer more in communal nests due to lower nestmate relatedness. To test whether resource availability differentially influences the costs of rivalry for A- and B-offspring, we tested the interaction between size rank and per-capita provisioning rate across all two-nestling broods, with the prediction that lower resource availability might more greatly affect B-offspring. Second, given that resource availability may differentially affect the costs of offspring rivalry in non-communal and communal broods, we tested two further interactions across all two-nestling (i.e. non-communal and communal) broods. To test whether resource availability differentially affects offspring in different nest types, we tested the interaction between nest type and per-capita provisioning rate. To test whether variation in the number of caregivers influences offspring costs, we tested the relationship between offspring rivalry costs and the number of caregivers. Less than 5% of the broods in our dataset were provisioned by >1 helper so we considered helper presence or absence in binary terms. We modelled the number of caregivers as a three-level factor: non-helped non-communal broods (2 caregivers), helped non-communal broods (3 caregivers) and communal broods (always at least three caregivers), using communal broods as the reference group.

3.4 Results

3.4.1 Resource availability

Per-capita provisioning rate varied over the day and increased with nest age (Table 3.1a). Controlling for these factors, nest type had a significant effect on per-capita provisioning rate

(Table 3.1a). Per-capita provisioning rate was lower in non-communal broods than in singleton broods, but per-capita rate to communal broods was not different to singletons (Table 3.1a, Fig. 3.1a). Singleton nests tended to occur in higher quality territories than communal nests, though this was marginally non-significant (P = 0.06, Table 3.1a). Territory quality was not different between singleton and non-communal broods (Table 3.1a, Fig. 3.1b). Singleton, non-communal and communal nests did not occur in years of different island-wide food availability (Table 3.1a, Fig. 3.1c).

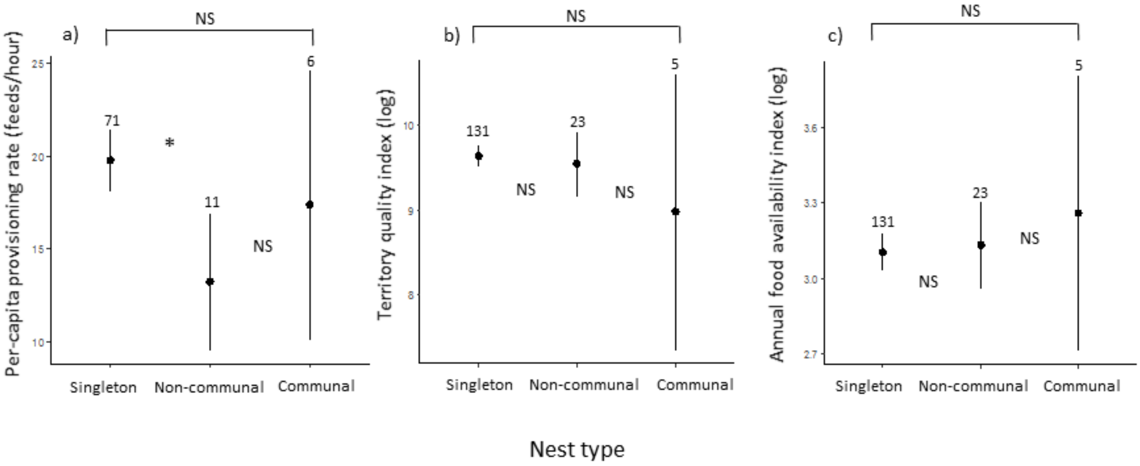


Figure 3.1 Differences in resource availability in terms of a) per-capita provisioning rate, b) territory quality and c) island-wide food availability between singleton and non-communal, or singleton and communal broods in the Seychelles warbler. Dots and lines denote mean and 95% CI respectively, sample sizes per group are denoted beside each group. Significant ("*") and non-significant ("NS") differences between groups at P < 0.05 are displayed.

3.4.2 Brood-level differences

Nestlings were less related to each other in communal than in non-communal nests (Table 3.1b, Fig. 3.2a). There was no difference in nestling size asymmetry between the two nest types (Table 3.1b, Fig. 3.2b), though asymmetry decreased with nest age (Table 3.1b). Total brood mass tended to be higher in communal broods, but this was marginally non-significant (P = 0.07, Table 3.1b, Fig. 3.2c).

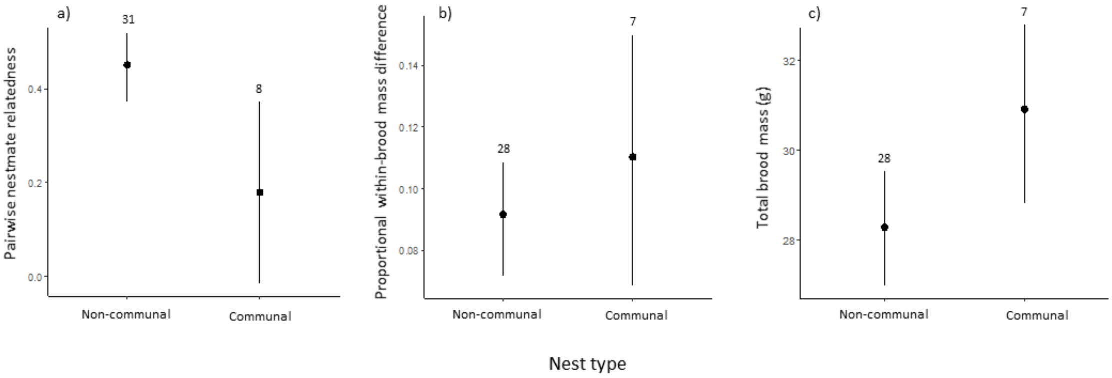


Figure 3.2 Brood-level differences in a) relatedness, b) nestling size asymmetry and c) total brood mass between non-communal and communal nests (each with two offspring) in the Seychelles warbler. Dots and lines denote mean and 95% CI respectively, sample sizes per group are denoted beside each group. Significant (“*”) and non-significant (“NS”) differences between groups at P < 0.05 are displayed.

3.4.3 Costs of offspring rivalry

Nest type had a significant effect on body mass (Table 3.2). Nestlings in non-communal broods were of significantly lower body mass than those in singleton broods, whereas the mass of nestlings in communal broods was not different to that of singletons (Table 3.2, Fig. 3.3a). Neither territory quality nor food availability influenced nestling mass (Table 3.2) and neither showed an interaction with nest type (Supplementary Table S3.1).

Telomere length decreased with tarsus length (Table 3.2) but did not vary with nest type: singletons did not have different telomere length to either non-communal or communal nestlings (Table 3.2, Fig. 3.3b). Telomere length was not significantly related to nest age, island-wide food availability or territory quality (Table 3.2) and neither food availability nor territory quality showed an interaction with nest type (Supplementary Table S3.1).

Table 3.1. The effect of a) resource availability and b) brood-level differences between singleton broods and non-communal or communal broods in the Seychelles warbler. F and P values for main effects of categorical variables are reported from an ANOVA. Significant predictors are highlighted in bold.

Hypothesis	Response	Predictor	*F*	Estimate ± SE	*P*
a) Resource availability	Per-capita provisioning rate (*n* = 88)	**Nest type[1]**	**5.28**		**0.02**
		- Non-communal		**-5.46 ± 1.96**	**<0.01**
		- Communal		-1.08 ± 2.52	0.67
		Observation time[2]	2.68		0.08
		- Midday		0.50 ± 1.59	0.76
		- Late		**3.49 ± 1.63**	**0.04**
		Nest age		**0.41 ± 0.18**	**0.02**
	Non-communal vs singleton (*n* = 154)	Territory quality		-0.20 ± 0.34	0.56
		Food availability		0.20 ± 0.50	0.69
	Communal vs singleton (*n* = 136)	Territory quality		-1.09 ± 0.57	0.06
		Food availability		0.67 ± 0.78	0.39
b) Brood-level differences	Relatedness (*n* = 39)	**Communal[3]**		**-0.27 ± 0.09**	**<0.01**
	Size asymmetry (*n* = 35)	**Nest age**		**<-0.01 ± <0.01**	**0.02**
		Communal[3]		0.01 ± 0.03	0.74
	Total brood mass (*n* = 35)	Communal[3]		2.61 ± 1.40	0.07
		Nest age		0.11 ± 0.20	0.57

Reference groups
[1] 'Singleton
[2] 'Early'
[3] 'Non-communal'

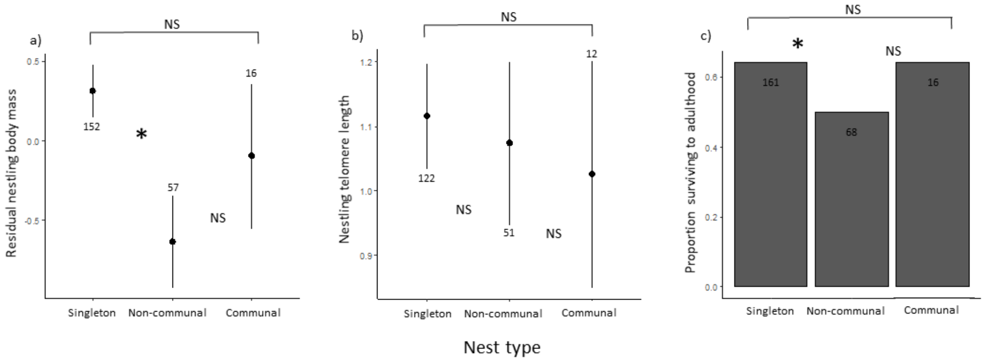


Figure 3.3 Differences in individual costs of offspring rivalry in terms of a) residual body mass (controlling for tarsus length, sampling time and date), b) telomere length and c) survival to adulthood, between singleton and either non-communal or communal broods in the Seychelles warbler. Dots and lines denote mean and 95% CI respectively, sample sizes per group are denoted beside each group. Significant ("*") and non-significant ("NS") differences between groups at P < 0.05 are displayed.

Nest type did not have a significant effect on survival to adulthood (*P* = 0.09, Table 3.2), suggesting that any differences between nest types are marginal. Nonetheless, nestlings in non-communal broods were slightly less likely to survive to adulthood than those raised singly, but the survival of nestlings from communal broods did not differ from that of singleton broods (Table 3.2, Fig. 3.3c). Nestling survival did not vary with nest age, island-wide food availability or territory quality (Table 3.2), and neither food availability nor territory quality interacted with nest type (Supplementary Table S3.1). Survival increased with tarsus length (Table 3.2).

3.4.4 Differential influences of competitive ability and resource availability

There was an interaction between nest type and size rank on nesting body mass: B-offspring were of lighter mass than A-offspring in non-communal broods, but not in communal broods (β ± SE = -0.67 ± 0.28, P = 0.01, Fig. 3.4a). No interacting effect of nest type and nestling size rank was observed for telomere length or survival to adulthood (Supplementary Table S3.2).

Across all non-communal and communal broods, there was also an interaction between per-capita provisioning rate and nestling size rank on body mass: B-offspring were lighter than A-offspring when per-capita provisioning rate was low, but not when it was high (β ± SE = 0.05 ± 0.02, P = 0.04, Fig. 3.4b). This interaction was not significant for either telomere length or survival to adulthood (Supplementary Table S3.2).

No interaction was detected between per-capita provisioning rate and nest type: the influence of per-capita provisioning rate on body mass, telomere length and survival to adulthood did not differ between non-communal and communal broods (Supplementary Table S3.2).

Table 3.2 The effect of nest type (non-communal or communal, compared to singletons) and additional predictors on three hypothesised costs of offspring rivalry in Seychelles warbler nestlings. Significant terms are highlighted in bold.

Response	Predictor	*F*	Estimate ± SE	P - value
Body mass	**Nest type[1]**	**14.75**		**<0.01**
(*n* = 225)	**- Non-communal**		**-1.00 ± 0.19**	**<0.01**
	- Communal		**-0.53 ± 0.36**	**0.14**
	Tarsus length		**0.74 ± 0.04**	**<0.01**
	Catch time[2]	**3.68**		**0.03**
	- Afternoon -		**0.35 ± 0.17**	**0.05**
	Evening		**0.52 ± 0.20**	**0.01**
	Catch month		**0.18 ± 0.06**	**<0.01**
	Sex[3]		0.16 ± 0.14	0.25
	Nest age		-0.01 ± 0.02	0.46
	Territory quality		0.07 ± 0.13	0.61
	Food availability		-0.19 ± 0.22	0.41
	Tarsus length * sex		0.10 ± 0.07	0.18
Telomere length	**Tarsus length**		**-0.04 ± 0.02**	**0.03**
(*n* = 185)	Nest type[1]	0.21		0.81
	- Non-communal		-0.06 ± 0.08	0.49
	- Communal		-0.12 ± 0.15	0.43
	Sex[3]		-0.05 ± 0.06	
	Nest age		<0.01 ± <0.01	0.33
	Territory quality		<0.01 ± 0.06	0.99
	Food availability		-0.02 ± 0.12	0.88
Survival to adulthood	**Tarsus length**		**0.27 ± 0.10**	**<0.01**
(*n* = 245)	Nest type[1]	2.41		0.09
	- Non-communal		**-0.78 ± 0.39**	**0.04**
	- Communal		-0.47 ± 0.67	0.48
	Nest age		< 0.01 ± 0.04	0.89
	Territory quality		0.11 ± 0.30	0.70
	Food availability		0.30 ± 0.53	0.57

Reference groups

[1] 'Singleton'

[2] 'Early'

[3] 'Female'

Compared to nestlings in communal broods (*n* = 16), nestlings in non-communal broods with no helper (n = 10) were of lighter body mass (β ± SE = -0.81 ± 0.38, P = 0.04, Fig. 3.4c). Nestlings in non-communal broods with a helper (n = 12) also tended to have lighter body mass than those in communal broods, but this relationship was marginally non-significant (β ± SE = -0.69 ± 0.34, P = 0.06; Fig. 3.4c). The number of caregivers had no effect on nestling telomere length or survival to adulthood (Supplementary Table S3.2).

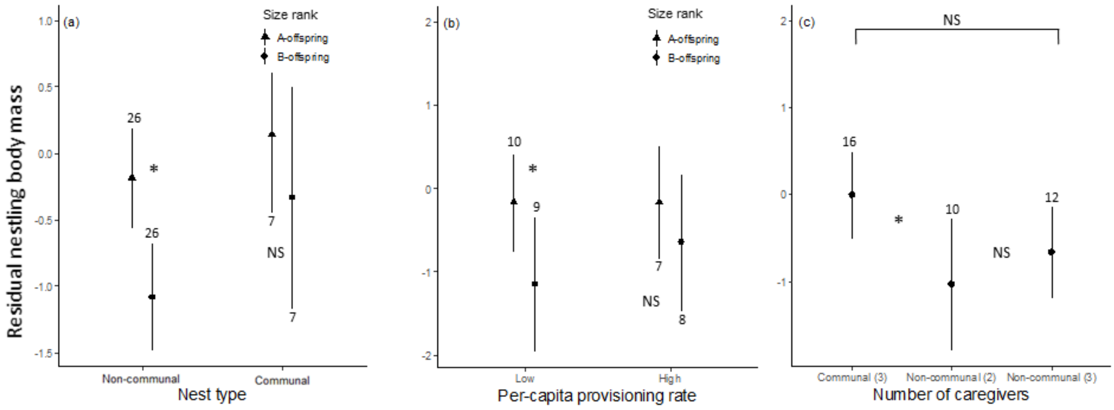


Figure 3.4 Interactions involving size rank and resource availability on residual nestling body mass (corrected for tarsus length, sampling time and date) in two-nestling broods of the Seychelles warbler. a) Influence of nest type on body mass according to size rank. b) Influence of per-capita provisioning rate on body mass according to size rank. Note that per-capita provisioning rate was modelled as a continuous variable but grouped here for visual clarity. c) Influence of additional caregivers in non-communal nests. Non-communal nests are split according to those that were provisioned by a helper-at-the-nest (caregivers = 3) and those that were provisioned only by the breeding pair (caregivers = 2) and both are compared to communal nests, which are always provisioned by three parents. Dots and lines denote mean and 95% CI respectively, sample sizes per group are denoted beside each group. Significant ("*") and non-significant ("NS") interactions at P < 0.05 are displayed.

3.5 Discussion

In this study, we determined whether nestlings in non-communal and communal nests suffered costs of offspring rivalry and investigated the degree to which resource availability and competitive ability influenced those costs. We found that the two nestlings in non-communal broods received less food per-capita than singleton broods and appeared to suffer body mass- and survival-based costs to offspring rivalry that were absent for the two nestlings in communal broods. Size rank played a more prominent role in determining the condition of individuals in non-communal broods (versus communal broods) and in all two-nestling broods when per-capita provisioning rate was lower. Furthermore, the presence of a helper in non-communal nests appeared to mitigate some offspring rivalry costs in terms of body mass, which is known to predict offspring survival in this species. In combination, these findings suggests that resource availability to individual nestlings, rather than within-nursery relatedness, is the principle driver of offspring rivalry costs in this species. However, it is important to note that these findings stem from a relatively small number of communal broods and thus should be interpreted carefully. Below we discuss the implications of these findings for our understanding of how offspring conflict can be resolved in communal-breeding systems.

Relatedness between nursery-mates has the potential to influence the degree to which parents disagree over the outcome of offspring rivalry (Parker 1989). Not surprisingly, nestlings in communal Seychelles warbler broods are significantly less related to each other than those in non-communal nests (Fig. 3.2a), suggesting that there should be some degree of conflict between communally-breeding mothers over the distribution of offspring rivalry costs within the brood. In non-communally breeding species, parents often influence the distribution of rivalry costs by increasing prenatal investment to, or initiating the earlier hatching of, preferred offspring (e.g. Mock and Plodger 1987). In a similar way, parents of communal broods should be selected to increase the competitive ability of their own offspring such that the majority of costs fall on other, unrelated offspring (Riehl 2010). The resulting conflict, where each parent would "prefer" for their co-parents to bear the majority of offspring rivalry costs, has a clear parallel with sexual conflict over parental investment in species with biparental care. While the latter has received a great deal of both theoretical (Houston and Davies 1985; Lessells and McNamara 2012) and empirical (e.g. Schwagmeyer et al. 2002; Bebbington and Hatchwell 2016) attention, the resolution of parental conflict over offspring rivalry costs in communally breeding species remains a key point for future research.

Brood or litter size is assumed to be limited by, amongst other things, the availability of parental resources at the time of reproduction (Wilbur et al. 1974). Surprisingly, we found no evidence that the occurrence of either non-communal or communal broods was related to increases in temporal food availability or greater territory quality (Fig. 3.1). Resource availability is apparently also not more important for non-communal than communal broods, which is surprising given that the reduced provisioning rate to non-communal broods apparently reduces offspring fitness (see below); perhaps provisioning of non-communal broods is limited not by absolute resource availability but by physiological constraints on the caregivers' ability to supply that food. The high prevalence of singleton broods and relatively long lifespan found in this species (Komdeur 1994) may mean that caregivers' own future reproduction and survival prospects weigh heavier than resources in determining parental investment decisions (Trivers 1974).

Assuming that parental condition limits investment in individual offspring (e.g. Hodge et al. 2009), we envision two potential outcomes of conflict over the distribution of offspring rivalry costs in communal nurseries. Where extra, communally breeding parents are typically "subordinate" to a main breeding pair, such as in moorhens *Gallinula chloropus* (McRae 1995) and meerkats *Suricata suricatta* (Young et al. 2006), differences in social status and condition may lead to a natural competitive hierarchy in the nursery, similar to that found in many non-communally breeding species (Mock and Parker 1997). Where extra parents are of the same social status with no clear dominance hierarchy, such as in the banded mongoose *Mungos mungo* (Gilchrist et al. 2004) and groove-billed anis *Crotophaga sulcirostris* (Vehrencamp 1978), the ability to invest in competitive offspring phenotypes should result in equal distribution of offspring rivalry costs within the nursery. We present two lines of evidence to support the latter outcome in Seychelles warblers. First, size asymmetry between nestlings in a brood was not significantly greater in communal than in non-communal nests (Fig. 3.2b), suggesting that nestlings of different mothers did not tend to be more divergent in terms of quality. Second, B-offspring appeared to pay a greater cost to offspring rivalry in non-communal nests, while B-offspring in communal nests performed as well as A-offspring in terms of body mass (Fig. 3.4a). It seems likely therefore that Seychelles warbler parents are unable to skew the costs of offspring rivalry away from their own offspring, but under what general circumstances this is the case is a highly interesting question that remains to be answered.

In non-communal breeders, asymmetry within the brood probably evolves as a mechanism to ensure that at least some offspring are not exposed to the full costs of offspring rivalry (Mock and Parker 1997). However, non-communal broods are also likely to exhibit a greater degree of hatching asynchrony than communal broods simply due to physiological constraints on egg-laying. In the Seychelles warbler, non-communal broods are typically completed over 24 hours (Komdeur et al. 2002) but communal broods can potentially be completed in one morning (Komdeur 1994). Since hatching asynchrony would reduce the combined age of nestlings in non-communal broods when compared to communal broods, an alternative explanation for our finding that non-communal broods receive less per-capita food than communal broods is that the lower energetic requirement of younger non-communal nestlings reduce the total amount of food parents need to provide. However, several lines of evidence lead us to reject this explanation. First, the nestling period is relatively long in the Seychelles warbler (17-19 days, Komdeur 1992) so two nestlings that differ in age by one day are unlikely to have fundamentally different total resource requirements than two of the same age. Second, we show that the proportion of size asymmetry between A- and B-offspring is not different between non-communal and communal nests (Fig. 3.2b), suggesting that any systematic differences in hatching asynchrony between non-communal and communal broods do not have a detectable effect on offspring size differences. Finally, if hatching asynchrony is influencing size differences in non-communal broods, we would expect a consistent difference in body mass between A- and B-offspring in these broods. The fact that B-offspring are only lighter than A-offspring when provisioning rate is low (Fig. 3.4b) suggests that resource availability, rather than nestling age, drives the observed differences in body mass between A- and B-offspring in non-communal broods.

The fact that B-offspring tend to suffer when provisioning rate is low suggests that when nursery-mates are forced to compete for more limited resources, they tend to diverge in quality with respect to competitive ability. Similar patterns have recently been found with respect to milk transfer in spotted hyenas *Crocuta crocuta* (Hofer et al. 2016). It could be argued that the link between the high provisioning rate and apparent lack of offspring rivalry costs in communal nests is driven by some unknown factor that influences both of these variables. The fact that the number of caregivers seems to influence offspring body mass suggests that this is not the case: non-communal nestlings who were provisioned by two parents were lighter than those in communal broods (three parents), whereas the body mass of non-communal nestlings with a helper was not significantly different from communal nestlings. It is worth noting that the addition of a third carer in non-communal nests did not entirely mitigate the body mass cost for communal nestlings. This is likely due to non-breeding helpers provisioning less than females who have produced offspring in the nest (see Richardson et al. 2002), but could also result from other, undetected differences between non-communal and communal nests, such as egg quality (e.g. Cariello et al. 2006). By combining direct comparisons between non-communally and communally reared nestlings and broader tests of variation in resource availability and competitive ability across all two-nestling broods, we find evidence to support the hypothesis that any negative effects of reduced relatedness on offspring-level costs of rivalry are entirely mitigated by the additional food provisioning associated with communal breeding. While this conclusion relies partly on a relatively small number of communal broods, the fact that all our results point to the same pattern does suggest an important role for resource availability in this respect.

While we found evidence that body mass and survival differed with nest type, nestling telomere length did not differ between singleton, non-communal and communal broods. It is worth noting that this may be due to our relatively low sample size in this analysis, but could also arise if the relationship between somatic costs and telomere length only manifests after some time. We generally sample nestlings on day 10 of the nestling period, which is just over half-way through the growth phase (when telomere loss tends to be greatest [Heidinger et al. 2012]). It is possible that telomere length differences associated with varying costs of offspring rivalry would be more visible towards the end of the nestling period when, based on the patterns we find using body mass and survival, the most telomere shortening should have occurred in non-communal nestlings. It is also possible that a measure of telomere change, rather than length, would allow us to better detect costs of offspring rivalry. In the present study, we were unable to measure changes in telomere length during the nestling period due to issues with repeatedly disturbing nesting attempts in this rare species. However, aside from any inherited differences in telomere length (which appear to be relatively low in birds [Reichert et al. 2015]), the measurement taken during sampling is likely to provide a reasonable approximation of telomere loss between hatching and sampling. In addition, nestling telomere length measured at a similar developmental stage has been shown elsewhere to vary according to brood size (Boonekamp et al. 2014) and also in relation to size rank (Nettle et al. 2015), suggesting that any differences in telomere loss between nest types should also be visible in this study. Perhaps the degree of differences between singleton, non-communal and communal nests are not sufficient to cause differences in telomere length in the Seychelles warbler, but telomeres could potentially be used to measure differential costs of offspring rivalry in other facultatively communal breeders.

