The evolution of cooperation in an arid-zone bird: bet-hedging, plasticity and constraints

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……………………………………………………………..

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Summary

The evolution of cooperation represents a long-standing puzzle in evolutionary biology. From insects to mammals, this behaviour has evolved multiple times in separate lineages. Even though inclusive fitness theory provides a solid theoretical framework to understand the evolution of cooperation, there are still many research challenges in the evolutionary study of cooperation. First, most of the evidence for the effects of cooperation on the reproductive success of beneficiaries in vertebrate societies is based on correlational studies, which can be confounded by several environmental factors. Second, there are recent theoretical formulations to explain the evolution of cooperation that have not been empirically tested yet but could shed new light on the selective pressures that facilitate the evolution of cooperation. Third, we still have a poor understanding of the sources of among individual variation in cooperative behaviours. In particular, few studies have investigated whether the level of cooperation expressed by individuals is heritable and, therefore, could respond to natural selection. In this thesis, I combine nine years of life-history and behavioural information with field experiments and genomics to investigate (i) the routes for non-breeding individuals to acquire indirect fitness benefits and (ii) the sources of among-individual variation in cooperation in white-browed sparrow-weavers (*Plocepasser mahali*), an arid-zone cooperative breeder. After a general introduction to the subject of cooperative breeding, in Chapter 2 I test a novel hypothesis for the evolution of cooperation, the 'altruistic bet-hedging' hypothesis. There, I show that non-breeding helpers reduce variation in the reproductive success of breeders without affecting their arithmetic mean reproductive success. Furthermore, I show that this reproductive variance compression appears to arise because helpers specifically reduce unpredictable rainfall-induced variation in reproductive success, just as hypothesised by global comparative studies of the evolution of cooperative breeding in birds. Then, I investigate alternative routes through which helpers may gain indirect fitness benefits. Specifically, in Chapter 3 I investigate the effects of helpers on pre- and post-natal maternal investment in reproduction. The findings in Chapter 3 provide clear evidence for maternal plasticity in pre-natal investment in reproduction (egg volume) in response to the number of helpers. Moreover, the helper effect of increased pre-natal maternal investment is associated with a decrease in post-natal maternal investment. In Chapter 4, I test the philopatry hypothesis for the evolution of sex differences in cooperation within animal societies and find strong support for this hypothesis in white-browed sparrow-weavers. Furthermore, Chapter 4 highlights the need to consider both sex differences in direct fitness *benefits* and *costs* when trying to understand sex differences in cooperation. Finally, in Chapter 5 I investigate among-helper variation in cooperative generosity, finding consistent individual differences and providing evidence for heritable variation in this trait. To conclude, in Chapter 6 I discuss the implications of these results for our general understanding of the evolution of cooperation in animal societies and highlight methodological approaches for future empirical studies of cooperation in the wild.

For Natalia, Teresa, José Luis C. S. and José Luis C. L.

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Writing a PhD thesis was never the purpose *per se* but rather an opportunity to spend four years of my life doing what I have felt passionate about since I was a young teenager. I owe this opportunity to many people. Once that opportunity was given, many others helped me complete the journey successfully and made my life a lot more joyful.

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Author's declaration

All chapters of this thesis have been written by Pablo Capilla-Lasheras with comments and editing by Dr. Andrew Young. Guidance was provided throughout by Dr. Andrew Young. Prof. Alastair Wilson provided additional guidance for Chapter 5. Prof. Alastair Wilson, Dr. Xavier Harrison and Dr. Emma Wood commented and edited Chapter 2 and Chapter 5. Antony Brown, Melanie Weedon, Dr. Natalia Bravo-Santano and Nina Bircher provided comments that improved Chapters 3, 4 and 5. Dr. Simon Evans provided useful comments that improved Chapter 5.

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Chapters 2, 3, 4 and 5 are the result of the scientific endeavour and collaboration of several people throughout many years. Therefore, I acknowledge their contribution at the beginning of each chapter and use the pronoun ‘we’ throughout. This grammatical use by no measure means that any part of this thesis is not my own work but instead aims to appreciate the collaborative aspect of the mentioned thesis chapters.

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Chapter 1

General Introduction

1.1. Theoretical framework for the evolution of cooperative behaviour

As Darwin intuited and the modern synthesis in evolutionary biology formalised, natural selection filters populations by removing genes, and hence traits, of those individuals that are not well adapted to their environment (Darwin, 1859; Gould, 2002). How is it then possible that natural selection maintains or even favours the existence of altruistic genes in individuals that sacrifice their own reproduction and instead assist to raise the offspring of others? A solution for this paradoxical question was proposed in 1964, when W. D. Hamilton published a pivotal theoretical formulation and defined the concept of inclusive fitness (Hamilton, 1964). Inclusive fitness theory acknowledges that individuals can transmit copies of their own genes not only via direct reproduction (i.e. 'direct fitness') but also by increasing the reproductive success of related individuals, with whom they share genes (i.e. 'indirect fitness'). Hamilton thus proposed that selection acts upon 'inclusive fitness', the combination of direct and indirect fitness (Hamilton, 1964). Hamilton predicted that cooperation would be favoured by natural selection when the reproductive costs to the altruistic individual ('c') did not exceed the reproductive benefits to the recipient ('b') weighed by the genetic relatedness ('r') between the altruistic actor and the recipient individual (i.e. Hamilton's rule: $r \times b > c$, Hamilton, 1964). While Hamilton's rule provided a powerful model to explain the evolution of cooperation in nature, more than 50 years after Hamilton's pivotal papers many questions about the evolution and maintenance of cooperative behaviours are still unanswered (West & Griffin, 2007). In particular, we are still lacking a clear understanding of the origins of variation in cooperative tendencies, how environmental factors affect the evolution of cooperation and the role of direct fitness benefits in shaping cooperation in animal societies.

In cooperatively breeding species, defined as species in which some individuals help to rear the offspring of others (Griffin & West, 2002; West *et al.*, 2007; Koenig & Dickinson, 2016), there is variation in the level of cooperation expressed among and within individuals (Komdeur, 2006; Bergmüller *et al.*, 2010). Among species, genetic relatedness between breeders and helpers has been found to positively predict the amount of help provided by helpers (Griffin & West, 2003; Green *et al.*, 2016), consistent with the predictions of inclusive fitness theory (Hamilton, 1964). Within species, individual roles (e.g. whether individuals act as breeders or helpers) and the level of help that individuals provide can also vary markedly (Bergmüller *et al.*, 2010). Crucially, differences in genetic relatedness among individuals are often low within species and social groups (e.g. Clutton-Brock *et al.*, 2002; but see Russell & Hatchwell, 2001 for an exception), suggesting that individual diversity in cooperative behaviour may be a product of variation in other factors.

To set the scene for the empirical chapters that follow I summarise below our current understanding of the role that (i) the environment, (ii) indirect fitness benefits, and (iii) direct fitness payoffs have played in the evolution of cooperative breeding, highlighting in the process a number of complications that represent current research challenges in this field. I then outline the aims and structure of this thesis, which seeks to address these challenges.

1.2. The role of the environment in the evolution of cooperative breeding

Hamilton himself noted that cooperation could only be selected for when complementary factors created the conditions for related individuals to interact (Hamilton, 1987). Indeed, the evolution of cooperation needs to be understood as a two-step process: first, the evolution of the delayed dispersal of offspring and the consequent origin of family living and / or kin neighbourhoods (e.g. Hatchwell, 2016) and, second, the evolution of cooperative activities *per se* (Griesser *et al.*, 2017).

Environmental factors have been traditionally suggested to play an important role promoting delay dispersal and family living. Early studies trying to explain the existence of cooperatively breeding species acknowledged the role of the environment imposing high costs to dispersal and / or independent breeding and, therefore, promoting local dispersal or no dispersal at all (Emlen, 1982b, 1994; Cockburn, 1998; Hatchwell, 2009). The environment can constrain dispersal and independent breeding, thereby creating extended family living and kin neighbourhoods, if environmental conditions (i) impose high survival costs to dispersal (Ridley *et al.*, 2008; Bonte *et al.*, 2012) or (ii) promote habitat saturation and a shortage of breeding positions (Koenig *et al.*, 1992; Komdeur, 1992; Kingma *et al.*, 2016). It should be noted that the selective pressures favouring the evolution of delayed dispersal and kin-structured populations need not match those that select for the evolution of cooperation *per se* (Emlen, 1982b; a; Griesser *et al.*, 2017). Failure to appreciate this nuance may have contributed to confusion about the general role of the environment in the evolution of cooperatively breeding societies, which typically show both delayed dispersal and cooperation (e.g. Gonzalez *et al.*, 2013; Cornwallis *et al.*, 2017). Indeed, in a global comparative analysis of cooperative breeding in birds, Griesser *et al.* (2017) showed that the environmental factors promoting group living (i.e. delayed dispersal) are different from those that promoted the evolution of helping *per se* (i.e. cooperation). Once delayed dispersal has evolved, individuals can accrue indirect fitness benefits by helping relatives, leading to the evolution of cooperative behaviours (Brown, 1987; Emlen, 1991).

The fact that cooperative breeding is associated with unpredictable environments led to the idea, first conceptually proposed by Rubenstein (2011), that helping acts as a bet-hedging strategy (Rubenstein, 2011). Bet-hedging is an adaptive strategy that increases the long-term fitness of a lineage not by increasing its (arithmetic) mean reproductive success but by reducing its variance in reproductive success (see Starrfelt & Kokko, 2012, and references therein). Bet-hedging is of particular relevance in unpredictable environments, where plasticity cannot evolve as organisms do not have reliable cues (i) to predict the environment in which a given trait will be expressed and (ii) to predict the relationship between fitness and the expression of a given trait across different environments (Simons, 2011). Bet-hedging strategies for adaptation to unpredictable environments have been, and still are, intensively study both theoretically (Lehmann & Balloux, 2007; Starrfelt & Kokko, 2012) and empirically (Beaumont *et al.*, 2009; Olofsson *et al.*, 2009; Simons, 2011). However, until very recently, no studies had integrated bet-hedging theory and the general Hamiltonian framework for the evolution of cooperation.

Such integration between bet-hedging theory and Hamiltonian social theory was recently carried out by Kennedy *et al*. (2018), proposing altruistic bet-hedging as a new formulation to understand the evolution of cooperation in unpredictable environments (Kennedy *et al.*, 2018). Altruistic bet-hedging integrates traditional bet-hedging theory and inclusive fitness theory, and acknowledges that the reproductive benefits of cooperation to the recipient and the reproductive costs of cooperation for the actor ('b' and 'c' in classical Hamilton's rule) can also be affected by changes in variation of reproductive success and not only by changes in (arithmetic) mean reproductive success (Kennedy *et al.*, 2018). Altruistic bet-hedging describes a specific scenario where even in the absence of positive effects of cooperation on mean reproductive success, cooperation can still be selected for if it reduces variation in the reproductive success of breeding individuals (Kennedy *et al.*, 2018). Cooperative individuals can therefore gain indirect fitness benefits by increasing the mean reproductive success of related breeders (as proposed by classical Hamilton's rule, Hamilton, 1964) and / or by reducing variation in the reproductive success of related breeders (as envisaged by altruistic bet-hedging theory, Kennedy *et al.*, 2018).

1.3. Routes to gain indirect fitness benefits for helpers

1.3.1. Empirical evidence for positive effects of cooperation on the (arithmetic) mean reproductive success of related breeders

Helpers in cooperatively breeding species can gain indirect fitness benefits by engaging in cooperative activities if they increase the reproductive success of related reproductive individuals (Hamilton, 1964; Brown, 1987; Emlen, 1991). There are several potential routes to acquire these indirect benefits.

First, helpers can increase the survival of offspring produced by breeding individuals. In many animal societies, helpers provide food for offspring of other individuals (i.e. 'alloparental care'), positively impacting offspring survival (Brown, 1987; Emlen, 1991; Field *et al.*, 2000; Clutton-Brock *et al.*, 2001; Magrath, 2001; Russell *et al.*, 2003a; Dickinson & Hatchwell, 2004; Young *et al.*, 2015; Koenig & Dickinson, 2016). Helper contributions can not only increase offspring survival but also increase the future reproductive success of offspring (Hatchwell *et al.*, 2004; Russell *et al.*, 2007b; Meade *et al.*, 2010). However, experimental approaches are rarely used in the study of cooperative breeders (for an exception, see Liebl *et al.*, 2016) and a number of confounding factors may affect the conclusions of correlational studies regarding the impact of helpers on the reproductive success of breeders. In particular, variation in territory quality can create spurious correlations between the presence or number of helper and the reproductive success of breeders (Cockburn, 1998). Territory quality is likely to positively affect the size of the group that occupies it by offering more resources for successful upbringing of offspring or by reducing competition among helpers and breeders and, thus, allowing more individuals to delay dispersal (Cockburn, 1998; Ekman *et al.*, 2001). If this is the case, high quality territories may accumulate more helpers and, independently, increase the reproductive success of breeders (Cockburn, 1998).

Second, helpers may lighten offspring provisioning workloads of related breeders (i.e. 'load-lightening', Heinsohn, 2004). Load lightening occurs when the presence of helpers is associated with a decrease in the workload of breeders after offspring hatch or are born (Hatchwell, 1999; Russell *et al.*, 2003a; Heinsohn, 2004). Load lightening may allow breeding individuals to re-allocate resources for other reproductive bouts or for own survival (Heinsohn, 2004). In meerkats (*Suricata suricatta*), for example, breeding females have more litters per year when they are assisted by more helpers (Russell *et al.*, 2003a). Alternatively or in combination with the latter effect, there is evidence from several species suggesting that load lightening by helpers can also lead to increase survival of breeding individuals (Khan & Walters, 2002; Kingma *et al.*, 2010; Langmore *et al.*, 2016).

Load-lightening effects by helpers have been traditionally investigated at a post-natal stage, measuring helper contributions towards offspring food provisioning (Hatchwell, 1999). In recent years, however, there has been an increasing interest in understanding the causes and consequences of load-lightening at a pre-natal stage, when helpers lighten the workloads of breeders before offspring hatch or are born (Russell *et al.*, 2007a; Dixit *et al.*, 2017). For example, in superb-fairy wrens (*Malurus cyaneus*) helpers are associated with decreases in maternal pre-natal investment (egg size) under certain environmental conditions (Russell *et al.*, 2007a; Langmore *et al.*, 2016). However, as it is the case for studies on helper effects on reproductive success of breeders (see above), most studies of cooperative breeders investigating load-lightening at a pre- or post-natal stage do not adequately control for variation in territory quality or, indeed, breeder quality. As detailed above, high quality territories can accumulate a high number of helpers and, independently, allow females to, for example, lay larger eggs (Cockburn, 1998; Lejeune *et al.*, 2016). In the latter scenario, a correlation between helper number and pre-natal maternal investment would arise, potentially leading to the erroneous conclusion that females lay larger eggs when assisted by more helpers. To circumvent this problem, the effect of helpers on the reproductive success of breeders should be assessed by experimentally manipulating cooperative contributions of helpers (e.g. Taborsky *et al.*, 2007) or by statistically controlling for variation in reproductive success among territories (van de Pol & Wright, 2009; Lejeune *et al.*, 2016).

1.3.2. Empirical evidence for effects of cooperation on the variance in reproductive success of related breeders

Evidence that cooperation reduces variance in the reproductive success of related breeders, as envisaged under altruistic bet-hedging (Kennedy *et al.*, 2018), is much scarcer than evidence for effects of cooperation on arithmetic mean reproductive success. Such variance reductions effects have been suggested in birds (Rubenstein, 2011; Koenig & Walters, 2015), mammals (Ebensperger *et al.*, 2014) and some invertebrates (Stevens *et al.*, 2007; Kocher *et al.*, 2014). Two studies of cooperatively breeding species have investigated whether groups with more helpers show lower variance in reproductive success, resulting in some evidence to suggest that they may (superb starlings, *Lamprotornis superbus*, Rubenstein, 2011; and acorn woodpeckers, *Melanerpes formicivorus*, Koenig & Walters, 2015). However, whether such patterns arise because helping behaviour *per se* reduces variance in the reproductive success of relatives is unknown. Individuals in larger groups may have lower variance in reproductive success not because larger groups contain more helpers but due to benefits of group size that arise independent of helping (e.g. improved foraging success) or because larger groups of these particular species may also contain more breeders (which could itself reduce variance in reproductive success via a range of mechanisms, see Stevens *et al.*, 2007).

1.4. Individual variation in contributions to cooperation

1.4.1 Sex differences in contributions to cooperation

Strikingly, in many animal societies, there is a difference in the contributions of male and females helpers to cooperation within their natal group (Cockburn, 1998; Clutton-Brock *et al.*, 2002; Young *et al.*, 2005; Hodge, 2007; Williams & Hale, 2007). This is the case despite the fact that both subordinate males and females are often equally related to breeding individuals and to the future generations of offspring they help rear (Clutton-Brock *et al.*, 2002). It follows that both subordinate males and female should accrue similar indirect fitness benefits by helping and thus kin selection alone cannot readily explain sex differences in cooperation in this context (Clutton-Brock *et al.*, 2002).

Several hypotheses have been proposed to explain sex differences in cooperation in the absence of sex differences in relatedness to the recipients of help (Whitney, 1976; Charnov, 1978; Clutton-Brock *et al.*, 2002). Among them, the philopatry hypothesis has received the strongest support to date (Clutton-Brock *et al.*, 2002). This hypothesis predicts that the more philopatric sex will show higher levels of cooperation due to increased direct fitness benefits or reduced direct fitness cost (Clutton-Brock *et al.*, 2002). Indeed, sex differences in direct fitness benefits and / or costs of cooperation can generate sex differences in cooperation (Field *et al.*, 2006; Downing *et al.*, 2018). The role of sex differences in direct fitness *benefits* has been particularly highlighted (Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). In general, if survival and breeding opportunities for helpers increase with group size (Kokko *et al.*, 2001; Kokko & Ekman, 2002; Kingma *et al.*, 2014), those individuals that stay in their natal territories as helpers for longer are expected to gain more direct benefits from cooperation (assuming that cooperation has a positive effect on offspring production and, therefore, increases group size). The role of sex differences in the direct fitness *cost* of cooperation has been much less investigated but it could, however, play a key role in generating sex differences in cooperation (Young *et al.*, 2005; Hodge, 2007). Understanding the relative importance of direct fitness effects in the generation of variation in cooperation would shed new light to our comprehension of the evolution of cooperative breeding.

1.4.2. Genetic and environmental variation in individual cooperative generosity

After accounting for sex differences in levels of cooperation, it is common that helping individuals still vary in their levels of cooperation (Komdeur, 2006). While variation in abiotic (e.g. weather) and biotic (e.g. social interactions) factors can impact individual contributions to cooperation (Hatchwell & Russell, 1996; Hatchwell, 1999; Clutton-Brock *et al.*, 2002, 2003; Russell *et al.*, 2003b; Johnstone, 2011; Adams *et al.*, 2015; Wiley & Ridley, 2016), helpers often show consistent cooperative levels throughout their lives (Bergmüller *et al.*, 2010). The origin of such consistent individual variation in cooperation is unclear but two general explanations are proposed: the existence of additive genetic variation for cooperative generosity and / or the existence of early-life environmental effects that persists throughout an individual's life (English *et al.*, 2015; Taborsky, 2017). The existence of heritable genetic variation for cooperative generosity is especially relevant for our understanding of the evolution of cooperation (Kasper *et al.*, 2017b). Whether heritable variation for cooperation exists will determine its potential to respond to natural selection. Despite its evolutionary importance, compelling evidence that heritable variation contributes appreciably to inter-individual variation in cooperative generosity is lacking.

1.5. Study system and study area

In this thesis, I investigate the evolution of cooperation in white-browed sparrow-weavers (*Plocepasser mahali* Smith 1836, subspecies *mahali*) using a long-term data set, behavioural experiments and molecular tools. White-browed sparrow-weavers live in semi-arid regions of East and Southern Africa (Collias & Collias, 1978; Lewis, 1981, 1982a; Ferguson, 1988). The study population is located in Tswalu Kalahari Reserve in the Kalahari Desert of South Africa. This site is placed in the southern belt of the Kalahari Desert, close to the Korannaberg mountain range, a savannah-like habitat, where camelthorn (*Vachellia erioloba*) and shepherd's tree (*Boscia albitrunca*) are the predominant tree species. Between 2007 and 2016, Tswalu received on average 357 mm (standard deviation = 153 mm; range = 213-670) of total rainfall per year, mostly between December and March, corresponding to the wet season. Rainfall conditions were very variable both within and among years (Chapter 2). Average temperature per year between 2007 and 2016 was 21.1 Celsius degrees (°C) (standard deviation = 0.83 °C), with maximum average temperatures in January (27.9 ± 4.52 °C) and minimum average temperatures in July (12.2 ± 4.95 °C).