3.6 Conclusions

Previous work has demonstrated that Seychelles warbler nestlings who are raised with a competitor have reduced body mass and suffer survival costs compared to those raised alone (Bebbington et al. 2016a; Chapter 2 of this thesis). Here, we show that both these costs are limited to nestlings reared in non-communal broods and appear to be largely absent in communal broods. While relatedness between nestlings was considerably lower in communal than in non-communal broods, the absence of within-brood competitive asymmetry or differential offspring rivalry costs in the former suggests that this competitive equality does not lead to escalated offspring rivalry costs. The patterns we report here rely on small sample sizes; validation of our findings in other facultative communal breeders are needed before any strong conclusions are drawn. However, the fact that resource availability appears to mitigate offspring rivalry costs more generally does support the hypothesis that escalated costs of competition among non-kin may be mitigated by the increased resource availability to communally-reared nestlings. We suggest that increased parental resources in communal broods, which likely arises as a consequence of a greater number of provisioning female parents, overrides any additional costs of increased competition between offspring of different parents. This finding could help explain how communal breeding can remain stable in the context of costly offspring rivalry and selfish genes.

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3.8 Supplementary information

Supplementary Table S3.1 Nonsignificant interactions between nest type and spatial or temporal resource availability with regard to the three measures of sibling rivalry cost in Seychelles warbler nestlings.

Response	Predictor	Estimate ± SE	*P*
Body mass	Nest type * food availability	Non-communal 0.24 ± 0.42	0.75
		Communal -0.10 ± 0.71	0.89
	Nest type * territory quality	Non-communal 0.02 ± 0.31	0.95
		Communal 0.18 ± 0.46	0.69
RTL	Nest type * food availability	Non-communal <0.01 ± 0.17	0.98
		Communal -0.06 ± 0.32	0.84
	Nest type * territory quality	Non-communal -0.01 ± 0.13	0.94
		Communal -0.16 ± 0.18	0.36
Survival to adulthood	Nest type * food availability	Non-communal -0.32 ± 0.85	0.71
		Communal -1.51± 1.43	0.29
	Nest type * territory quality	Non-communal -0.04 ± 0.68	0.95
		Communal -0.72 ± 0.94	0.44

Supplementary Table S3.2 Nonsignificant interactions regarding the influence of competitive ability and resource availability on costs of sibling rivalry in non-communal and communal Seychelles warbler nests.

Response	Predictor	Estimate ± SE	*P*
Body mass	Nest type * per-capita provisioning rate (versus non-communal)	0.15 ± 0.09	0.11
RTL	Nest type * size rank (versus non-communal)	-0.06 ± 0.19	0.75
	Per-capita provisioning rate * size rank (versus A-offspring)	<-0.01 ± 0.02	
			0.66
	Nest type * per-capita provisioning rate (versus non-communal)	<0.01 ± 0.03	0.89
	Number of caregivers (versus communal)	Non-communal, no help: -0.08 ± 0.17	0.66
		Non-communal, help: 0.09 ± 0.17	0.62
Survival to adulthood	Nest type * size rank (versus non-communal)	0.39 ± 1.42	0.78
	Per-capita provisioning rate * size rank (versus A-offspring)		
		0.09 ± 0.15	0.52
	Nest type * per-capita provisioning rate (versus non-communal)	0.07 ± 0.18	0.71
	Number of caregivers (versus communal)	Non-communal, no help: 0.70 ± 1.45	0.63
		Non-communal, help: 0.07 ± 1.24	0.96

Table S3.3 Post-hoc model outputs showing the difference between non-communal and communal nests in each analysis (see main text for full details). Non-communal nests were the reference group in all cases, expect for the row marked * where non-communal nests with 3 carers was the reference group.

Analysis	Response	Contrast	Estimate ± SE	*P*
Resource availability	Per-capita provisioning rate	Communal	4.58 ± 3.82	0.15
Costs of offspring rivalry	Body mass	Communal	0.47 ± 0.38	0.23
	Telomere length	Communal	0.07 ± 0.17	0.68
	Survival to adulthood	Communal	0.32 ± 0.70	0.65
Differential costs of resource availability*	Body mass	Non-communal, 2 carers	0.12 ± 0.47	0.79

Chapter 4

Telomere length reveals cumulative and transgenerational inbreeding effects in a passerine bird

A version of this manuscript is published in *Molecular Ecology* doi: 10.1111/mec.13670

Seemingly healthy Seychelles warblers can suffer from hidden costs of inbreeding. Photo by K Bebbington.

4.1 Abstract

Inbreeding results in more homozygous offspring that should suffer reduced fitness, but it can be difficult to quantify these costs for several reasons. First, inbreeding depression may vary with ecological or physiological stress and only be detectable over long time periods. Second, parental homozygosity may indirectly affect offspring fitness, thus confounding analyses that consider offspring homozygosity alone. Finally, measurement of inbreeding coefficients, survival and reproductive success may often be too crude to detect inbreeding costs in wild populations. Telomere length provides a more precise measure of somatic costs, predicts survival in many species and should reflect differences in somatic condition that result from varying ability to cope with environmental stressors. We studied relative telomere length in a wild population of Seychelles warblers (*Acrocephalus sechellensis*) to assess the lifelong relationship between individual homozygosity, which reflects genome-wide inbreeding in this species, and telomere length. In juveniles, individual homozygosity was negatively associated with telomere length in poor seasons. In adults, individual homozygosity was consistently negatively related to telomere length, suggesting the accumulation of inbreeding depression during life. Maternal homozygosity also negatively predicted offspring telomere length. Our results show that somatic inbreeding costs are environmentally dependent at certain life stages but may accumulate throughout life.

4.2 Introduction

In inbred individuals, increased homozygosity leads to the expression of deleterious recessive alleles and the reduction of any heterozygote advantage, and has been shown to reduce fitness across a broad range of taxa (Keller and Waller 2002; Brekke et al. 2010; Simmons 2011; Lacy and Alaks 2013). Inbreeding depression may result through suboptimal cell functioning: both metabolic efficiency (Kristensen et al. 2006; Ketola and Kotiaho 2009) and immune responses (Reid et al. 2003) decline with increased homozygosity. Disruption to such physiological processes as a result of inbreeding can lead to the increased production, or inefficient processing, of damaging oxidant molecules (Nemoto et al. 2000; Balaban et al. 2005; Massudi et al. 2012), the effects of which are normally mitigated by upregulation of antioxidant production. Inbred individuals may be further limited in their ability to produce antioxidant defences if they are less able to access food and other key energetic resources (Ketola and Kotiaho 2009), for example, through reduced competitive ability (Sharp 1984). We therefore expect inbreeding to reduce fitness. However, the costs of inbreeding reported in natural systems vary hugely among individuals and populations (Armbruster and Reed 2005). This lack of consistency could result from variation in the available power to detect effects (Huisman et al. 2016), but may also indicate that certain individuals and populations experience low costs of inbreeding.

There are various reasons why the fitness costs of inbreeding might remain undetected in natural systems (e.g. Keane et al. 1996; Kalinowski et al. 1999). First, inbreeding depression is usually measured in terms of survival or reproductive success (Walling et al. 2011; Kennedy et al. 2014; reviewed in Chapman et al. 2000). These ultimate components of fitness might, however, be confounded by other factors, such as variation in habitat quality and stochastic mortality (Miller and Coltman 2014). Second, inbreeding depression in offspring could be confounded by parental effects, which may in turn be affected by the inbreeding level of either or both of the parents. Thus offspring fitness may suffer as a result of having inbred parents regardless of their own level of inbreeding (Keller 1998; García-Navas et al. 2014). Such indirect costs of inbreeding could manifest as reduced parental investment by inbred parents, for example through poor prenatal nutrition (Wetzel et al. 2012), or the attraction of a poor-quality mate (Sheridan and Pomiankowski 1997). Third, inbreeding variance in natural populations has often been investigated using individual homozygosity across a panel of neutral molecular markers. The results of such studies are inconsistent (Hansson and Westerberg 2002), which is likely due to the potential inaccuracy of measuring genome-wide homozygosity using a limited number of markers (Balloux et al. 2004; Slate et al. 2004; reviewed in Miller and Coltman 2014)

– this method is therefore mainly suited to studies of isolated populations with high inbreeding variance (Slate et al. 2004). Finally, inbreeding effects are easily confounded by variation in external factors. For example, it may only be possible to detect inbreeding costs during periods of heightened environmental or physiological stress (Keller et al. 2002; Marr et al. 2006; Auld and Relyea 2009) or when there is sufficient variation in individual success (Harrison et al. 2011). Furthermore, any negative effects of stressful periods may be cumulative, so that inbreeding-related damage accrued during unfavourable conditions builds up in cells and tissues but may only be detectable above a certain threshold level (Grueber et al. 2010). Such cumulative effects of inbreeding can only be studied with longitudinal data on environmental conditions, ideally collected across individuals' entire lifespans. The rarity of such data from wild populations, combined with the potential for somatic damage to remain undetected until survival effects are visible, might create a substantial gap in our understanding of the costs of inbreeding.

The complications in measuring inbreeding depression may be alleviated by using telomere dynamics to capture individual variation in inbreeding effects. Telomeres are regions of non-coding DNA that protect chromosomes from DNA damage during meiosis (Blackburn 1991). Telomere loss occurs during cell replication, but is also driven by metabolic oxidant by-products that damage DNA (Finkel and Holbrook 2000; von Zglinicki 2000). Oxidative stress (an imbalance in favour of oxidant molecules over defensive antioxidant molecules) arises when individuals do not produce sufficient levels of antioxidants (Finkel and Holbrook 2000), often in periods of elevated somatic stress such as during reproduction (van de Crommenacker et al. 2011) or long-distance travel (Constantini et al. 2007). Telomere length, while probably not causative, appears to be linked to cell-level oxidants and is a useful biomarker for somatic damage (Simons 2015).

Recent studies have linked telomere dynamics to individual life histories and survival in a range of vertebrates (reviewed in Barrett and Richardson 2011), and telomere shortening has been found to reflect energetic costs in relation to factors including reproductive investment (Bauch et al. 2013), chronic infection (Asghar et al. 2015) and early life conditions (Heidinger et al. 2012). Given the links between impaired somatic function and inbreeding (e.g. Teska et al. 1990; Norman et al. 1995) and between rates of telomere shortening and somatic stress (von Zglinicki 2002; Epel et al. 2004), inbred individuals should have shorter telomeres than outbred individuals. Unlike fitness measures such as survival and reproductive success, telomere lengths reflect exposure to factors that have influenced an individual's intrinsic condition up to any given point in time. For example, if inbreeding depression in the parental generation limits the amount of investment in offspring (reviewed in Keller and Waller 2002), then offspring telomere length should be negatively associated with parental homozygosity, at least in early life when parental investment is key. Similarly, if inbreeding depression mainly manifests during stressful periods (Keller et al. 2002; Marr et al. 2006; Auld and Relyea 2009), then telomere loss during environmental stress will be greater among inbred than outbred individuals. Furthermore, the difference in telomere loss between inbred and outbred individuals should increase with age as more stressful periods are experienced.

The Seychelles warbler *Acrocephalus sechellensis* provides an excellent system in which to investigate the costs of inbreeding in a natural setting. The population on Cousin Island, Seychelles, has been extensively monitored, with birds regularly caught and sampled, since 1994. Virtually no migration to or from the island occurs (Komdeur et al. 2004), creating a small (ca. 320 adults), closed population with excellent longitudinal data on individual environmental conditions. Inbreeding occurs frequently in the Seychelles warbler; ca. 5% of all offspring have parents that are first-order relatives (Richardson et al. 2004). Individual homozygosity, as assessed at a panel of microsatellite loci, does not directly influence adult survival in this species, but in poor environmental conditions maternal (but not paternal) homozygosity predicts juvenile survival (Richardson et al. 2004, Brouwer et al. 2007). Importantly, both juvenile and adult telomere length predict survival in the Seychelles warbler (Barrett et al. 2013; Spurgin et al. submitted), while juvenile telomere length is also strongly positively correlated with the availability of insect prey in the year of hatching and the social environment (Spurgin et al. submitted).

In this study we investigate how the telomere length of individual Seychelles warblers varies with individual and parental homozygosity in order to quantify the somatic cost of inbreeding in a natural setting. Specifically, more rapid telomere loss in inbred individuals should lead to a negative relationship between individual homozygosity and telomere length. Parental investment is crucial in altricial bird species such as the Seychelles warbler and, given the extremely long period of offspring dependence in this species (Eikenaar et al. 2007) and our previous finding that offspring survival is related to maternal homozygosity (Richardson et al. 2004), we also predict that individual telomere length will vary with maternal and paternal homozygosity. Finally, we hypothesise that the relationship between telomere length and homozygosity is environmentally dependent and will accumulate over individuals' lifetimes.

4.3 Materials and methods

4.3.1 Study species and system

We use data collected as part of a long-term study of Seychelles warblers on Cousin Island, Seychelles (Komdeur 1992; Hammers et al. 2013). The Cousin population is saturated at approximately 320 individuals in ca. 110 territories (Komdeur 1996; Brouwer et al. 2009). Each year during the main breeding season (June – September) and in some years during the minor breeding season (January to March), a census is carried out, all breeding attempts are followed and birth dates are obtained to give accurate age estimates for all individuals in the population. During each season, as many birds as possible are caught using mist nets and (if not already ringed) given a metal BTO ring and a unique combination of three colour rings for individual identification. As a result, many birds are caught on their natal territories as dependent fledglings, and subsequently sampled multiple times during their lives. A small (25 µl) blood sample is taken by brachial venipuncture from all captured individuals and stored in 0.8 ml of absolute ethanol. The age class of each bird (juvenile versus adult) is confirmed using eye colour (Komdeur 1992).

In this study, we used a total of 1064 samples from 592 individuals caught between 1995 and 2009, for which we had both telomere length measures and detailed lifelong ecological data. Our dataset included both juveniles (aged under one year at sampling: 90 males, 82 females) and adults (aged over one year: 248 males, 229 females).

Seychelles warblers defend year-round territories and their diet consists entirely of insects taken from leaves within the territory (Komdeur 1996). There is annual variation in insect availability on Cousin (Komdeur 1992), which is measured each year as the island-wide mean number of insects per unit leaf area counted across all territories on the island (termed “annual food availability”). Since telomere length should be a function of past as well as present experiences, we also calculated mean island-wide insect food availability (termed “lifetime food availability”) across the lifespan of each individual up to the point of sampling.

4.3.2 Molecular methods

DNA for sexing and microsatellite analysis was extracted from blood samples using ammonium acetate, following Richardson et al. (2001). Sex was determined using the PCR method developed by Griffiths et al. (1998). To measure individual homozygosity we used individual genotype data from a panel of 30 polymorphic microsatellite loci previously developed in the Seychelles warbler (Richardson et al. 2001; Spurgin et al. 2014). Although not all individuals were typed at all 30 loci, 99% were typed at 26 or more loci and 100% were typed at 20 or more loci. To determine parentage, we used the same 30 microsatellites to assign within-group parentage using maximum-likelihood estimation in MASTERBAYES 2.52 (Hadfield et al. 2006) with Wang's (2004) genotyping error model, following the MbG_Wang method of Patrick et al. (2012). Genotyping error rates were set to 0.005. We ran 15,001,000 iterations, discarding the first 1,000 and applying a thinning interval of 15,000. Autocorrelation between successive iterations was <0.1. Tuning parameters were set to 0.01 for unsampled sires and 0.005 for unsampled dams to ensure the Metropolis Hasting values ranged from 0.2–0.5. To maximise assignment confidence, we used only individuals for which the candidate father (assigned with an acceptance threshold of 80%) was the social partner of the dominant breeding female in the territory. Full details of the parentage assignment protocol can be found in Wright et al. (2014).

We used the R (2014) package Rhh 1.0.1 (Alho and Välimäki 2012) to calculate individual standardised heterozygosity between 0 and 2 (Coltman et al. 1999; Alho et al. 2010). We henceforth refer to homozygosity (i.e. 2 – standardised heterozygosity) in accordance with the hypothesised negative effect of inbreeding on telomere length. Offspring homozygosity at 14 of these markers correlates well with parental relatedness in this species (Richardson et al. 2004). We used two methods to test the ability of our extended microsatellite panel (30 loci) to reflect genome-wide levels of homozygosity and thus inbreeding. Using the Rhh package in R (Alho and Välimäki 2012) we calculated a mean homozygosity–homozygosity correlation coefficient (genotyped loci are randomly assigned to one of two groups correlated against each other to determine similarity) from 5000 iterations of the correlation (Balloux et al. 2004; Alho et al. 2010). We also estimated identity disequilibrium (g_2) from 5000 bootstraps for our typed loci using RMES (David et al. 2007).

4.3.3 Telomere measurement

For telomere measurement we used quantitative PCR (qPCR), following the reaction protocol developed previously for the Seychelles warbler (Barrett et al. 2013). Briefly, DNA was extracted using a DNeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions with modification of overnight lysis at 37˚C and a final DNA elution volume of 80 µl. DNA integrity was verified visually using electrophoresis on a 1.2% agarose gel and the concentration was quantified using a NanoDrop 8000 Spectrophotometer (ThermoScientific). We used a relative measure of telomere length that describes the amount of telomeric DNA in a sample relative to that of GAPDH, a constantly expressed reference gene. LinRegPCR 2014.2 was used to correct baseline fluorescence, determine the window-of-linearity for each amplicon and calculate individual well efficiencies. Threshold values (Nq) were set in the centre of the window-of-linearity per amplicon for all samples. We then calibrated quantification cycle (Cq) values per amplicon across different plates by pooling six blood samples as a 'golden sample' inter-plate calibrator (inter-plate repeatability for telomere amplicon = 0.94). We calculated the mean Cq value for each sample, excluding samples where Cq values differed by >0.5 between the two repeats. We then calculated relative telomere length (RTL) for each sample using equation 1 in Pfaffl (2001). We chose to use RTL rather than continuing with the previously used method for calculating absolute telomere length (Barrett et al. 2012), as i) using RTL enabled us to run more samples per plate (as an oligo standard is not required) and ii) most other studies have adopted the RTL method, very few have calculated absolute telomere length (which we developed to allow cross species comparisons), and our experience now suggests that such comparisons are unlikely to be reliable.

4.3.4 Statistical analyses

To investigate the effect of individual and parental homozygosity on RTL, we first constructed minimal models in R 3.2.2 (R Core Team 2014) containing all variables that have been associated with either juvenile or adult telomere length in the Seychelles warbler (detailed below). We then constructed full models by adding homozygosity measures and biologically relevant interactions (separately for individual and parental homozygosities) to the minimal models, but removing any non-significant interactions. Effect sizes and P-values (calculated using likelihood ratio tests) for non-significant interactions were obtained by re-introducing them into the final model (reported in Tables 4.1 and 4.2). We determined whether the final model better described the data than the minimal model by comparing AICc values, considering differences of >2 to be significant (Symonds and Moussalli 2011). R^2 values were calculated using MuMIn (Bartoń 2013). We checked for collinearity between explanatory variables by calculating variance inflation factors and correlating variables with each other.

Telomere length and its predictors are different in adults and juveniles (Barrett et al. 2013; Spurgin et al. submitted), so we tested the relationship between individual homozygosity and RTL separately for juveniles and adults. In all models, RTL, which was normally distributed, was used as a response variable. For juveniles (n = 187), we used general linear models without random effects, since individuals were sampled only once as juveniles. We included annual food availability (the only known predictor of juvenile RTL; Spurgin et al. submitted) in the minimal model. Previous studies in this species have reported sex-biased inbreeding depression (Richardson et al. 2004), so we also included sex in the minimal model in order to test for an interaction with homozygosity. In the full model, we added individual homozygosity and tested for interactions between individual homozygosity and annual food availability, and homozygosity and sex.

For adults we used mixed models with “individual ID” as a random effect, since some adults had multiple measurements of RTL (n = 737 samples from 420 individuals). We included age in the minimal model for adults, which is the only variable known to predict adult Seychelles warbler telomere length (Barrett et al. 2013). We also included sex, so we could test for sex-biased effects of homozygosity on RTL in the full model. The effect of lifetime food availability on RTL has not been previously investigated in adult Seychelles warblers but we included it in the minimal model because annual food availability has a strong influence on RTL in early life (Spurgin et al. submitted). In the full model, we added individual homozygosity as a fixed effect and tested for interactions between individual homozygosity and age, individual homozygosity and sex, and individual homozygosity and lifetime food availability.

For a subset of individuals (77 juveniles and 127 adults) for which both parentage and telomere data were available, we tested for an association between maternal and paternal homozygosities and offspring RTL. We first performed a linear model to assess the relationship between offspring and parental homozygosities. We then tested the effects of maternal and paternal homozygosity on RTL separately for offspring sampled as juveniles and adults. Minimal models were constructed as in the previous paragraph but also included any additional predictors arising from the individual homozygosity analyses. In the full models, we added maternal and paternal homozygosity as fixed effects. For juvenile offspring, we also included the interactions between both parental homozygosities and annual food availability. For adult offspring, we included food availability in the first year of life, and interactions with parental homozygosities. This allowed us to determine whether effects of parental homozygosity on adult RTL were driven by early-life conditions or arose independently in adulthood.

4.4 Results

4.4.1 Homozygosity–homozygosity correlation and g_2 estimation

Standardised homozygosity was similar in juveniles and adults (juvenile mean ± SD = 0.97 ± 0.21; adult mean ± SD = 0.99 ± 0.23). The homozygosity–homozygosity correlation was significantly positive (mean Pearson's correlation coefficient ± SE = 0.124 ± 0.001, $P < 0.001$). The g_2 parameter estimate was significantly greater than zero (g_2 ± SD = 0.009 ± 0.003, $P < 0.001$) – comparable to the mean g_2 value (0.007 ± 0.022 SD) from a recent meta-analysis (Miller and Coltman 2014). Together these results indicate that our panel of microsatellite markers reflects genome-wide homozygosity in the Seychelles warbler. These parameters are comparable to those reported in other organisms, including trees (Rodriguez-Quillon et al. 2015) and mammals (Annavi et al. 2014). A recent review suggested that g_2 measures should be generally meaningful when $g_2 \geq 0.005$ and $P \leq 0.01$ (Kardos et al. 2014); both criteria are met by our microsatellite panel.

4.4.2 Individual homozygosity and RTL

In juveniles (*n* = 137), there was a significant interaction between annual food availability and individual homozygosity (Table 4.1a): there was a negative effect of individual homozygosity on RTL in years of low food availability but no effect in years of high food availability (Fig. 4.1a and c). Sex and its interaction with homozygosity were non-significant (Table 4.1a). The final model ($R^2 = 0.11$) including individual homozygosity was better supported than the minimal model (Δ AICc = 3.79).

In adults (*n* = 568), RTL was negatively related to individual homozygosity. This relationship between homozygosity and RTL was weak ($R^2 = 0.011$; Fig. 4.2a), but significant (Table 4.2a). RTL decreased with age, as previously demonstrated in this species, and males had longer telomeres than females (Table 4.2a). Lifetime food availability was positively related to RTL (Table 4.2). All interaction terms were non-significant and were dropped from the final model. The final model ($R^2 = 0.35$) including individual homozygosity did not differ in fit from the minimal model (Δ AICc = 1.22).

4.4.3 Parental homozygosity and offspring RTL

There was a positive relationship between maternal, but not paternal, homozygosity and offspring homozygosity, but the relationship was weak (maternal: β ± SE = 0.16 ± 0.07, $P = 0.03$; paternal: β ± SE = -0.01 ± 0.07, $P = 0.85$). Consequently, both offspring and parental homozygosities could be considered within the same model when testing for relationships between homozygosity and offspring RTL.

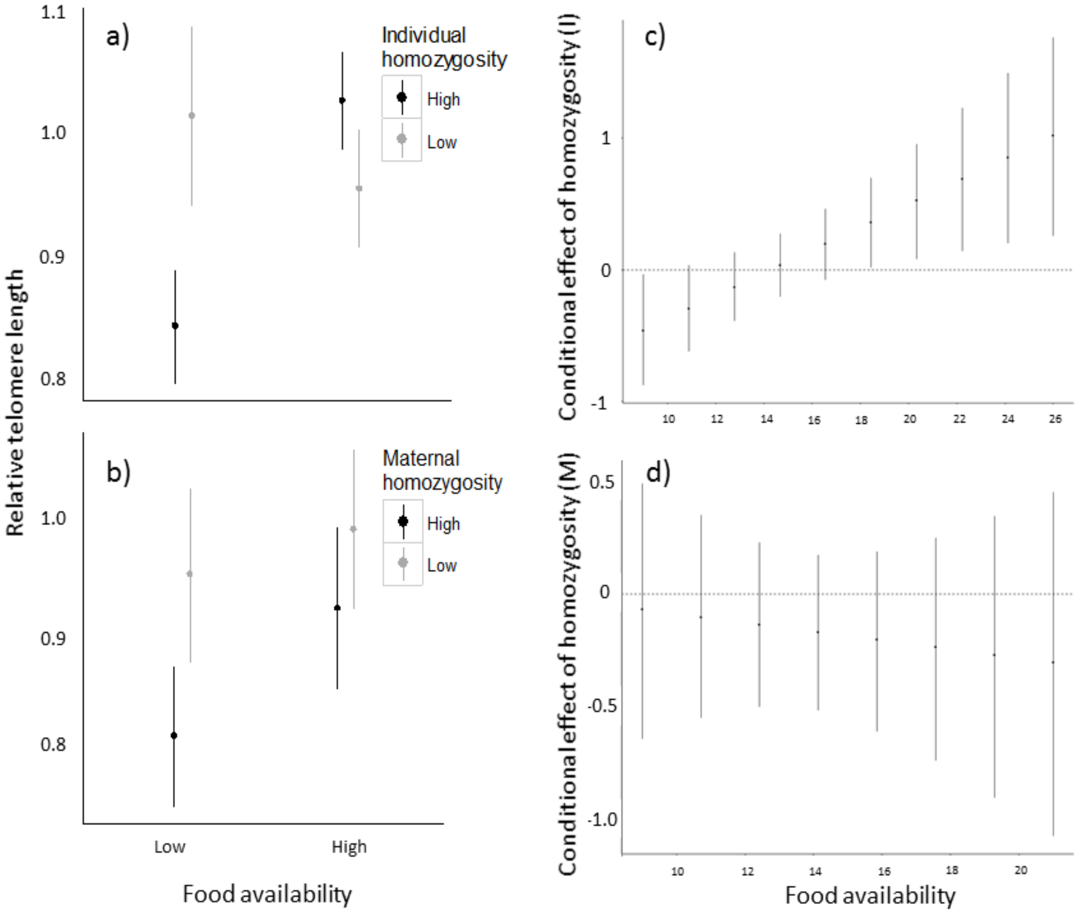

Figure 4.1 Relationship between standardised individual (I) homozygosity (graphs a) and c)) or maternal (M) homozygosity (graphs b) and d)) and relative telomere length of juveniles born in years of high and low food availability. In the left-hand plots, food availability was split into a factor according to the median value for visual clarity, but was modelled as a continuous variable. Right-hand plots display the conditional effect of homozygosity on RTL, across the range of food availability values. The values on the *y*-axis indicates the direction of the homozygosity effect on RTL, given the value on the *x*-axis. Bars represent 95% confidence limits.

In the subset of juveniles with known parentage (*n* = 77), neither maternal nor paternal homozygosity predicted offspring RTL nor interacted with food availability (Table 4.1b). There was a non-significant trend showing that the RTL of offspring of inbred and outbred mothers differed more in years of low food availability (Fig. 4.1b and d), but this was nonsignificant. The null model containing only individual homozygosity (R^2 = 0.16) was better supported than a model also containing maternal and paternal homozygosity (Δ AICc = 2.37). Finally, in the subset of adults with known parentage (*n* = 182) maternal homozygosity was negatively related to offspring RTL (Fig. 4.2b). Neither paternal homozygosity, food availability in birth year nor any interactions significantly predicted offspring RTL. The final model (R^2 = 0.22) including both individual and maternal homozygosity had a lower AICc than the minimal model (Δ AICc = 3.90).

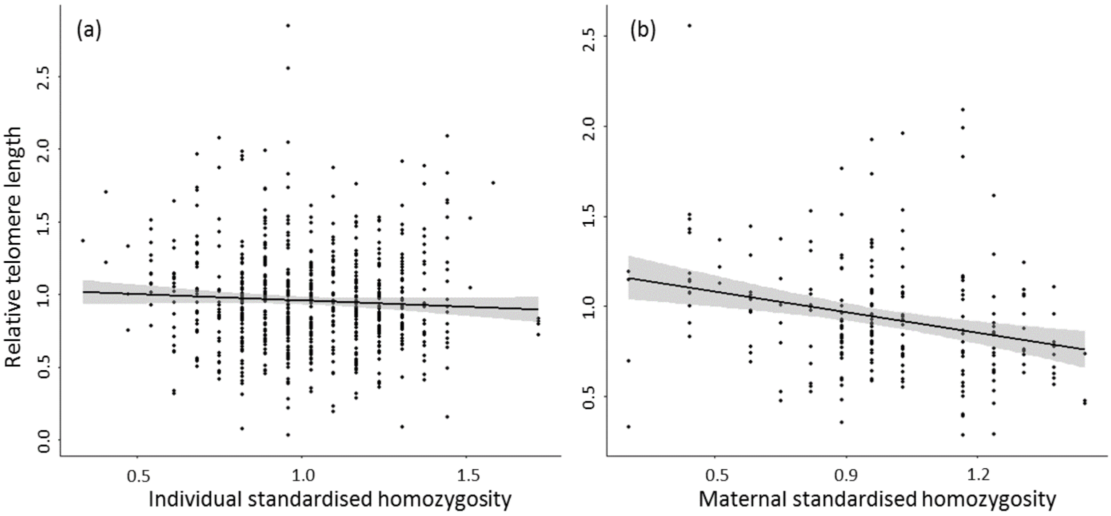

Figure 4.2 Relationship between (a) standardised individual homozygosity and (b) standardised maternal homozygosity and relative telomere length in adult Seychelles warblers. Points represent raw data, lines represent fitted values (linear regression) and shading represents credible intervals.

Table 4.1 Parameter estimates from models of juvenile relative telomere length in relation to (a) individual (I) homozygosity and (b) maternal (M) and paternal (P) homozygosity. Significant terms in the final models are in bold.

Homozygosity	Model	Parameter	Estimate ± SE	*P*
(a) Individual *n* = 137	Minimal	Annual food availability	0.02 ± <0.01	<0.01
		Sex (male)	0.06 ± 0.05	0.19
	Final	**Homozygosity (I)**	**-1.24 ± 0.47**	**<0.01**
		Annual food availability	**-0.07 ± 0.03**	**0.04**
		Annual food availability*Homozygosity (I)	**0.09 ± 0.03**	**<0.01**
		Sex (male)	0.06 ± 0.05	0.23
		Sex*homozygosity (I)	-0.14 ± 0.24	0.57
(b) Parental *n* = 77	Minimal	Annual food availability	0.02 ± 0.01	0.05
		Homozygosity (I)	-0.32 ± 0.17	0.07
		Annual food availability*Homozygosity (I)	0.08 ± 0.05	0.14
	Final	**Annual food availability**	**0.02 ± 0.01**	**0.05**
		Homozygosity (I)	-0.32 ± 0.17	0.07
		Annual food availability*Homozygosity (I)	-0.08 ± 0.05	0.14
		Homozygosity (P)	0.18 ± 0.14	0.20
		Homozygosity (M)	-0.16 ± 0.17	0.35
		Homozygosity (M)*Annual food availability	-0.04 ± 0.05	0.43
		Homozygosity (P)*Annual food availability	<0.01 ± 0.04	0.91

Table 4.2 Parameter estimates from models of adult relative telomere length in relation to (a) individual (I) homozygosity and (b) maternal (M) and paternal (P) homozygosity. Significant terms in the final models are in bold.

Homozygosity	Model	Parameter	Estimate + SE	*P* value
(a) Individual *n* = 568	Minimal	Lifetime food availability	0.03 ±<0.01	<0.01
		Age	-0.03 ± <0.01	<0.01
		Sex (male)	0.08 ± 0.03	<0.01
	Final	**Lifetime food availability**	**0.03 ± <0.01**	**<0.01**
		Age	**-0.03 ± <0.01**	**<0.01**
		Sex (male)	**0.08 ± 0.03**	**<0.01**
		Homozygosity (I)	**-0.14 ± 0.07**	**0.04**
		Lifetime food availability*Homozygosity (I)	-0.01 ± 0.02	0.50
		Age*Homozygosity (I)	0.01 ± 0.02	0.61
		Sex*Homozygosity (I)	0.01 ± 0.13	0.93
(b) Parental *n* = 182	Minimal	Lifetime food availability	0.02 ± <0.01	<0.01
		Homozygosity (I)	-0.36 ± 0.12	<0.01
		Age	-0.03 ± 0.01	0.02
		Sex (males)	0.10 ± 0.05	0.05
	Final	**Lifetime food availability**	**0.01 ± <0.01**	**0.01**
		Homozygosity (I)	**-0.26 ± 0.12**	**0.03**
		Homozygosity (M)	**-0.32 ± 0.10**	**<0.01**
		Age	**-0.03 ± 0.01**	**<0.01**
		Sex (male)	**0.10 ± 0.05**	**0.03**
		Homozygosity (P)	0.16 ± 0.11	0.17
		Food availability at birth	<0.01 ± 0.01	0.89
		Homozygosity (P)*Food availability at birth	0.05 ± 0.03	0.12
		Homozygosity (M)*Food availability at birth	0.02 ± 0.02	0.51

4.5 Discussion

Our results demonstrate a negative relationship between an individual's homozygosity and relative telomere length, revealing inbreeding costs using a more sensitive measure compared to power- and resolution-limited survival and reproduction measures. In early life this relationship was dependent on environmental conditions (i.e. annual food availability), whereas inbred adults had shorter telomeres regardless of the food availability they experienced across life. This suggests that the effect of inbreeding on telomeres may accumulate, as more stressful factors are experienced, so that by adulthood shorter RTL is consistently associated with higher homozygosity. Maternal, but not paternal, homozygosity was also linked to adult RTL, indicating trans-generational impacts of inbreeding. Although the relationships we report are weak, we believe they offer useful insight into the fine-scale mechanics of inbreeding depression in the wild.

Telomere length is an established biomarker of somatic costs (Hall et al. 2004; Ujvari and Madsen 2009; Boonekamp et al. 2014) and as such is a good candidate to detect inbreeding depression. Previous studies of inbreeding depression in the Seychelles warbler have reported no relationship between individual homozygosity and survival (Richardson et al. 2004; Brouwer et al. 2007). This previous underestimation of inbreeding depression in this species probably reflects the fact that inbreeding damage accumulates in cells and tissues; whereas reduced survival may only be detected at some threshold of damage, shorter telomeres can be detected at any point. Thus, our finding that RTL varies with individual homozygosity suggests that inbred individuals have worse somatic condition, which could arise through two non-mutually exclusive pathways. First, inbred individuals may have suboptimal cell functioning that directly increases oxidant levels and increases damage to telomeres (Nemoto et al. 2000; Balaban et al. 2005; Massudi et al. 2012). Second, inbred individuals could mitigate poor cell functioning under normal circumstances, but experience greater-than-normal damage during periods of stress due to poor physiological (Armario et al. 1995) or behavioural (Bleakley et al. 2006) responses.

In line with the second of these pathways, we hypothesised that inbreeding damage to telomeres would be cumulative and vary as a function of the number of stressful events experienced over an individual's entire lifetime. In juvenile Seychelles warblers, the strength of the relationship between inbreeding and telomeres varied with food availability at birth – a key early-life stressor in this species (Spurgin et al. submitted). By calculating lifetime food availability for adults across their pre-sampling life and testing for an interaction between this and homozygosity, we hoped to capture some of the variation in stress exposure over life. Adults with lower lifetime food availability have logically experienced more food-poor periods and the resulting stress accumulation should have impacted inbred birds to a greater extent. Our finding that individual homozygosity and lifetime food availability have consistently negative (rather than interacting) relationships with adult RTL does not support this. This may be because adults face an increased number of different types of stressors, linked to factors such as reproductive effort and social status. An interaction between age and homozygosity on RTL would provide more unequivocal support for the prediction that inbreeding costs accumulate across life, as older adults should have (on average) experienced more (generic) stressors than younger adults. However, there is strong selective mortality of individuals with shorter telomeres in this species

(Barrett et al. 2013), which likely confounds the interaction between age and inbreeding. Nonetheless, our finding that homozygosity as a main effect is significantly related to telomere length in adults, but not juveniles, provides some evidence that the effect of inbreeding on telomere attrition is cumulative over an individual's lifetime. If early life was the key driver of inbreeding depression, we would expect the interaction between food availability in year of birth and homozygosity to be present even in adulthood. The fact that the negative effect of homozygosity is continuous in adulthood suggests that (multiple) further stressful periods experienced in the post-juvenile period have compounded the effects of inbreeding that commence in early life.

A previous study on the Seychelles warbler showed that maternal (but not paternal) homozygosity was negatively related to juvenile survival, but that this effect only occurred during low quality breeding seasons and arose through differences in genetics or egg provisioning (Brouwer et al. 2007). Although there was no significant interaction between food availability and maternal homozygosity on juvenile RTL, the difference between the RTL of offspring from inbred and outbred mothers when food availability was low (Fig. 4.1b) was in the same direction as the significant trend with individual homozygosity (Fig. 4.1a). We may find that with increased sample size and power, the effect becomes significant. We also found that maternal (but not paternal) homozygosity was related to offspring RTL in adulthood. In accordance with individual homozygosity, maternal homozygosity therefore became a consistent predictor of RTL by adulthood. This further supports the idea of accumulating inbreeding costs: as for individual homozygosity, the cost of poor maternal investment (e.g. egg resources which control development [Schwabl 1996]) may reduce an offspring's ability to mitigate costs of external stressors throughout life.

We are only aware of three studies testing the relationship between inbreeding and telomere length. Two studies compared telomere lengths of inbred and outbred strains of laboratory mice and reported extreme elongation of telomeres in inbred strains (Hemann and Greider 2000; Manning et al. 2002). These studies considered between- rather than within-population inbreeding variation, and the results cannot easily be compared with those from wild systems. The third study, in a natural population of white-throated dippers *Cinclus cinclus*, reported no significant relationship between inbreeding and telomere length (Becker et al. 2015), but addressed this only as an aside to questions regarding heritability of telomere length. Given that the study did not consider the potential environmental-dependency or cumulative nature of inbreeding effects, it is difficult to make conclusions regarding the reported results. There is a clear need for more tests of individual level inbreeding effects on telomeres in both wild populations and laboratory organisms if we are to understand the impact of inbreeding in the soma.

We show several relationships between RTL and homozygosity at different life stages in this study, but it is important to note that these relationships only explain a limited amount of variation (Figures 4.1 and 4.2). They must be confirmed in other systems before any general conclusions about this relationship are drawn. We believe that the low explanatory power of homozygosity arises through the inherent noise in homozygosity measures and also in telomere data. Telomere length is used as a biomarker of biological cost because it is predicted to vary in response to individual physiology, behaviour and environment, but this very useful property means that the relationship with any one given factor is logically weakened by all others. It is extremely difficult to account statistically for all possible drivers of telomere length; laboratory studies where the environmental drivers of telomere length can at least partially be standardised may prove extremely valuable in this sense.

Finally, we present one result that contrasts with previous findings in the Seychelles warbler. We found that adult males had longer telomeres than females, whereas Barrett et al. (2013) found no sex difference. The dataset used in this manuscript is approximately double the size used by Barrett et al. (2013) which, combined with the fact that we report a previously undetected result (rather than failing to support a previously reported result), suggests that our data provide greater power to detect sex differences. Limiting our analysis to only those samples used by Barrett et al. (2013) resulted in the relationship between sex and RTL no longer being significant, suggesting that the discrepancy arises through the inclusion of more samples in the current study. Supporting this, the sex effect in our study appears to be more pronounced among cohorts born after 2000 (Supplementary Fig. S4.1), which were not included in Barrett et al. (2013). It therefore seems likely that the discrepancy between the two studies arises through a combination of difference in power, and potentially some cohort-level differences.

4.6 Conclusions

To the best of our knowledge, this is the first study to demonstrate a negative relationship between genome-wide homozygosity (and thus inbreeding) and telomere length in a natural system. Given the strong link between telomere length and future survival in this and other species, our results suggest that telomeres are able to detect subtle costs of inbreeding that may not be detectable with life-history data alone. Our results also suggest that inbreeding costs accumulate with age as individuals experience a greater number of stressful periods, but this remains to be tested more thoroughly. Nonetheless, our findings present novel insights into previously unexplored somatic damage that occurs as a result of inbreeding in wild populations.

4.7 References

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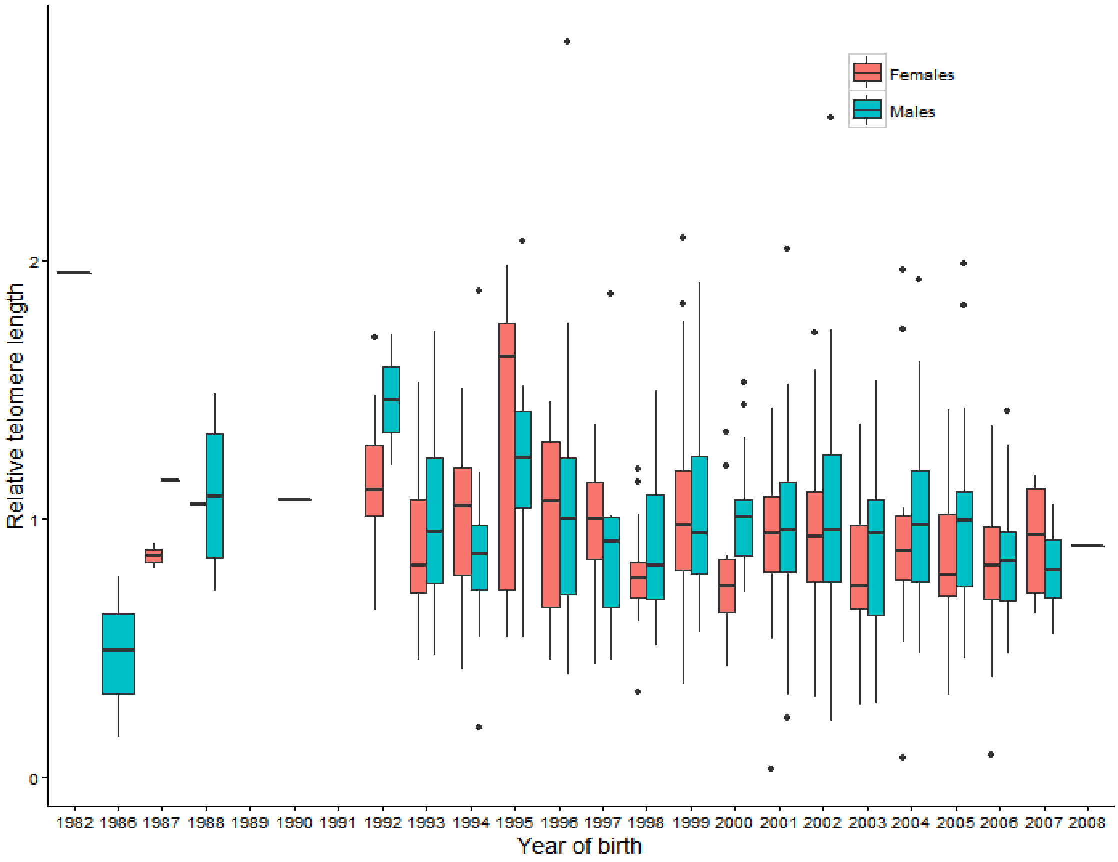
4.8 Supplementary information



Supplementary Figure S4.1 Sex differences in relative telomere length of Seychelles warblers across years of sampling, showing median (middle line) and second and third quartiles below and above respectively.

Chapter 5

Status-dependent costs of group-living in a cooperative breeder

Life in a group can be more difficult for some individuals than others. Photo by Seychelles Warbler Project.

5.1 Abstract

Animals that live in stable groups gain many fitness benefits, but may also compete for limited resources in the shared territory. Quantifying the costs of such competition is therefore a key step in understanding how group living remains evolutionarily stable, but obtaining an accurate measure of group living costs can be problematic for two reasons. Firstly, it is unclear whether costs of group living arise through direct competition over food in the territory, or through socially-induced stress related to the maintenance of social hierarchies and reproductive rights. Second, the costs arising through these two mechanisms may be very different for individuals of socially dominant and socially subordinate status. In this study, we used three physiological measures that reflect different levels of physiological cost (body mass, telomere loss and survival) to tease apart costs of direct competition for food and costs of social stress in dominant and subordinate Seychelles warblers *Acrocephalus sechellensis.* We found that both dominants and subordinates had lower body mass when per-capita food availability was lower. This suggests that while competition for food is an important component of group living costs, it does not impact individuals differently according to their social rank. After accounting for territorial food availability, we found that dominant individuals living in larger groups had greater mass and less telomere shortening. However, while subordinate telomere shortening was unrelated to properties of the social group, subordinate females had lower body mass in large groups. Our results suggest that competition for food is costly for all group members, while social stress mainly impacts subordinate females. These results demonstrate the need for a comprehensive understanding of both mechanistic and social factors in order to understand the stability of group living.

5.2 Introduction

Individuals may improve their fitness by living with conspecifics in stable social groups. Group living can increase the survival of group members (in terms of reduced predation risk or enhanced foraging ability), but also provides benefits in terms of direct and indirect reproductive success (Krause and Ruxton 2002; Whitehouse and Lubin 2005). Such benefits are believed to drive the evolution of group living in cooperative breeders that defend exclusive territories. However, competition between group members over limited resources, such as access to food or reproduction (Vehrencamp 1983; Clark and Mangel 1986), can introduce a costly component to group life. In order to understand the widespread occurrence of group living and cooperative breeding in animal taxa and also the considerable intraspecific variation in group size, it is therefore important to investigate the potential costs of group membership.

Many studies have highlighted the importance of social status when studying the costs of group living (reviewed in Creel 2001). Hierarchical social structures are common to many group-living species; an individual’s position in that hierarchy may have considerable influence on the relative costs of group membership (e.g. Cavigelli et al. 2003; Bender et al. 2006). Many studies to date have used rank-related differences in the secretion of stress-related hormones to make inferences about the physiological cost of group living for individuals of different social status (reviewed in Creel et al. 2013). However, empirical work has demonstrated a remarkably varied set of patterns with respect to physiological costs of group living for socially dominant and subordinate individuals: in many cooperatively breeding species there is a general tendency for dominants to exhibit higher stress (e.g. Creel et al. 1996; 1997; 2001; Goyman and Wingfield 2004; Mileva et al. 2010), but some species show the opposite tendency (Young et al. 2006; reviewed in Creel et al. 2013). One reason for this variation is that the costs of a given social status may vary with resource availability (Rubenstein 2007), reproductive costs (Cram et al. 2015) or fluctuations in group composition (Rubenstein and Shen 2009).

There are two broad mechanisms by which costs of living in groups can arise. First, where groups make use of an exclusive territory, costs of group living may arise through direct competition for food (Krause 1994; Brouwer et al. 2006). Under this hypothesis, per-capita food availability, rather than absolute group size, dictates the costs of group living. If direct competition for food occurs, we therefore predict that weaker competitors – usually subordinates (Forrester 1991; Maclean and Metcalfe 2001; Cafazzo et al. 2010) – will suffer the greatest costs when food becomes scarce. The second way that costs of group living might arise is indirectly through aggression related to challenging social status or conflict over the right to reproduce (Goymann and Wingfield 2004). Under this hypothesis, the number and potentially the identity of group members, when controlling for resource availability, dictate group-living costs. If social stress occurs as a result of group living, we predict that dominants will suffer more in large groups because they must invest more in maintaining their position and suppressing subordinate group members (Rubenstein and Shen 2009). With respect to subordinates, we can predict two scenarios under the social stress hypothesis: aggression towards subordinates could be more common in larger groups (because the cost to dominants of subordinate reproduction are higher), but aggression towards any one individual could be diluted if dominants have a greater number of subordinates to control (Rubenstein and Shen 2009). Testing such hypotheses about how group living costs are distributed among group members require accurate measurement of physiological stress or condition. Recently, researchers have turned to physiological biomarkers of cost, in particular oxidative stress, in order to explore differences in costs of group living among group members (van de Crommenacker et al. 2011; Cram et al. 2015). While studies of oxidative stress are an important first, a range of measures is needed to fully understand both the immediate physiological responses to social conditions and longer-term fitness consequences of group membership.

In this study, we test the *direct competition* and *social stress* hypotheses of the costs of group living in the facultatively cooperatively breeding Seychelles warbler *Acrocephalus sechellensis*. Previous work in the Seychelles warbler demonstrated that individuals living in larger groups have lower survival probabilities, which appeared to be due to absolute group size rather than territorial resource availability (Brouwer et al. 2006). While this finding supports the social stress hypothesis, it remains unclear whether costs arising through direct competition or social stress affect dominants and subordinates equally. In the Seychelles warbler, groups consist of two dominants and 0-5 subordinates (Komdeur 1992; Kingma et al. 2016). We can therefore make clear predictions about the distribution of costs within groups, outlined in Table 5.1. Under the direct competition hypothesis, subordinates should suffer the most from low territorial food availability because dominants are likely to out-compete them for access to limited food. Under the social stress hypothesis, dominants should suffer more in larger groups because there are a greater number of subordinates to control (Goymann and Wingfield 2004; Rubenstein and Shen 2009).