The study population consists of 30-40 white-browed sparrow-weaver social groups occupying an area of approximately 1.5 Km^2 that have been monitored since 2007. White-browed sparrow-weavers breed cooperatively in social groups that consist of a dominant pair and up to ten subordinate helping individuals of both sexes (Lewis, 1982a; Harrison *et al.*, 2013a). Dominance was assessed weekly throughout the study period based on dominant-related behavioural observations (Walker *et al.*, 2016; York *et al.*, 2019). Dominant individuals are readily identified in the field as they are often in close association with each other and often display a conspicuous singing duet (York *et al.*, 2019). The dominant male displays a characteristic dawn song (York *et al.*, 2014) and is the individual that invests most in sentinelling within groups (Walker *et al.*, 2016). Molecular work on the study population has revealed that the dominant pair monopolises reproduction within each social group, while 12-18% of offspring sired by extra-group dominant males (Harrison *et al.*, 2013a; b). The dominant female completely monopolises reproduction; she is the only individual within each social group ever known to lay or incubate eggs (Harrison *et al.*, 2013a). Furthermore, this molecular work confirmed that dominance status assigned using behavioural observations also accurately identifies the breeding pair. Individuals in our study population were observed in the field, their reproductive activity monitored and their cooperative contributions quantified between September and April of the following year, period that covers the wet season in Tswalu when birds are more likely to breed.

In white-browed sparrow-weavers, offspring of both sexes typically delay dispersal from their natal group for a variable amount of time (median = 1.91 years; inter-quantile range = 1.17 – 2.43 years) during which they engage in several cooperative activities as non-breeding subordinates. Subordinates of both sexes cooperatively contribute to nest and roost weaving (Collias & Collias, 1978), vigilance against predators (Walker *et al.*, 2016), territorial defence (Collias & Collias, 1978; Walker *et al.*, 2016; York *et al.*, 2019) and offspring food provisioning (Lewis, 1982a; Walker, 2015). Early work on another subspecies (subspecies *pectoralis*) in a different location suggests that the presence of helpers may increase the total rate of food delivery to broods (i.e. there may be 'additive helper effects', Lewis, 1982a) and lighten the workload of the dominant female (i.e. 'load lightening', Lewis, 1982a). Consistently with these findings, (i) offspring reared in groups with more helpers in our study population appear to show lower rates of telomere erosion during development (Wood, 2017; which could have beneficial effects on performance, Young, 2018), and (ii) experimental work on our study population suggests that rearing offspring entails a physiological cost (body mass loss and a rise in oxidative damage) and that this cost appears to be lower in groups with more helpers (Cram *et al.*, 2015).

1.6. Thesis aims and outline

In this thesis, I utilise the white-browed sparrow-weaver as a model system for (i) testing the altruistic bet-hedging hypothesis for the evolution of cooperation, by investigating whether helping in white-browed sparrow-weaver societies affects the (arithmetic) mean and variance in reproductive success of related breeders, (ii) investigating pre- and post-natal maternal investment responses to the presence of helpers, to shed light on the mechanisms through which helpers impact recipients, (iii) investigating whether sex differences in direct fitness payoffs from cooperation (arising from sex differences in philopatry) could explain sex differences in cooperation, and (iv) whether there is evidence of consistent individual differences in helper contributions to cooperative provisioning, and to what extent these reflect heritable (additive genetic) variation in this trait. To accomplish these aims, I combine a 9-year data set containing life-history and behavioural observations of a natural population of white-browed sparrow-weavers with field experiments and genomic information. Throughout this thesis, I apply an analytical technique (within-subject centring, van de Pol & Wright, 2009) that allows me to investigate the relationship between helper number and a given life-history or behavioural trait while controlling for potentially confounding trait variation attributable instead to variation in quality among territories and/or parents (Cockburn, 1998; van de Pol & Wright, 2009). Briefly, the chapters will address the following:

Chapter 2 tests the altruistic bet-hedging hypothesis for the evolution of cooperation (Kennedy *et al.*, 2018). Specifically, I investigate whether helping affects the (arithmetic) mean and variance in reproductive success of related breeders. This work provides strong support for this hypothesis, revealing that helping appears to have no effect on the mean reproductive success of related breeders but instead compresses their variance in reproductive success. Moreover, this reproductive variance compression appears to arise because helpers specifically reduce unpredictable *rainfall*-induced variation in reproductive success, just as hypothesised by global comparative studies of the evolution of cooperative breeding in birds (Griesser *et al.*, 2017).

Chapter 3 investigates pre- and post-natal maternal investment responses to the presence of helpers. Utilising repeated observations of egg volume (i.e. pre-natal investment) and feeding rates to the offspring (post-natal investment) per dominant female, this chapter provides evidence for opposing effects of (female) helpers on pre- and post-natal maternal investment in reproduction.

Chapter 4 tests the philopatric hypothesis for the evolution of sex differences in cooperation within animal societies. I find support for this hypothesis in white-browed sparrow-weavers: natal subordinates of the more philopatric sex (females) contribute more towards cooperative care of offspring than the less philopatric sex (males). Furthermore, I highlight the importance of sex differences in both direct fitness *benefits* and direct fitness *costs* of cooperation for the evolution of sex differences in helping behaviours in animal societies.

Chapter 5 investigates the existence of consistent individual differences in helping behaviour and quantifies additive genetic variance for cooperation generosity. This chapter provides evidence for consistent among-individual differences in cooperative generosity and for the existence of additive genetic variation in this trait.

Chapter 6 summarises the results of this thesis, places them in a broader context and discusses their implications for our understanding of the evolution of cooperation in the wild.

Chapter 2

Altruistic bet-hedging in an arid zone cooperative breeder

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2.1. Abstract

Analyses of the global biogeography of altruism suggest that unpredictable environments have favoured the evolution of altruistic helping behaviour (helping to rear the offspring of others, Jetz & Rubenstein, 2011; Griesser *et al.*, 2017). It has therefore been hypothesised that selection for altruism may frequently arise because helping reduces variance in the reproductive success of relatives in unpredictable environments (a scenario termed 'altruistic bet-hedging', Kennedy *et al.*, 2018). Here we show that helping behaviour does reduce environmentally-induced variance in the reproductive success of relatives in a wild cooperative bird, the white-browed sparrow-weaver (*Plocepasser mahali*). Our decade-long study in the Kalahari desert reveals that non-breeding helpers have no overall effect on the mean reproductive success of related breeders, but instead reduce *variance* in the reproductive success of related breeders. Moreover, this variance reduction arises in part because helpers specifically reduce unpredictable rainfall-induced variance in reproductive success, just as hypothesised by global comparative analyses (Jetz & Rubenstein, 2011; Griesser *et al.*, 2017). Our novel analytical approach implicates effects of helping *per se* rather than correlated effects of group size and isolates within-mother effects of helping from potentially confounding among-mother variation in performance. Our findings lend new strength to the leading explanation for the global biogeography of altruism and highlight the wider importance of considering the impacts of altruism on both the mean and variance in performance of recipients.

2.2. Main text

Explanations for the evolution of altruism via kin selection typically focus on scenarios in which altruistic acts yield indirect fitness benefits to the actor by increasing the *mean* reproductive success of relatives (Hamilton, 1964). However, recent theory has highlighted that kin selection can also favour altruistic acts that decrease *variance* in the reproductive success of relatives in unpredictable environments; a scenario termed 'altruistic bet-hedging' (Kennedy *et al.*, 2018). Altruistic bet-hedging thus integrates the rationale of kin selection (Hamilton, 1964) with the long-recognised potential for selection to favour bet-hedging strategies that decrease variance in performance in unpredictable environments (Starrfelt & Kokko, 2012).

This theoretical development was stimulated by the empirical observation that altruistic helping behaviour (helping to rear the offspring of others) is globally associated with unpredictable environments (Jetz & Rubenstein, 2011; Griesser *et al.*, 2017; see also Sheehan *et al.*, 2015) and the ensuing hypothesis that selection for helping arises in part from it reducing environmentally-induced variance in the reproductive success of relatives. While these global associations highlight the potentially widespread importance of altruistic bet-hedging, compelling evidence that altruistic helping behaviour does indeed reduce variance in the reproductive success of relatives in unpredictable environments remains elusive (Cockburn & Russell, 2011). Breeders in larger social groups of two cooperative bird species do appear to show lower reproductive variance under some circumstances (Rubenstein, 2011; Koenig & Walters, 2015) but whether such patterns reflect effects of helping on environmentally-induced variance in reproductive success is unknown.

White-browed sparrow-weavers live in extended family groups in which a single dominant female (hereafter the ‘mother’) and male completely monopolise within-group reproduction and 0-10 non-breeding subordinates (hereafter ‘helpers’) of both sexes help to feed the dominants’ nestlings (Figure 1a; Harrison *et al.*, 2013a). Helping behaviour has the potential to yield indirect fitness benefits, as helpers are typically offspring of the dominant pair, leaving them closely related to the offspring that they help to rear (Figure 1b). As this species’ environment is characterised by highly unpredictable rainfall patterns (see Supplementary Information A & B and Figure S1 for the high inter-annual rainfall variation and low temporal auto-correlations for our Kalahari study population), helping could well yield indirect fitness benefits in part via reductions in environmentally-induced variance in the reproductive success of related breeders, as envisaged under altruistic bet-hedging (Kennedy *et al.*, 2018).

While helpers of both sexes feed nestlings, female helpers do so at substantially higher rates than males (Figure 1c). Accordingly, partitioning natural variation in helper numbers into its within- and among-mother components (to isolate the effects of *within*-mother variation in helper number from the potentially confounding effects of variation *among* mothers or their territories; see methods), revealed that *within*-mother variation in female helper number strongly positively predicts the total rate at which her brood is fed (Figure 1d, Table S1). By contrast, *within*-mother variation in male helper number did not predict total provisioning rate (Table S1). Indeed, the experimental removal of helpers from wild social groups confirmed that the presence of female helpers has a causal positive effect on total provisioning rate (Figure 1e), while the same is not true for male helpers (Table S2).

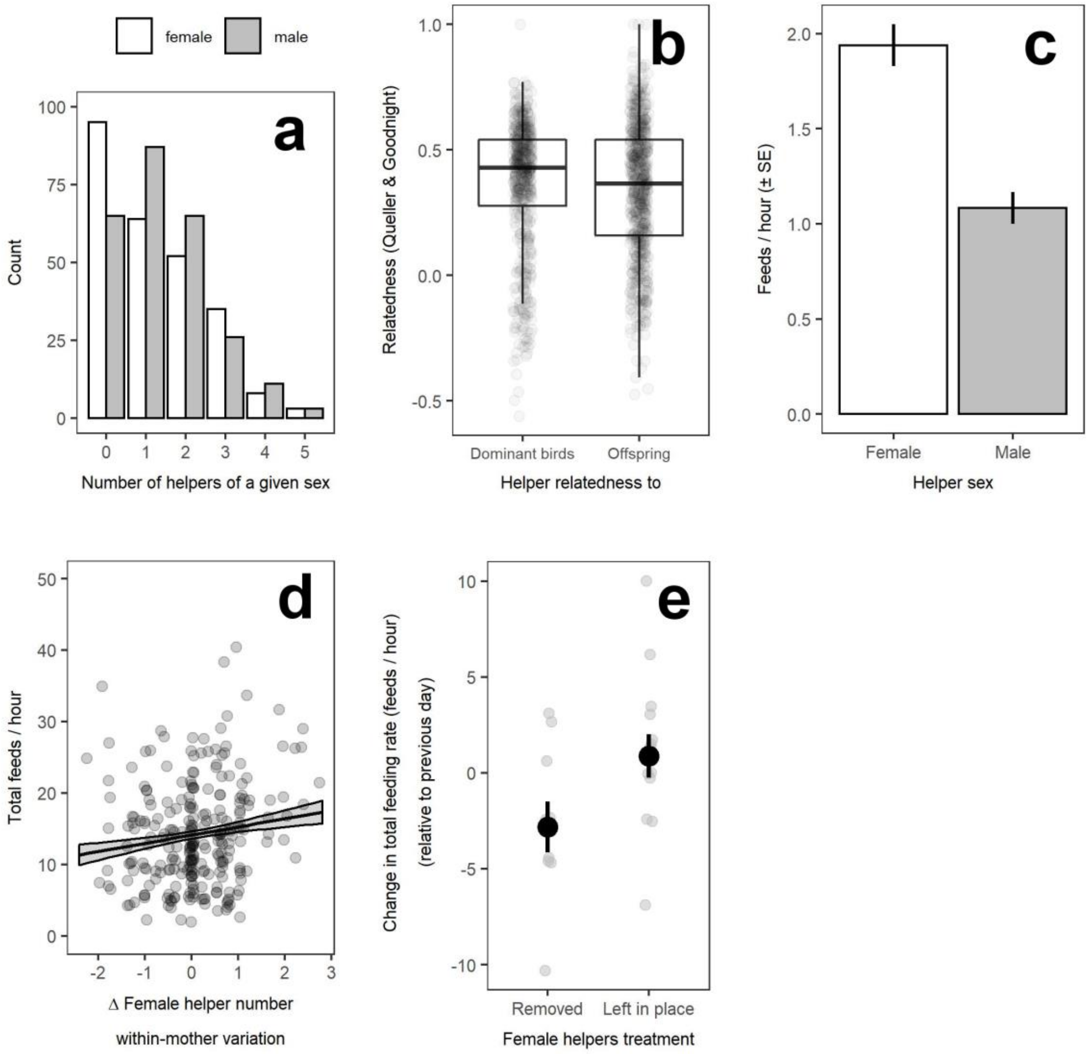


Figure 1. Helping and its impact on offspring provisioning. (**a**) Frequency of female and male helpers in 400 broods in 36 social groups. (**b**) Genetic relatedness of helpers to the dominant (breeding) pair and their offspring (see methods). (**c**) Female helpers feed nestlings at a significantly higher rate than male helpers (linear model of log-transformed provisioning rate: estimate for sex effect (± SE) = 0.17 (± 0.02); ΔAIC on removal of helper sex predictor = 52.43). (**d**) Within-mother variation in female helper number (Δ female helper number) predicted total brood provisioning rate (Table S1). (**e**) Experimental removal of female helpers reduced total provisioning rate (relative to preceding day) significantly more than a non-removal treatment (Table S2). Grey dots show raw data. Mean model predictions (± SE) are shown in d and e.

To investigate the effects of female and male helpers on both the mean and variance in reproductive success of mothers, we used Bayesian bivariate statistical modelling. This approach allows the effect of helpers on variance in reproductive success to be directly assessed by comparing the variance components of reproductive success across categories of mothers with contrasting numbers of helpers (e.g. for mothers with no versus some female helpers; see methods). As sparrow-weaver helpers only contribute to post-hatching offspring care (they do not feed the mother or incubate), our bivariate models investigated the effects of helpers on the number of nestlings that fledged from a given breeding attempt (hereafter 'reproductive success') while controlling for variation in the initial number of hatchlings (see methods for rationale). Our analyses reveal that mothers with female helpers do not have a higher *mean* reproductive success than those without (n = 400 broods of 68 mothers in 36 social groups; estimate [95% CI] for the intercept difference = -0.09 [-0.41, 0.22]; Figure S2), but instead show markedly lower *variance* in reproductive success (Figure 2a). These patterns cannot be attributed to confounding effects of variation among mothers (or territories), as within-mother variation in female helper number also predicted the mother's variance in reproductive success (Figure 2b) and not her mean reproductive success (estimate [95% CI] for the intercept difference = -0.16 [-0.44, 0.12]; Figure S2). Univariate modelling confirms that continuous variation in female helper number (avoiding the categorisation of helper numbers required for this bivariate approach) does not predict mean maternal reproductive success either (Figure 2c; Table S5). Conducting these same analyses for male helper numbers revealed no detectable effects of male helpers on the mother's mean or variance in reproductive success (Table S3 and Table S4). That these relationships are all detectable for female helper numbers but not male helper numbers implicates helping behaviour *per se* as the driver, rather than correlated effects of group size (that could arise independent of helping), as females helpers help at substantially higher rates than males (Figure 1c) and only female helpers have causal additive effects on the group's total provisioning rate (Figure 1d,e). Our inability to detect a helper effect on the *mean* reproductive success of mothers cannot be attributed to correlated variation in maternal egg investment concealing a helper effect (*sensu* Russell *et al.*, 2007a), as no mean helper effect is revealed when variation in egg volume or hatchling mass are statistically controlled (Table S4).

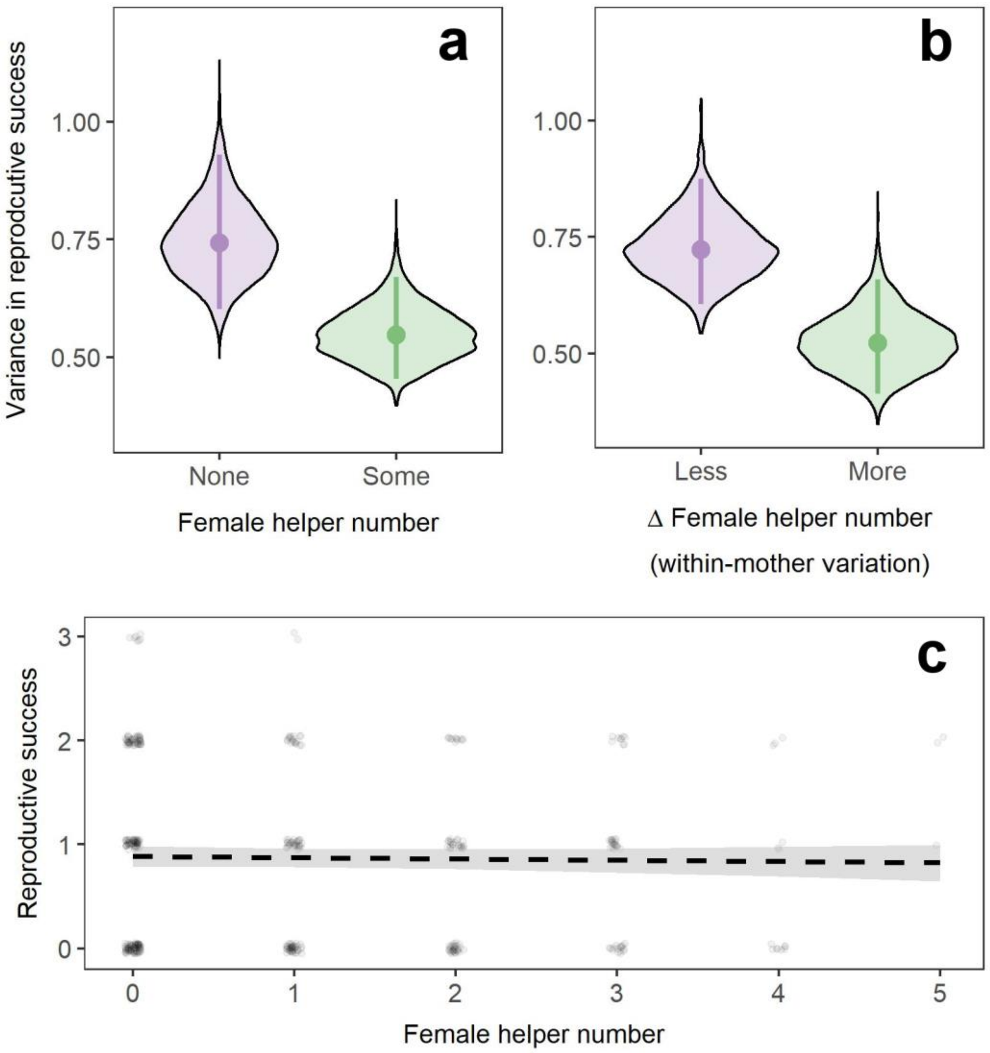


Figure 2. Female helper effects on mean and variance in reproductive success of related breeders. (**a**) Bivariate modelling revealed that mothers with some female helpers experience lower variance in reproductive success than mothers with no female helpers. (**b**) This result holds when we isolate the effect of within-mother variation in female helper number (Δ female helper number). Panels **a** and **b** show estimates of the residual variance in reproductive success for mothers assisted by (**a**) 'None' or 'Some' female helpers, and (**b**) 'Less' or 'More' female helpers than each mother's mean female helper number within the data set. In both cases we found a significant difference between the variance estimates for the two helper number classes (difference estimate [95% CI]: (**a**) 'None'–'Some' = 0.197 [0.002, 0.392]; (**b**) 'Less'–'More' = 0.205 [0.025, 0.386]). Shaded areas reflect the posterior distributions from two MCMC runs, and the points and error bars inside the shaded areas reflect the median values and 95% CIs for the estimate of residual variance. (**c**) Univariate modelling confirmed that continuous variation in female

helper number does not predict mean reproductive success either (Figure S4). Raw data (transparent grey dots) and mean model predictions (± SE) are shown.

The above findings suggest that altruistic helping behaviour in sparrow-weaver societies reduces variance in the reproductive success of related breeders. However, 'altruistic bet-hedging' refers to a more specific scenario in which altruism reduces reproductive variance arising from unpredictable environmental variation (Kennedy *et al.*, 2018) and global comparative studies of the evolution of cooperation in birds hypothesise that the relevant environmental variable is rainfall (Jetz & Rubenstein, 2011; Griesser *et al.*, 2017). To investigate whether sparrow-weaver helpers specifically mitigate rainfall-induced variation in reproductive success, we tested for the required statistical interaction between female helper number and rainfall in a univariate model of nestling survival to fledging (see methods). As rainfall could impact reproductive success over a range of timescales in this arid-zone species, we first used a sliding window optimisation approach (Supplementary information D; van de Pol *et al.*, 2016) to establish that nestling survival was most strongly predicted by the total rainfall that fell between 36 days pre-hatching and 9 days post-hatching (Figure S3). Our modelling then confirmed that the statistical interaction between the total rainfall during this window and female helper number strongly predicts nestling survival (Figure 3a; Table S5), as expected under altruistic bet-hedging. The number of female helpers positively predicted nestling survival in dry conditions but negatively predicted nestling survival in wet conditions, an interaction that leaves mothers with markedly lower rainfall-related variation in nestling survival when they have more female helpers (Figure 3a). Again, this pattern holds when we isolate the effects of *within*-mother variation in female helper number (Figure 3b; Figure S6). Male helper numbers, by contrast, did not predict nestling survival, either in isolation or via interactions with rainfall (Table S6).

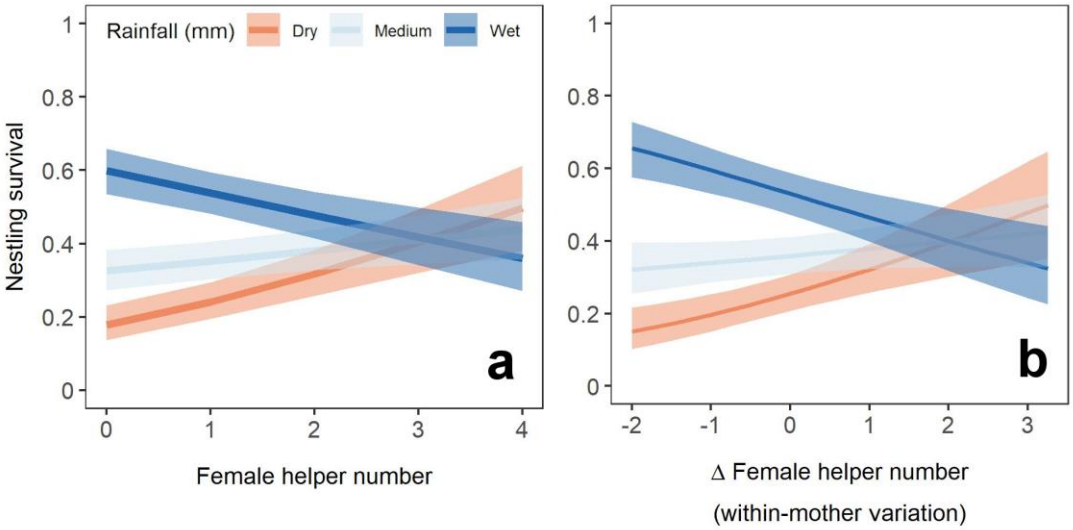


Figure 3. Helpers mitigate rainfall-induced variation in reproductive success. (**a**) While rainfall-induced variation in nestling survival to fledging is high in the absence of female helpers (zero on the x axis), mothers with more female helpers experience lower rainfall-related variation in nestling survival because nestling survival is strongly predicted by an interaction between rainfall and female helper number: under dry conditions nestling survival increases with increasing female helper number, while in wet conditions it decreases with increasing female helper number. This interaction was apparent both (**a**) when modelling the effects of population-level variation in female helper number and (**b**) when isolating the effect of within-mother variation in female helper number (Δ Female helper number). Rainfall and Female helper number (or Δ Female helper number) were not correlated (Pearson's correlation, r [95%CI] = 0.006 [-0.093, 0.104]). The plots present mean model predictions ± SE (thick coloured lines and shaded area respectively) for broods that experienced either (i) no rainfall during the focal rainfall window ('Dry', orange), (ii) a medium level of rainfall ('Medium', light blue, the prediction for total rainfall = 47.5 mm, the lower tertile of the non-zero total rainfall distribution) or (iii) a high level of rainfall ('Wet', dark blue, the prediction for total rainfall = 115 mm, the upper tertile of the non-zero total rainfall distribution).

This striking interaction suggests that the apparent negative effect of female helpers on variance in maternal reproductive success revealed by our bivariate analyses (Figures 2a,b) may indeed arise in part from helpers reducing rainfall-induced variance in reproductive success, as envisaged by global comparative studies of the evolution of cooperation in birds (Jetz & Rubenstein, 2011; Griesser *et al.*, 2017). To demonstrate that this is the case, we then integrated rainfall (in the focal window) into our original bivariate models as an additional fixed effect predictor. This confirmed that rainfall has a stronger effect on reproductive success in groups with fewer female helpers than in groups with more female helpers (Figure S4; as expected given Figure 3). Allowing for this effect reduces the residual variance in reproductive success for mothers with fewer female helpers, without affecting the residual variance for mothers with more female helpers (Figure S4), thereby accounting in part for the overall effect of female helpers on residual reproductive variance shown in Figure 2.

Together, our findings strongly suggest that helping behaviour in sparrow-weaver societies reduces variance in the reproductive success of related breeders arising from unpredictable environments, as envisaged for an altruistic bet-hedging strategy (Kennedy *et al.*, 2018), and that it does so in part by mitigating rainfall-related reproductive variance, as hypothesised by global comparative analyses of the evolution of cooperation in birds (Jetz & Rubenstein, 2011; Griesser *et al.*, 2017). While selection for helping as an altruistic bet-hedging strategy (*sensu* Kennedy *et al.*, 2018) need not arise *solely* from beneficial effects on the reproductive *variance* of relatives, the absence here of a detectable net effect of helpers on the *mean* reproductive success of relatives highlights the likely importance of such variance compression effects in this species. Indeed, while the above analyses focus specifically on helper effects on nestling survival to fledging, additional analyses reveal that helpers also have no detectable effect on the mean clutch size or re-clutching rate of mothers (Chapter 3) or on the apparent reproductive value of fledglings (i.e. alternative routes through which helping could yield indirect fitness benefits; Table S7). Helping might also yield direct fitness benefits to helpers, which could act in concert with kin selection to favour helping in this species. The most credible mechanism through which helping could yield direct benefits across the social vertebrates is if it increases group size by improving offspring survival or by increasing breeders' reproductive rate, as helpers may stand to benefit from living in a larger group ('group augmentation' hypothesis, Kingma *et al.*, 2014). It is notable then that helping in sparrow-weaver societies actually does *not* improve offspring survival on average (Figure 3) or reproductive rates (Chapter 3), and so will not tend to augment group size. Helpers do appear to reduce the *variance* in offspring survival though (Figure 2a, b), highlighting the possibility that helping yields hitherto unexplored direct fitness benefits that also arise via bet-hedging processes (i.e. helping may reduce rainfall-related variance in the helper's future group size).

The lack of a net effect of helpers on the mean reproductive success of relatives arises because the apparent positive effect of helping in dry conditions is countered by a surprising negative effect of helpers under wet conditions (see Simons, 2009, 2011 for a discussion about bet-hedging and fluctuating selection). We suspect that this negative effect reflects a change in the primary cause of nestling mortality from starvation under dry conditions (which would be mitigated by the causal positive effects of female helpers on provisioning rate; Figure 1) to nest predation under wetter conditions (which could conceivably be exacerbated when more helpers are visiting the nest). While nest predators (snakes, mongooses, birds and ants, Wood & Young, 2019) rarely leave signs of their actions in our study population, analyses of the subset of cases in which the circumstances of nestling mortality do suggest a particular cause of death are consistent with this view. With increasing rainfall, the probability of nestling starvation decreases, while the probability of whole-brood predation increases (Figure S5). The overall negative effect of rainfall on nestling mortality suggests that the former effect typically dominates (Figure S3).

Theoretical treatments of bet-hedging typically envisage that selection for strategies that reduce reproductive variance arises because additional offspring contribute disproportionately to relative fitness when competitors are producing few (Starrfelt & Kokko, 2012; Kennedy *et al.*, 2018) (i.e. that helping's positive effect on offspring production in 'dry' conditions will outweigh its negative effect on offspring production in 'wet' conditions when expressed in relative fitness terms [Kennedy *et al.*, 2018], in Figure 3). While such a mechanism is certainly plausible here, ecological processes will also impact selection for variance reduction strategies. For example, the ability to produce offspring in harsh conditions could have even stronger effects on relative fitness if harsh conditions also reduce adult survival. Biological traits of the species will also have an impact on the importance of selection for variance reduction strategies (e.g. iteroparity – (Kennedy *et al.*, 2018).

Our findings also highlight an important general point: whenever helping behaviour shows the properties of altruistic bet-hedging, the extent to which (kin) selection for helping arises via helper effects on the mean versus the variance in reproductive success of relatives will be extraordinarily sensitive to environmental conditions. This is because helping behaviour can only compress environmentally induced variance in reproductive success by having different effects on performance in different environments (e.g. see Figure 3). Where this is the case, changes in the relative frequency of these environments over space (e.g. a species range) or time (e.g. under historical and future climate change) will impact whether selection for helping arises principally from reproductive variance compression or instead from classical net effects of helping on the mean reproductive success of relatives. For example, white-browed sparrow-weavers range throughout the semi-arid regions of East and Southern Africa, and while rainfall throughout the region is unpredictable, there is appreciable spatial variation in both its mean and variability (Figure 4). Selection for helping behaviour in this species could therefore *currently* arise from (i) classical net positive effects of helping on *mean* reproductive success in more consistently arid regions (as helping improves offspring survival in dry environments; Figure 3), coupled with (ii) weaker positive selection arising more from reproductive variance compression (as envisaged under altruistic bet-hedging) in the more variable and intermediate conditions currently experienced at our Kalahari study site ('X' symbol in Figure 4). Selection could also conceivably act *against* helping behaviour in more mesic parts of the species' range. Indeed, such a pattern of positive selection for helping in both dry and variable environments concords well with the frequent finding in large-scale comparative analyses that cooperatively breeding species are globally associated with both arid *and* unpredictable rainfall regimes (Cornwallis *et al.*, 2017; Griesser *et al.*, 2017; Lukas & Clutton-Brock, 2017). Recognising the potential for marked temporal variation in climatic conditions over evolutionary timescales also highlights the difficulty of evaluating the role of reproductive variance compression in the evolutionary *origins* of helping in the cooperative breeders observed today (Cockburn & Russell, 2011; Koenig, 2017). Our findings do nevertheless highlight that the evolutionary history of helping could well have been characterised by fluctuations in the relative importance of classical net effects of helping on the mean reproductive success of relatives (Hamilton, 1964) and the reproductive variance compression effects more recently envisaged under altruistic bet hedging (Kennedy *et al.*, 2018).

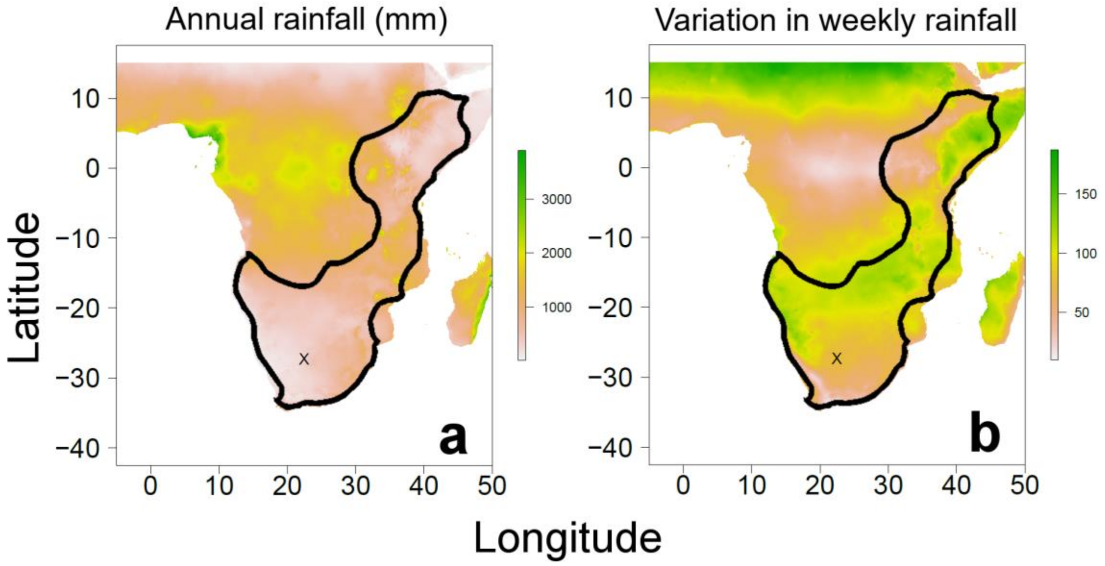


Figure 4. Environmental variation across the white-browed sparrow-weaver's range. Approximate range of the white-browed sparrow-weaver (area within the solid black line) in Africa, showing (**a**) annual rainfall (mm) and (**b**) coefficient of variation in weekly rainfall (%). Across its range, the species experiences a wide range of rainfall regimes. As helping has rainfall-dependent effects (Figure 3), the extent to which selection on helping at any given locality arises from effects on the reproductive variance of breeders versus classical net positive effects on the mean reproductive success of breeders will likely vary across the species range. Species distribution drawn based on GBIF records (www.gbif.org, accessed on 23/01/2019). 'X' shows the location of our study population. Climatic data extracted from WorldClim v2 (Fick & Hijmans, 2017).

2.2. Methods

2.2.1. Study species, population and general field monitoring

White-browed sparrow-weavers live in semi-arid regions of South and Southeast Africa. Our study population is located in Tswalu Kalahari Reserve in the Northern Cape Province of South Africa (27°16'S, 22°25' E). Data were collected continuously from September/October to April/May, coinciding with the wet season, between 2007 and 2016 (nine consecutive field seasons). Approximately 40 social groups of white-browed sparrow-weavers were monitored every year, each one inhabiting a small territory in an area of approximately 1.5 Km^2 (Harrison *et al.*, 2014). White-browed sparrow-weaver social groups contained a dominant pair and a varying number of subordinate individuals of both sexes, ranging from none to ten subordinate birds (Harrison et al., 2013; Lewis, 1982; Figure 1a). Individual dominant pairs and social groups were easily monitored and distinguished in the field as all group members foraged together, engaged in weaving, territory defence and roosted together in individual woven chambers in a single tree or cluster of trees close to the centre of their territory (Harrison *et al.*, 2013a; Walker *et al.*, 2016). All birds in the population were fitted with a single metal ring and three colour rings for identification from the beginning of the study period (under SAFRING license 1444).

Every white-browed sparrow-weaver group contained a single dominant (reproductive) female and male (Harrison *et al.*, 2013a). Previous studies carried out in the same population of white-browed sparrow-weavers showed that only dominant females laid eggs (i.e. there is no in-group or extra-group maternity; Harrison *et al*., 2013a). Genetic analysis confirmed that nests always contained eggs from a single female and that that individual could be readily identified as the dominant female using behavioural observations (Harrison *et al.*, 2013a). Dominant males commonly gained paternity in their social groups, with a low percentage of extra-group, but not extra-pair within-group, paternity (Harrison *et al.*, 2013a). Dominant pairs were easily identified in the field because they displayed a distinct set of behaviours (Walker *et al.*, 2016). In every group, dominant female and male are in very close association with each other and these two birds spend much time foraging and duetting together (Walker *et al.*, 2016). Dominant and subordinate individuals are, therefore, easily identified in the field. The sex of every individual could be determined after the first six months of life as our study population present beak colour sexual dimorphism (Leitner *et al.*, 2009).

White-browed sparrow-weaver groups were monitored every one or two days to detect new clutches. Once a new clutch was found, nests were checked daily until the clutch had been completed (i.e. when no new eggs appeared in two consecutive nest checks). Then, clutches were checked eight days after the first egg was laid to confirm successful progress of the incubation. Following this latter mid-incubation check, clutches were checked daily starting 15 days after the first egg was laid until the fate of every egg was determined (hatched or failed). Nestlings were monitored in the nest every four days, ringed at age 13 and followed until their 17th day of life. After the nest check on day 17th, we discontinued nest checks to avoid premature fledging. Behavioural observations as well as targeted catching and ringing sessions were carried out in the population and we determined successful fledging for individual nestlings if they were observed or caught outside their nests. Fledglings do not disperse in their first six months of life but remain in their natal territories where they typically become subordinate individuals.

White-browed sparrow-weaver group composition was assessed every week throughout every field season. Social groups were observed at least once every week and birds were identified on the basis of their colour-ring combination. Additionally, birds were routinely caught while roosting at night and this information was also used to define group memberships. For every brood in our analysis, number of male and female helpers were calculated based on field observations over a 24-day period spanning from egg laying to the predicted fledgling date. Young individuals hatched in a given season, and whose contribution towards provisioning offspring is very small (Lewis, 1982a), were not considered as helpers until they reach their second season of life and were, therefore, at least six month old.

When entire broods or individual nestlings disappeared from nests, we determined the most likely cause of death between predation or starvation as follows:

- Predation:
    I. Presence of signals of predation. Mongooses and goshawks are common predators of white-browed sparrow-weaver broods and they leave visible damage to the nest structure.
    II. In the absence of nest damage and in broods larger than one nestling, when every nestling disappeared in the same time interval (e.g. between two consecutive nest-checks, within less than four days). Snakes are also a potential predator of weaver nests (Spottiswoode, 2007). They predate whole broods but normally do not leave signs of damaged in the nest.
- Starvation:
    I. In broods larger than one nestling, when individual nestlings died (most often the lightest individual) leaving another sibling alive.

II. In single-nestling broods, when field observations suggested starvation as the most probable cause of death; e.g. finding a nestling dead inside the nest when both dominant adults were alive and holding social dominance.

As our ability to categorise broods between these two causes of death depends on brood size, we restricted our analysis of the temporal variation of starvation *versus* predation to broods of two nestlings (n = 113). Then, we fitted a binomial generalised linear mixed model to the probability of predation (value = 1) *versus* starvation (value = 0), controlling for among-mother and among-season variation (included as random effect intercepts). Rainfall, as calculated for the analysis of fledgling success was included as fixed predictor.

Out of 764 nestlings detected in the field with ± 4 days of uncertainty in their hatching date, 374 survived to fledging and 48 of them (12.83%) became dominant individuals between October 2007 and April 2016. We modelled the probability of dominance acquisition as a binomial trait using a GLMM including clutch ID, year and dominant female ID as random terms. Additionally, we included as fixed effect predictors: the numbers of female and male helpers, brood size, total rainfall (as a linear and quadratic terms - see sliding window analysis) and the interactions between female and male helper number and rainfall.

2.2.2. Provisioning behaviour

Provisioning behaviour was recorded for breeding attempts between the 2007 and 2016 (180 broods in 35 social groups and a total of 459 days of recordings). We collected provisioning behaviour data using video-recordings between 8 and 13 days after the first egg of a given clutch hatched. Video recordings were watched using VLC media player and data were extracted from every provisioning event: time of the feed, duration of the feed, feeder sex (based on beak coloration, Leitner *et al.*, 2009), feeder identity based on a unique vent pattern and colour-ring combination, and feeder social rank (dominant or subordinate). For every recorded breeding event, we always followed a standardised protocol: at least five days before data collection started, we (i) caught and marked the vent of every bird in the group apart from the dominant female using black hair dye to aid video identification and (ii) deployed tripods in the field to acclimatise the birds to its presence. On recording days, video cameras were set up in the morning at standard times that tracked monthly changes in sunrise. Provisioning behaviour was recorded for approximately three hours per brood and day. Within-nest cameras have confirmed that all nest visits in which the birds are not conspicuously carrying grass entail the visiting bird carrying a single food item to the nest and delivering it to the chicks (Walker, 2015). No visits were non-provisioning visits and in no cases were visiting birds observed to eat the delivered food item themselves (Walker, 2015).