However, there may also be differences between males and females with respect to our two hypotheses. (1) Seychelles warbler females are smaller and lighter than males (e.g. van de Crommenacker et al. 2011), which may reduce their ability to compete with larger group members. Under the direct competition hypothesis, we therefore expect dominant and subordinate females to suffer more than males when territory food availability is low.

Table 5.1 Summary of predictions and supporting evidence in relation to two hypotheses about costs of group living in the Seychelles warbler.

Hypothesis	Prediction	Prediction supported?	Evidence
Direct competition	I) Low food availability is more costly for subordinates than for dominants	**Partly –** Influence of per-capita territory quality on body mass is slightly stronger in subordinates, but per-capita territory quality also affects telomere loss in dominants	Table 5.2a, Table 5.3a, Fig. 5.1, Fig. 5.2
	II) Low food availability is more costly for females than for males	**No** – the relationship between per-capita territory quality and body mass/ΔRTL is the same for males and females	Results
Social stress	I) Large groups are more costly for dominants than for subordinates	**No** – large group size reduces subordinate, but not dominant, body mass. Dominants experience less telomere shortening in large groups	Table 5.2, Table 5.3a, Fig. 5.2, Fig 5.3a
	II) Large groups have sex-dependent costs for subordinates	**Yes** – female subordinates in larger groups have lower mass, but this is not true for male subordinates	Fig. 5.1b

(2) Subordinate Seychelles warblers almost never evict the established breeder in their group (Richardson et al. 2007), but subordinate females sometimes lay an egg in the nest alongside the single egg of the dominant female (Richardson et al. 2001). Male subordinates rarely gain parentage in the group (Richardson et al. 2001; Hadfield et al. 2006), but this may be due to suppression of male reproductive behaviour by dominants. Subordinate reproduction has the potential to jeopardise the reproductive success of both male and female dominants; under the social stress hypothesis, we therefore predict that male and female dominants experience similar costs in terms of maintaining their social rank. It is more difficult to make predictions regarding differences between male and female subordinates with respect to the social stress hypothesis. Given their potential to cuckold dominant males, subordinate males may be subject to more aggression than females. However, while recent work suggests that subordinate female reproduction is not detrimental to offspring fitness (Chapter 3 of this thesis), both male and female dominants presumably benefit from preventing joint nesting if the territory does not hold sufficient resources to support two offspring.

We test our hypotheses (Table 5.1) by incorporating information from three measures that reflect physiological cost on different timescales. We use (1) body mass (controlled for structural size) to measure immediate responses to social conditions in terms of fat and protein storage (Schulte-Hostedde et al. 2005), (2) telomere dynamics to measure long-term accumulation of oxidative damage (Epel et al. 2004) and provide an indicator of future survival prospects (Haussmann and Marchetto 2010) and (3) survival probability to measure the direct survival cost of group living. In the Seychelles warbler, dominants and subordinates often occupy different age classes (Komdeur 1992; Kingma et al. 2016), which makes it difficult to separate effects of social status from those of age. In addition, approximately half of all dominants live on territories without subordinates (Komdeur 1992; Kingma et al. 2016), but the converse (subordinates living on territories without dominants) is never true, meaning that (on average) subordinates live in much larger groups. Rather than attempting problematic direct comparisons between dominants and subordinates, we therefore explore the relationship between group properties and physiological costs separately for individuals occupying each status.

5.3 Materials and methods

In this study, we consider individual Seychelles warblers that were caught and sampled between 2003 and 2014 from the population on Cousin Island, Seychelles. This population has been studied intensively since 1997 (Komdeur et al. 2016) but we chose to focus on recent years when sampling frequency is greatest and information regarding group properties and individual social status is most accurate (Kingma et al. 2016). We restricted our analyses to birds who were over three months of age and therefore independent of parental care (Komdeur 1996).

5.3.1 Measuring body mass, telomere length and survival

During each main breeding season (June-September) and some minor (January-March) seasons, as many birds as possible were caught and sampled using mist nets. Any unringed individuals were given a unique combination of three colour rings and a British Trust for Ornithology metal ring for individual identification. Since all individuals and nesting attempts have been identified and monitored over many years (Komdeur et al. 2016), we were able to accurately determine an individual's age when caught based on nesting and ringing records. At each catch, we recorded body mass and tarsus length (to nearest 0.1g and 0.1mm respectively) and the date and time (early: 06.30-11.00 h; midday: 11.00-15.00 h; late: 15.00-18.00 h) of capture. A 25µl blood sample was taken by venipuncture and stored in absolute ethanol. DNA was extracted from blood samples using a Qiagen DNeasy blood and tissue kit and used to determine individual sex (following Griffiths et al. 1998) and relative telomere length (using real-time qPCR to determine the concentration of telomeric DNA relative to a references gene, see Barrett et al. [2013]; Bebbington et al. [2016a]; Chapter 4 of this thesis for full details). After catching an individual, we monitored its location and group membership (see below) every year until it disappeared from the population. Seychelles warblers virtually never leave the island (Komdeur 2004); any individuals not observed for two consecutive years could therefore be assumed dead (Brouwer et al. 2006; Barrett et al. 2013; Hammers et al. 2015).

5.3.2 Measuring components of group living

During every main breeding season, a compete census of the Seychelles warbler population on Cousin Island was followed by (at least) weekly monitoring of the ca. 110 territories on the island, during which time group composition was recorded. Seychelles warblers are entirely insectivorous and forage for insect prey exclusively within their own territory, so group membership could be determined by behavioural observations of spatial movements and interactions with other group members within a territory (Komdeur 1992; Kingma et al. 2016). We distinguished between socially dominant and socially subordinate group members: the dominant breeding pair was recognised through courtship and pair-bonding behaviour (Komdeur 1992; Richardson et al. 2003). In territories where subordinates were present, we performed a one hour provisioning watch to determine whether each subordinate helped in nestling provisioning (for full details see Bebbington et al. [2016b]; Chapter 2 of this thesis). In order to test whether direct competition or social stress are important in determining the costs of group living, we distinguished between the number of individuals in the group (social stress) and the amount of food available per individual in the territory (direct competition). Each main breeding season, we also recorded territory quality, calculated as a log measure of the number of insects per area of leaf in the territory (Komdeur 1992; Brouwer et al. 2006), to quantify spatial variation in resource availability between territories. We calculated a measure of per-capita territory quality as total territory quality divided by the number of independent (>3 months old) individuals in the group.

5.3.3 Cross-sectional and longitudinal data

For each individual caught between 2003 and 2014, we recorded the resident group size in the season of sampling. We used this cross-sectional dataset to determine how social conditions in the season of sampling influenced current physiological costs (body mass). However, many individuals were repeatedly sampled while occupying the same social status (dominant or subordinate) over many seasons. We used these longitudinal samples to determine how group size influenced long-term physiological costs (telomere length and survival) over a longer period of time. For dominants and subordinates that were caught more than once with at least six months between the first and last catch (the minimum time between breeding seasons), we calculated mean values for group size and per-capita territory quality across all breeding seasons between and including the season of the first and final time we sampled the individual in that status. We then calculated a measure of total telomere change over the same period as the difference between the last telomere measurement and the first, henceforth ΔRTL, such that negative values indicate telomere loss and positive values indicate increases in telomere length. Lastly, to measure differences in survival between individuals, we recorded whether the individual was present in the population in the year following the final sampling.

5.3.4 Statistical analyses

All analyses were conducted in R (version 3.3.1, R Core Team 2016). To test whether variation in group size differently affects individuals of different social status (Table 5.1), we performed separate analyses for dominants and subordinates throughout. We built separate models for each of the three measures of physiological cost: body mass and ΔRTL were modelled as Gaussian responses and survival as a binomial response. Variance inflation factors (VIF) were checked prior to model fitting and found to be suitably low (all $VIF < 4$, Dormann et al. 2012) as to not influence our analyses. We included all predictors (see below) in the final model, but removed any nonsignificant interactions and quadratic effects. For group size, we report effect sizes relative to the smallest groups, but we report parameters for all contrasts in full in Supplementary Table S5.1. For all three responses, we tested for effects of per-capita territory quality and group size. Including these two variables (which were not strongly correlated, $r = 0.09$) in the same model allowed us to distinguish between our two hypotheses. In order to test for direct competition, we asked whether the amount of food per individual – controlling for absolute group size – influenced physiological costs. To test for social stress, we asked whether the number of individuals – controlling for per-capita food availability – influenced physiological costs. To explore sex-specific costs of group living (Table 5.1) we also tested interactions between these two variables and individual sex. Previous work has demonstrated physiological differences between subordinates that do and do not help provisioning offspring (van de Crommenacker et al. 2011), so we included status (helped/did not help) in all subordinate analyses.

In the models of body mass, we included time and month of capture and the interaction between sex and tarsus length (to account for sex-specific scaling of mass with tarsus length) to control for temporal variation in mass and structural size differences, respectively (Bebbington et al. 2016b; Chapter 2 of this thesis). Since there were very few groups with more than four independent individuals, we modelled group size in the season of sampling as a three-category factor (3, 4 or 5+). Visual inspection of the raw data suggested a quadratic relationship between body mass and age, so we included both age and age^2 as predictors. For subordinates, we included each individual's helping status in the season of sampling. Previous work has shown that helping status differently affects subordinate mass throughout the season (van de Crommenacker et al. 2011), but we also included month of capture in the model (see above) to control for this. We included 3 random effects: individual identity, territory identity and year of sampling to account for repeat measures of individuals, territories and years, respectively.

In the models of ΔRTL and survival, we modelled mean group size across the sampling period as both a linear and a quadratic (group size^2) predictor, since previous work has suggested a non-linear relationship between group size and survival (Brouwer et al. 2006). For subordinates, we included the proportion of seasons (0-1) in which the individual was observed helping across the sampling period. We also included time between samples in models of ΔRTL (mean years ± SE dominants: 3.58 ± 0.19; subordinates: 1.80 ± 0.17) to account for differences in telomere change according to the length of the sampling period - this is a preferable approach to modelling telomere change as a rate (Spurgin et al. submitted). For each individual, values across multiple years were considered as a single independent data point, so there was no need to correct for individual identity or year of sampling; however we did include territory identity as a random effect as some individuals in our dataset lived in the same territory.

5.4 Results

5.4.1 Dominants

After controlling for significant effects of tarsus length, time of capture and month of capture (Table 5.2a), dominant mass increased with per-capita territory quality (Table 5.2a, Fig. 5.1a) but did not vary with group size (Table 5.2a, Fig. 5.2a). Dominant mass initially increased, then decreased, with age (significant quadratic term, Table 5.2a). Males were heavier than females, but we found no interaction between sex and either group size or per-capita territory quality.

Dominant ΔRTL increased linearly with mean group size (Table 5.2b, Fig. 5.3a) – mean group size^2 had no significant effect (Table 5.2b). Individuals who experienced higher mean per-capita territory quality across the sampling period also experienced less telomere loss (Table 5.2b, Fig. 5.3b), but ΔRTL did not vary with age, sex or time between samples (Table 5.2b). We found no interactions between sex and mean group size, mean group size^2 or mean per-capita territory quality. Dominant survival to the year following final sampling was not related to any of the predictors, although older individuals were slightly more likely to die before the following season (P = 0.07, Table 5.2c). There were no interactions between sex and group size, group size^2 or per-capita territory quality.

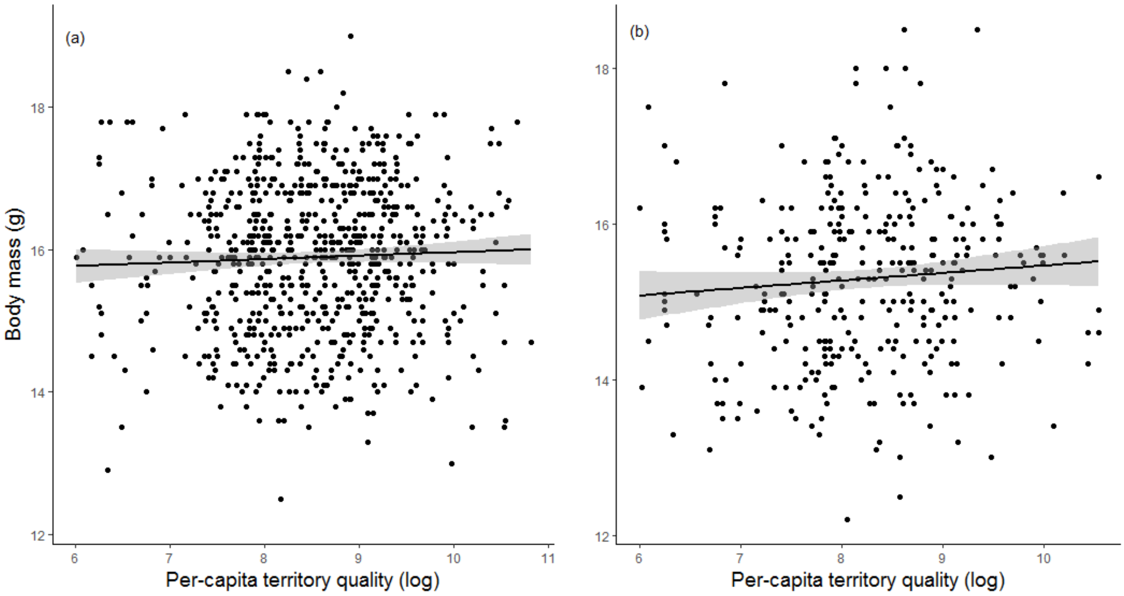


Figure 5.1 Relationship between body mass and per-capita territory quality in (a) dominant and (b) subordinate Seychelles warblers. Dots represent raw values, line and shading represent estimates and standard error from a linear regression.

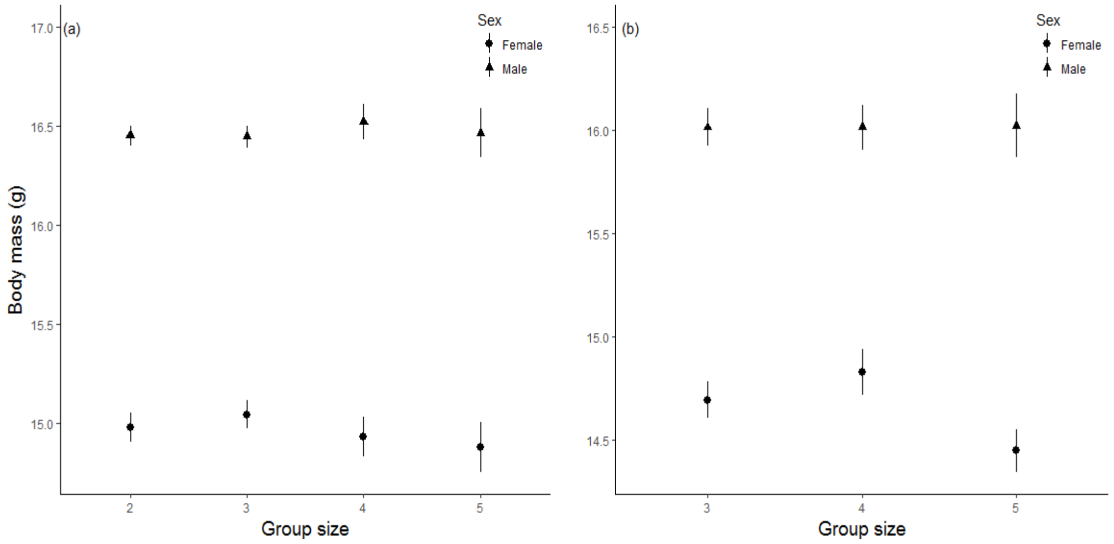


Figure 5.2 Relationship between body mass and group size according to individual sex in (a) dominant and (b) subordinate Seychelles warblers. In dominants, neither males nor females differ in mass according to group size, whereas in subordinates, females in groups of five or more are lighter than those in smaller groups. Dots and lines show mean and standard error per group, respectively.

Table 5.2 Predictors of (a) body mass, (b) change in relative telomere length (ΔRTL) and (c) survival in dominant Seychelles warblers. *F* and *P* values for main effects of categorical variables are reported from an ANOVA. Significant predictors are in bold font. Main effects are reported from models without interactions, linear terms are reported in the absence of non-significant quadratic terms.

Response	Predictor	*F*	Estimate ± SE	*P*
(a) Body mass	**Tarsus length**		**0.29 ± 0.05**	**<0.01**
(*n* = 417)	**Time of capture**[1]	**4.07**		**0.02**
	- Midday		0.08 ± 0.07	0.22
	- Late		**0.22 ± 0.06**	**<0.01**
	Month of capture		**0.09 ± 0.02**	**<0.01**
	Sex[2]		**1.01 ± 0.10**	**<0.01**
	Age		**0.12 ± 0.03**	**<0.01**
	Age ^ 2		**<-0.01 ± <0.01**	**<0.01**
	Per-capita territory quality		**0.08 ± 0.04**	**0.03**
	Group size[3]	1.91		0.13
	- 3		0.09 ± 0.06	0.13
	- 4		0.07 ± 0.09	0.40
	- 5+		-0.15 ± 0.12	0.22
	Tarsus length * sex		0.13 ± 0.10	0.18
(b) ΔRTL	**Mean group size**		**0.14 ± 0.06**	**0.01**
(*n* = 141)	**Mean per-capita territory quality**		**0.12 ± 0.06**	**0.04**
	Age		-0.02 ± 0.02	0.20
	Sex[2]		-0.09 ± 0.07	0.21
	Mean group size ^ 2		0.05 ± 0.05	0.31
	Time between samples		<0.01 ± 0.02	0.63
(c) Survival	Age		-0.10 ± 0.06	0.07
(*n* = 141)	Mean per-capita territory quality		-0.21 ± 0.31	0.49
	Sex[2]		-0.21 ± 0.36	0.57
	Mean group size		-0.02 ± 0.29	0.94
	Mean group size ^ 2		0.02 ± 0.28	0.96
Reference groups	[1] ‘Early’ [2] ‘Female’ [3] ‘2’			

5.4.2 Subordinates

After controlling for significant effects of tarsus length, time of capture and month of capture (Table 5.3a), per-capita territory quality had a positive effect on subordinate mass (Fig. 5.1b). There was no main effect of group size on subordinate body mass, but subordinates from groups of 5 or more were slightly lighter than those from groups of 3 (Table 5.3a) but not 4 (Supplementary Table S5.1). However, this relationship was mainly true for female subordinates (significant sex * group size interaction: Table 5.3a, Fig. 5.2b). Subordinate body mass scaled quadratically with age (Table 5.3a). Non-helpers were heavier than helpers and males were heavier than females (Table 5.3a). There was no interaction between sex and per-capita territory quality.

Subordinate ΔRTL was negatively related to age, but did not vary according to group size, sex or any other variables (Table 5.3b). There were also no interactions between sex and mean group size, mean group size^2 or per-capita territory quality. Subordinate survival was not related to any of the predictors (Table 5.3c) and we found no interactions between sex and group size, group size^2 or per-capita territory quality.

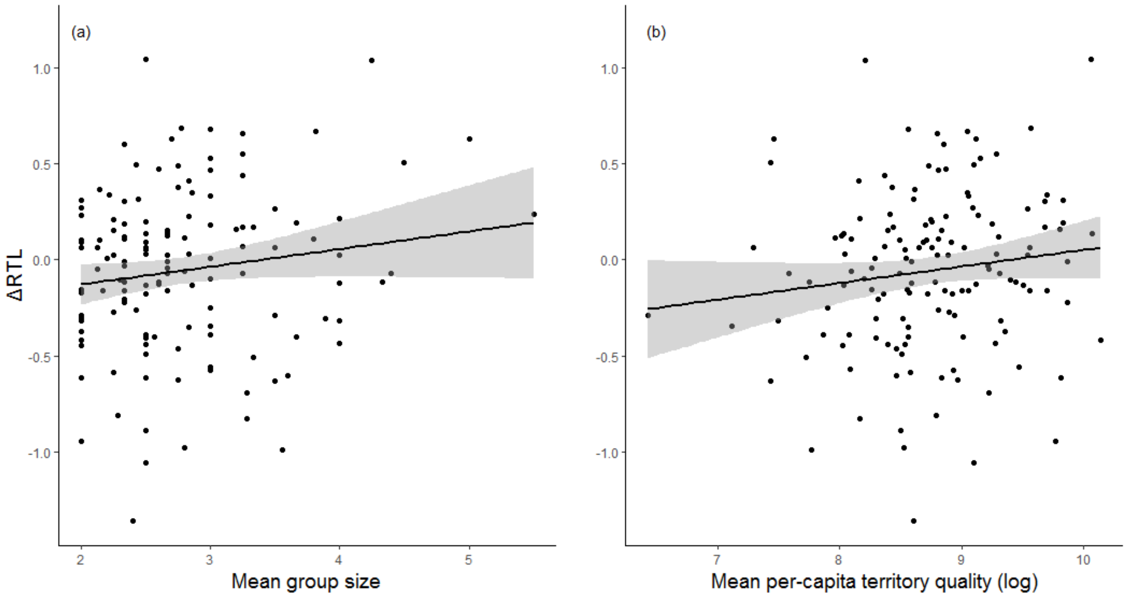


Figure 5.3 Relationship between ΔRTL and (a) mean group size and (b) mean per-capita territory quality in dominant Seychelles warblers. Lines and shading represent fitted values and standard error from a linear regression, dots represent raw values.

Table 5.3 Predictors of (a) body mass, (b) change in relative telomere length (ΔRTL) and (c) survival in subordinate Seychelles warblers. *F* and *P* values for main effects of categorical variables are reported from an ANOVA. Significant predictors are in bold font. Main effects are reported from models without interactions, linear terms are reported in the absence of non-significant quadratic terms.

Response	Predictor	*F*	Estimate ± SE	*P*
(a) Body mass	**Tarsus length**		**0.29 ± 0.07**	**<0.01**
(*n* = 323)	**Time of capture**[1]	**14.18**		**<0.01**
	- Midday		**0.27 ± 0.10**	**0.01**
	- Late		**0.56 ± 0.11**	**<0.01**
	Month of capture		**0.16 ± 0.04**	**<0.01**
	Sex[2]		**0.85 ± 0.14**	**<0.01**
	Age		**0.37 ± 0.10**	**<0.01**
	Age ^ 2		**-0.03 ± 0.01**	**0.01**
	Non-helper[3]		**0.25 ± 0.10**	**0.01**
	Group size[4]	2.12		0.12
	- 4		-0.04 ± 0.10	0.69
	- 5+		**-0.26 ± 0.13**	**0.04**
	Per-capita territory quality		**0.14 ± 0.06**	**0.04**
	Tarsus length * sex		**-0.32 ± 0.13**	**0.01**
	Sex * group size		4: 0.04 ± 0.19	0.85
			5: 0.58 ± 0.23	**0.01**
(b) ΔRTL	**Age**		**-0.19 ± 0.08**	**0.02**
(*n* = 48)	Proportion of seasons as helper		0.35 ± 0.21	0.11
	Sex[2]		0.17 ± 0.14	0.23
	Mean group size		-0.10 ± 0.10	0.35
	Time between samples		0.06 ± 0.07	0.37
	Mean group size ^ 2		-0.09 ± 0.14	0.55
	Mean per-capita territory quality		-0.03 ± 0.09	0.76
(c) Survival	Sex[2]		-0.71 ± 0.69	0.31
(*n* = 48)	Age		-0.26 ± 0.26	0.31
	Mean group size		-0.27 ± 0.47	0.57
	Proportion of seasons as helper		0.54 ± 1.14	0.64
	Mean per-capita territory quality		0.13 ± 0.45	0.79
	Mean group size ^ 2		<-0.01 ± 0.72	0.99

Reference groups
[1] 'Early'
[2] 'Female'
[3] 'Helper'
[4] '3'

5.5 Discussion

In social species, individuals gain many benefits from living in groups (Whitehouse and Lubin 2005). However, depending on the degree to which group members must compete for limited resources or spend energy on agonistic interactions, group living may also incur physiological costs (Krause and Ruxton 2002). In this study, we tested whether the costs of living in large groups vary with social status and sex. We found that all individuals suffered from low per-capita resource availability regardless of social status, although subordinates seemed to pay slightly higher costs when food was limited. Only subordinate individuals suffered a cost of increasing group size and this cost was more severe for female than male subordinates. Below, we discuss these results with respect to the hypotheses and predictions outlined in Table 5.1.