2.2.3. Field removal experiment

To investigate the causal link between the number of helpers in a given group and the total feeding rate that the offspring received, we carried out a field removal experiment in February and March in 2017. During this period, broods were included in the experiment and allocated to a ‘removal’ or ‘control’ treatment sequentially once a focal brood reached 10 days after hatching. Provisioning behaviour was monitored in every brood following the standard protocol (see above).

On the last day of provisioning recordings (day 12 or 13 of the breeding attempts, N = 18 and 5 broods respectively), we caught every subordinate individual in ‘removal’ broods from their sleeping chambers within one hour before sunrise time. White-browed sparrow-weavers roost in individual chambers at night and catching individuals while roosting is readily done with tailored catching poles. Dominant males were identified from underneath the roost by their vent marks and were not caught. Dominant females, who roosted inside nests, were not disturbed either. Caught subordinate birds were kept in individual cages following approved ethical guidelines until the end of provisioning data collection 3-4 hours after sunrise. Every caught subordinate bird was released back in their territories. Field observations confirmed that these birds did always establish back in their territories immediately or within a few hours after release. Subordinate birds in 'control' broods were not caught and caged. However, the experimenter (PC-L) walked underneath the roosting tree without catching the birds in order to expose dominant individuals in 'control' broods to the same disturbance as in 'removal' broods.

We then investigated the change in total feeding rate between the day of experimentation and the previous provisioning day. Brood size did not change across sequential days within treatments or between treatments (linear model: $N_{control} = 16$, $N_{removal} = 13$, $t = 0.67$, $df = 26.81$, $p = 0.510$). Likewise, provisioning behaviour was recorded for similar durations within breeding attempts (linear model: $t = -0.64$, $df = 27$, $p = 0.528$) and between experimental groups (linear model: $t = -0.33$, $df = 27$, $p = 0.745$).

Using these experimental data, we investigated the effect of female helpers on total provisioning rates. We compared the change in total feeding rate pre- and post-manipulation between groups with female helpers not caught (n = 14) and groups where all female helpers had been removed (n = 9). Likewise, we isolated the effect of male helpers on total provisioning rates by comparing the change in total feeding rate between groups with male helpers present (n = 9) and groups were male helpers were experimentally removed (n = 9).

2.2.4. Rainfall data

Daily rainfall data were collected from two rainfall gauges located in the west (27° 16’ 58.9’’ S, 22° 23’ 02.1’’ E) and east (27° 17’ 42.1’’ S, 22° 27’ 34.9’’ E) of our study, 7.60 Km apart from each other. These two rainfall measurements were highly correlated during the study period (Pearson’s product-moment correlation: r = 0.875, 95% CI = 0.867 – 0.882, df = 3,347). We, therefore, calculated average daily values across both gauges and used this as a proxy for rainfall conditions at our study site.

2.2.5. Statistical analysis

2.2.5.1. Subordinate contributions to provisioning offspring

Total feeding rate to the brood was analysed using linear mixed models including date of observation, social group ID and brood ID as random intercept terms. Number of female helpers, number of male helpers, brood age and brood size were included as fixed effect predictors. For the removal field experiment, we analysed the change in feeding rate caused by our experimental removal of helpers. We tried to explain variation in that change associated with the manipulation (removal of all female or male helpers) and the age of the brood using linear mixed models. Social group ID was included as a random intercept term.

2.2.5.2. Effects of female helper number on variance and mean of reproductive success

To investigate the effects of female helpers on the variance of reproductive success as well as on its mean, we fitted a bivariate linear mixed model in which the response variables where:

1. Number of nestlings that fledged from breeding attempts with no female helpers (185 broods, 63 dominant females)
2. Number of nestlings that fledged from breeding attempts with some female helpers (215 broods, 51 dominant females).

46 dominant females had broods across both response terms. We carried out an additional analysis using a bivariate model that contained two response terms as (excluding breeding females with only one breeding event and, therefore, no within-mother variation in the number of female helpers, n = 388):

1. Number of nestlings that fledged from breeding attempts in which the within-mother number of female helpers was lower or equal to the within-mother mean (227 broods, 56 dominant females).
2. Number of nestlings that fledged from breeding attempts in which the within-mother number of female helpers was higher than the within-mother mean (161 broods, 49 dominant females – all of which also appeared in the data set for the first response).

The last model aimed to investigate if differences in variance components found in our first analysis were due to within-mother variation in the number of female helpers.

In every case, bivariate models contained an independent intercept for each response term as well as independent random effect (co)variances for breeding year (season) and mother ID (i.e. dominant female ID). We also allowed for response-specific residual variances (i.e. within mother variance in number of fledglings) that informed us about within-mother variance in reproductive success across different response terms. In these models, we controlled for number of hatchlings; thus, investigating the number of fledged nestlings conditional upon hatching, phase in which we show that helping females are important to rear offspring.

We fitted bivariate linear mixed models using the 'MCMCglmm' R package (Hadfield, 2010), running two independent MCMCs of 210,000 iterations with an initial burn-in period of 10,000 iterations and a thinning interval of 100 iterations. MCMC effective sample size for every model estimate always higher than 1,000. We assessed the convergence of MCMC models by visualising MCMC traces and calculating the Gelman-Rubin diagnostic (upper confidence interval < 1.01 in every case). Statistical differences between model coefficients and variance components were extracted calculating the 95% credible interval for the difference between pairs of estimates. Default priors were used for fixed effects (Normal distribution, $\mu = 0$, $\sigma^2 = 10^8$) and residual variances (inverse Wishart, V = 1, nu = 0.002). Parameter expanded priors were used for random (co)variances with $\mu_\alpha = 0$ and $\sigma^2_\alpha = 25^2$).

2.2.5.3. Sliding window approach for rainfall effects on proportion of nestlings that fledged

White-browed sparrow-weavers can breed continuously during the wet season in the Northern Cape of South Africa, between September and April, and can show high among-female asynchrony in laying dates. Hence, we used a relative sliding window approach to find the rainfall windows most associated with the proportion of nestlings that fledged from individual breeding attempts. Personal observations (Capilla-Lasheras & Young personal observations) suggested that rainfall was linked with reproduction in white-browed sparrow-weavers in a short time scale (Lewis, 1982a). We investigated relative sliding windows backdating up to 80 days from the predicted fledging date (18 days after hatching). Sliding rainfall windows were inspected in one-day intervals. To decrease the likelihood of false positive results, that are more probable for very short windows, only sliding windows longer than four days were considered.

For each sliding window, total amount of rainfall was calculated and used as a rainfall index. Rainfall index was then included as a predictor in an appropriate statistical model (see below for baseline model specifications) to test the importance of a given rainfall window. The AIC of models including rainfall index was compared to the AIC of a baseline model not containing this environmental predictor (but keeping a similar model structure), yielding a ΔAIC value ('AIC support'). We investigated linear and quadratic rainfall effects. For each of these model structures (linear and quadratic), the sliding window protocol was carried out independently.

Several factors may compete with rainfall to explain variation in fledging success. We, therefore, built a baseline model that included, as fixed terms: number of female helpers, number of male helpers, brood size, number of female helpers x Brood size, number of male helpers x Brood size; and, as random effects: year and mother ID.

To assess the likelihood of a false positive result regarding the rainfall window in the quadratic model structure (model structure with highest rainfall support), we carried out 10 randomisations of the data set. In each randomisation, the biological reference date (predicted fledging date) for the sliding window approach was randomised by re-shuffling – similar to the approach implemented in the R package ‘climwin’ (Bailey & van de Pol, 2016). For each randomisation, the sliding window protocol was fully applied and the AIC support for the top-performing rainfall window recorded. This analytical routine was implemented using an customised R script ‘Sliwin_routine.R’ available at: https://github.com/PabloCapilla/WeaverTools (inspired by the R package ‘climwin’ v1.2.0, Bailey & van de Pol, 2016).

2.2.5.4. Effects of rainfall and the number of female helpers on the proportion of nestlings that fledged

After applying the sliding window approach, we fitted a binomial mixed model to investigate the relative contribution of variables explaining variation in the proportion of nestlings that fledged (‘nestling survival’). The initial (‘global’) model contained every fixed and random effect in the baseline sliding window model and total amount of rainfall (‘rainfall’ hereafter) calculated for the most supported sliding window. We also included the interactions between rainfall and number of i) male and ii) female helpers. This global model, incorporating every variable of interest, was not over-dispersed (Residual deviance = 668.82, df = 383) and a simulation of scaled model residuals, using the R package ‘DHARMa’ (Hartig, 2018), suggested a uniform distribution of model residuals (500 model simulations, Kolmogorov-Smirnov Test, D = 0.057, p = 0.156). The global model was, then, subjected to AIC model selection (see more details below).

2.2.5.5. Confounding effect of territory quality: within-mother centering

A common concern in studies of cooperative species is that group size can be associated with territory quality, creating a spurious correlation between reproductive output and group size (both of which can be positively associated with territory quality) (Cockburn, 1998). We addressed this concern in three different ways:

1. Showing experimental evidence for a positive causal link between the number of female helpers and the total provisioning rate to the offspring
2. Excluding young individuals from our calculations of the number of male and female helpers
3. Carrying out our analyses using number of helpers and then partitioning this variable to untangle effects born of within-mother and among-mother variation in number of helpers, Δ number of helpers and μ number of helpers respectively (van de Pol & Wright, 2009). Within-mother (Δ) number of helpers accounts for variation in number of helpers within-mother and per territory and, therefore, its effects cannot be attributed to maternal or territory quality, which operates at the among-mother level. We carried out within-mother centering per territory, thus, simultaneously accounting for among-territory and among-dominant female differences in quality. Within-group centering was carried out using a R function, *d_centering*, available here: https://github.com/PabloCapilla/WeaverTools

2.2.5.6. General modelling approach

All statistical models and visualisations were carried out in R (version 3.5.1 to 3.6.1., R Core Team, 2019). The importance of single predictors and statistical hypotheses (e.g. a given combination of model predictor) in univariate models was assessed using an information-theoretic (IT) approach. Starting from a global model, containing every predictor of interest (as described above), simpler combinations of fixed predictors were fitted to the data and ranked based on Akaike's Information Criterion (AIC, Burnham & Anderson, 2002). Linear mixed models compared by AIC were fitted using maximum likelihood (ML). ΔAIC values were then calculated for every model (i.e. AIC difference between a given model and the best model – thus, ΔAIC for the best model equals 0).

We gave further consideration to models within a ΔAIC value of six (Richards, 2008) and subsequently reduced this Δ6 top model set by applying the 'nesting rule' described by Richards (2008). This rule aims to avoid the retention of overly-complex models that do not improve model fit by discarding models that are more complex versions of simpler (nested) models with lower AIC support. Adding variables with no or little explanatory power to a well-supported model is known to decrease AIC by less 6 points, therefore, being retained in our Δ6 top-model set (Arnold, 2010). The nesting rule tries to reduce the chance of considering models with such un-informative variables. When quadratic terms were included in a given model, linear coefficients were always present. Every model set included intercept-only models. Unless otherwise stated, model selection tables show standardised model coefficients (mean centered and scale to one standard deviation) to aid comparison of effect sizes across predictors (Gelman & Rubin, 1992). In model selection tables each line presents a model from the top model set (within ΔAIC =6 of the 'top model' [the model with the lowest AIC]), while each of the fixed effect cells presents the estimate for that fixed effect in that model (or is blank if the focal fixed effect was absent from that model).

Chapter 2: Supplementary Material

Supplementary Information Chapter 2

A – Rainfall conditions in our study site are highly variable

Rainfall conditions in our study site in the Kalahari Desert of South Africa were highly variable both within and among years. Average total amount of rainfall between 2007 and 2016 was 357 mm (standard deviation = 153 mm) with a minimum of 194 mm in 2007 and a maximum of 670 mm in 2011. The among-year coefficient of variation in total rainfall was 42.86%, a value 5 and 39% higher than those reported by Koenig and Walters (2015) (CV = 40.8%) and Rubenstein (2011) (CV = 26.09%). Among-year variation in rainfall conditions during the wet season (October to April) was also high, with average values for years ranging between a maximum of 585 mm in 2011 to a minimum of 174 mm in 2015 (mean ± standard deviation among years: 321 mm ± 137 mm). Within years, January was the wettest month (mean ± sd: 3.35 mm ± 11.4 mm) and August the driest (mean ± sd: 0.05 mm ± 0.45 mm), with monthly coefficients of variation ranging between 339% in January to 1,231% in July.

B – Rainfall cannot be predicted based on current conditions

Auto-correlation analyses strongly suggested that current rainfall conditions did not provide information about future rainfall (Figure S1). The correlation between the amount of rainfall on a given day was very weakly correlated with that of the previous day (r = 0.068). Allowing for auto-correlation lags of up to 1,095 days (three years), 0.12 was the highest rainfall correlation found, for a time lag of 1,042.

C – Subordinate contributions to provisioning offspring

We analysed 180 broods between 2007 and 2016. On average, provisioning data were collected 2.55 days for every brood.

Within-mother and among-mother number of female helpers was positively associated with total provisioning rate to the offspring (controlling for brood age and brood size, whose effect on total provisioning rate was also positive; Table S1). Within-mother variation in number of female helpers appeared in every model with ΔAIC < 8.86, providing strong statistical support for a positive effect of the number of female helpers on total provisioning rates (Table S1). Likewise, total provisioning rate in groups that were, on average, large were higher than the rate of small groups ('μ Number of female helpers' and 'μ Number of female helpers', Table S1).

Effect sizes for within-mother number of male helpers were estimated as three times smaller than the size of the effect of within-mother number of female helpers and did always receive much less statistical support that the terms for female helpers (Table S1). Including within-mother number of male helpers ('Δ male helper number') in the top model shown in Table S1 did not change the estimates of within- or among-mother number of female helpers. Carrying out a complementary analysis that did not separate within- and among-group effects of number of helpers, we found similar results, with strong support for a positive effect of the number of helping females.

Results from the removal field experiment, indicated that temporarily removing the contribution of female helpers towards provisioning offspring caused a significant decrease of total provisioning rate compared to a previous day of provisioning. This factor was associated with an increase in AIC of 2.66 (Table S2). To further support the differential effect of female and male helpers, we repeated the analysis of the field experiment. This time, we compared total provisioning rate of groups where every male helper was removed and groups with subordinate male helpers present. The removal of male helpers did not impact total provisioning rate to the offspring (Table S2b). For the experimental manipulation of the number of male helpers, the intercept-only model scored the lowest AIC, with experimental treatment (i.e. removal of male helpers) appearing in the model set after ΔAIC > 1.43 and not being retained after application of the nesting rule (Table S2b).

D - Rainfall effects on the proportion of nestlings that fledged

Total amount of rainfall between 36 days prior hatching and 9 days after hatching strongly predicted the proportion of nestlings that fledged following a quadratic relationship (Figure S3). A quadratic rainfall relationship between rainfall and nestling survival to fledgling was much more supported by the data than a simpler linear association (the AIC for quadratic model was in both cases approximately 5 points lower than the AIC for the linear relationship).

None of the top performing models in ten randomisations of the data showed stronger AIC support than the -29.07 AIC points achieved by the quadratic model using the real data set (Figure S3b). The strongest AIC support across the ten randomisations ranged from -21.91 to -11.65. The distribution of values for AIC support within each randomisation also suggested that the likelihood of achieving -29.07 AIC support was very unlike under random conditions (Figure S3b).

The most-supported rainfall model suggested that rainfall had a strong positive effect on the proportion of nestlings that fledged between 0 mm and approximately 185 mm of total rainfall. Rainfall negatively impacted fledging success in broods associated with more than 185 mm of total rainfall. Only a low percentage of broods in our 9-season data set experienced such high rainfall conditions (14.75%, 59 out of 400). Negative rainfall effects in our system are very likely due to heavy rainfall events, causing nests to collapse. Field observations provided some evidence for this hypothesis and our data also supported this idea. Maximum daily rainfall (i.e. highest rainfall amount fell on a single day) during the nestling phase was higher for clutches with total rainfall above 185 (mean ± standard error = 44.30 mm ± 3.0 mm) than for those broods with total rainfall below 185 mm (mean ± standard error = 10.30 mm ± 0.78 mm; Mann-Whitney-Wilcoxon test, $W = 17{,}381$, $p < 0.001$). Brood failure due to very intensive rainfall events is likely to be independent from helping effects on offspring survival. In contrast, helping effects have been hypothesised to be differentially important when environmental conditions are harsh *versus* benign (i.e. dry *versus* moderately wet). Therefore, we focused our attention here on the range of circumstances where rainfall had a positive effect on the proportion of nestlings that fledged (341 clutches with total rainfall below 185 mm). However, note that an additional analysis of the whole data set, including every rainfall level, yielded similar results that when focusing on positive rainfall conditions, with the interaction between the number of female helpers and rainfall appearing in the model with lowest AIC (i.e. strongest statistical support).

Supplementary Figures Chapter 2

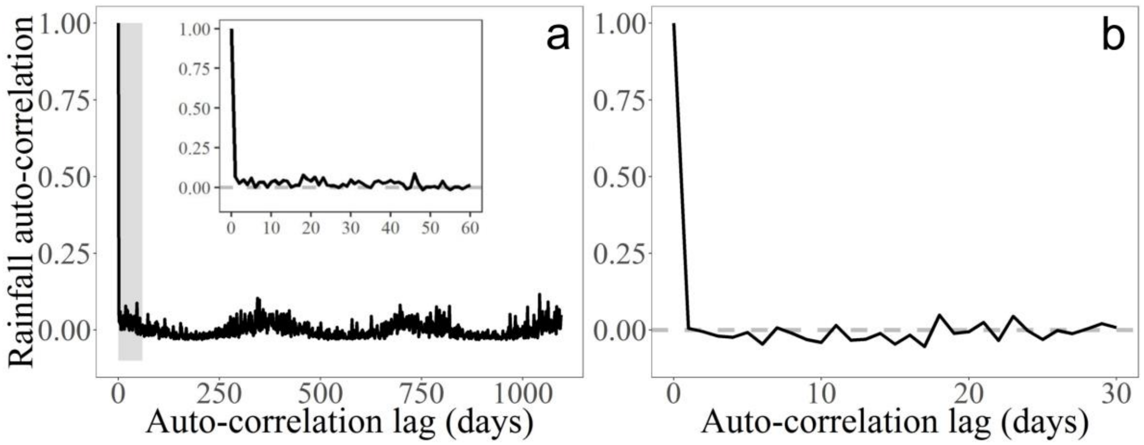


Figure S1. Temporal predictability of rainfall. (a) Rainfall auto-correlation for day lags from 0 (present day) to 1,095 days (three years). Rainfall on a given day was not correlated with that of previous days. The inset shows an amplification of the grey-shaded area, representing lags of up to 60 days. (**b**) Rainfall auto-correlation calculated over the window of 45 days in which rainfall was found to be most associated with the proportion of nestlings that fledged (see results section on rainfall effects on fledging success). The black line represents the average rainfall auto-correlation across all broods.

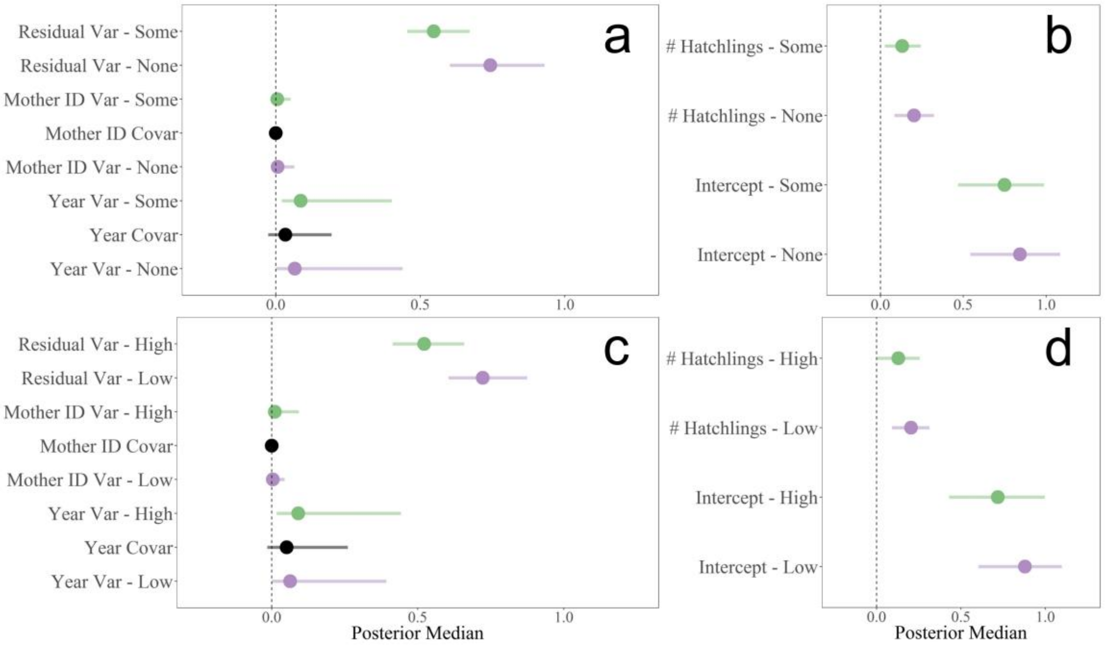


Figure S2. Female helper effects on mean and variance of reproductive success. Model estimates and 95% credible intervals from bivariate mixed models explaining variation in reproductive success for mothers assisted by none or some helping females (**a**, **b**) and higher or lower number of female helpers than the average for individual females (**c**, **d**). The left-hand side column (**a**, **c**) shows estimated and 95% CI for (co)variance components, whereas fixed term estimations are shown on the right-hand column (**b**, **d**).

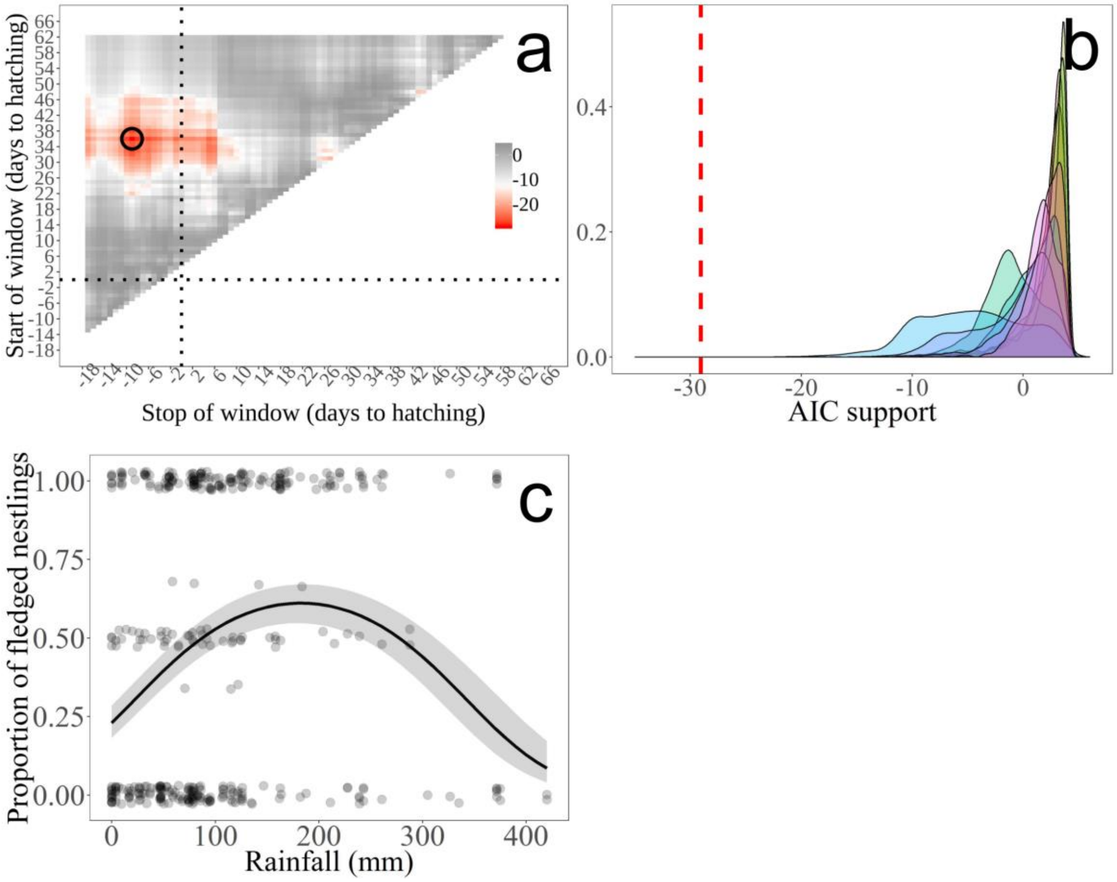


Figure S3. Sliding window analysis for the effects of rainfall on the proportion of nestlings that fledged. (**a**) AIC support for rainfall models of varying start and stop values (in days to hatching). Tile colour represents AIC support, the redder the tile, the stronger the support for a given rainfall window. Dotted lines mark hatching dates. (**b**) Density distribution of AIC support values for ten randomised sliding window analyses. These density distributions illustrate AIC support values expected by chance (i.e. assuming no relationship between total rainfall and nestling survival). Each randomisation is shown in a different colour. The vertical red dashed line gives the AIC support for the quadratic rainfall model fitted to the real data, a value that is highly unlikely assuming no relationship between total rainfall and nestling survival. (**c**) Predicted mean effect (± SE) of total rainfall over the best sliding window on the proportion of fledged nestlings (i.e. 'nestling survival'). Shaded area represents one standard error around the mean prediction (black line). Raw data points are illustrated as transparent grey dots with a slight offset in the y-axis to aid visualisation of overlaying points.

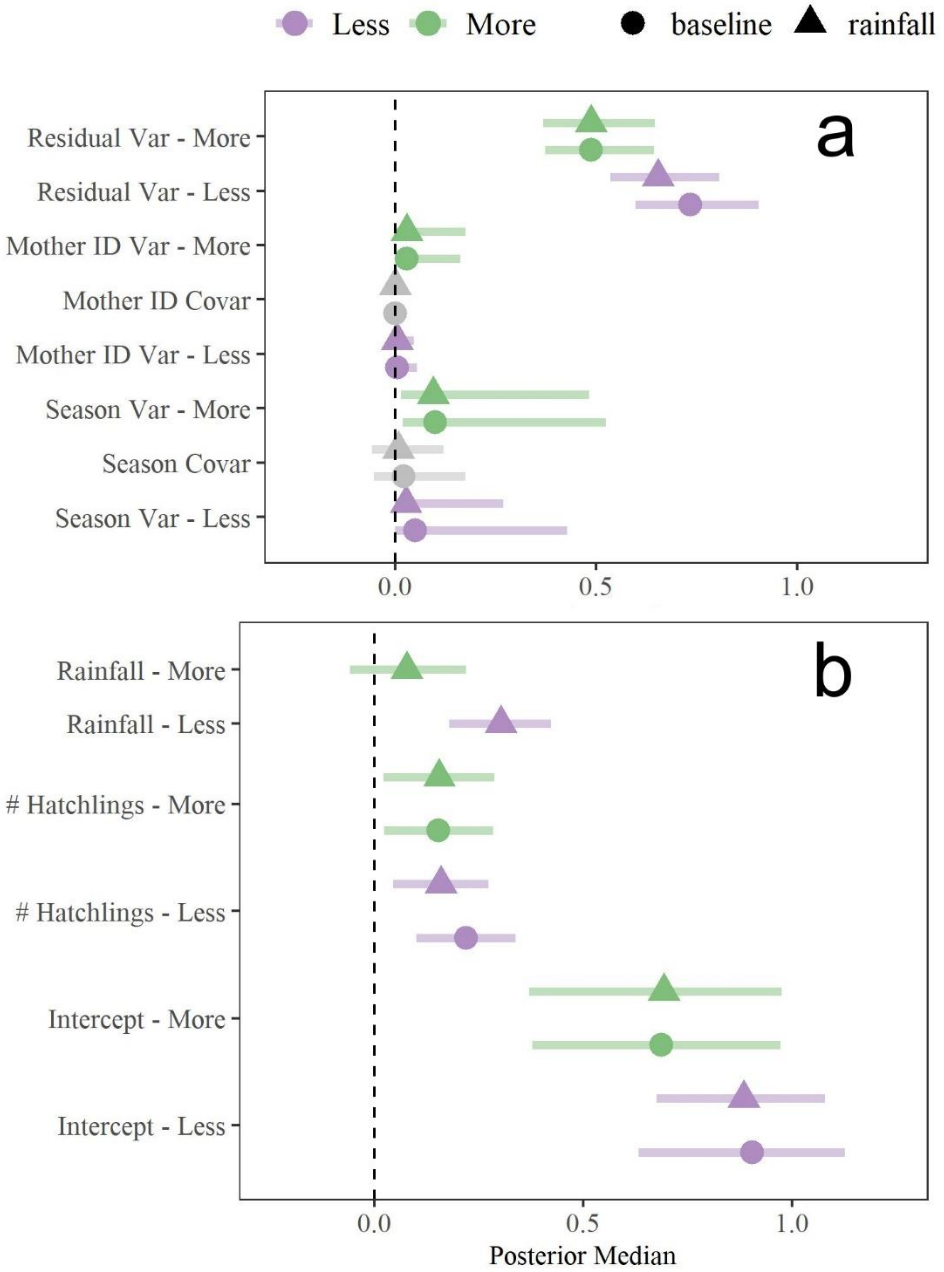


Figure S4. Effect of rainfall on variance of reproductive success. Bivariate model estimates and 95% credible intervals for fixed effects (**a**) and (co)variance components (**b**) explaining variation in the number of fledged nestlings per brood. The inclusion of the rainfall variable (triangles) in the baseline model (circles) caused a reduction of residual variance for broods with a low within-mother number of female helpers ('less' - purple) but did not change the variance estimation for broods with a high within-mother number of female helpers ('more' - green), suggesting that within-mother variance differences between large and small groups are, in part, explained by their response to rainfall. Rainfall positively predicted reproductive success, but this effect was stronger when within-mother number of helper females was low compared to broods assisted a high number of helper females (rainfall estimate for ‘less’ and ‘more’ number of female helpers [95% CI]: ‘less’ = 0.30 [0.18, 0.422], ‘more’ = 0.08 [-0.06, 0.22]. Difference between model estimates, ‘more’ – ‘less’ [95% CI] = -0.22 [-0.41, -0.04]). Random effect co-variances are illustrated in grey. Baseline and rainfall bivariate mixed models were fitted using MCMCglmm in R (Hadfield, 2010) (for MCMC details see Methods).

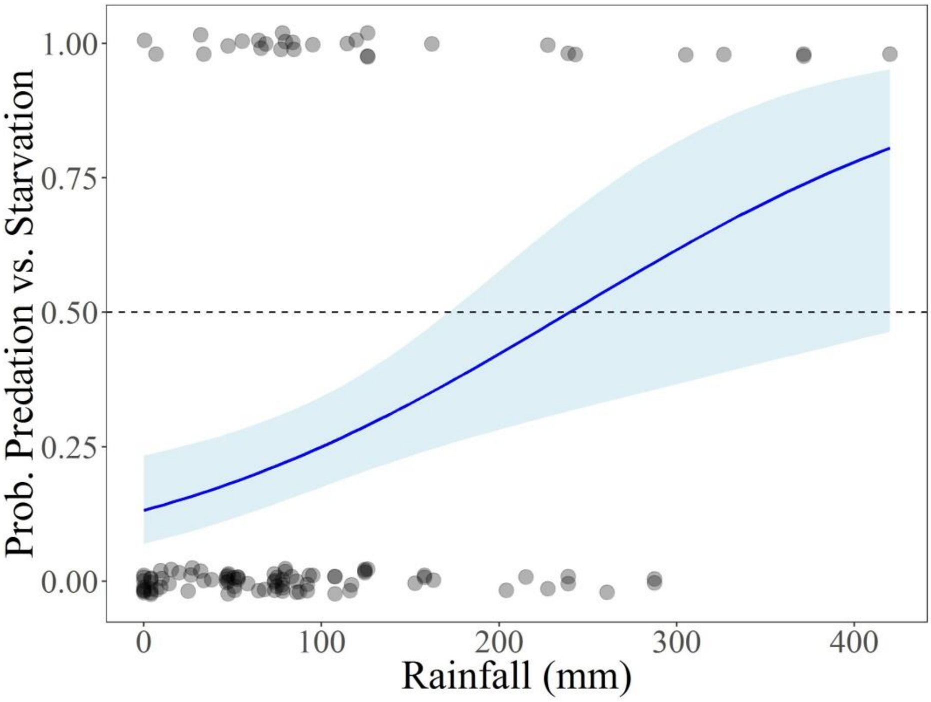


Figure S5. Mortality analysis. Probability of an entire brood being predated (i.e. y-axis value = 1) against the probability of starvation for nestlings (i.e. y-axis value = 0). When a mortality event occurred, there was an increasing probability of it being a predation event as rainfall increased (GLMM: ΔAIC after dropping the 'Rainfall' predictor = 9.67; see methods). We did not detect an effect of helpers on the probability of predation under any environmental conditions. The dashed black line represents an equal probability of predation or starvation (y-intercept = 0.5) whereas the blue line gives model predictions ± se. Black dots illustrate raw data points (n = 113).

Supplementary Tables Chapter 2

Table S1. Analysis of the effect of the number of male and female helpers on total provisioning rate to the brood, following the partitioning of variation in helper numbers in to its within- (Δ) and among-mother (μ) components (see methods). Models within ΔAIC of six of the top model are shown. We found strong support for an effect of Δ female helper number but not Δ male helper number on total provisioning rate. k = number of model parameters. w = Akaike's weights. N = 459 provisioning days.

Intercept	Δ Female helper number	Δ Male helper number	μ Female helper number	μ Male helper number	Brood Age	Brood Size	k	AIC	ΔAIC	w
-0.006	0.212		0.087	0.094		0.342	9	1157.5	0	0.115
-0.008	0.210			0.113		0.334	8	1157.6	0.18	0.105
-0.005	0.193	0.077	0.089	0.098		0.356	10	1157.8	0.30	0.099
-0.008	0.192	0.074		0.118		0.347	9	1158.1	0.60	0.085
-0.006	0.215		0.109			0.339	8	1158.1	0.66	0.083
-0.004	0.221		0.092	0.093	-0.048	0.338	10	1158.2	0.75	0.079
-0.004	0.202	0.073	0.094	0.097	-0.045	0.351	11	1158.7	1.21	0.063
-0.007	0.218			0.114	-0.043	0.330	9	1158.7	1.22	0.063
-0.005	0.197	0.071	0.112			0.352	9	1158.7	1.23	0.062
-0.004	0.224		0.114		-0.049	0.335	9	1158.9	1.40	0.057
-0.006	0.200	0.071		0.118	-0.040	0.342	10	1159.2	1.78	0.047
-0.004	0.207	0.067	0.117		-0.046	0.347	10	1159.6	2.11	0.040
-0.009	0.213					0.327	7	1159.7	2.21	0.038
-0.009	0.197	0.066				0.339	8	1160.4	2.98	0.026
-0.008	0.220				-0.042	0.323	8	1160.8	3.30	0.022
-0.007	0.205	0.063			-0.039	0.334	9	1161.7	4.19	0.014

Table S2. Analysis of a helper removal experiment to test the effect of (**a**) female and (**b**) male helpers on total provisioning rate (feeds / hour) to the brood. Model coefficients are not standardised to ease biological interpretation. (**a**) The change in total provisioning rate between two consecutive days of experiment was 13 feeds / hour lower when all female helpers in a group were removed compared to when female helpers were left in place (N = 23). (**b**) With a similar sample size (N = 18), we did not find statistical evidence for a causal effect of removing all male helpers on the total provisioning rate (the removal treatment effect was not present in the top model). k = number of model parameters. w = Akaike weights.

a

Intercept	**Brood age**	**Female helper removal treatment effect**	**k**	**AIC**	**ΔAIC**	**w**
3.668		-13.000	4	193.5	0	0.494
85.300	-6.700	-11.560	5	194.8	1.32	0.256
140.700	-11.570		4	196.2	2.66	0.131
-1.062			3	196.4	2.84	0.119

b

Intercept	**Brood age**	**Male helper removal treatment effect**	**k**	**AIC**	**ΔAIC**	**W**
-6.717			3	157.6	0	0.469
-3.563		-5.908	4	159.0	1.43	0.230
89.180	-7.930		4	159.2	1.64	0.207
80.240	-6.966	-5.174	5	160.8	3.20	0.095

Table S3. Male helper effects on mean and variance of reproductive success (number of nestlings fledged, while controlling for variation in the number of hatchlings). The posterior distributions are presented for a bivariate model in which the two response terms correspond to the number of fledged nestlings in broods fed by (i) no male helpers (the 'No MH' estimates in the table; n = 137 broods) or (ii) some male helpers (the 'Some MH' estimates in the table; n = 263 broods). There was no evidence of a difference between the two contexts (i.e. with and without male helpers) in either the mean reproductive success of mothers (i.e. no difference in the 'Intercept' estimates; 95% CI for the difference, 'Some MH' - 'No MH' = -0.228 to +0.154) or variance in reproductive success of mothers (i.e. no difference in the 'Residual Variance' estimates; 95% CI for the difference, 'Some MH' – 'No MH' = -0.198 to +0.068). The model controlled for a positive effect of the number of hatchlings in the brood (as a fixed effect) and for variation in reproductive success among seasons and mothers (using random effects). Median model estimates and their 95% credible intervals are shown for each term based on two independent MCMCs (see methods). 'Covariance' refers to the covariance between the two response terms for a given random effect.

Fixed effect estimates

	Lower	Median	Upper
Intercept (No MH)	0.481	0.659	0.843
Intercept (Some MH)	0.443	0.623	0.805
Number of hatchlings (No MH)	0.314	0.412	0.513
Number of hatchlings (Some MH)	0.246	0.329	0.408

Variance component estimates

Residual Variance (No MH)	0.437	0.535	0.661
Residual Variance (Some MH)	0.408	0.476	0.560
Season (No MH)	0.000	0.023	0.195
Season (Some MH)	0.011	0.044	0.205
Season Covariance	-0.010	0.022	0.128
Mother ID (No MH)	0.000	0.007	0.059
Mother ID (Some MH)	0.000	0.003	0.032
Mother ID Covariance	-0.014	0.000	0.014

Table S4. Summary of results for the analysis of reproductive success (i.e. number of nestlings fledged) controlling for variation in (**a**) maternal egg investment (‘egg volume’ in mm^3; N = 348 clutches) and (**b**) hatchling mass (g; N = 310 broods). Egg volume was averaged for each clutch, and hatchling mass for each brood, to allow their fitting as predictors in a clutch-level or brood-level analysis respectively. Including these predictors did not reveal an association between either female or male helper number and mean reproductive success. Models within ΔAIC of six of the top model are shown. k = number of model parameters. w = Akaike’s weights. Extending the analysis also yielded no support for interactions between either female or male helper number and either egg volume or hatchling mass.

a	**Intercept**	**Brood size**	**Female helper number**	**Male helper number**	**Egg volume (mm^3)**	**k**	**AIC**	**ΔAIC**	**w**
	0.284	0.293				5	850.5	0	0.307
	-0.152	0.298			0.0001	6	851.6	1.06	0.181
	0.314	0.292	-0.026			6	852.1	1.59	0.139
	0.261	0.294		0.018		6	852.4	1.86	0.121
	-0.157	0.297	-0.031		0.0001	7	853.0	2.48	0.089
	-0.163	0.299		0.015	0.0001	7	853.5	2.95	0.070
	0.29	0.294	-0.028	0.021		7	853.9	3.39	0.056
	-0.171	0.298	-0.033	0.019	0.0001	8	854.8	4.31	0.036
b	**Intercept**	**Brood size**	**Female helper number**	**Male helper number**	**Hatchling mass**	**k**	**AIC**	**ΔAIC**	**w**
	-0.228	0.261			0.170	6	753.3	0	0.403
	-0.199	0.259	-0.030		0.172	7	754.8	1.52	0.188
	-0.273	0.266		0.031	0.170	7	754.8	1.55	0.185
	-0.247	0.265	-0.033	0.035	0.173	8	756.3	2.97	0.091

0.346	0.250				5	756.8	3.49	0.070
0.302	0.254		0.031		6	758.3	5.05	0.032
0.377	0.248	-0.025			6	758.4	5.16	0.031

Table S5. Summary of results for the analysis of the proportion of hatchlings per brood that successfully fledged. Only one model (the top model) appeared within ΔAIC of six of the top model. This model contained an interaction between the number of female helpers and total rainfall during the rainfall window. The removal of this interaction caused an increase of AIC of 7.14, suggesting very strong support for this interaction. k = number of model parameters. w = Akaike's weights. N = 341 broods (those broods that experienced <185mm total rainfall during the focal window (see methods); repeating the analysis with the whole data set and the quadratic rainfall effect on fledging success revealed the same result, with the interaction between number of female helpers and rainfall appearing in the top model, improving AIC 1.65 points compared to second best model).