5.5.1 Hypothesis I: costs of group living arise through direct competition for food

When food is limited, stronger individuals may gain preferential access to resources (e.g. Alanärä et al. 2001; Cafazzo et al. 2010). We therefore predicted that per-capita territory quality would have a larger influence on subordinate costs than on dominant costs (Table 5.1). The relationship between mass and per-capita food availability was, although significant, rather weak in both dominants and subordinates (Fig 5.1), but the relationship was slightly stronger for subordinates (Tables 5.2a & 5.3a). We interpret this result as, at best, partial support for the direct cost hypothesis: dominants are only slightly (if at all) more robust to increased competition for food than subordinates. However, we had predicted a much stronger effect of social rank on vulnerability to food shortage. One possible explanation for the fact that dominants seem to suffer (almost) as much as subordinates may be that it is simply too difficult for dominants to monopolise food. Seychelles warblers take their insect prey from the undersides of leaves that are distributed throughout the territory (Komdeur 1992) and, since vegetation typically covers the whole territory, subordinates displaced from a food patch may be able to forage in other areas. Importantly, controlling subordinate access to food may leave dominants with less time to forage themselves (Caraco 1979), especially when resources are evenly distributed to begin with. Resources tend to be spread across territories in many social species; lack of ability to control access to food may therefore be an important mediator of group living in social species more generally (Johnstone et al. 2002). An alternative, but not mutually exclusive, explanation is that within-group relatedness increases the indirect fitness benefits of sharing food. Many social species, including the Seychelles warbler (Richardson et al. 2002), form predominantly family-based groups (Brown 1987; Emlen 1995) who may be less inclined to withhold resources from offspring or other relatives (Hamilton 1964; Ekman et al. 2000). It seems plausible that a combination of both these mechanisms explain the relative parity of resource-based costs of group living among dominant and subordinate Seychelles warblers.

Under the hypothesis that direct competition for food drives costs of group living, we also predicted that females, who tend to be lighter and smaller than males, would suffer more when food became scarce (Table 5.1). However, we found no evidence of sex differences with respect to per-capita territory quality in dominants or subordinates. Perhaps the relative size difference between males and females does not constitute a genuine difference in competitive ability or aggression. Female Seychelles warblers instigate territorial chases as often as males (Kingma et al. under review) and are regularly observed fighting with both territory intruders and members of the same social group (*pers. obs.*), suggesting that they are capable of initiating antagonistic behaviours. However, the lack of sex differences can also be explained according to the principles of resource dispersion and nepotism outlined above – even if females are weaker competitors, they are unlikely to suffer disproportionately as long as resources are difficult to defend.

5.5.2 Hypothesis II: costs of group living arise through social stress

Studies of stress hormone excretion have shown that the cost of dominance status depends on the amount of energy that must be spent on maintaining that status (reviewed in Goymann and Wingfield 2004). Factors such as group size and the number of subordinate individuals, which presumably reflect the likelihood of challenges to dominance and associated aggression, have also been shown to drive variation in costs for dominant individuals (Rubenstein and Shen 2009). Under the hypothesis that costs of group living arise through social stress, we therefore predicted that dominant Seychelles warblers would experience greater costs in large groups where they have to control more subordinates. However, our results point towards the opposite: in the short term, dominant body mass was unrelated to group size (Fig. 5.2a) and over the entire sampling period, dominant individuals who lived in large groups experienced less telomere shortening (Fig. 5.3a). Clearly, we have no evidence that dominants pay a higher cost to their social status when they must control a greater number of subordinates.

Dominants may not suffer in large groups for two reasons. First, we suggest that the positive influence of group size on dominant telomere dynamics might be an artefact of differences in individual quality. The majority of subordinate Seychelles warblers are retained offspring from previous breeding attempts (Komdeur 1992; Kingma et al. 2016); higher-quality dominants who are able to invest more in maintaining somatic condition may also be more successful breeders. Large group sizes would then simply be a reflection of that success – such a mechanism quickly becomes self-fulfilling (Kokko et al. 2001; Kingma et al. 2014). Second, dominants may benefit from larger group sizes because a greater number of subordinates increases the likelihood of receiving help in raising offspring, allowing dominants to reduce their own reproductive workload (Brown 1978; Heinsohn 2004) and minimise oxidative damage or telomere loss. In support of this, previous work in the Seychelles warbler has shown that helpers can lighten the load of dominants (Komdeur 1994). We suspect that a combination of these two mechanisms drive the positive effects of group size on dominant Seychelles warbler telomere dynamics and suggest that, if dominants experience any costs associated with increased need for social control, they are outweighed by the reproductive benefits of retaining subordinates.

We predicted that agonistic interactions with dominants could have important consequences for subordinates, but the directional effect of group size on subordinate costs could be positive or negative depending on the mechanism underlying social stress (Table 5.1). On one hand, subordinates in larger groups might experience less aggression from dominants because dominant aggression is diluted over a greater number of subordinates (Rubenstein and Shen 2009). One the other hand, subordinates may experience more aggression in larger groups because subordinate reproduction in such groups may jeopardise the dominants' own reproduction (e.g. Pettay et al. 2016) or survival (Brouwer et al. 2006). Our results partially support the latter of these two mechanisms - female, but not male, subordinates were lighter in groups of 5 or more (Fig 5.2b). This suggests that female subordinates living in large groups are subject to more agonistic interactions than male subordinates. Supporting the idea that benefits of philopatry might be relatively low for females in larger groups, recent work has shown that subordinates from larger groups are more likely to be evicted, while female subordinates are also more likely to perform extra-territorial forays than their male counterparts (Kingma et al. 2016). Our results suggest that the benefits of philopatry in large groups might indeed be smaller for females, a conclusion that might partly explain the female-biased dispersal (Eikenaar et al. 2008) and foraying behaviour (Kingma et al. 2016) reported in this species.

5.5.3 Comparing physiological measures of cost

Our three components of physiological condition provided different information about the costs of group living for individuals of different social status. We were able to detect differences in body mass according to components of group living in both dominants and subordinates (Tables 5.2a & 5.3a), but territory quality and group size only seemed to influence telomere dynamics in dominants (Table 5.2b). Telomere shortening occurs more rapidly under higher levels of oxidant molecules (Von Zglinicki 2002), which accumulate in response to heightened metabolism and reduced antioxidant capacity (Finkel and Holbrook 2000). One interpretation of our results is therefore that, while per-capita territory quality and group size appear to be important predictors of subordinate mass, the subsequent long-term somatic damage caused by group living are minimal. However, it is important to note that, because Seychelles warblers usually occupy subordinate positions in early adulthood before gaining a breeding position (Komdeur et al. 2016), the period of time over which we measured subordinate telomere change was necessarily small (mean ± SE length of sampling period for dominants: 3.58 ± 0.19, subordinates: 1.80 ± 0.17). This reduced sampling period may also be compounded by the fact that our sample size for subordinate telomere change was smaller than that for dominant telomere change (n = 48 vs 141 samples), although we have been able to detect telomere loss with respect to other components of the social environment elsewhere with a similar sample size (Bebbington et al. under review; Chapter 6 of this thesis). The lack of resolution to detect telomere change over such a small sampling period may explain our finding that subordinate telomere dynamics do not vary according to group living, but it would be extremely interesting to test this in species where the duration of subordinate tenures is longer.

While previous work demonstrated an effect of group size on total lifespan (Brouwer et al. 2006) we were unable to detect any short-term survival differences according to group properties, either in dominants or subordinates. We suspect that one reason for the discrepancy between previous and current findings is that costs of group living are not great enough to produce significant short-term survival differences between individuals. It is possible that our survival measure suffers from the same issue as subordinate telomere dynamics – perhaps effects would be detected over a longer sampling period. Nonetheless, group living presumably remains stable because the associated benefits drive selection for individuals who remain in groups of optimal size (Alexander 1974). It is therefore perhaps not surprising that we failed to find short-term survival differences in our population.

5.6 Conclusions

In this study, we measured three components of physiological condition in Seychelles warblers of different social status and sex in order to test whether costs of group living arise through direct competition for food or through social stress. Our results provide only very weak support for the hypothesis that subordinates may suffer greater costs due to reduced ability to compete for food. While dominants appeared to benefit from living in large groups, our results suggest that subordinate females suffer when living in large groups, possibly as a result of aggression from other group members. These findings highlight the importance of considering the mechanisms driving differences in costs of group living in social species and suggest that differences in the benefits of group membership can have important implications for dispersal behaviour and population structure.

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5.8 Supplementary information

Supplementary Table S5.1 Post-hoc model outputs showing the differences between categories of group sizes with respect to dominant and subordinate Seychelles warbler body mass.

Status	Contrast	Estimate ± SE	*P*
Dominants	3 vs 4	<0.01 ± 0.08	0.97
	3 vs 5+	-0.21 ± 0.12	0.08
	4 vs 5+	-0.20 ± 0.13	0.11
Subordinates	4 vs 5+	-0.22 ± 0.13	0.09

Chapter 6

Kinship and familiarity mitigate costs of social conflict between neighbours

A version of this manuscript is under review at *Proceedings of the National Academy of Sciences USA*

A Seychelles warbler displays aggressively in preparation for a territorial dispute. Photo by S Walsh.

6.1 Abstract

Since virtually all organisms compete with others in their social environment, mechanisms that reduce conflict between interacting individuals are crucial for the evolution of stable families, groups and societies. Here, we tested whether costs of social conflict over territorial space between Seychelles warblers *Acrocephalus sechellensis* neighbours are mitigated by kin-selected (genetic relatedness) or mutualistic (social familiarity) mechanisms. By measuring longitudinal changes in individuals' body mass and telomere length, we demonstrate that the fitness costs of territoriality are driven by a complex interplay between relatedness, familiarity, local density and sex. Physical fights were less common at territory boundaries shared between related or familiar males. In line with this, male territory owners gained mass when living next to related or familiar males, and also showed less telomere attrition when living next to male kin. Importantly, these relationships were strongest in high-density areas of the population. Males also had more rapid telomere attrition when living next to unfamiliar neighbours, but mainly when relatedness to those neighbours was also low. In contrast, neither kinship nor familiarity influenced body mass or telomere loss in female territory owners. Our results indicate that resolving conflict over territorial space through kin-selected or mutualistic pathways can reduce both immediate energetic costs and permanent somatic damage, thus providing an important mechanism to explain fine-scale population structure and cooperation between different social units across a broad range of taxa.

6.2 Introduction

In nature, conflict between individuals occurs because organisms are selected to pursue selfish interests that rarely align with those of the individuals with whom they interact. If there are indirect genetic benefits of promoting genes shared with a relative, conflict can be mitigated by kinship (Hamilton 1964). However, conflict resolution can also be achieved if interacting individuals gain direct benefits (either mutualistic or reciprocal) from cooperating with each other (Trivers 1972).

Understanding whether and how conflict is resolved within families, groups and societies is important for understanding a wide range of behavioural phenomena (Clutton-Brock 2009), but it is often unclear if and when kin-selected or mutualistic pathways to conflict resolution are important in animal societies. The respective influences of kinship and mutualistic benefits on conflict resolution could be simultaneous or even interactive (Ward and Hart 2003); both could also be dependent on other aspects of the social environment (Clutton-Brock 2002). Furthermore, the degree to which these processes mitigate the observed costs of conflict may depend on the timescale over which those costs are measured. The benefits of reduced conflict between individuals may only be observable after repeated interactions over a long period of time (Lehmann and Keller 2006).

One situation where both kin-selected and mutualistic processes might play important interacting roles is in the resolution of conflict over territorial space. Territoriality is widespread throughout the animal kingdom (Stamps 1994) and – unlike frequently-studied situations like cooperative hunting, breeding or vigilance, where conflict is seemingly resolved – is not specific to a particular social system or population structure. Territory boundary defence is costly (Stamps and Buechner 1985), so cooperative maintenance of a boundary should be beneficial as it reduces the need for costly policing. Kin selection could help prevent escalated conflict over territory boundaries, but there is also a well-established mechanism by which mutualistic benefits between interacting individuals could resolve conflict. The “dear enemy” phenomenon predicts that conflict is lower between familiar neighbours (Fisher 1954). This is because it is less costly to maintain territory boundaries with existing neighbours, with whom agreements about space use have already been reached, than renegotiate territory boundaries with new neighbours (Getty 1987). Thus, familiarity between neighbours can be considered one form of mutualism-based conflict resolution.

The costs of territoriality do indeed appear to be moderated to some extent by both kinship and familiarity. Individuals living in close proximity to kin in “kin neighbourhoods” (Dickinson and Hatchwell 2004) often have higher reproductive success, which has largely been attributed to reduced aggression towards the offspring of related neighbours (e.g. Mappes et al. 1995; MacColl et al. 2000; Lee et al. 2009; reviewed in Hatchwell 2010). There is also evidence that familiarity between neighbours decreases territorial aggression (Eason and Hannon 1994) and improves reproductive success (Beletsky and Orians 1989; Grabowska-Zhang et al. 2011). However, several unanswered questions hinder our understanding of conflict resolution with respect to territoriality. First, the respective influences of kinship and familiarity are rarely studied simultaneously in the same system, so conclusions about their relative importance in minimising territorial conflict, and the degree to which they interact, are missing. Second, it is unclear whether the influence of kinship and familiarity depend on other aspects of the social environment, such as the intensity of conflicts and level of local competition. Third, remarkably little is known about the effect of territorial conflict on fitness-linked physiological markers of individual condition. Without information about physiological costs of social conflict, it is difficult to make inferences about the overall benefit that conflict resolution between neighbours has for individual fitness.

Here, we investigate how kinship and familiarity act and interact with each other as well as the broader social environment to influence the immediate and long-term physiological costs of territorial conflict in the cooperatively breeding Seychelles warbler *Acrocephalus sechellensis*. In this species, dominant breeding pairs (accompanied by up to 5 subordinate individuals) occupy stable year-round territories which the pair vigorously defend against conspecific intruders (Komdeur 1992; Kingma et al. 2016). The relatively long lifespan of this species (mean = 5 years, Hammers et al. 2015) and stability of territories in space and time (Komdeur 1992) means that dominant territory owners usually occupy a territory for several consecutive years and hence repeatedly interact with the same neighbours. However, breeder displacement (Richardson et al. 2007) or death, and the occasional appearance and disappearance of territories, also creates temporal variation in the social neighbourhood. This variation allows us to examine longitudinal changes in individual physiological condition in response to changing levels of conflict over territory boundaries.

We first quantify variation in conflict at territory boundaries by investigating the occurrence of physical fights between focal individuals and their neighbours in relation to kinship and familiarity. Previous work has shown intraspecfic aggression at boundaries to be an important component of territorial behaviour in this species (Komdeur and Edelaar 2001; Kingma et al. 2016). We then test whether and how properties of the social neighbourhood, including neighbour density, genetic relatedness and social familiarity, interact to influence three fitness-related components of physiological cost in focal individuals. We measure (i) ΔMass (change in body mass between two sampling points), as a function of change in the social neighbourhood between the same two points. The use of body mass as a linear measure of physiological condition can be problematic in species where flight manoeuvrability is linked to predation risk (Lima 1986, but see also Walters et al. 2017). However, adult Seychelles warblers have no predators (Komdeur and Kats 1999) and body mass has been linked to various other components of physiological condition (van de Crommenacker et al. 2011a,b), suggesting that this measure is a useful indicator of current territorial costs. (ii) ΔRTL, (change in focal individual telomere length) as a function of mean social neighbourhood properties over the sampling period. Telomere length is a widely-used bioindicator of somatic stress (Epel et al. 2004) that predicts survival in the Seychelles warbler (Barrett et al. 2013) and has also been shown to reflect the accumulation of somatic damage (Bebbington et al. 2016a; Chapter 4 of this thesis) arising through heightened metabolic costs and oxidative stress (von Zglinicki 2002). (iii) Survival to the year directly following the sampling period in relation to mean neighbourhood properties over the sampling period.

6.3 Materials and methods

6.3.1 Field data

We collected data from the population of ca. 250 adult Seychelles warblers living on ca. 110 territories on Cousin Island, Seychelles, which has been subject to intensive individual-level monitoring since 1997 (Komdeur et al. 2016). In this period the population has been the source of two conservation-based translocations to other islands (in 2004 [Richardson et al. 2006] and 2011 [Wright et al. 2014]). In order to exclude potential disruption of the population density and resource availability after these translocations, here we focus exclusively on the period between 2006 and 2010, during which translocation-related population disturbance was minimal. Across the five years of the study, fieldwork was conducted during the main breeding season (June-September) and, in some years, the minor breeding season (January-March). Each season, as many birds as possible were caught using mist nets and, if not already ringed, given a unique BTO metal ring and three colour rings for individual identification. A ca. 25 µl blood sample was taken at each catch. Body mass (to 0.1 g), tarsus length (to 0.1 mm) and time of catch (early: 06.30-11.00 h; midday: 11.00-15.00 h; late: 15.00-18.00 h) were also recorded and used to calculate residual body mass, which we used in analyses of immediate physiological condition (see below). This sampling regime is identical to that used in previous years of the long-term study, meaning that ca. 96% of independent birds in the population were already ringed and genotyped at the start of the current study (Richardson et al. 2001; Hammers et al. 2015).

Our dataset contained longitudinal samples from 58 focal breeding males and 38 focal breeding females (henceforth focal males and focal females, respectively) that were initially caught (within this study period) between 2006 and 2008, with at least one repeat catch by 2010. We monitored the presence of each focal individual in the population after their final catch. Since dispersal from the island is virtually absent (Komdeur et al. 2004) and resighting probability extremely high, focal individuals could be confidently assumed dead if they were not seen for two successive main breeding seasons after their final catch (Brouwer et al. 2006; Barrett et al. 2013).

In every main breeding season, each territory was surveyed to determine the identity of the dominant breeding pair (easily recognised through courtship and pair behaviour) and the presence of any subordinate birds living in the territory (Komdeur 1992). We distinguished between males and females based on molecular sexing (see below). Groups forage exclusively within their own territory and defend territory boundaries from conspecifics, meaning that we could accurately identify these boundaries based on behavioural observations (Komdeur 1992, Kingma et al. 2016). During surveys of territory boundaries and group composition, we also opportunistically observed physical fights at boundaries. Fights are extremely fast and hard to follow but, where possible, we recorded the identity of the birds involved in the fight and the boundary at which the fight occurred. Under this sampling regime, the likelihood of observing a fight increases with the amount of time spent surveying a territory, but surveying effort is relatively equal across territories, so we do not expect this to bias our analysis. Seychelles warblers are insectivorous and territory quality is measured in each main breeding season as insect density per unit of foliage in each territory, following Brouwer et al. (2006). In each main season, we produced a map specifying each territory's location (Supplementary Fig. S6.1), drawn based on a grid system of static poles that cover the island at 50m intervals (Eikenaar et al. 2008) and georeferenced in ArcMap 10.3. Spatial and territorial data were only collected in the main seasons due to a lack of resolution in the (relatively brief) minor seasons but, since territories are relatively stable (Komdeur 1992), data collected in the main breeding season are likely to be a good representation of the year-round territorial environment.

6.3.2 Molecular data

DNA was extracted from blood samples with a Qiagen DNeasy blood and tissue kit and used to determine sex following Griffiths et al. (1998), and individual genotypes at a panel of 30 microsatellite markers already developed for the Seychelles warbler (Richardson et al. 2001; Spurgin et al. 2014). We compared the suitability of two pairwise relatedness estimators, Queller and Goodnight (QG) (Queller and Goodnight 1989) and Lynch and Ritland (Lynch and Ritland 1999), in the R package 'related' (Pew et al. 2015) and determined that QG was most suitable in our microsatellite panel (Supplementary Table S6.1; Supplementary Fig. S6.2). QG relatedness estimates between all dominant breeders in the population over the study period (including all focal individuals and all neighbouring breeders) were calculated in GenAlEx 6 (Peakall and Smouse 2006). Pairwise relatedness has previously been shown to reflect pedigree relatedness in the Seychelles warbler (Richardson et al. 2004) and heterozygosity across our microsatellite panel is also known to reflect genome-wide heterozygosity (Bebbington et al. 2016a; Chapter 4 of this thesis).

We measured telomere length in each blood sample according to the protocol described in detail elsewhere (Barrett et al. 2013; Bebbington et al. 2016a; Chapter 4 of this thesis). Briefly, we calculated a relative measure of telomere length as the concentration of telomeric DNA relative to that of a normalizer gene, GAPDH, using quantitative real-time PCR. We then calculated each individual's change in relative telomere length over the sampling period (henceforth referred to as ΔRTL) as the difference between telomere length at first and final sampling, such that positive values indicate increases in telomere length and negative values indicate decreases in telomere length.

6.3.3 Defining neighbourhood properties

We used the territory map produced in each main season to define a focal individual's "social neighbourhood", which consisted of the dominant breeding male and female owners (henceforth male and female neighbours) of all territories that directly bordered part of the focal individual's territory (Supplementary Fig. S6.1). We then calculated five parameters of the social neighbourhood. The first parameter was associated with the expected intensity of territorial interactions: neighbour density, calculated as the number of territories in the social neighbourhood (which is unrelated to territory size; see Supplementary Fig. S6.3). The second and third parameters were associated with kinship: relatedness to male neighbours and relatedness to female neighbours, calculated as the mean pairwise genetic relatedness between the focal individual and all other dominant males and females, respectively, in the neighbourhood. The final two parameters were associated with familiarity: number of new male neighbours and number of new female neighbours, calculated as the number of male and female neighbours, respectively, that did not occupy the dominant position in their territory in the previous year and hence were new in the focal individual's social neighbourhood. If the focal bird was itself newly dominant in that year, we considered all its neighbours to be new. We calculated these five variables separately for each main season, but also calculated the mean male and female relatedness values and total number of new male and female neighbours across the longitudinal sampling period for each focal individual.

6.3.4 Statistical analyses

For a subset of focal individuals (*n* = 19 focal males and 16 focal females) that we were able to positively identify taking part in a single territorial fight at a boundary, we tested whether relatedness to and familiarity with the neighbours being fought differed from that expected by chance. To investigate the effect of kinship, we used paired t-tests to compare the relatedness between the focal individual and the owner of the territory where the fight took place against the focal individual´s mean relatedness to all other neighbouring territory owners (that were not observed fighting with the focal individual), separately for each sex. To investigate the effect of familiarity, we performed a chi-square goodness-of-fit test to determine whether the proportion of fights observed between new neighbours (observed value) was greater than the population-wide mean proportion of new neighbours per social neighbourhood (expected value).

We examined variation in immediate and long-term territoriality costs as a function of kinship and familiarity, creating separate models for focal males and females throughout in order to determine whether social neighbourhood properties have differential influences on the sexes. Collinearity between all variables was checked prior to modelling using variance inflation factors (VIF). In no case was the VIF large enough to cause issues in the analysis (all VIF < 4, [Dormann et al. 2012]). We used a model selection approach to determine which properties of the social neighbourhood influenced residual body mass, telomere dynamics and survival. Using the package MuMIn (Bartoń 2011) in R (version 3.3.1, R Core Team 2016), we created a global mixed-model that contained all variables, plus selected interactions of interest, as standardized predictors so that both main effects and interactions could be interpreted (Grueber et al. 2011). We also included two to three random effects: in all models we included the age of the focal individual at first sampling to account for age-related differences in physiological measures; in models of residual body mass and telomere dynamics we included the number of years between samples (mean ± SE = 1.70 ± 0.07, range = 1-4) and in the model of male residual body mass we also included individual identity, as some males had multiple measures of body mass change. We report conditional averages of each parameter across the top model set, which contained all models where delta AICc ≤ 2 (Burnham and Anderson 2002). Complete outputs for top models sets are presented in the Supplementary information (Table S6.2).

To measure the immediate influence of the social neighbourhood on individual costs, we tested whether the change in residual body mass (ΔMass) was related to changes in each of the five social neighbourhood properties between the same two points. To determine whether the influence of kinship and familiarity depends on the intensity of territorial interactions, we also tested whether the change in these components had varying effects according to whether neighbour density decreased or increased (change in relatedness or change in number of new neighbours * change in neighbour density). To investigate the interplay between kinship and familiarity, we tested whether change in the number of new neighbours had a varying effect on ΔMass according to whether the focal individual became more or less related to those neighbours (change in relatedness * change in number of new neighbours, separately for male and female neighbours).

To measure the long-term influence of the social neighbourhood on individual costs, we tested whether (i) telomere change (ΔRTL) and (ii) survival to the year following the final sample (binomial response) were related to the mean of each of the five social neighbourhood properties across the sampling period. We also included two covariates: the focal individual´s mean territory quality (accounting for environmentally-induced differences in physiological costs [van de Crommenacker et al. 2011a]) and mean group size (the number of independent resident birds in the territory, accounting for potential effects of social support on territoriality costs [Earley et al. 2006]) across the sampling period. We tested for interactions between neighbour density and each of the other social neighbourhood properties. We also tested whether the number of new neighbours had a differential effect on ΔRTL and survival depending on mean neighbour relatedness (mean relatedness * number of new neighbours, separately for male and female neighbours).