Intercept	**Brood size**	**Female helper number**	**Male helper number**	**Rainfall**	**Female helpers x Rainfall**	**Male helpers x Rainfall**	**k**	**AIC**	**ΔAIC**	**w**
-0.161	-0.348	-0.036		0.532	-0.316		7	687.7	0.00	0.424

Table S6. Analysis of the proportion of nestlings that survived to fledging (per brood), partitioning variation in female number in to their within- (Δ) and among-mother (μ) components. This analysis confirms that the results shown in Table S5 were principally due to effects of *within*-mother variation in female helper number, whose interaction with rainfall appeared here in the top three models (ΔAIC < 3.33). k = number of model parameters. w = Akaike's weights. Standardised model estimates are presented to aid the comparison of effect sizes across predictors. N = 341 broods (those that experienced rainfall below 185 mm; see methods).

Intercept	Brood size	ΔFemale helper number	μFemale helper number	Rainfall	ΔFemale helpers x Rainfall	μFemale helpers x Rainfall	k	AIC	ΔAIC	w
-0.160	-0.351	-0.080	0.051	0.540	-0.250	-0.204	9	690.8	0.00	0.399
-0.162	-0.334	-0.060		0.502	-0.255		7	691.5	0.69	0.283
-0.164	-0.333	-0.056	0.033	0.504	-0.251		8	693.4	2.57	0.110
-0.131	-0.338		0.086	0.570		-0.197	7	694.2	3.32	0.076
-0.131	-0.320			0.530			5	694.9	4.03	0.053
-0.128	-0.341	-0.059	0.080	0.568		-0.203	8	695.8	5.00	0.033
-0.134	-0.320		0.064	0.533			6	696.4	5.57	0.025
-0.129	-0.321	-0.042		0.527			6	696.7	5.86	0.021

Table S7. Summary of results within a ΔAIC value of six for the analysis of the probability of dominance acquisition for nestlings fledged before May 2014 (i.e. every individual had at least two years to become dominant; N = 330). Neither the number of female helpers, male helpers or rainfall (or their interaction) predicted whether a fledgling acquired dominance between 2007 and 2016. Brood size was not either associated with dominance acquisition. The intercept-only model scored the lowest AIC by ~1 point. Similar results were obtained when modelling the probability of dominance acquisition for hatchlings (i.e. combining probability of survival to fledging and of acquiring dominance – n = 620). k = number of model parameters. w = Akaike's weights.

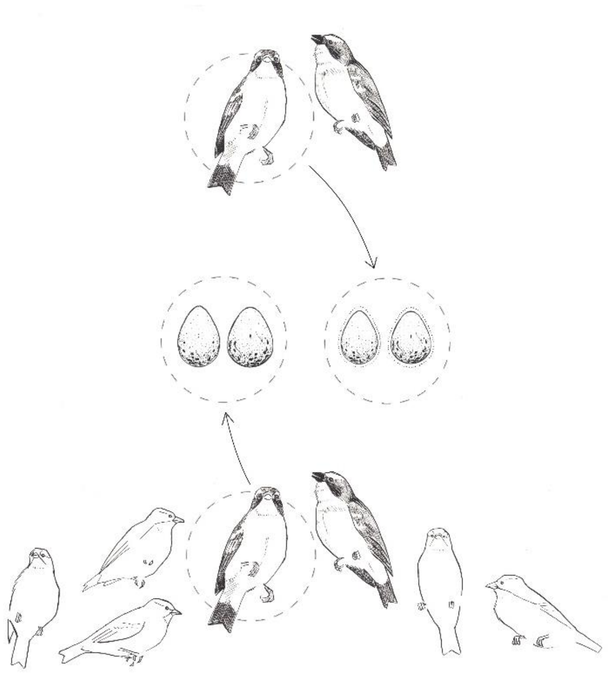
Intercept	**Brood Size**	**Number female helpers**	**Number male helpers**	**Rainfall[1]**	**Rainfall[2]**	**Number female helpers x Rainfall[1]**	**Number female helpers x Rainfall[2]**	**Number of male helpers x Rainfall1**	**Number of male helpers x Rainfall2**	**k**	**AIC**	**ΔAIC**	**w**
-2.234										4	262.30	0	0.105
-2.269				-0.219						5	263.20	0.92	0.066
-2.209		-0.226								5	263.20	0.96	0.065
-2.249		-0.272		-0.265						6	263.70	1.46	0.051
-2.208	0.130									5	263.80	1.50	0.049
-2.215					-0.096					5	264.00	1.79	0.043
-2.233			-0.025							5	264.20	1.99	0.039
-2.243	0.148			-0.235						6	264.50	2.29	0.033
-2.256				-0.289	-0.197					6	264.60	2.31	0.033
-2.287		-0.378		-0.257		-0.245				7	264.70	2.40	0.032
-2.187	0.129	-0.224								6	264.70	2.46	0.031

-2.227	0.150	-0.273		-0.283					7	265.00	2.79	0.026
-2.268			-0.058	-0.228					6	265.10	2.85	0.025
-2.200		-0.215			-0.062				6	265.10	2.87	0.025
-2.209		-0.231	0.021						6	265.20	2.95	0.024
-2.244		-0.252		-0.319	-0.159				7	265.30	3.07	0.023
-2.198	0.118				-0.072				6	265.60	3.38	0.019
-2.249		-0.270	-0.010	-0.267					7	265.70	3.45	0.019
-2.208	0.130		-0.007						6	265.80	3.50	0.018
-2.215			-0.020		-0.095				6	266.00	3.78	0.016
-2.267	0.144	-0.378		-0.277		-0.244			8	266.00	3.78	0.016
-2.238	0.130			-0.300	-0.176				7	266.10	3.82	0.015
-2.291		-0.371		-0.330	-0.184	-0.269			8	266.20	3.95	0.015
-2.310			-0.115	-0.226				-0.177	7	266.30	4.04	0.014
-2.256			-0.052	-0.296	-0.195				7	266.50	4.25	0.013
-2.243	0.144		-0.037	-0.240					7	266.50	4.26	0.012
-2.286		-0.379	0.003	-0.257		-0.245			8	266.70	4.40	0.012
-2.186	0.134	-0.234	0.041						7	266.70	4.42	0.011
-2.183	0.124	-0.218			-0.035				7	266.70	4.43	0.011
-2.227	0.136	-0.256		-0.330	-0.135				8	266.80	4.53	0.011
-2.295		-0.279	-0.073	-0.269				-0.194	8	266.80	4.54	0.011
-2.276		-0.271		-0.344	-0.203		0.137		8	267.00	4.76	0.010
-2.212		-0.223			-0.081		0.064		7	267.00	4.78	0.010

-2.226	0.151	-0.276	0.014	-0.281						8	267.00	4.79	0.010
-2.199		-0.220	0.022		-0.063					7	267.10	4.86	0.009
-2.244		-0.250	-0.009	-0.320	-0.159					8	267.30	5.07	0.008
-2.309			-0.132	-0.313	-0.240			-0.230		8	267.40	5.18	0.008
-2.198	0.118		-0.005		-0.072					7	267.60	5.38	0.007
-2.285	0.150		-0.098	-0.239				-0.186		8	267.70	5.40	0.007
-2.275	0.130	-0.373		-0.341	-0.160	-0.265				9	267.70	5.45	0.007
-2.218			-0.024		-0.099				0.031	7	268.00	5.75	0.006
-2.266	0.147	-0.384	0.027	-0.274		-0.245				9	268.00	5.76	0.006
-2.238	0.127		-0.035	-0.304	-0.176					8	268.00	5.79	0.006
-2.273	0.159	-0.285	-0.053	-0.285				-0.205		9	268.10	5.81	0.006
-2.302		-0.257	-0.093	-0.343	-0.208			-0.241		9	268.20	5.91	0.005
-2.290		-0.372	0.004	-0.329	-0.184	-0.269				9	268.20	5.95	0.005
-2.291		-0.371		-0.330	-0.184	-0.270	-0.001			9	268.20	5.95	0.005
-2.312		-0.366	-0.048	-0.261		-0.192		-0.142		9	268.20	5.97	0.005

Chapter 3

Maternal plasticity in a cooperative bird: opposing effects of helping on pre- and post-natal maternal investment



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3.1 Abstract

It has been hypothesised that mothers in cooperatively breeding societies adjust their pre-natal investment in offspring according to the availability of help with post-natal care, with important implications for our understanding of both maternal and helper effects. While relationships between helper number and egg size have been reported in some cooperative birds, whether such patterns reflect *within*-mother plasticity rather than *among*-mother correlations between egg size and helper numbers is unknown. Here we provide the first formal evidence of helper-related maternal plasticity in egg size in a cooperative bird. Using a reaction-norm approach we show that individual mothers lay larger eggs when they are assisted by more female helpers. That this plastic response is predicted by female helper numbers and not male helper numbers implicates post-natal helping *per se* as the likely cause of this relationship, rather than general effects of group size, as female helpers feed nestlings at substantially higher rates than males. This plastic response could reflect an adaptive maternal response to post-natal effects of helpers on the mother's net payoff from egg investment. Indeed, further reaction-norm analyses reveal that female (but not male) helpers lighten the post-natal workloads of mothers, which may reduce the *cost* of egg investment. The known causal positive effect of female (but not male) helpers on total nestling provisioning rate could also increase the *benefits* of egg investment in this species. Both explanations require the post-natal social environment to be predictable at the time of egg laying, which we demonstrate is very much the case.

3.2. Introduction

Maternal effects are widely recognised to have downstream fitness consequences for mothers and offspring (Mousseau & Fox, 1998; Lindström, 1999; Krist, 2011; Pick *et al.*, 2016) and there is growing evidence for plastic variation in maternal pre-natal investment (i.e. egg production or gestation, Russell *et al.*, 2003a; Kvalnes *et al.*, 2013; Langmore *et al.*, 2016). In social organisms, social conditions can be highly variable and mothers are predicted to evolve investment strategies that maximise their fitness returns on investment according to their social environment (Russell & Lummaa, 2009). Cooperatively breeding species are of particular interest in this context. In these species, helpers typically contribute to the post-natal feeding of the offspring of breeding females (hereafter 'mothers') and thus have the potential to impact the net payoffs to mothers from pre-natal investment (Russell *et al.*, 2007a; Russell & Lummaa, 2009). That mothers can be assisted by variable numbers of helpers throughout their lives leads to an expectation of selection for plastic strategies in which mothers adjust pre-natal investment in offspring according to the likely availability of help during post-natal care. Different maternal strategies for adjusting pre-natal investment to the presence of helpers are hypothesised to arise depending on how helpers impact the net payoff to mothers from pre-natal reproductive investment.

Helpers have the potential to reduce the marginal *benefit* to mothers of increasing pre-natal investment, leading to strategies in which mothers *reduce* pre-natal investment when assisted by more helpers (Russell & Lummaa, 2009; Savage *et al.*, 2015). For example, such a strategy might be favoured if helpers compensate for maternal reductions in pre-natal investment by increasing the overall provision of post-natal care (i.e. additive care, Savage *et al.*, 2015). Indeed, it has been highlighted that such compensatory reductions in egg size in the presence of helpers have the potential to conceal beneficial effects of helpers on the offspring that they raise if empirical studies fail to control for associated variation in pre-natal maternal investment (Russell *et al*. 2007a). Notably though, the rationale for predicting such a compensatory maternal response requires that helper-derived post-natal investment can indeed compensate for reductions in maternal pre-natal investment, which may not always be the case. Indeed, there is ample evidence highlighting the potential for pre-natal conditions, and pre-natal maternal investment more specifically, to have formative effects on offspring phenotype and performance (Henry & Ulijaszek, 1996; Hales & Barker, 2001; Krist, 2011; Pick *et al.*, 2016, 2019).

Helpers also have the potential to reduce the marginal *cost* to mothers of increasing pre-natal investment, leading to strategies in which mothers instead *increase* pre-natal investment when assisted by more helpers (e.g., Woxvold & Magrath, 2005). For example, helpers are often thought to lighten the post-natal provisioning workloads of mothers (Hatchwell, 1999; Heinsohn, 2004; Meade *et al.*, 2010), which could allow mothers to pre-emptively increase their levels of pre-natal investment, to which helpers cannot directly contribute. Indeed, where maternal investment *in utero/ovo* does have formative effects on offspring fitness (see above), allowing higher maternal pre-natal investment could constitute an important and otherwise cryptic mechanism through which helpers impact offspring fitness. Notably, this hypothesis highlights the potential for empirical studies of helper effects that control for variation in pre-natal maternal investment (so as to reveal potentially 'concealed' helper effects; *sensu* Russell et al. 2007a; see above) to instead underestimate helper effects, by shrouding beneficial helper effects on offspring that arise indirectly by allowing mothers to increase pre-natal investment.

In cooperatively breeding birds, relationships between the presence or number of helpers and pre-natal maternal investment in the egg (typically egg volume) have now been detected in at least nine species, with both negative and positive associations reported (Dixit *et al.*, 2017). Typically, such relationships suggest that females with (more) helpers lay smaller eggs (*Malurus cyaneus,* Russell *et al.*, 2007a; *Corvus corone corone,* Canestrari *et al.*, 2011; *Philetairus socius,* Paquet *et al.*, 2013), with just one study reporting the reverse relationship (i.e. females with helpers lay larger eggs; *Cyanopica cooki,* Valencia *et al.*, 2017). However, no study to date has provided compelling evidence that such patterns arise specifically from plasticity in egg size within individual mothers, rather than among-mother variation in egg size being correlated with among-mother variation in helper presence or number (e.g. females on higher quality territories might simply lay larger eggs and have more offspring that survive to become helpers). Indeed, the one study to date that has utilised within-individual centring statistical techniques to explicitly tease apart the effects of within and among-mother variation in helper number found that the negative relationship detected between helper number and egg volume in red-winged fairy wrens (*Malurus elegans*) arose from among-mother differences rather than maternal plasticity, illustrating the importance of taking this approach (Lejeune *et al.*, 2016). The rationale typically invoked to explain relationships between egg volume and helper number rests on the central importance of the cooperative contributions that helpers provide to post-natal care. However, correlations between egg size and helper number could equally reflect maternal responses to variation in social group size which could itself impact the optimal level of pre-natal investment independent of the availability of post-natal help *per se* (Taborsky *et al.*, 2007).

Here we use a long-term field study of cooperatively breeding white-browed sparrow-weaver, *Plocepasser mahali*, societies to (i) explicitly test for maternal plasticity in pre-natal egg investment in response to the availability of help, and (ii) investigate the selective pressures that may have shaped the plastic response that we detect. Throughout, we use a statistical approach ('within-individual centring', van de Pol & Wright, 2009) that allows us to isolate effects of *within*-mother variation in social environment on egg investment (i.e. maternal plasticity) from potentially confounding effects of variation among mothers. White-browed sparrow-weavers live in social groups of 2-12 birds, in which a single dominant male and female (hereafter 'the mother') monopolise within-group reproduction and non-breeding subordinates of both sexes help to rear the dominant pair's young by feeding nestlings (Lewis, 1982a; Harrison *et al.*, 2013a). Female helpers feed nestlings at approximately twice the rate of male helpers (Chapter 2) and, accordingly, within-mother variation in the number of female helpers has a causal positive effect on the total rate at which broods are provisioned, and positively predicts offspring survival under dry conditions (Chapter 2). The greater effect of female helpers on the group's overall offspring provisioning rate provides an unusual opportunity to tease apart maternal responses to the availability of post-natal help *per se* from maternal responses to group size (which could readily influence maternal investment independent of effects of help, Kokko *et al.*, 2001; Kingma *et al.*, 2014). We focus our attention on variation in egg volume, which in this species strongly predicts both egg mass at laying and nestling mass at hatching (see results, Figure 1). Maternal variation in egg volume may therefore have fitness implications for offspring, not least because hatchling mass also predicts nestling survival in this species (Chapter 2). White-browed sparrow-weavers have a modal clutch size of two eggs (range 1-3) and so marginal adjustments in pre-natal maternal investment per clutch may be more readily achieved through changes in egg size than clutch size, leaving this species a strong candidate for maternal plasticity in egg size.

First, we utilise a large longitudinal data set of 271 clutches (490 eggs) laid by 62 mothers (1-21 clutches per mother; median 7) to investigate whether within-mother variation in the number of female and/or male helpers at laying predicts (within-mother) variation in egg volume. Experimental evidence has already confirmed additive effects of female (but not male) helper number on the total rate at which offspring are provisioned in this species (Chapter 2). Therefore, if additive helper effects on post-natal care do indeed reduce the marginal *benefit* to mothers from increasing egg size (e.g. by compensating for maternal reductions in egg investment, Russell *et al.*, 2007a), all else being equal, we would predict that mothers should *reduce* egg volume when they have more female (but not male) helpers. However, if mothers show plastic reductions in their own *post-natal* feeding of nestlings when they have more (female) helpers (i.e. maternal load-lightening occurs, Hatchwell, 1999), this could reduce the marginal *cost* to mothers from increasing pre-natal investment, given the prospect of a reduced need to invest in post-natal care. Where the latter effect dominates, we would instead predict that mothers should *increase* egg volume when they have more (female) helpers. Second, we thus utilise a large longitudinal data set of maternal post-natal nestling provisioning behaviour (48 mothers making 4,621 feeding visits to 108 broods; 1-10 clutches per mother; median 4 clutches) to establish whether such post-natal maternal workload-lightening occurs in this species. Specifically, we investigate whether within-mother variation in the number of female and/or male helpers predicts (within-mother) variation in maternal nestling feeding rate. Third, we investigate whether the maternal egg plasticity findings could be explained instead by parallel maternal adjustments of clutch size or the number of clutches laid per season according to helper numbers, as might be expected if helpers instead impact the maternal resolution of trade-offs between egg volume and clutch size or number. In our analyses, we also control for variation in environmental conditions (rainfall and temperature) as these have been suggested to influence maternal investment in reproduction (Bennion & Warren, 1933; Blanckenhorn, 2000; Langmore *et al.*, 2016). As female helpers contribute substantially more to post-natal care than male helpers (Chapter 2), we predict that plasticity in all maternal traits should be more sensitive to the number of female helpers than the number of male helpers (a finding that would also implicate maternal responses to help *per se* rather than group size). Finally, plastic maternal adjustments in egg size at laying according to the likely availability of female and male helpers during the post-natal provisioning period (approximately 16-40 days later in this species) require that the latter is predictable at the time of laying. Hence, we also investigate whether this is indeed the case.

3.3. Methods

3.3.1. White-browed sparrow-weaver study population

White-browed sparrow-weavers live in semi-arid regions of South and Southeast Africa. Our study population is located in Tswalu Kalahari Reserve in the Northern Cape Province of South Africa (27°16'S, 22°25' E). Fieldwork was carried out from September to May between 2007 and 2016. Approximately 40 social groups of white-browed sparrow-weavers (where its group is a reproductive unit) were monitored, each one dominating a small territory in an area of approximately 1.5

km^2. Sparrow-weaver reproductive groups were easily monitored and distinguished in the field as all group members foraged together, engaged in weaving and territory defence, and roosted together in individual woven chambers in a single tree or cluster of trees close to the centre of their territory. All birds in our population are fitted with a single metal ring and three colour rings for identification from the beginning of the study period, or from the time they are first detected in the population (under SAFRING license 1444). The sex of every individual could be determined after the first six months of life as our study population present beak colour sexual dimorphism (Leitner *et al.*, 2009).

Every white-browed sparrow-weaver group contains a single dominant (reproductive) female (i.e. mother) that lays every egg in the group (Harrison *et al.*, 2013a). Mothers are easily identified in the field because they display a distinct set of behaviours. In every group, mothers are in very close association with a single dominant male. These two birds spend much time foraging and duetting together (Walker *et al.*, 2016). Only mothers have been observed incubating the eggs or entering the nest during the incubation phase. Furthermore, genetic analysis confirmed that, in our study population, nests always contain eggs from a single female and that this individual could be readily identified as the mother using behavioural observations (Harrison *et al.*, 2013a).

White-browed sparrow-weaver groups were regularly monitored every one or two days to detect new clutches. Once a new clutch was found, egg length and maximum width were measured with a plastic calliper to the nearest 0.1 mm. Nests were checked daily until the clutch had been completed. Then, clutches were checked 8 days after the first egg was laid to confirm successful progress. Following this latter mid-incubation check, clutches were checked daily starting 15 days after the first egg was laid until the fate of every egg was determined (hatch or failure). Hatchlings were weighed on their first day of life using a portable scale to the nearest 0.01 g. White-browed sparrow-weaver group composition was assessed every week throughout every field season. Each social group was observed at least once every week and birds were identified on the basis of their colour-ring combination. Additionally, birds were routinely caught while roosting at night and this information was also used to define group memberships. For every egg and clutch in our analysis, number of male and female helpers were calculated based on field observations as individuals present in a given group on the day of laying (for eggs) or the day of clutch initiation (for clutches). Young individuals hatched in a given breeding year, and whose contribution towards provisioning offspring is very small (Lewis, 1982a), were not considered as helpers until they were at least 6-month old.