The spatial nature of our data posed a risk of non-independence: if two focal individuals lived in adjacent territories, they would be included in each other’s neighbourhood and hence have the potential to influence each other. There is also the potential for spatial autocorrelation between the social neighbourhood and undetected environmental factors. We therefore tested whether similarity in individual body mass and telomere dynamics was related to the spatial proximity of two individuals. Using ArcMap 10.3, we calculated the centre point of each territory using the spatial map of 2006 as a template, and calculated the distance in meters between each of these centre points. Using the “ncf” package in R, we calculated Moran’s I (Moran 1950) and significance values of the residuals of regression models of each response variable on all neighbourhood properties. Moran’s I was not significantly different from zero for the residuals of any of the predictor variables (Supplementary Fig. S6.4) and a visual inspection of the distribution of neighbourhood properties across the island did not reveal any spatial grouping of neighbourhood relatedness, familiarity or neighbour density (Supplementary Fig. S6.5), so we conclude that spatial structure is unlikely to be influencing the results of our analyses.

6.4 Results

6.4.1 Physical aggression between neighbours

Focal males were significantly more likely to fight at borders where they were less related to the male neighbour (Table 6.1, Fig. 6.1a), but also fought with new neighbouring males more often than expected by chance (Table 6.1, Fig. 6.1b). The likelihood of focal males fighting at boundaries was not related to relatedness or familiarity between the focal male and neighbouring females (Table 6.1). Focal female fights did not vary with the relatedness or familiarity of their neighbours of either sex (Table 6.1).

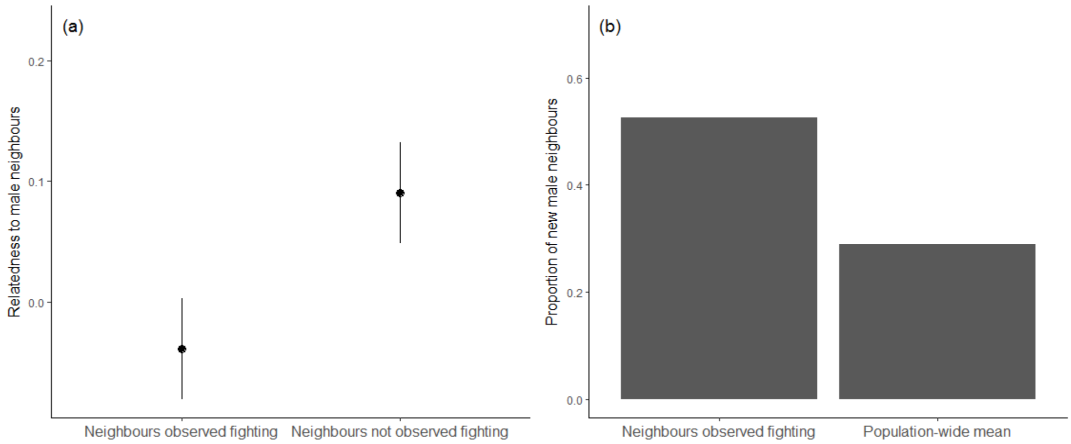

Figure 6.1. Territorial fights involving focal male Seychelles warblers in relation to kinship and familiarity among neighbours. (a) Focal males are significantly less related to male neighbours that own a territory where a fight was observed (n = 19) than they are to other male neighbours in their social neighbourhood. Dots and bars represent mean pairwise relatedness and standard error, respectively. (b) The proportion of observed fights (bars) that involved new neighbours (n = 10 of 19 fights) is significantly greater than the population-wide mean proportion of new neighbours (n = 210 of 735 neighbours) in the social neighbourhood.

6.4.2 Immediate influences of the social neighbourhood: body mass

Among males who experienced an increase in neighbour density, those who became more related to their male neighbours gained more mass than those that became less related (Table 6.2a, Fig 6.2a, c). Males who experienced an increase in the number of new neighbours (i.e. neighbour familiarity decreased) lost more mass, but again only when total neighbour density increased (Table 6.2a, Fig. 6.2b, d). The top model set for focal male ΔMass also contained changes in all five properties of the social neighbourhood as main effects, but none apart from neighbour density were significant predictors of ΔMass (Table 6.2a).

Table 6.1 The influence of neighbour relatedness and familiarity on the likelihood of Seychelles warblers being observed fighting at a territory boundary. Relatedness was examined using a paired t-test and familiarity was examined using a chi-squared test (see methods). Bold font indicates a significant difference between neighbours that were observed fighting and those not observed fighting.

Focal individual sex	Neighbourhood property	*t* (relatedness) or χ^2 (familiarity)	df	*P*
Male (*n* = 19)	**Relatedness to fought male neighbour vs. other male neighbours**	**2.88**	**18**	**0.01**
	New vs. familiar male neighbour	**5.15**	**1**	**0.02**
	New vs familiar female neighbour	<0.01	1	0.98
	Relatedness to fought female neighbour vs other female neighbours	2.04	18	0.06
Female (*n* = 16)	Relatedness to fought male neighbour vs other male neighbours	0.24	15	0.82
	New vs familiar male neighbour	0.00	1	0.99
	New vs familiar female neighbour	0.05	1	0.83
	Relatedness to fought female neighbour vs other female neighbours	0.06	15	0.95

Focal female ΔMass did not vary with changes in any of the social neighbourhood properties. The top model set contained a single predictor, change in neighbour density, but this was not significant (Table 6.3a).

6.4.3 Long-term influences of the social neighbourhood: telomere dynamics and survival

Among focal males living at high neighbour densities, relatedness to male neighbours had a positive influence on ΔRTL (Table 6.2b), but this was not the case for males living at medium and low neighbour densities (Fig. 6.3). The effect of new male and female neighbours on focal male ΔRTL varied with relatedness to those neighbours (Table 6.2b). Males who experienced a higher number of new male and female neighbours experienced more telomere shortening, but mainly when relatedness to those neighbours was low (Fig. 6.4). Individuals who lived in smaller groups across the sampling period experienced more telomere shortening (Table 6.2b). The top model set also contained all five properties of the social neighbourhood as main effects, but none of these were significant predictors of ΔRTL (Table 6.2b).

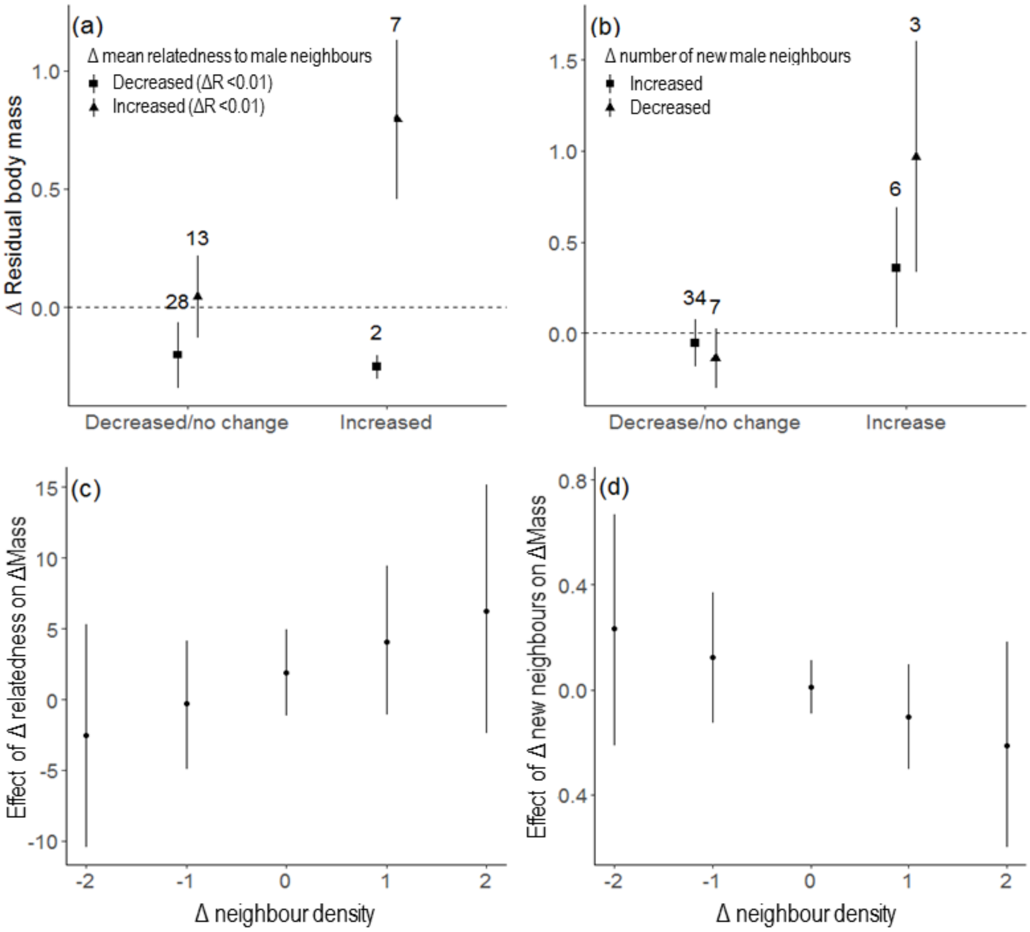

Figure 6.2. Interacting effects of relatedness, familiarity and neighbour density on ΔMass of focal male Seychelles warblers (*n* = 50). In the top row, ΔMass is more positive when male neighbours become more (a) related or (b) familiar (the number of new male neighbours decreases), but only when neighbour density also increases. Dots and error bars represent means and standard errors, with sample sizes per group denoted above each bar. The bottom row displays the same information using continuous data. When neighbour density increases, the conditional effect of (c) relatedness on ΔMass (*y* axis) becomes more positive and (d) new neighbours on ΔMass (*y* axis) becomes more negative.

ΔRTL of focal females was not related to any properties of the social neighbourhood. The top model set included neighbour density, number of new male and female neighbours, relatedness to female neighbours, mean territory quality and mean group size, but none of these predictors were significant (Table 6.3b). Relatedness to male neighbours and all interaction terms were absent from the top model set.

In the year directly following final sampling, 40% of focal males and 24% of focal females in our dataset had died. However, neither focal male nor focal female survival was predicted by any of the social neighbourhood properties across the sampling period. In focal males, relatedness to male neighbours and number of new male neighbours were present in the top model set, but not significant (Table 6.2c). In focal females, the number of new male neighbours, neighbour density and relatedness to male neighbours were in the top model set, but again not significant (Table 6.3c).

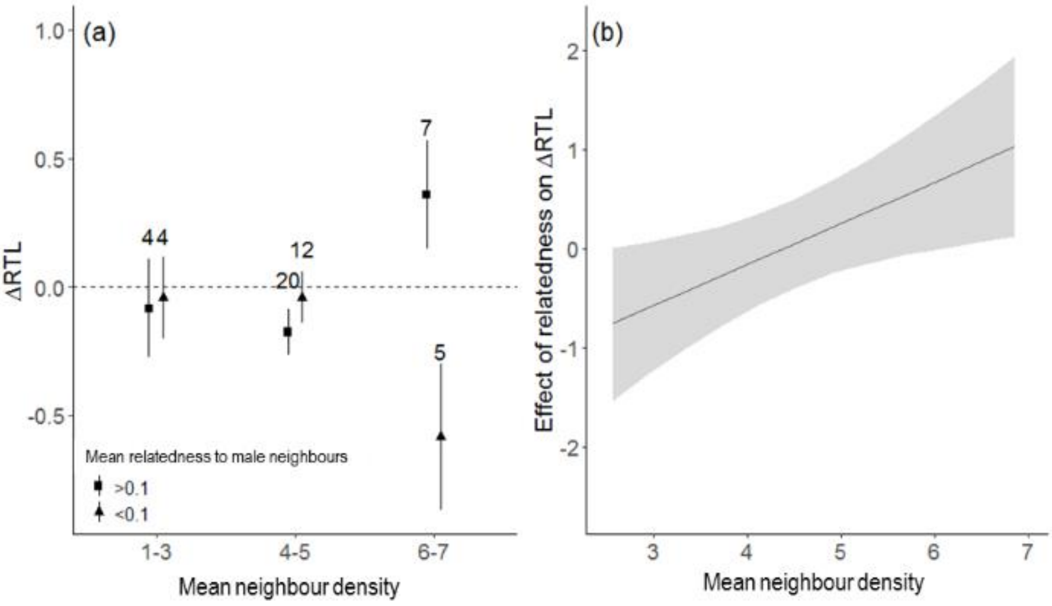

Figure 6.3. Interacting effects of relatedness to male neighbours and neighbour density on telomere change (ΔRTL) in focal male Seychelles warblers (n = 52). In (a), ΔRTL is more positive when relatedness to male neighbours is high, but only at high neighbour density. Dots and error bars represent means and standard errors, with sample sizes per group denoted above each bar. In (b), the conditional effect of relatedness on ΔRTL (*y* axis) becomes more positive as mean neighbour density increases.

Table 6.2 Model-averaged estimates and relative importance (RI) of social neighbourhood properties in relation to (a) change in residual body mass, (b) change in telomere length and (c) survival of focal male Seychelles warblers. Significant predictors are in bold, variables not included in the top model set are not reported. Sample sizes vary as not all measured variables were available for all individuals and are therefore given separately for each model.

Response	Predictor	Estimate ± SE	RI	*P*
(a) ΔMass (*n* = 50, 8 models in top set)	**Change in neighbour density**	**0.47 ± 0.22**	**0.90**	**0.04**
	Change in relatedness to male neighbours * change in neighbour density	**0.95 ± 0.40**	**0.12**	**0.02**
	Change in number of new male neighbours * change in neighbour density	**-1.40 ± 0.63**	**0.12**	**0.03**
	Change in number of new male neighbours	-0.68 ± 0.35	0.45	0.06
	Change in number of new female neighbours	0.69 ± 0.39	0.56	0.08
	Change in relatedness to male neighbours	0.40 ± 0.23	0.47	0.09
	Change in relatedness to female neighbours	-0.23 ± 0.21	0.10	0.30
(b) ΔRTL (*n* = 52, 8 models in top set)	**Group size**	**0.35 ± 0.12**	**1.00**	**<0.01**
	Relatedness to male neighbours * neighbour density	**0.66 ± 0.23**	**0.22**	**<0.01**
	Relatedness to male neighbours * number of new male neighbours	**0.40 ± 0.16**	**0.29**	**0.02**
	Relatedness to female neighbours * number of new female neighbours	**0.67 ± 0.30**	**0.13**	**0.02**
	Relatedness to female neighbours	0.16 ± 0.12	0.31	0.19
	Number of new male neighbours	-0.18 ± 0.14	0.56	0.20
	Relatedness to male neighbours	-0.14 ± 0.13	0.51	0.29
	Number of new female neighbours	-0.09 ± 0.12	0.13	0.46
	Neighbour density	<0.01 ± 0.12	0.22	0.99
(c) Survival (*n* = 58, 3 models in top set)	Relatedness to male neighbours	-0.36 ± 0.57	0.23	0.54
	Number of new male neighbours	0.28 ± 0.59	0.21	0.64

Table 6.3 Model-averaged estimates and relative importance (RI) of social neighbourhood properties in relation to a) change in body mass, b) change in telomere length and c) survival in focal female Seychelles warblers. Variables not included in the top model set are not reported. Sample sizes vary as not all measured variables were available for all individuals and are therefore given separately for each model.

Response	Predictor	Estimate ± SE	RI	*P*
a) ΔMass (*n* = 31, 2 models in top set)	Change in neighbour density	0.46 ± 0.35	0.35	0.22
	Intercept	-0.09 ± 0.28	1.00	0.75
b) ΔRTL (*n* = 32, 7 models in top set)	Number of new female neighbours	-0.24 ± 0.17	0.16	0.16
	Neighbour density	-0.23 ± 0.16	0.16	0.18
	Number of new male neighbours	-0.20 ± 0.17	0.12	0.25
	Territory quality	0.18 ± 0.16	0.11	0.29
	Relatedness to female neighbours	-0.16 ± 0.16	0.10	0.34
	Group size	-0.16 ± 0.16	0.10	0.34
c) Survival to next year (*n* = 38, 4 models in top set)	Neighbour density	0.80 ± 0.84	0.22	0.36
	Number of new male neighbours	0.65 ± 0.89	0.18	0.49
	Relatedness to male neighbours	-0.55 ± 0.77	0.17	0.49

6.5 Discussion

Territory boundaries represent an agreement over the division of space but, depending on the degree of conflict between neighbours, can be costly to maintain. In the Seychelles warbler, male territory owners are more often observed fighting at borders shared with unrelated and/or unfamiliar male neighbours. In line with this, males appear to gain an immediate benefit (in terms of increased body mass) of becoming more related to or familiar with male neighbours, but only when the total number of neighbours increases. Males also gain long-term benefits (in terms of reduced telomere loss) from having related male neighbours when living at high densities. Additionally, males lose more telomere repeats when living next to unfamiliar male and female neighbours, but only if these neighbours are not relatives. In contrast, focal females do not appear to respond to neighbour relatedness or familiarity with respect to territorial conflict or the associated physiological costs, suggesting that the social neighbourhood is less important for females than for males. Our findings provide evidence for a complex interplay between kinship, familiarity, sex and other aspects of the social environment that determines the cost of territorial conflict in wild animals. We discuss these results and their implications below.

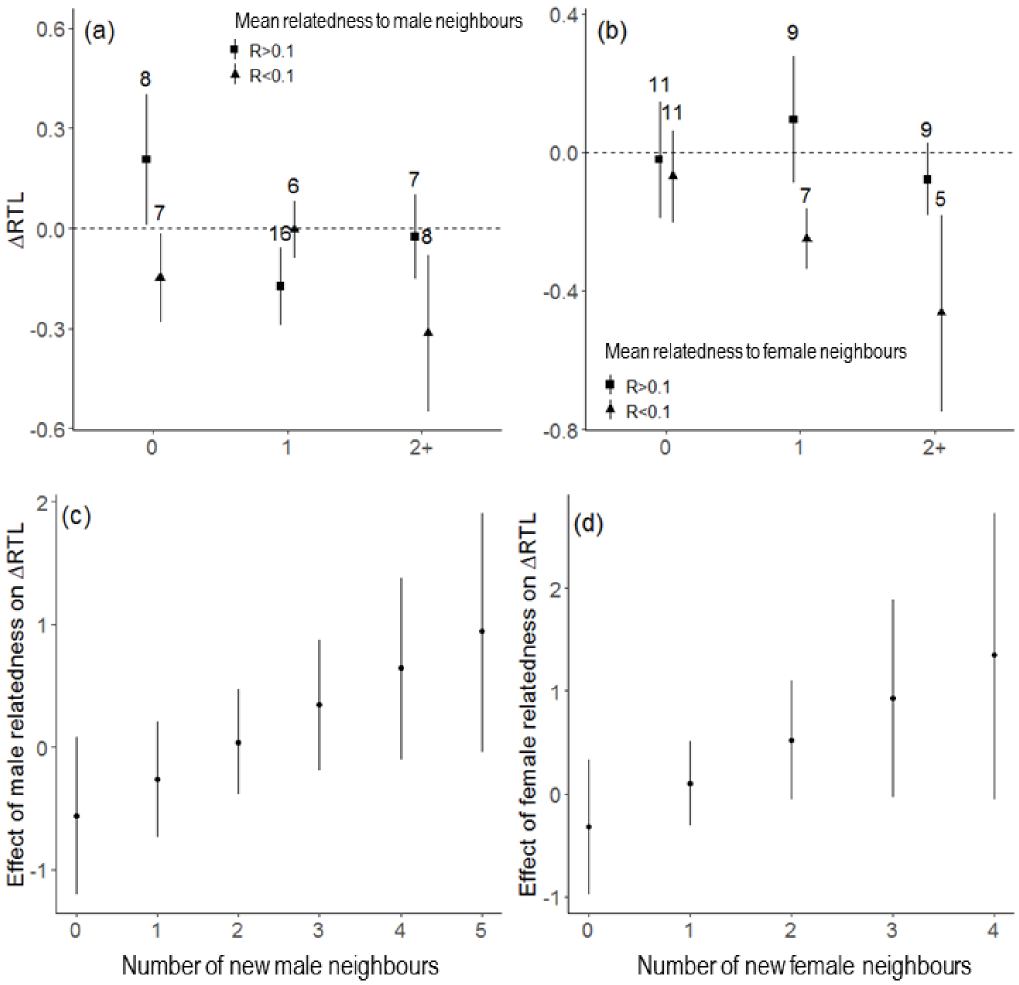

Figure 6.4. Interacting effects of relatedness and familiarity on focal male change in telomere length (ΔRTL) in Seychelles warblers (*n* = 52). In the top row, ΔRTL is more negative when the number of (a) new male neighbours increases or (b) new female neighbours increases, but only when relatedness to those neighbours is low. Dots and error bars represent means and standard errors, with sample sizes per group denoted above each bar. The bottom row displays the same information using continuous rather than binned data. When the number of new (c) male or (d) female neighbours increases, the effect of relatedness to those neighbours on ΔRTL (*y* axis) becomes more positive.

6.5.1 Physical aggression between neighbours

Males were more likely to be observed fighting male neighbours that they were less related to, or that were new in the social neighbourhood (Fig. 6.1). Although this analysis consisted of fairly small sample sizes and should therefore be interpreted with caution, our results support those found in other species. For example, in willow ptarmigans *Lagopus lagopus*, males were more likely to fight unfamiliar neighbours (Eason and Hannon 1994) and were less aggressive towards related neighbours (Watson et al. 1994), while several studies in salmonid fish demonstrate reduced aggression in kin shoals (Brown and Brown 1993a,b; Brown and Brown 1996). It would be extremely interesting to investigate the exact function of kinship and familiarity in this context: are fights less common between cooperating neighbours because each individual is less willing to "cheat" by crossing the agreed boundary, or because cooperating neighbours do not enforce the boundary so strictly, leading to more territory overlap? Evidence from a handful of studies point to the latter, at least with respect to kinship between neighbours (e.g. Mappes et al. 1995; Griffiths and Armstrong 2002; Stoen et al. 2005; reviewed in Hatchwell et al. 2010), but it currently unclear whether territory overlap (accepted cheating) or reduced incentive to trespass (assured cooperation), also occurs between more familiar neighbours.

6.5.2 Immediate benefits of neighbour kinship and familiarity

Given that body mass reflects an individual's physiological state in the Seychelles warbler (van de Crommenacker et al. 2011b), as is broadly assumed across many species (Schulte-Hostedde et al. 2005, but see [Labocha and Hayes 2012] for limitations of this metric), individuals who spend more time finding food and/or less energy on territorial defence should have greater mass. We found that males who experienced an increase in either relatedness to, or familiarity with, male neighbours gained mass (Fig. 6.2). These patterns suggest an immediate within-individual response to changes in levels of territory boundary conflict. Somewhat similar results have been reported in zebrafish *Danio rerio*, in which juveniles grew to a greater size when shoaling with familiar kin than with unfamiliar non-kin (Gerlach et al. 2007). In the current study, both familiarity and relatedness play independent roles in mitigating territorial costs. However both these relationships were present only when focal males had a high number of neighbours – this result is intuitive under the assumption that territorial costs, and hence the degree to which kinship and familiarity can be influential, accumulate with the number of boundaries to maintain.

6.5.3 Long-term benefits of neighbour kinship and familiarity

Telomere shortening is exacerbated by the damaging effect of oxidants that arise in the body as a result of metabolic processes and other factors (von Zglinicki 2002). In wild animals, more rapid telomere shortening can arise through poor internal state (Bebbington et al. 2016a; Asghar et al. 2015; Bebbington et al. 2016b; Chapters 2 and 4 of this thesis) or suboptimal environmental conditions (e.g. Hall et al. 2004; Boonekamp et al. 2014) and is known to predict survival in the Seychelles warbler (Barrett et al. 2013) and other species (Haussmann and Marchetto 2010). We found that focal males experienced less telomere attrition when they were more related to their male neighbours, but only when they had many neighbours (Fig. 6.3). We suggest that exacerbated telomere shortening results from the physiological stress of continued conflict with neighbours, which reduces the amount of time and resources that can be spent creating antioxidant defences and maintaining somatic condition more generally. Males who are in greater conflict with neighbours over territory boundaries must spend more time patrolling borders, singing and engaging in physical competition; it seems logical that the degree to which these costly activities hinder somatic maintenance depends on the number of borders to maintain. In the same way that body mass appears to reflect variation in territorial conflict in the immediate term, telomere dynamics appear to reflect more long-term somatic damage. Perhaps more intriguing is the finding that neighbour familiarity had different influences on telomere dynamics according to the focal male’s relatedness to his neighbours (Fig. 6.4). High numbers of new male and female neighbours were associated with greater telomere shortening, but in both cases high neighbour relatedness appeared to mitigate that relationship. This complex interaction between relatedness and familiarity illustrates the difficulty of separating kin-selected and non-kin-selected pathways to cooperation: depending on the relative strength of each mechanism, one may obscure the other.