3.3.2. Provisioning behaviour

Natural maternal provisioning behaviour was recorded for 174 breeding attempts between September 2007 and April 2016. We collected provisioning behaviour data using video-recordings between the 10th and 12th day after the first egg of a given clutch hatched. Video recordings were watched using VLC media player and data were extracted from every provisioning event: time of the feed, duration of the feed, feeder sex (based on beak coloration; Leitner *et al.*, 2009) and feeder identity based on a unique vent pattern (see below) and colour-ring combination. For every recorded breeding event, we always followed a standardised protocol: at least five days before data collection started, we (i) caught and marked the vent of every bird in the group apart from the dominant female using hair dye (Chapter 2) to aid video identification and (ii) deployed tripods in the field to acclimatise the birds to their presence. On recording days, video cameras were set up in the morning at standard times that tracked monthly changes in sunrise. Provisioning behaviour was recorded for approximately three hours per brood and day.

We then calculated the provisioning rate of mothers (feeds / hour). In a few cases, we did not get reliable bird identifications from the provisioning videos, adding uncertainty to our estimation of the maternal provisioning rate. We cleaned the original data set to retain only those days of observation where i) there were fewer than 3 feeds / hour of uncertainty in maternal feeding rate and ii) where the maternal feeding rate estimated based on reliable observations was higher than 75% of the maximum maternal feeding rate including all the uncertainty in feeder identity. We analysed the cleaned data set containing 108 broods in 34 social groups for 48 different dominant females with reliable maternal provisioning rate data. To avoid pseudo-replication, we calculated the mean maternal provisioning rate for a given brood (i.e. averaging over successive days of video recording).

3.3.3. Environmental data

Daily rainfall data were collected from two rainfall gauges located to the west (27° 16’ 58.9’’ S, 22° 23’ 02.1’’ E) and east (27° 17’ 42.1’’ S, 22° 27’ 34.9’’ E) of the study site, 7.60 km apart from each other. These two rainfall measurements were highly correlated during the study period (Pearson’s product-moment correlation: $r = 0.875$, 95% CI = 0.867 – 0.882, df = 3,347). We therefore calculated average daily values across both gauges and used this as a proxy for rainfall conditions at the study site.

Temperature data for a 0.25-0.25 (latitude-longitude) area that encompassed the study site was extracted from the GLDAS-2.1 Noah 0.25 degree 3-hourly data set (Rodell *et al.*, 2016), accessed via the NASA's Goddard Earth Sciences Data and Information Services Center online data system (Giovanni; http://disc.sci.gsfc.nasa.gov/giovanni). We calculated daily maximum and mean temperatures (i.e. the original data set contained eight temperature recordings per day). We inspected the daily correlation between this data set and temperature readings directly collected on our study site (2700 Watchdog weather station, Spectrum Technologies, Inc.) every 10 to 30 minutes for a limited period of time (partially between 2010 and 2015). Daily mean temperatures from these two data sources were highly correlated (Pearson's product-moment correlation: r = 0.973, 95%CI = 0.970 – 0.975, df = 1,771) validating the use of the GLDAS-2.1 Noah 0.25 degree 3-hourly data set to study temperature variation in the study site.

3.3.4. Statistical analysis

3.3.4.1. Identification of environmental windows of importance: sliding window approach

White-browed sparrow-weavers can breed continuously during the wet season, between September and April, and can show high among-female asynchrony in laying dates. Therefore, we used a relative sliding window approach to find the environmental windows most associated with egg volume and maternal provisioning rate (e.g. Kruuk *et al.*, 2015). We investigated relative sliding windows backdating up to 80 days from egg laying. Sliding windows were inspected in 1-day intervals (van de Pol *et al.*, 2016) and, given a maximum of ± 4 days of uncertainty in exact laying dates in the data set, sliding windows of at least 4 days of length were investigated. The number of days with maximum temperatures above 35°C ('heat wave' index hereafter) and total amount of rainfall ('rainfall' hereafter) in a given temporal window were used as environmental indexes. We investigated linear and quadratic effects of rainfall and linear effects of heat waves.

We applied the sliding window approach for heat waves and rainfall independently. For each temporal window, an appropriate statistical model (see below for baseline models) was fitted including rainfall or heat wave index. The AIC of models including an environmental index was compared to the AIC of a baseline model (see below) not containing the environmental predictor under investigation, resulting in a ΔAIC value (also termed 'AIC support' hereafter). The environmental window yielding the highest reduction in AIC compared to the baseline model (i.e. lowest ΔAIC) was chosen and used in subsequent analyses if it represented an improvement of more than 6 AIC points (a conservative threshold – Richards, 2008). We further tested the results of the sliding window analysis for heat waves and rainfall by carrying out 25 randomisations of the data set and assessing the probability of false positive results (van de Pol *et al.*, 2016). In each randomisation, the biological reference date (egg laying date) for the sliding window approach was randomised by re-shuffling – this approach is used in the R package 'climwin' (Bailey & van de Pol, 2016). For each randomisation, the sliding window protocol was fully applied and the AIC support for the top-performing model recorded. This analytical routine was implemented using our own R scripts, available at:
https://github.com/PabloCapilla/WeaverTools; that were inspired by the R package 'climwin' v1.2.0 (Bailey & van de Pol, 2016). Baseline models were linear mixed models for egg size and maternal provisioning rates. Due to the need to control for

multiple factors affecting egg size and clutch size, we fitted a global baseline model with every variable *a priori* predicted to explain variation in the trait of interest.

3.3.4.2. Structure of statistical models

Egg volume.

Linear mixed models were employed to explain variation in egg volume, calculated based on length and maximum breadth following the formula given by Narushin (2005). Four terms were included as random intercepts: breeding year (a 9-level factor from 2007 to 2015), social group ID, clutch ID and maternal ID. We also included, as fixed effects: egg position within the clutch, clutch size, number of female helpers, number of male helpers and the interaction between helper number (both females and males) and (i) egg position, and (ii) clutch size. Environmental indexes for heat waves and rainfall were included as determined by the sliding window approach.

Between 2007 and 2016, we collected length and width information, and therefore volume, from 906 eggs that were detected in the field with less than four days of uncertainty around their laying date. Out of these, laying order information was available for 490 eggs (i.e. final sample size for analysis), with an average of 54 eggs per season (range: 23 – 93) from 271 clutches in total sampled from 62 dominant females across 37 social groups (mean = 7.90 eggs per female; range 1 - 21).

Provisioning rate of breeding females.

Linear mixed models were used to explain variation in maternal provisioning rate. Three terms were included as random intercepts: breeding year (a 9-level factor from 2007 to 2015), social group ID, and maternal ID. Fixed effects included brood size, number of female helpers, number of male helpers as well as the interactions between helper number and brood size. Following the sliding window analysis and the calculation of the heat wave and rainfall indexes, we included these two environmental predictors as fixed terms. The final data set for maternal provisioning rate contained 108 broods in 34 social groups for 48 different dominant females.

Clutch size.

Clutch size was modelled as a Gaussian trait (ranging from 1 to 3) using linear mixed models. Three terms were included as random intercepts: breeding year (a 9-level factor from 2007 to 2015), social group ID and maternal ID. As fixed terms, we included clutch order within the breeding year, number of female helpers and number of male helpers. This analysis comprised clutches that were found in the field with less than four days of uncertainty in laying dates; thereby reducing the probability that any egg in the clutch disappeared before we recorded it. Out of a total of 344 clutches, 284 clutches (82.56%) were found the day the first egg was laid. For 37 clutches (10.76%), there was one day of uncertainty around the date the first egg was laid, two days for 16 clutches (4.65%) and three days of 7 clutches (2.03%). Clutches for 66 different dominant females in 37 distinct territories (i.e. social groups) appeared in the final data set (n = 344 clutches). The number of female and male helpers for each clutch was calculated as adult birds (i.e. those not hatched in the focal breeding year and, therefore, older than six months) on the day that the 1st egg of the clutch was laid. Repeating this analysis utilising a zero-truncated Poisson error structure yielded qualitatively identical results.

Number of breeding attempts per year.

We calculated the number of clutches laid by mothers between the beginning and the end of every breeding year. For this analysis, we only retained observations for females that remained breeders for the whole breeding season (208 observations of 56 breeding females in 38 social groups). We explained variation in the number of clutches laid per year using a generalised mixed model (for Poisson-distributed data), including social group ID and female ID as random effects. We also included total rainfall in the whole breeding year (i.e. from 1st of September to 30th of April) as well as the mean number of female and male helpers.

3.3.4.3. General statistical procedures

To investigate the importance of factors explaining variation in egg volume, maternal provisioning rate, clutch size, number of breeding attempts per year and maternal pre-laying body condition we employed an information-theoretic (IT) approach. Starting from a global model (that contained only those variables and interactions predicted to have an effect – see above), simpler combinations of fixed predictors were fitted to the data and ranked based on Akaike's Information Criterion (AIC, Burnham & Anderson, 2002; Burnham, 2004). ΔAIC values were then calculated for every model (i.e. AIC difference between a given model and the best model – thus, the ΔAIC for the best model equals zero). We gave further consideration to models within a ΔAIC value of six (Richards, 2008; Richards *et al.*, 2011) and reduced this "Δ6 model set" by applying the 'nesting rule' described in (Richards, 2008). This rule aims to avoid the retention of overly complex combinations of predictor by discarding models that are more complex versions of simpler (nested) models with poorer AIC support. Adding variables with no or little explanatory power to a well-supported model is known to decrease AIC (Arnold, 2010) and, therefore, the nesting rule tries to reduce the chance of considering models with such uninformative variables. When quadratic terms were included in a given model, linear coefficients were always present. Intercept-only models were always considered. For AIC comparisons, models were fitted using maximum likelihood (ML). Model coefficients were standardised by mean centering and dividing by one standard deviation; effect sizes are therefore comparable across different predictors. Statistical analyse were performed in R version 3.6.1. (R Core Team, 2019).

A common concern in studies of cooperative species is that group size can be associated with territory quality, creating a spurious correlation between fitness traits and group size (both of which can be positively associated with territory quality; Cockburn, 1998; Cockburn *et al.*, 2008). We addressed this concern in two different ways. First, we excluded young individuals with less than six months of life from our calculations of the number of male and female helpers. Second, we carried out the analyses using number of helpers and then partitioned this variable to untangle effects born of within-mother and among-mother variation in number of helpers, Δ number of helpers and μ number of helpers respectively (van de Pol & Wright, 2009). Within-mother (Δ) number of helpers accounts for variation in number of helpers experienced by a given dominant female and, therefore, its effects cannot be attributed to territory quality, which operates at the among-mother level. We carried out within-mother centering per territory, thus, simultaneously accounting for among-mother and among-territory differences in quality. Within-mother centering was carried out using a customised R function, *d_centering*, available here: https://github.com/PabloCapilla/WeaverTools

3.4. Results

3.4.1. Patterns and implications of maternal plasticity in egg volume

We analysed the volume of 490 eggs laid in 271 clutches of 1-3 (median = 2) eggs by 62 mothers in 37 social groups, with 1-21 clutches (median = 7) measured per mother. Our data set reveals substantial within-mother variation in egg volume (Figure 1a x axis) in addition to among-mother variation in egg volume (Figure 1a y axis). Moreover, within-mother variation in egg volume positively predicted egg mass on the day of laying (LM: $\beta = 0.484 \pm 0.016$; $\Delta AIC = 456.40$; Figure 1c) as well as nestling mass on the day of hatching (LM: $\beta = 0.282 \pm 0.048$; $\Delta AIC = 31.32$; Figure 1d). It is quite plausible that sparrow-weaver mothers could adjust their egg volume according to the likely future availability of help during the post-natal period, because helper numbers at laying strongly predict those during the post-natal period (Figure 1b; for female helper number, LM: $N = 271$, $\beta = 0.94 \pm 0.017$; and for male helper number, LM: $N = 271$, $\beta = 0.93 \pm 0.022$).

3.4.2. Maternal plasticity in egg volume: individual mothers lay larger eggs when they have more female helpers

Mothers that had more female helpers at laying laid larger eggs ($\Delta AIC = 3.71$; Table 1). Partitioning variation in female helper number into its within- and among-mother components revealed evidence of maternal plasticity: individual mothers laid larger eggs when they had more female helpers (Δ female helper number effect; $\Delta AIC = 3.79$; Table 2; Figure 2). We found much weaker evidence that male helper numbers predicted variation in egg volume, both at the population level (Table 1; the best supported model containing a 'male helper number' effect was 3.71 AIC points below the top model) and within mothers (Table 2; the top model containing a 'Δ male helper' effect after applying the nesting rule, see methods, was 3.79 AIC points below the top model). We also found evidence for consistent and large among-mother variation in egg volume: the 'maternal ID' random effect in both models explained approximately 70% of the variation in egg volume ('maternal ID' random effect variance = 0.569; likelihood ratio-test: $\chi_1^2 = 136$, $p < 0.001$).

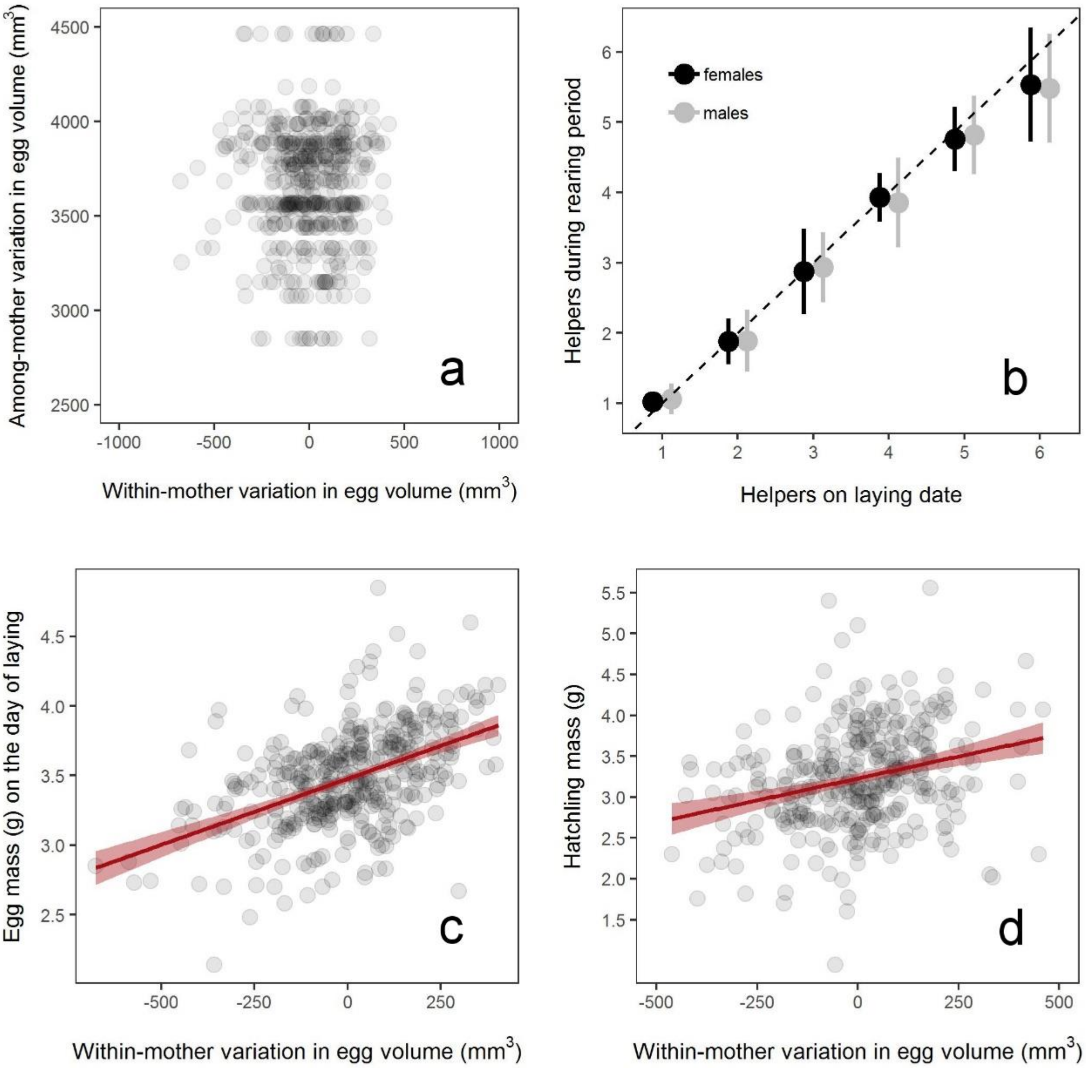

Figure 1. Patterns and implications of maternal plasticity in egg volume. (**a**) Egg volume showed high variation both within (x-axis) and among females (y-axis). Among-mother variation in egg volume was calculated as the mean egg volume per female, while within-mother variation in egg volume represents deviations from the female-specific mean egg volume (hence the negative and positive values). (**b**) Helper number at laying strongly predicted helper number during the nestling

rearing phase (mean ± standard deviation (SD) are presented for both male and female helpers; dashed line indicates a 1:1 relationship). Within-mother variation in egg volume positively predicted (**c**) egg mass (g) on the day of laying and (**d**) hatchling mass (g). Mean model predictions ± standard error (SE) are plotted in red.

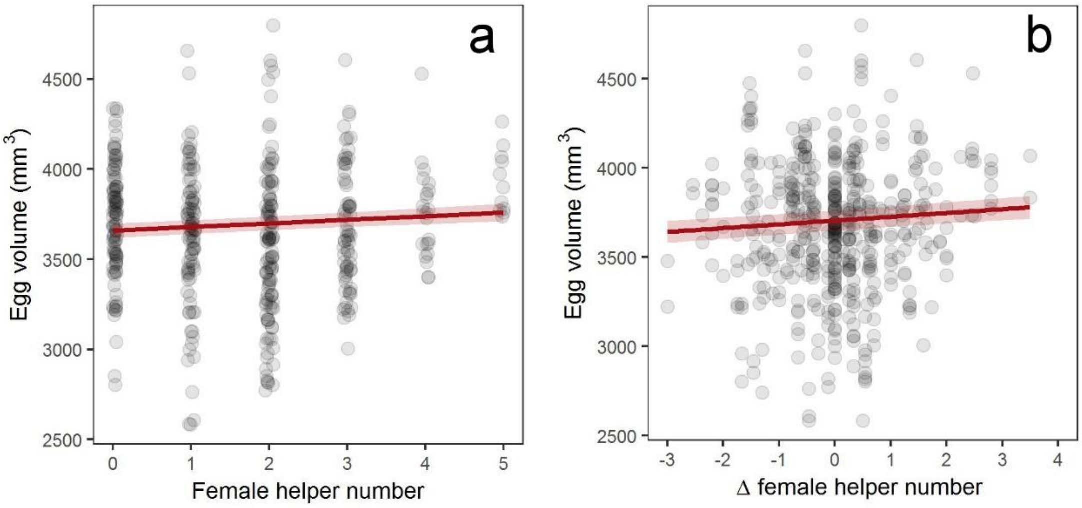

Figure 2. **Maternal plasticity in egg volume.** (**a**) Female helper number positively predicts egg volume at the population level (prior to partitioning variation in helper number). (**b**) Within-mother variation in female helper number ('Δ female helper number') also positively predicts egg volume, providing evidence of maternal plasticity. Grey dots illustrate raw data points and red lines present model predictions for mean egg volume (± SE).

Egg volume was also negatively predicted by the number of days above 35°C in a window of 13 days before egg laying ('heat wave' index; see methods for window optimisation): the higher the heat wave index, the smaller the eggs (Tables 1 & 2; Supplementary information A; Figure S1; Table S1). We also found that total rainfall 44 to 49 days before laying negatively predicted egg volume (negative linear and quadratic terms; Table 1). However, our sliding window analyses suggest that this result has a fair likelihood, 12%, of being generated by chance (i.e. it could well be a false positive; Supplementary information A; Figure S2, Table S1). Clutch size did not predict egg volume (Tables 1 & 2) but egg position within the clutch did: later laid eggs were smaller than first laid eggs (Tables 1 & 2). Egg volume was not affected by the interactions between the number of male or female helpers and clutch size or egg position (Table 1 & 2).

3.4.3. Maternal plasticity in nestling provisioning: individual mothers provision less when they have more female helpers

Mothers that had more female helpers during the nestling period showed lower nestling provisioning rates (ΔAIC = 3.23; Figure 3a; Table 3). Partitioning variation in female helper number into its within- and among-mother components revealed evidence of maternal plasticity: individual mothers decreased their nestling provisioning rate when they had more female helpers (Δ female helper number effect; Table S5). Again, we found much weaker evidence that male helper numbers predicted variation in maternal provisioning rate, both at the population level (Table 3; the best supported model containing a 'male helper number' effect was 3.04 AIC points below the top model) and within mothers (Δ male helper number effect; Table S5; the best supported model containing a 'Δ male helper number' effect was 1.79 AIC points below the top model).