Each new neighbour has a unique set of spatial demands depending on its resource requirements, competitive ability and personality traits, and these require potentially costly renegotiation of the territory boundary (Getty 1987). If the new neighbour is a relative, negotiation costs may be less severe as the neighbour’s interests are likely to be more aligned with that of the focal individual. If not, the focal individual will benefit most by keeping that neighbour for as long as possible in order to avoid constant renegotiation (which is potentially harmful and costly) with new neighbours. Selection for maintenance of relationships with familiar competitors is known more formally as the “dear enemy” phenomenon (Fisher 1954). With regard to the current study, we suggest that dear enemy-type interactions are responsible for the observed relationships between neighbour familiarity and both body mass and telomere dynamics.

Despite convincing evidence that the social neighbourhood influences both body mass and telomere dynamics, we found no effect of any neighbourhood properties on the short-term survival of either focal males or focal females. This is perhaps not surprising given the stochasticity and potential confounds inherent to survival measures (Miller and Coltman 2014) and the fact that our relatively small sample size may only be sufficient to detect very strong survival differences. It is also possible that a relationship between territorial cooperation and survival would manifest over longer timespans: in this study we considered the effect of the social neighbourhood on survival over a specific period of an individual's life, but survival-based consequences of heightened territorial conflict may manifest over entire lifetimes. Given the established association between telomere length and survival in the Seychelles warbler (Barrett et al. 2013), it can nonetheless be reasonably concluded that the observed physiological stress associated with heightened territorial conflict detrimentally impacts individual fitness.

6.5.4 Genetic and social relatedness measures

Evidence to date suggests that kin discrimination in birds is based on indirect cues of relatedness, such as matching phenotype (e.g. recognising similarity in song) or spatial location (e.g. assuming individuals in a natal territory are kin) (Komdeur and Hatchwell 1999). Unfortunately we were unable to accurately assess social relatedness in the current dataset; we therefore used genetic pairwise relatedness to infer kinship between focal individuals and their neighbours. However, high levels of extra-pair paternity in this species (44% [Komdeur et al. 2016]) lessen the extent to which genetic relatedness matches social relatedness. This raises the question of why we found that genetic relatedness between neighbours affects not only their propensity to fight, but also the degree to which they benefit from each other. We envisage two possible reasons: (i) Seychelles warblers use indirect phenotypic and spatial cues to discriminate kin, but the effect of perceived (social) relatedness on territorial costs is so strong that we were still able to detect it using genetic relatedness; or (ii) Seychelles warblers use genetic cues to determine kinship and are responding directly to genetic relatedness. Previous work in the Seychelles warbler suggests that relatedness is estimated using association cues (Richardson et al. 2003; Komdeur et al. 2004), suggesting that the former is more likely. However, there is some evidence in other bird species for direct use of allelic similarities to discriminate kin, especially in regard to odour recognition (Bonadonna and Sanz-Aguilar 2012; Krause et al. 2012). It would be extremely interesting to repeat the analyses we describe here with measures of social relatedness, both in this and other social species.

6.5.5 Sex-differences in costs of territoriality

In all components of our investigation, we found that territorial costs are mediated by kinship and familiarity for males, but not for females. Focal female body mass and telomere length did not vary with any properties of the social environment and females did not appear to fight more with certain types of neighbour (although the small sample size admittedly limits our interpretation of this result). In addition, focal male territoriality costs appear to be much less dictated by the identity of female neighbours than that of male neighbours, at least in terms of immediate costs. Interestingly, our finding that only males seem to respond to the social environment fits with the pattern of female-biased dispersal distance previously reported in the Seychelles warbler (Eikenaar et al. 2008) – perhaps selection favours reduced dispersal distance in males in order to promote kinship with neighbours.

6.6 Conclusions

The degree to which kin selection and mutualistic benefits can resolve conflicts between interacting animals remains one of the most intriguing puzzles in evolutionary biology. In Seychelles warblers, we show that it is not only possible for both these pathways to reduce the costs of conflict over space, but also that they can interact with each other and with the social environment to differentially affect costs of territoriality. We also show that the benefits of peaceful boundaries can even influence the rate at which an individual accumulates physiological damage (as measured through telomere attrition), suggesting that interactions between neighbours have the potential to significantly affect fitness and population structure. Our results also highlight the importance of sex differences in determining how mitigating conflict can be beneficial, and suggest a complex interplay between kinship and familiarity that can help explain the causes and consequences of behavioural conflict more generally.

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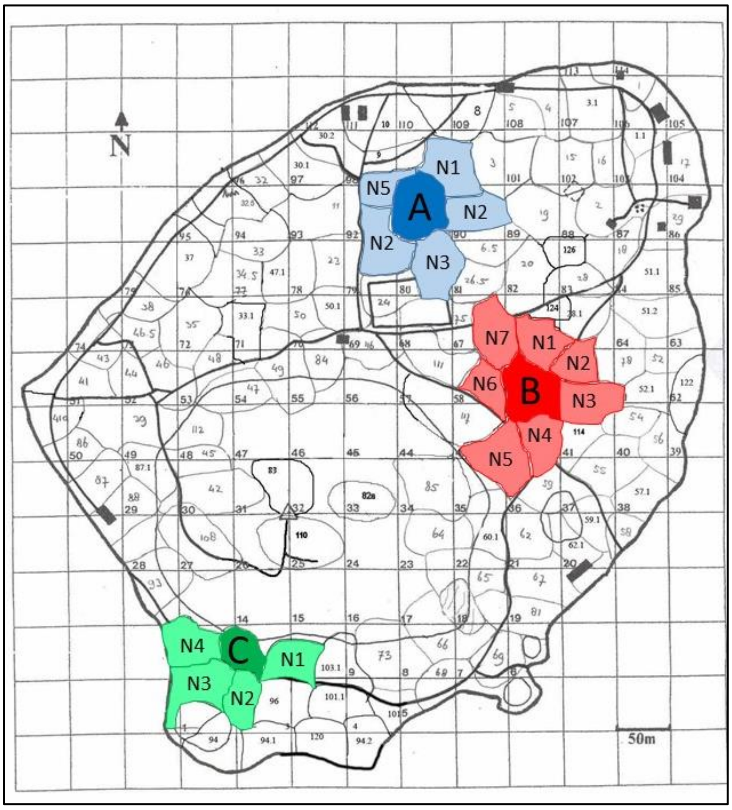
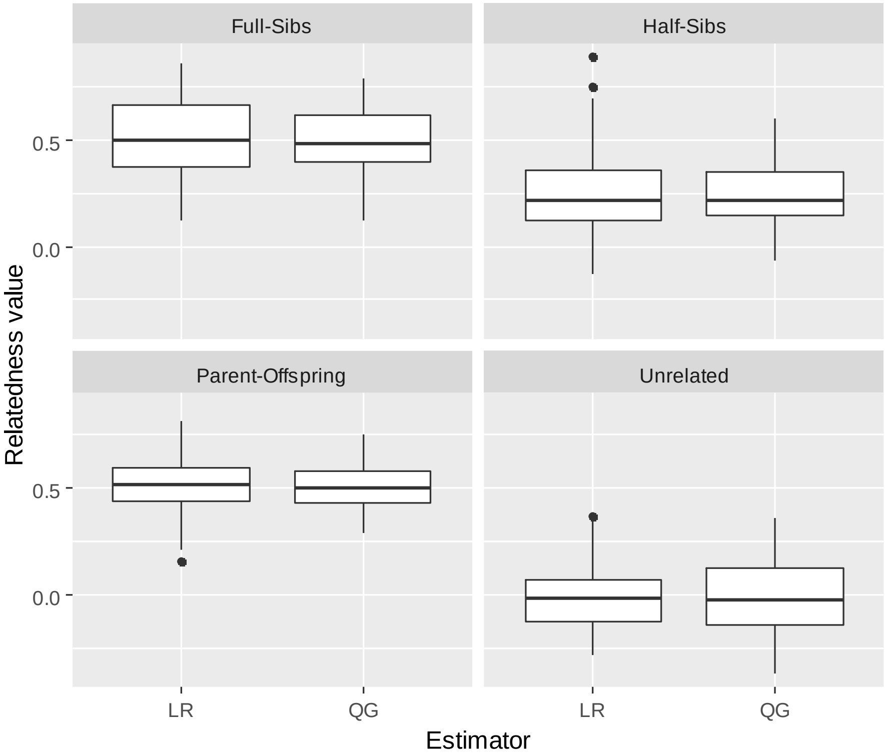
6.8 Supplementary information



Supplementary Figure S6.1. Example of a spatial territory map of the Seychelles warbler population on Cousin Island during the 2006 main season, showing the distribution of territories across the island and the method by which a "social neighbourhood" was defined. In this example, three focal individuals were sampled in their respective territories A, B, and C and the territories that border part of the focal territory (highlighted in blue, red and green respectively) make up that individual's social neighbourhood in the given season.

Supplementary Table S6.1 Correlation coefficients between observed and expected relatedness values for two relatedness estimators, LR (Lynch & Ritland 1999) and QG (Queller and Goodnight 1989). To obtain the correlations, we simulated 100 pairs of individuals using an input file that contained the genotypes of all male Seychelles warblers in our dataset (n=168) in the R package 'related' (Pew et al. 2014) and used the function 'compareestimators' to produce correlation coefficients.

Relatedness estimator	Reference	Correlation coefficient
LR	Lynch & Ritland (1999)	0.792
GQ	Queller & Goodnight (1989)	0.803



Supplementary Figure S6.2 Box plots comparing the relatedness estimates for pairs of simulated individuals of known relatedness using LR (Lynch & Ritland 1999) and QG (Queller & Goodnight 1989) relatedness estimators.

References

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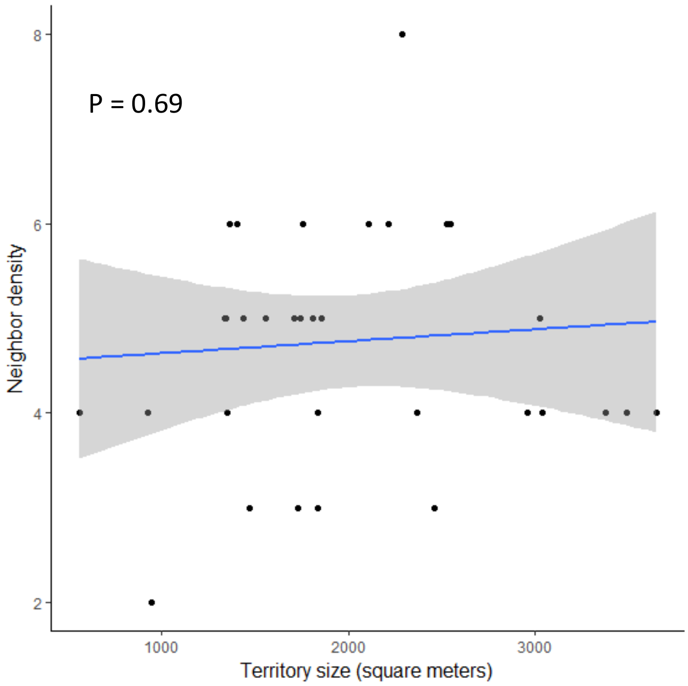
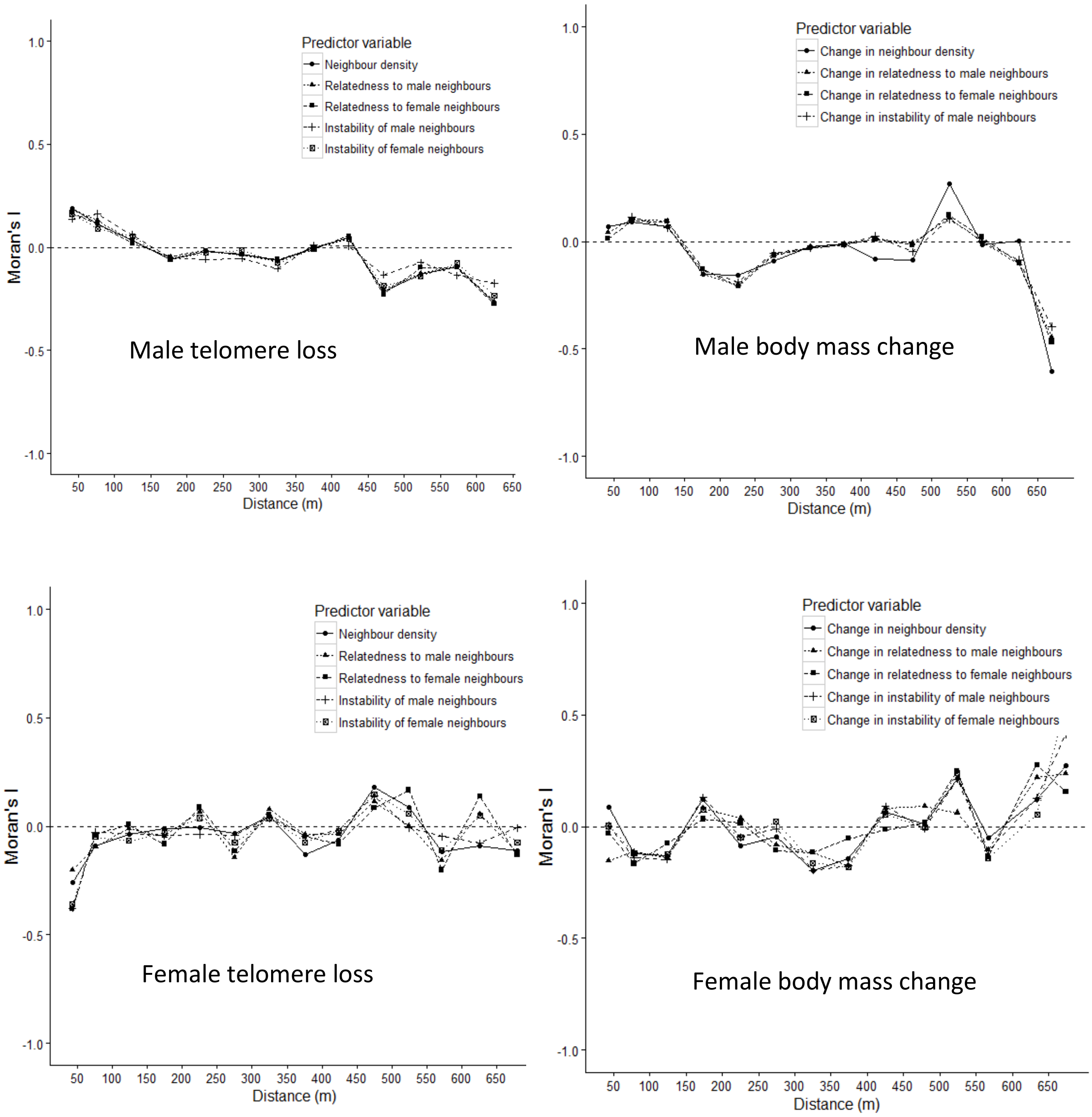
Queller DC, Goodnight KF 1989. Estimating relatedness using molecular markers. *Evolution* 43: 258–275.



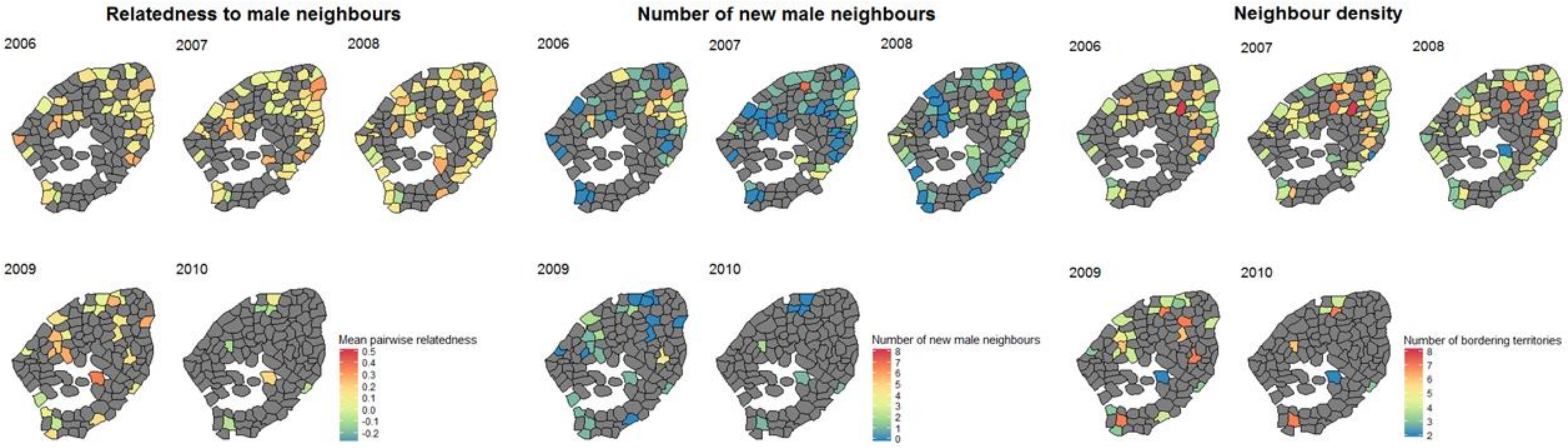
Supplementary Figure S6.3 Correlation between territory size and neighbour density (number of territories in the social neighbourhood) in Seychelles warblers sampled in the 2006 main breeding season (*n* = 34). Larger territories do not have a greater number of bordering territories and hence this relationship is unlikely to influence the reported effects of neighbour density on individual territoriality costs reported in the main text. Dots represent raw data, line and shading represents the predicted slope and 95% confidence limits respectively from a linear regression.

Supplementary Table S6.2 Candidate models derived from global models of territoriality costs according to neighbour relatedness and familiarity in the Seychelles warbler, where AICc ≤ 2. Den = neighbour density, RelM = relatedness to male neighbours, RelF = relatedness to female neighbours, NewM = number of new male neighbours, NewF = number of new female neighbours, GS = mean group size, TQ = mean territory quality.

Analysis	Rank	Model	AICc	ΔAIC_c	w_i
Male Δ body mass	1	ΔDen	125	0	0.06
	2	ΔDen+ΔRelM+ΔNewF+ΔNewM	126	1	0.04
	3	ΔDen+ΔRelM+ΔNewF+ΔNewM+ΔDen*ΔRelM+ΔDen*ΔNewM	126.2	1.3	0.03
	4	ΔDen+ΔRelM	126.4	1.4	0.03
	5	ΔDen+ΔNewF	126.5	1.5	0.03
	6	ΔDen+ΔRelF	126.6	1.6	0.03
	7	ΔRelM+ΔNewF+ΔNewM	126.6	1.6	0.03
	8	ΔDen+ΔNewF+ ΔNewM	126.9	2	0.02
	Null	1	127.1	2.1	0.02
	Global	ΔDen+ΔRelM+ΔRelF+ΔNewM+ΔNewF+ΔDen*ΔRelM+ΔDen*ΔRelF+ΔDen*ΔNewM+ΔDen*NewF+ΔRelM*ΔNewM+ΔRelF*ΔNewF	141.9	16.9	0
Female Δ body mass	Null	1	97.9	0	0.21
	1	ΔDen	99.1	1.2	0.11
	Global	ΔDen+ΔRelM+ΔRelF+ΔNewM+ΔNewF+ΔDen*ΔRelM+ΔDen*ΔRelF+ΔDen*ΔNewM+ΔDen*NewF+ΔRelM*ΔNewM+ΔRelF*ΔNewF	142.2	44.3	0
Male ΔRTL	1	Den+GS+TQ+RelF+RelM+NewF+NewM+RelM*NewM	71.2	0	0.05
	2	Den+GS+TQ+RelF+RelM+NewM	71.6	0.4	0.04
	3	GS+RelF+NewF+RelF*NewF	72	0.8	0.04
	4	GS	72.4	1.2	0.03
	5	Den+GS+RelM+Den*RelM	72.4	1.2	0.03
	6	Den+GS+RelM+NewM+Den*RelM	72.5	1.2	0.03
	7	GS+RelF+RelM+NewM+RelM*NewM	72.7	1.5	0.02
	8	GS+RelF	72.9	1.7	0.02
	Null	1	76.8	5.6	0
	Global	Den+GS+TQ+RelF+RelM+NewF+NewM+Den*RelF+Den*RelM+Den*NewF+Den*NewM+RelF*NewF+RelM*NewM	89.7	18.5	0
Female ΔRTL	Null	1	52.5	0	0.08
	1	Den	53.4	0.9	0.05
	2	NewF	53.4	0.9	0.05
	3	NewM	54.1	1.5	0.04
	4	TQ	54.1	1.6	0.04
	5	RelF	54.4	1.9	0.03
	6	GS	54.4	1.9	0.03
	Global	Den+GS+TQ+RelF+RelM+NewF+NewM+Den*RelF+Den*RelM+Den*NewF+Den*NewM+RelF*NewF+RelM*NewM	101.9	49.4	0
Male survival	Null	1	81.7	0	0.14
	1	RelM	83.5	1.8	0.06
	2	NewM	83.7	2	0.05
	Global	Den+GS+TQ+RelF+RelM+NewF+NewM+Den*RelF+Den*RelM+Den*NewF+Den*NewM+RelF*NewF+RelM*NewM	109.9	28.2	0
Female survival	Null	1	45.9	0	0.13
	1	Den	47.3	1.4	0.06
	2	RelM	47.7	1.8	0.05
	3	NewM	47.8	1.9	0.05
	Global	Den+GS+TQ+RelF+RelM+NewF+NewM+Den*RelF+Den*RelM+Den*NewF+Den*NewM+RelF*NewF+RelM*NewM	78	32.1	0



Supplementary Figure S6.4 Correlograms showing the pairwise similarity in model residuals between focal Seychelles warblers as a function of the distance between their respective territories. Model residuals were derived from a linear regression of each neighbourhood property on the response and correlograms of residuals as a function of distance were produced in R package "ncf" and run with 1000 resamples. Distance was arranged into 50m bins, which corresponds with the average territory diameter in this species. Filled circles throughout denote that Moran's I did not significantly differ from zero at any distance class for any combination of response and neighbourhood properties, thus spatial autocorrelation is unlikely to be influencing the results reported in the main text.



Supplementary Figure S6.5 Heat maps showing the distribution of (left to right) variation in relatedness to male neighbours, number of new male neighbours and neighbour density across the population of Seychelles warblers on Cousin Island, 2006-2010.

Appendix

No evidence that kin selection increases the honesty of begging signals in birds

A version of this manuscript is published in *Evolution Letters* doi: 10.1002/evl3.18

The evolution of begging signals is a major source of debate in behavioural ecology. Photo by K Bebbington.

A.1 Abstract

A recently published research article used comparative analyses to demonstrate that competition between offspring and kin selection are important drivers of interspecific variation in the honesty of begging signals in birds. Providing plausible mechanisms to explain this variation is fundamental to our understanding of parent-offspring conflict and the evolution of family life, and evidence for kin-selected honesty has major implications for understanding signalling behaviour more broadly. However, we feel that the evidence for a role of kin selection in this context is still missing. Below, we present a combination of arguments and empirical tests in order to demonstrate an alternative, more parsimonious interpretation of interspecific variation in begging honesty that does not rely on kin selection, but instead is based on established principles of scramble competition.

A.2 A short introduction to offspring begging signals

Across an enormously diverse range of taxa, offspring direct behaviourally complex begging displays towards caregiving parents. The function and evolution of such behaviour has intrigued biologists for decades, spawning a myriad of different explanatory hypotheses that make diverse assumptions about the balance of power between parents and their offspring (Royle et al. 2002), the reliability of information that begging signals convey to parents (Kilner and Johnstone 1997) and the roles of kin selection and competition among offspring (Trivers 1972).

If parents make active choices about how to partition resources within a brood, there are two scenarios where we can expect offspring begging to be an "honest" signal. Firstly, if the cost of expressing a begging signal outweighs the marginal fitness gained by successfully securing parental resources, begging signals should honestly reflect "need" (Godfray 1995). Second, if there is a high risk that not all family members survive to adulthood, such that offspring are selected to boast their own quality and/or parents are selected to invest in the most valuable offspring, begging signals should honestly reflect "quality" (Grafen 1990). However, the degree to which begging behaviours honestly reflect any information seems to vary greatly between species (Mock et al. 2011). While many studies appear to support honest begging (e.g. Redondo and Castro 1992; Andrews and Smiseth 2013), yet others suggest that begging is a form of scramble competition for resources passively allocated by parents to the most conspicuous display (e.g. Smith and Montgomerie 1991; Parker et al. 2002). One possible source of this variation may be interspecific differences in the degree of evolutionary conflict within the family over the allocation of parental resources. Specifically, where high relatedness between family members means that their evolutionary interests in terms of resource allocation are more aligned (Trivers 1974), honesty should prevail. Where evolutionary interests are less aligned, for example when the direct fitness benefit of acquiring resources outweighs the inclusive fitness benefit of sharing them with relatives, honesty becomes suboptimal and scramble competition should be more prevalent (Briskie et al. 1994).

In their recent comparative analysis across avian taxa, Caro et al. (2016) explore interspecific variation in honesty of begging signals in relation to variation in conflict between family members over the allocation of parental care. Caro et al. (2016) first test the hypothesis that begging honesty decreases with increasing competition for parental resources (Mock and Parker 1997). They show convincing evidence that the correlation between begging and need becomes weaker with the presence and increasing number of siblings in both current and future broods. These interspecific patterns provide important validation for the hypothesis that intense offspring competition for limited resources selects for exaggerated, and thus dishonest, begging signals (Royle et al. 2002). Moving onto a second hypothesis, Caro et al. (2016) test whether begging is more honest when relatedness to future offspring, and hence the inclusive fitness benefit of sharing parental resources, is higher (Trivers 1974). According to Caro et al.'s (2016) interpretations, the results they present support this second hypothesis; in doing so, they provide the first empirical evidence that relatedness between competitors can effectively reduce parent-offspring conflict and offer a solution for one of the most widely debated phenomena in behavioural ecology. In the next section, we explain why it is premature to embrace the conclusions of Caro et al. (2016) as evidence for a role for kin selection in this context.

A.3 Estimating the inclusive fitness value of future siblings

When an individual's parents can produce more offspring in the future, inclusive fitness benefits (i.e. the transfer of shared genes to future generations) may favour individuals that adopt strategies that facilitate the production of those offspring. Producing honest signals of current need in order to preserve excess parental resources (i.e. energy or food) for future broods (Trivers 1974) is one potential strategy. How then should we calculate expected inclusive fitness benefits from the perspective of current offspring? In their comparative analysis, Caro et al. (2016) suggest that relatively low inclusive fitness benefits arise when parents do not breed together to produce future broods, as is the case when a) one or both of the parents die, or b) parents divorce. By combining these two measures, Caro et al. (2016) show that offspring begging signals are less honest when parents have a lower likelihood of breeding together in the future, which they interpret as evidence that kin selection drives honesty of begging signals.

Although we agree that the death of one parent indeed reduces future indirect benefits, it is incorrect to assume the same for divorce, and we therefore question whether this conclusion is correct. As demonstrated in Fig. A.1, divorced parents will both go on to produce half-siblings, with a total inclusive fitness value equal to that produced when remaining together. In fact, the inclusive fitness benefits gained from offspring produced from divorced parents might actually be greater than those from parents who remain together. In another recent comparative study, Culina et al. (2015) show that divorce generally improves a parent's subsequent reproductive success, suggesting that offspring in species with high divorce rates should be under greater selection to beg honestly.

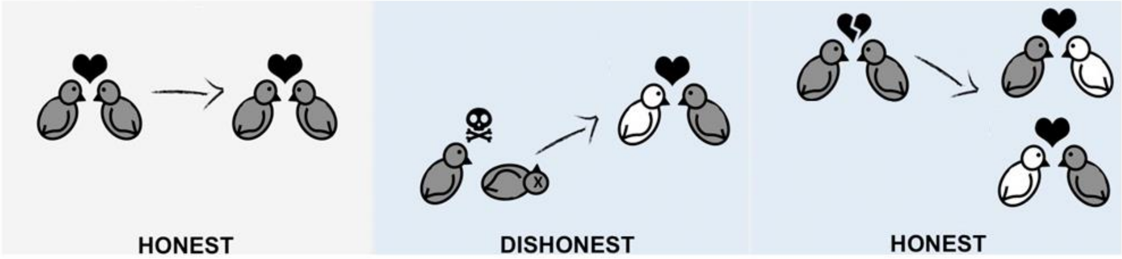


Figure A.1 Modified after Fig. 4 in Caro et al. (2016). Kin selection predicts that offspring should be honest about their need when parents are likely to produce full siblings in future (left-hand panel). If this is the case, the death of one parent (middle panel) should promote offspring dishonesty because of reduced relatedness to future offspring (relatedness = 1 * 0.25). However, we argue that divorce (right-hand panel) does not promote dishonesty in this way (as implied in Fig. 4 in Caro et al. [2016]) because both parents will continue breeding and hence produce two sets of half-siblings, which together have equal or even higher value than one set of full siblings (total relatedness ≥ 2 * 0.25 = 0.5).

Having established that parental divorce is unlikely to reduce the kin-selected incentives for current offspring to beg honestly, we retested the hypothesis that high inclusive fitness benefits of future offspring select for honest signalling. In order to provide a more accurate calculation of inclusive fitness benefits, we disregarded divorce rates and only used the likelihood of both parents surviving to reproduce next season to produce an estimated likelihood of full siblings being produced in future. Data were obtained from Caro et al. (2016), and Phylogenetic Generalized Least Square (PGLS) analyses were implemented in the caper package (Orme et al. 2013) in R 3.3.0 (R Core Team 2016). We accounted for phylogenetic uncertainty by applying the models to a set of 100 equiprobable phylogenetic trees (using the Hackett backbone with all species), obtained from http://www.birdtree.org (Jetz et al. 2012). In contrast to the results reported by Caro et al. (2016), we found no difference in the correlation between begging and need (i.e. begging honesty) according to whether parents are likely to produce full or half siblings in the future (PGLS: β ± SE = -0.026 ± 0.098, t = -0.271, P = 0.787, Fig. A.2). We also were unable to support the conclusion of Caro et al. (2016) if, instead of using the classification of full or half siblings, we tested the effect of absolute probability that both parents survive (range = 2 - 88 %) (PGLS: β ± SE = 0.073 ± 0.2071, t = 0.351 P = 0.727).

As we outline above and Fig. A.1, variation in divorce rates is unlikely to be linked to variation in future inclusive fitness. Since divorce accounted for on average (±SE) 49% ± 4 of the total likelihood that pairs did not breed together the following year (44 species, range = 0 to 99%), it is perhaps not surprising that when we omit divorce rates from the equation we cannot support the conclusion that kin selection plays a role in honest begging. The question remains, however: how then can we explain the relationship found by Caro et al. (2016)? The answer most likely lies in life-history differences that are associated with parental divorce, mortality rates and levels of begging honesty. One such difference may be that species with a faster pace of life (i.e. high mortality and divorce rates) may produce larger clutches in which begging is less honest due to greater competition with current siblings. We assess this idea in the section below.

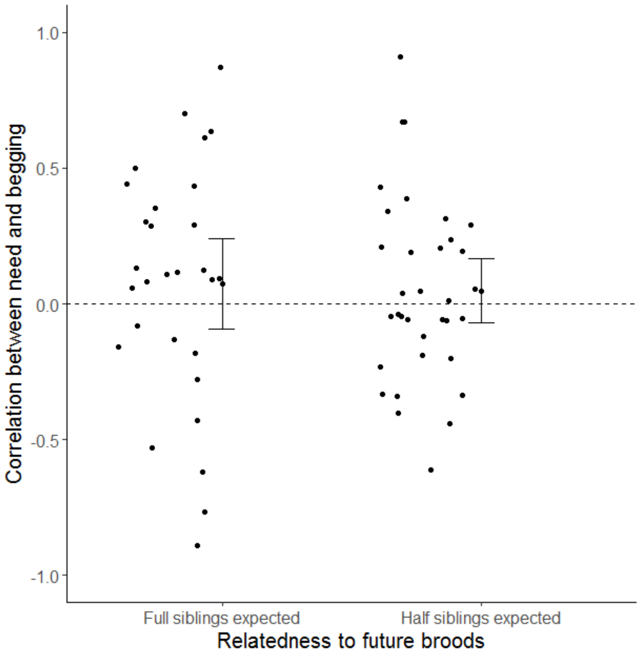


Figure A.2 Relationship between begging honesty (measured as the correlation between begging intensity and need) and relatedness to future broods in 63 bird species. Full siblings are expected when there is <50% chance of at least one parent death before next year (34 species) and half siblings are expected when there is >50% chance of at least one parent death before next year (29 species). Raw data were plotted and error bars represent 95% CIs.

A.4 Beyond kin selection: explaining variation in offspring begging honesty

One of the hypotheses that might explain variation in begging honesty proposes that competition for limited parental resources increases sibling rivalry and decreases honesty (Royle et al. 2002). As outlined above, evidence supporting the competition hypothesis is reported by Caro et al. (2016), who show that begging signals are less honest in the face of competition with co-existing offspring.

We argue that Caro et al.'s (2016) finding that species with greater mortality and divorce rates typically exhibit less honest begging can also be –arguably more parsimoniously - explained in terms of current competition for parental resources. Using the dataset from Caro et al. (2016), we used PGLS analyses (as described above) to test for a correlation between the likelihood that parents reproduce together in future and levels of current offspring competition in terms of clutch size. In line with the predictions of the scramble competition hypothesis, we found that species where parents have a higher probability of breeding together in the following year (calculated as (survival probability)^2 * (1-divorce rate)) produce smaller (log) broods (PGLS: β ± SE = -0.232 ± 0.773, t = -2.920, P = 0.031, Fig. A.3). In other words, the finding reported in Caro et al. (2016) that a low probability of breeding together in the following year leads to dishonest begging may alternatively be explained by the fact that competition in current broods of such species is higher.

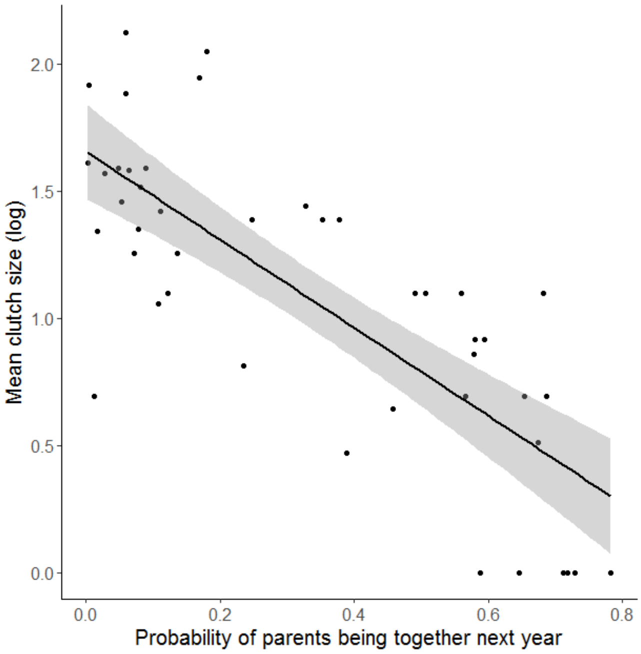


Figure A.3 Relationship between mean clutch size (log transformed) and the probability of parents reproducing together in the next year across 44 bird species. Untransformed raw data were plotted (with the regression line through the raw data) and shaded areas represent 95% CIs.

A.5 Concluding remarks

Interspecific variation in honesty of begging signals is an important source of information to make inferences about how selection acts according to social and ecological circumstances. The frequently-hypothesised role of kin selection in mediating intrafamilial conflict (Trivers 1974; Mock and Parker 1997; Royle et al. 2002; Chapter 3 of this thesis), and thus begging honesty, is intriguing and certainly merits further investigation. However, based on the logic and empirical testing outlined here, we argue that we currently lack any firm empirical evidence that kin selection is important in this context. In conclusion, we propose that the results of Caro et al. (2016) demonstrate convincing evidence that competition for limited resources, rather than kin selection, is the main driver of interspecific variation in the honesty of begging signals in birds. Thus, while kin selection is likely to play an important role in the evolution and stability of family life (Emlen 1995), it is crucial that we account for all sources of variation in inclusive fitness and develop an understanding of species-specific ecology in order to determine the mechanisms by which it acts.

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Chapter 7

General synthesis

"…a particular theory unifies seemingly disparate phenomena and, in effect, tells us to look more widely for patterns that we would not otherwise have sought." Joseph Travis, 2006.

Sunrise over Praslin Island, Seychelles. Photo by K Bebbington.

7.1 Social environments in the Seychelles warbler

In this thesis, I used a wild population of Seychelles warblers *Acrocephalus sechellensis* to test how different components of the social environment influence a suite of fitness-related metrics across individual lifespans. In order to gain a comprehensive understanding of social environmental effects, I also considered how these relationships varied depending on intrinsic individual characteristics and aspects of the physical environment. The combined results of the research presented here provide a clear overall message: the nature of an individual's social environment is important in determining the physiological costs of socially-mediated situations and behaviours. However, the multiple aspects of the social environment (including the abundance of social partners and their relatedness to the individual, relative competitive strength, and sex) exert differential costs on individuals in a complex, interactive manner. Further, the cost of the social environment depends on whether individuals can obtain the resources necessary to minimise, or capitalise on, the effects of its social partners. In this final chapter, I discuss some of the broad trends that persist across different social environments and life stages and provide some directions for future research.

7.1.1 The abundance of social partners

One conspicuous source of variation in the social environment is the number of social partners, or conspecifics with whom the individual interacts. In Chapter 2, I quantified the effect of nestmate presence in early life, under the hypothesis that individuals raised with a competitor suffer physiological costs of sibling rivalry (Mock and Parker 1997). I found clear costs in terms of reduced access to parental resources, reduced body mass and (for weaker competitors) reduced survival to adulthood among nestlings who shared the nest with a competitor. While the clutch size of Seychelles warblers on Cousin (1-2 eggs) provides limited variation in social partner abundance in early life, social environments in adulthood encompass much more variation in this respect. The number of independent Seychelles warblers living on a territory varies from 2-7 (Komdeur 1992); I hypothesised that this variation might be an important mediator of the costs of different social interactions in adult life. In Chapter 2, I found that individuals who grew up in larger groups had shorter reproductive tenures and shorter adult lifespan. In Chapters 5 and 6, I showed that group size has important implications for body mass and telomere length in adulthood, and that the number of extra-group social partners is an important mediator of territorial costs. Taken together, these results suggest that the number of social partners affects individual fitness at multiple levels of social organisation.

In the completely saturated habitat on Cousin Island, the number of social partners probably largely reflects the number of competitors for local resources (Brouwer et al. 2006); the fact that living in a large natal group negatively influences how fledglings perform in adulthood (Chapter 2) is indeed suggestive of poor resource acquisition under competition from a greater number of older group members. However, the fact that dominant individuals benefit from living in larger groups (Chapters 5 and 6) appears to contradict this argument. On Cousin, larger groups tend to occur in higher-quality territories (Brouwer et al. 2006); dominants from larger groups may use their competitive advantage to acquire more resources than those from smaller groups. Alternatively, dominants may benefit from a greater group size because it increases the chance that they will receive help from subordinates (Brown 1978; Heinsohn 2004). The varying effect of social group size across life highlights the importance of distinguishing between individuals at different life stages when considering social environmental effects. However, individual differences in the response to a social environmental factor may also depend on more subtle differences in competitive ability, which I discuss below.

7.1.2 Competitive ability

In early life, competitor presence had a greater influence on the physiological costs of the smaller of two nestmates (Chapter 2), suggesting that relative competitive ability regulates the importance of social partners during development (Drummond et al. 1986). Extending this reasoning to adult social environments, I predicted that dominant and subordinate individuals might experience different costs of group living (Chapter 5). I found that the effect of limited territorial resources had a slightly greater effect on the mass of subordinates than of dominants, which provides tentative evidence that subordinates may suffer more from social competition for food (Clutton-Brock et al. 1998). However, this pattern was less convincing than expected – if dominant individuals can out-compete subordinates, they should suffer no costs to reductions in food availability so long as the amount of food in the territory remains above the threshold dominants require to feed themselves. In Chapter 5, I propose that an even, low-density insect prey distribution throughout the territory might make it difficult for dominants to monopolise food when it becomes scarce (Johnstone et al. 2002). However, an alternative explanation is that high relatedness between group members (Richardson et al. 2002) reduces the degree to which individuals are inclined to exclude others from limited resources. Indeed, the effect of social or genetic relationships between social partners on individual fitness appeared to be important in many aspects of this thesis, as I describe in the next section.

7.1.3 Relationships with social partners

Arguably one of the most important theories to be developed in the context of social behaviour is that of kin selection, which proposes that altruistic behaviour towards related social partners promotes the success of genes shared by descent (Hamilton 1964). In Chapter 6, I demonstrated that males pay lower costs to maintaining borders that they share with relatives, which I interpret as support for kin-selected benefits of territorial cooperation. However, the importance of kinship with regards to other aspects of the social environment seems more equivocal. In Chapter 6 itself, I showed that a male's benefit of having related neighbours was most important when those neighbours were social strangers – in other words, kin-selected benefits are most detectable when there are no other mechanisms in place to reduce potential conflict. In Chapter 3, I hypothesised that the absence of kin-selected benefits of resource sharing in communal nests (where nestlings are unrelated) would lead to greater costs of sibling rivalry, but such costs seemed entirely absent. In the Appendix, I argued that the observed interspecific variation in the honesty of begging signals could be equally well, if not better, explained in terms of variation in resource availability as in terms of kin selection. This argument appears to be supported by the results presented in Chapter 3, where I showed that resource availability, rather than kinship, determined the costs of offspring competition. Lastly, the results presented in Chapter 4 demonstrate one context in which genetic relatedness between social partners is certainly not beneficial – individuals who were related to their mate produced offspring with shorter telomeres.

Kin selection is undoubtedly a powerful driver of group living, behavioural cooperation and other forms of sociality (Eberhard 1975). Considering the genetic relationship between interacting individuals is therefore very important when investigating the effect of social environments on social selection and fitness more generally. However, the results described in this thesis also draw attention to another side of kin selection – interactions between related individuals can lead to costly competition (West et al. 2002). For example, the results of Chapter 3 highlight one situation where it might be more advantageous for parents to raise their offspring in competition with non-kin, provided there are plentiful parental resources, than raising two of their own offspring under competition for limited food. In another example of a situation where interactions with kin are best avoided, Chapter 4 demonstrated the reproductive cost of choosing to mate with a related partner. However, even in such an intuitively negative phenomenon as inbreeding, relatedness between social partners can provide certain advantages. Individuals that reproduce with a genetically similar mate gain kin-selected benefits of promoting their genes through the reproductive success of their partner (Lehmann and Perrin

2003; Kokko and Ots 2006) and reduce the degree of conflict over parental care (Parker 1979). However, indirect genetic benefits of inbreeding are limited to situations where inbreeding depression is minimal (Kokko and Ots 2006). One such situation may be when the availability of resources is great enough to buffer the negative consequences of seemingly suboptimal social decisions. Below, I outline the role of resource availability in mediating social environmental influences more generally.

7.1.4 Social environments and resource availability

Life history theory assumes that resources that individuals can acquire from the environment are in limited supply and must be allocated towards either reproduction or maintenance of the soma (van Noordwijk and de Jong 1986). In this thesis, I focussed mainly on the latter. In order to understand how social environments influence physiological costs and somatic maintenance, it is therefore imperative that we take resource availability into account. Specifically, the resources that an individual can obtain is expected to influence its ability to buffer the costs, or capitalise on the benefits, of social interactions with other individuals. For example, if individuals gain thermoregulatory benefits from joining a communal roost, those who have the resources to successfully compete for a central position may benefit much more than those forced to occupy an edge position (Hatchwell et al. 2009). On the other hand, if communal roosting promotes the transfer of external parasites, those individuals with plentiful access to resources may be able to upregulate their immunological defences and mitigate the cost of parasite infection (Alaux et al. 2010).

In this thesis, I mainly focussed on the latter of these two scenarios: does resource availability mitigate (or compound) the costs imposed by the social environment? Two lines of evidence suggest that this is indeed the case: (1) In Chapter 4, I showed that incestuous reproduction was mainly costly (in terms of offspring telomere length) in years of low food availability. This finding may provide one explanation for the reasonably high prevalence of inbreeding in the Seychelles warbler (Richardson et al. 2004): because resources are plentiful for a sufficient proportion of the time, the average cost of inbreeding is not great enough to drive social selection with respect to mate choice. (2) In Chapter 2, I showed that being raised with a competitor substantially reduces the amount of food a nestling receives from its carers. Such resource limitation sets the stage for offspring competition (Mock and Parker 1997); in the Appendix demonstrated how this can influence the evolution of offspring begging signals. By comparing different breeding systems (Chapter 3), I found that parents can mitigate this competition by increasing the rate at which they provision nestlings. The results of Chapter 3 provide an intriguing example of how an individual's social environment (in this case, the presence of an extra carer) can influence its physical environment (resource availability) and fitness. A similar pattern emerged in Chapter 5, where the number of group members (a component of the social environment) influenced per-capita territorial resources (a component of the physical environment). Both cases show how interacting socio-physical environments are crucial for the evolution of life-history strategies, breeding systems and population structure.

7.2 Fitness-linked metrics for measuring physiological costs

In this thesis, I used a suite of different metrics to measure individual costs of the social environment, including body condition (Chapters 2-5 and 6), telomere length (Chapters 2-4), telomere change (Chapters 5 and 6) and survival (Chapters 2, 3, 5 and 6). Interestingly, the degree to which these metrics varied in relationship to aspects of the social environments was not entirely congruous. Body condition, which reflects immediate energetic state (e.g. Schulte-Hostedde et al. 2005) was the most consistent measure; in both early life and adulthood, I repeatedly found that individuals in less optimal social environments had lower condition. This suggests that social environments tend to have immediate influences on individual physiology – perhaps not surprising as social interactions often disrupt the acquisition of food (West-Eberhard 1979). However, this immediate physiological effect only translated into differences in telomere length with relation to inbreeding depression (Chapter 4). In Chapters 2 and 3, I found no effect of nestmate presence or relatedness on the telomere length of focal nestlings. It is possible that this null result arises through insufficient sample sizes. Telomere measurements are inherently noisy; they are prone to measurement error and, by virtue of the fact that telomere shortening occurs in response to all sources of physiological stress, may vary according to other, undescribed factors. However, other studies of nestling social environments have suggested that telomere change, rather than absolute length, accurately reflects nestling costs (Boonekamp 2014; Nettle et al. 2015). Indeed, the results of Chapters 5 and 6 demonstrate that longitudinal changes in telomere length can vary significantly according to social environmental conditions. Telomere loss over time, rather than length at a given time point, might provide more a more accurate assessment of the physiological stress an individual's environment has produced. Lastly, I was able to detect survival differences in relation to early life (Chapter 2 and 3), but not adulthood (Chapters 5 and 6), social environments. This is perhaps not surprising given the life history of the Seychelles warbler: survival is much lower in the juvenile period than during adulthood (Brouwer et al. 2006), meaning that there is much more variation in the former which can be linked to social environments.

7.3 General conclusions and directions for future research

The research presented in this thesis demonstrates that the social environment can drive intriguing patterns at the level of the individual, family, social group and population. However, it also highlights the need to consider interacting effects of other factors when aiming to understand these patterns. While I demonstrated that intrinsic competitive ability, social relationships and resource availability all influence an individual's physiological costs, there are doubtless many other factors that merit further investigation in this context. One increasingly-studied component of intrinsic state that is likely to have important implications for social interactions and social selection is individual's biological age, or in other words, the effect of senescence. While I addressed age on a broad scale by investigating social environments in early life and adulthood, it would be extremely interesting to consider how ageing and senescence affect the patterns described in Chapters 2-6. Is island-wide food availability as effective in mitigating inbreeding costs when parents are reaching the end of their lifespan? How does the presence of subordinates on a territory influence the costs of group living for young, inexperienced dominants compared to older ones? Could we investigate the possibility of a feedback loop between neighbour familiarity, which reduces territorial costs, and ecological constraints on breeding, which arise when territory owners have high longevity? The impact of age related patterns and senescence on social relationships might be extremely important in understanding how social selection works in wild systems.

Throughout the development of this thesis, I adopted an individual-based focus to answer questions about how different ecological circumstances influence fitness. My focus was passive: how is the individual affected by a given circumstance? This framework, where the individual is considered as a more or less helpless object that is shaped by its social and physical surroundings, is certainly unrealistic but constitutes an important first step because it essentially tells us where to go looking for social selection on traits. To give an example: in Chapter 6, I asked whether the costs of maintaining a territory were affected by an individual's relationship with its neighbours. The fact that territorial costs seem to be lower when territory boundaries are shared between relatives suggests that there should be social selection for traits that facilitate the development of kin neighbourhoods, such as short-distance dispersal between the natal and breeding territory (Dickinson and Hatchwell 2004). Armed with this a priori information, we can now turn to the reassuring guidance of Niko Tinbergen's (1963) 'four questions' in order to gain a comprehensive understanding of that trait and its place in shaping animal behaviour and physiology (Bateson and Laland 2013). We hypothesise that the current utility of short-distance dispersal is to promote kin neighbourhoods – what of the mechanism, development and evolution of that trait? All the research presented in this thesis can be treated in a similar way – traits that influence competitive ability in the nest (Chapters 2 and 3), mate choice (Chapter 4) and strategies regarding timing of dispersal (Chapter 5) would provide interesting topics for future research. Answering such questions is an important goal of our field; I hope that the research presented in this thesis facilitates the initial stage in the process of understanding how behaviour evolves in what has always been a very social world.

7.4 References

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