There was evidence to suggest that maternal provisioning rates increased with the number of days above 35°C (i.e. 'heat waves' index) 51 to 58 days pre-laying and with total rainfall 63 to 77 days pre-laying (Tables 3) but our analyses revealed a high probability that both of these results are false positives (Supplementary Information B; Table S1; Figure S3 & S4). The interactions between the number of male or female helpers and brood size did not explain appreciable variation in maternal provisioning rates (Tables 3).

3.4.4. No evidence of maternal adjustment of clutch size or number of clutches laid per year according to helper numbers

We did not find evidence that mothers adjusted their clutch size according to the number of female or male helpers in their group (Tables S6). The intercept-only model was the best supported model. We also found no evidence that mothers adjusted the total number of clutches that they laid in a given breeding season according to the number of female or male helpers in their group (Table S7). We did find strong evidence of a positive effect of the total rainfall during a given breeding season on the total number of clutches laid in that breeding season (ΔAIC = 10.12; Table S7).

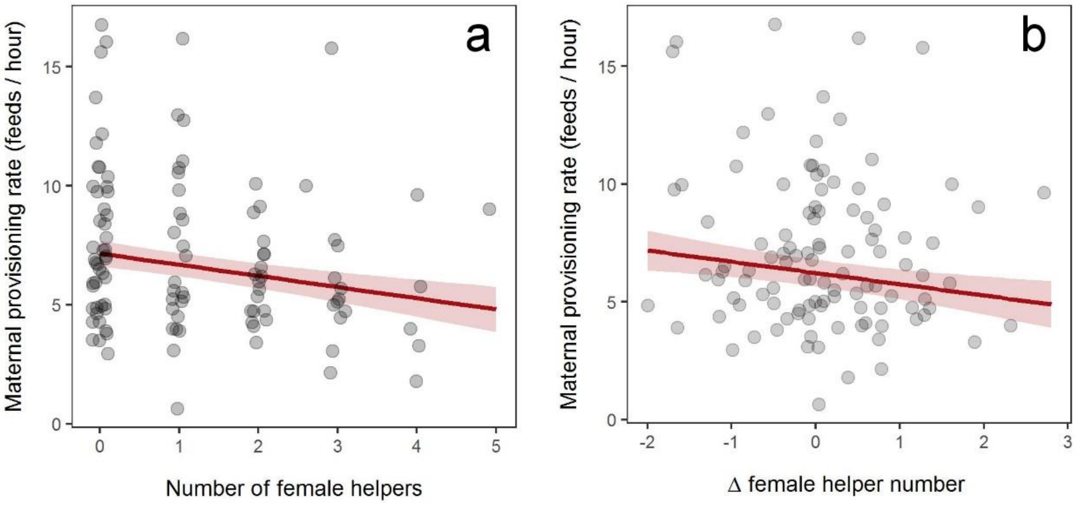

Figure 3. **Maternal plasticity in post-natal provisioning.** (**a**) The number of female helpers negatively predicts maternal nestling provisioning rate at the population level (Table 3). (**b**) Within-mother variation in the female helper number ('Δ female helper number') also negatively predicts maternal nestling provisioning rate, providing evidence of maternal plasticity. Grey dots illustrate raw data points and red lines present model predictions for mean egg volume (± SE). Grey dots illustrate raw data points and red lines present model predictions for mean maternal nestling provisioning rates (± SE).

Table 1. The top-performing models (i.e. those within ΔAIC < 6 of the top model) explaining variation in egg volume (linear mixed model; N = 490). Only those models retained after applying the nesting rule (see 'Methods'; Richards, 2008) are presented (for the full table see Table S2). Standardised model coefficients are shown along with number of model parameters ('k'), AIC, and ΔAIC. Other tested predictors not shown in this table (as they were not present in the models retained after applying the nesting rule) were as follows: clutch size, clutch size x female helper number, clutch size x male helper number, egg position x female helper number and egg position x male helper number.

Intercept	**Female helper number**	**Male helper number**	**Heat waves**	**Rainfall¹**	**Rainfall²**	**Egg position**	**k**	**AIC**	**ΔAIC**
0.006	0.078		-0.114	-0.069	-0.107	-0.119	11	890.4	0
-0.001		0.051	-0.115	-0.067	-0.105	-0.120	11	894.1	3.71
-0.008			-0.109	-0.071	-0.104	-0.121	10	894.4	4.05

Table 2. The top-performing models (i.e. those within ΔAIC < 6 of the top model) explaining variation in egg volume partitioning within- (prefix 'Δ') and among-mother (prefix 'μ') variation in male and female helper number. Only those models retained after applying the nesting rule (see 'Methods'; Richards, 2008) are presented (see full table in table S3). Standardised model coefficients are shown along with number of model parameters ('k'), AIC, and ΔAIC. Other tested predictors not shown in this table (as they were not present in the models retained after applying the nesting rule) were as follows: μ female helper number and μ male helper number.

Intercept	Δ Female helper number	Δ Male helper number	Heat waves	Rainfall^1	Rainfall^2	Egg position	k	AIC	ΔAIC
-0.006	0.061		-0.114	-0.068	-0.108	-0.119	11	890.5	0
-0.006		0.038	-0.115	-0.066	-0.106	-0.120	11	894.3	3.79
-0.008			-0.109	-0.071	-0.104	-0.121	10	894.4	3.90

Table 3. The top-performing models (i.e. those within ΔAIC < 6 of the top model) explaining variation in maternal provisioning rates. Only those models retained after applying the nesting rule (see 'Methods'; Richards, 2008) are presented (see full table in table S4). Standardised model coefficients are shown along with number of model parameters ('k'), AIC, and ΔAIC. Other tested predictors not shown in this table (as they were not present in the models retained after applying the nesting rule) were as follows: 'Female helper number X Brood size'.

Intercept	Female helper number	Male helper number	Brood size	Rainfall^1	Rainfall^2	Heat waves	Male helper number X Brood size	k	AIC	ΔAIC
0.004	-0.181		0.461	0.147	0.212	0.211		10	269.8	0
-0.019	-0.154	-0.128	0.436	0.192	0.237		-0.128	11	272.9	3.04
0.000	-0.175		0.461	0.208	0.237			9	272.9	3.07
0.013			0.463	0.157	0.213	0.199		9	273.1	3.23
-0.012		-0.147	0.432	0.200	0.237		-0.147	10	274.4	4.58
0.013	-0.182		0.417	0.156		0.238		9	274.9	5.02
0.013			0.461	0.216	0.235			8	275.5	5.61
0.023	-0.187		0.433			0.300		8	275.8	5.92

3.5. Discussion

We used a long-term data set containing extensive repeated measures of egg volume from mothers experiencing variable numbers of helpers to investigate whether mothers plastically adjust their pre-natal investment according to the availability of help with post-natal care. As helper number at laying strongly predicts helper number during post-natal care in this species, mothers should have sufficient information at laying to adjust their egg volume to the availability of post-natal help, were it adaptive to do so. Indeed, our analyses did reveal evidence of plasticity in maternal *pre*-natal investment according to helper numbers: individual mothers increased the volume of their eggs when assisted by more female helpers. While relationships between helper number and egg size have previously been reported in other cooperative breeders (see introduction), our analyses are the first to demonstrate that such a pattern arises from within-mother plasticity rather than variation among mothers. Our findings also revealed plasticity in maternal *post*-natal investment according to female helper numbers: individual mothers decreased their investment in post-natal nestling provisioning when assisted by more female helpers. Female helpers in white-browed sparrow-weaver societies provide post-natal care at twice the rate of male helpers (and only female helpers provide additive care; Chapter 2). Thus, the evidence of maternal plasticity in both pre- and post-natal investment according to variation in the number of female helpers, but not male helpers, implicates helping behaviour *per se* as the likely cause of these plastic responses, rather than correlated variation in group size. It seems unlikely that the observed maternal plasticity in egg volume reflects an effect of female helpers on the optimal resolution of a trade-off between egg volume and either clutch size or the number of clutches laid per year, as mothers did not vary clutch size or number according to helper numbers. Instead, it seems more likely that the presence of female helpers increases the marginal payoff to mothers of increasing egg investment, either by reducing the marginal *cost* of increasing egg investment (e.g. because they lighten the post-natal workloads of mothers) or by increasing the marginal *benefit* (e.g. if increasing egg investment has a greater effect on the reproductive value of offspring that will receive more post-natal care). We discuss these potential explanations for our findings in more detail below, along with their implications for our understanding of maternal and helper effects in cooperative breeders.

While relationships between egg size and helper number are typically interpreted as adaptive responses to effects of the anticipated availability of post-natal care on the payoffs from egg investment (see introduction), alternative explanations for such patterns exist. First, the relationship could arise as a by-product of trade-offs between egg volume and other pre-natal maternal investment traits (specifically clutch size or the number of clutches laid per year) that are themselves adjusted according to helper number (Lejeune *et al.*, 2016). This is unlikely to explain the relationship observed here, however, as our analyses reveal that neither clutch size nor the number of clutches laid per breeding season are related to helper number in this species. Indeed, white-browed sparrow-weavers show very little variation in clutch size (1-3 eggs; mode 2 [72.38% of clutches]) and so pre-natal adjustments in maternal investment may be more readily achieved via changes in egg size rather than clutch size. Second, maternal plasticity in egg size according to helper number could also arise not because mothers *pre-emptively* adjust egg investment to the likely availability of post-natal care for the eggs being laid, but because the *past* actions of helpers have impacted maternal condition (e.g. via lightening maternal workloads in previous breeding attempts; Russell *et al.*, 2003a), which itself affects the payoff from egg investment. This scenario cannot readily explain our findings either, as (i) helper numbers have no detectable effect on maternal body condition (see Supplementary material C), and (ii) the time since the last breeding attempt does not predict egg volume (either in isolation or via interactions with helper numbers), suggesting that egg volume is not appreciably impacted by carryover effects of past reproductive effort (see Supplementary material D). As such, it would seem quite plausible that the egg size plasticity observed here does reflect past selection for maternal strategies that adjust pre-natal investment to the likely future availability of post-natal help (Russell *et al* 2007a; Russell & Lummaa 2009). Indeed, as the number and composition of helpers at laying strongly predicts those during the ensuing post-natal care period in this species, sparrow-weaver mothers should have sufficient information at laying to adjust their egg volume according to the future availability of help, were it adaptive to do so.

The leading hypothesis for adaptive variation in maternal pre-natal investment in cooperative breeders proposes that where helpers increase the overall rate at which offspring are fed (i.e. provide additive care), selection may favour mothers that reduce their pre-natal investment, given the potential for the additive contributions of helpers to compensate for this (Russell *et al.*, 2007a). Our findings run precisely counter to this prediction and are consistent instead with female helpers reducing the marginal costs of pre-natal investment. We show that helpers lighten the post-natal workloads of mothers, potentially allowing mothers to invest more at the pre-natal stage of reproduction (i.e. reducing the marginal costs of increasing pre-natal investment in eggs). Indeed, mothers assisted by more female helpers increased their pre-natal investment laying larger eggs. Helpers load-lighten parental workload in a wide range of cooperative species (e.g. Hatchwell, 1999; Zöttl *et al.*, 2013a; Brouwer *et al.*, 2014) and, therefore, positive effects of helpers on maternal pre-natal investment may potentially be more common than previously thought.

It is also conceivable that the positive association between female helper numbers and egg volume arises in part because female helpers increase the marginal *benefits* of increasing maternal investment in the egg. For example, if female helpers increase juvenile survival (Clutton-Brock *et al.*, 2001; Russell *et al.*, 2002; Hatchwell *et al.*, 2004; Ridley, 2007; Kingma *et al.*, 2010), female helpers might also increase the probability of a return on maternal pre-natal investment in the egg. Such a mechanism may not apply in sparrow-weavers, however, as helpers actually have no net positive effect (when averaged across all ecological conditions) on offspring survival to fledging (Chapter 2). Additive helper effects (i.e. more post-natal care) might also increase the marginal benefits of pre-natal investment when the relationship between total investment in offspring (the sum of pre- and post-natal investment) and offspring fitness is accelerating (as envisaged in the 'silver spoon' hypothesis; Grafen, 1988). This possibility warrants closer attention but is likely to prove challenging to robustly test. One approach to test this hypothesis could be to experimentally allocate eggs of different sizes among different post-natal care environments (i.e. groups with different number of helpers) and demonstrate that the relationship between egg size and offspring fitness is steeper in groups with more helpers. Lastly, helpers might additionally increase the marginal benefits of pre-natal investment when mothers stand to gain the full benefits of increasing egg investment when additive care from helpers can cover the increased costs of meeting the potentially higher demand of the resulting nestlings (Clutton-Brock *et* *al.*, 1985; Klaassen & Bech, 1992; Figure 1d).

While we found a positive relationship between female helper number and pre-natal maternal investment, most studies of cooperative breeders to date have found negative relationships (Russell *et al.*, 2007a; Taborsky *et al.*, 2007; Canestrari *et al.*, 2011; Santos & Macedo, 2011; Paquet *et al.*, 2013; Sharp *et al.*, 2013; Dixit *et al.*, 2017). The apparent inter-specific diversity of relationships between helper number and egg size could well be attributable in part to the possibility in some species (i) that this relationship at the population level actually reflects among-mother variation rather than a plastic maternal response (e.g. Lejeune *et al.*, 2016; see Taborsky *et al.*, 2007, for one exception in a cooperatively breeding fish), and/or (ii) that this relationship actually reflects a response to correlated variation in group size rather than the availability of help *per se*. At least three key factors may ultimately help to explain inter-specific variation in the direction and magnitude of maternal plasticity in egg size according to the availability of help per se.

First, there may be variation among species in the relative importance of pre- and post-natal investment for offspring fitness. The potential for maternal reductions in pre-natal investment when helpers provide post-natal care for offspring relies on the assumption that pre-natal and post-natal investments are exchangeable (Savage *et al.*, 2015). While this assumption might be valid for some species, there is ample evidence highlighting the potential for *in ovo* and *in utero* conditions to have life-long formative effects on offspring phenotypes and fitness (Henry & Ulijaszek, 1996; Hales & Barker, 2001; Krist, 2011; Pick *et al.*, 2016, 2019) leaving it unlikely to be valid for all. Indeed, helpers in some species principally protect rather than provision offspring, leaving it unlikely that helping directly compensates for reductions in pre-natal investment (e.g. Taborsky *et al.*, 2007). Second, there may be variation among species in the shape of the relationship between total (pre- and post-natal) investment and offspring fitness; specifically, whether the relationship is accelerating or decelerating. When helpers have additive effects on the total amount of post-natal care that offspring receive, selection might favour mothers increasing their egg investment in the presence of helpers if the relationship is accelerating (as suggested above), but actually decreasing it if the relationship is decelerating. Third, there may be variation among species in the extent to which helpers lighten the workloads of mothers and/or provide additive care (see Hatchwell, 1999), given the key role that both phenomena could play in impacting the marginal payoff to mothers of increasing egg investment (see above). More empirical studies of maternal plasticity in pre-natal investment in cooperative breeders will ultimately be needed if we are to better understand the relative importance of these factors; in particular investigations that statistically or experimentally tease apart within-mother (i.e. plasticity) and among-mother variation in pre-natal investment (e.g Taborsky *et al.*, 2007; Lejeune *et al.*, 2016).

Like the social environment, abiotic environmental conditions also have the potential to modulate the net fitness payoff to mothers from pre-natal investment (Kruuk *et al.*, 2015; Langmore *et al.*, 2016), and our analyses did yield some evidence that abiotic conditions predict egg volume in this species. Total amount of rainfall between approximately 20 days pre-laying and 24 days post-hatching is a strong predictor of nestling survival in white-browed sparrow-weavers (Chapter 2). Therefore, rainfall in this window also has a strong potential to influence the payoff from egg investment. It is then surprising that we found no compelling evidence that mothers adjust their egg size at laying according to rainfall (as analysis of the outcomes of the moving window approach optimisation of the rainfall effects in our models suggests that they have a high likelihood of having arisen by chance). One potential explanation for the absence of maternal plasticity in this context is that rainfall is highly unpredictable for our Kalahari study population (Chapter 2). Additionally, most of the rainfall window that affects offspring survival occurs after laying and mothers may then be unable to accurately predict how rainfall will ultimately impact the fate of their offspring. In contrast to rainfall effects, we found strong evidence suggesting that the number of days with maximum temperatures above 35°C in the thirteen days before laying (i.e. the 'heat wave' index revealed by sliding window modelling; see methods) had a clear negative association with egg volume (a result that our analyses suggest is not likely to have arisen by chance). High temperatures have been shown to have associations with egg size and our results are in line with previous findings (Bennion & Warren, 1933; Blanckenhorn, 2000; Langmore *et al.*, 2016). It is conceivable that this pattern reflects an adaptive strategy (e.g. smaller eggs may be easier to keep cool) but it is also plausible this response rather reflects a detrimental effect of heat stress on pre-laying maternal condition or physiology (Ernsting *et al.*, 1997; Blanckenhorn, 2000).

By partitioning the effects of within- and among-mother variation in helper numbers on maternal egg volume, we have provided robust evidence for maternal plasticity in egg volume to the social environment. Mothers laid larger eggs when assisted by more female helpers, with the potential for fitness implications for offspring, given that egg volume predicts both egg and hatchling mass in this species and the latter predicts offspring survival (Chapter 2). We also demonstrate that mothers contributed less to post-natal care when assisted by more female helpers, providing a simple explanation for the egg size plasticity observed: mothers with more female helpers may invest more heavily in pre-natal investment *because* female helpers lighten their post-natal workloads. As such, our findings highlight a novel mechanism through which helpers might frequently accrue fitness benefits that remain virtually unexplored. While it has long been recognised that helpers may accrue indirect fitness benefits by lightening the workloads of related mothers if this improves maternal survival or reduces inter-clutch or inter-birth intervals (Woxvold & Magrath, 2005; Kingma *et al.*, 2010), our findings reveal that mothers might instead exploit post-natal load-lightening *pre-emptively*, reallocating maternal resources to pre-natal investment to which helpers cannot directly contribute. Indeed, while it has been suggested that studies of helper effects on offspring should control for variation in egg size in order to ensure that maternal reductions in egg size by helped mothers do not 'conceal' helper effects on offspring (Russell *et al.*, 2007a), our findings highlight a danger of this approach. If, as here, plastic mothers actually lay larger eggs in the presence of helpers, controlling for variation in egg size could lead instead to the underestimation of helper effects on offspring, by factoring out helper effects that arise indirectly from load-lightened mothers pre-emptively reallocating their resources from post-natal care to pre-natal investment in the egg. Indeed, given the widespread evidence across species that helpers lighten maternal workloads (e.g. Hatchwell, 1999; Kingma *et al.*, 2010), it is conceivable that such a pre-emptive reallocation of resources to pre-natal investment has actually contributed to the positive relationships already described in numerous species between helper numbers and offspring survival and/or performance.

Chapter 3: Supplementary Material

Supplementary Information Chapter 3

A - Egg volume: sliding window analysis

I. Heat waves (number of days above 35°C)

The sliding window analysis for heat waves revealed a significant negative effect on egg volume occurring in a window of 13 days pre-laying (Figure S1a). The model for this temporal window scored an AIC value 11.13 points lower than the baseline model. Furthermore, the analysis clearly identified a single and localised peak in the AIC landscape (Figure S1b) with no clear alternative windows supported by the data. None of our 25 randomisations scored a lower AIC value than the real data (Figure S1c – AIC support for randomised analysis ranged from 0.24 to -9.30).

II. Total rainfall

The sliding window analysis for total amount of rainfall indicated a negative quadratic effect on egg volume 49 to 44 days before egg laying (AIC support = -12.90 - Figure S2a). Simulating a random relationship between quadratic rainfall and egg volume 25 times (i.e. 25 randomisation tests as per the heat wave effect - van de Pol et al., 2016), we obtained equal or stronger AIC support for three randomisations, revealing a 12% probability of a false positive result (Figure S2c). Furthermore, the peak in AIC support in the sliding window landscape using the real data revealed scattered support for a number of different windows without clear localisation of maximum support (Figure S2b). It is also biologically unlikely that rainfall during only five days, 44 days before laying, affects egg volume.

B - Maternal provisioning rate: sliding window analysis

I. Heat waves (number of days above 35°C)

Mothers increased their provisioning rates with increasing heat wave index calculated between 58 and 51 days prior to hatching (AIC support = -6.33; Table S1; Figure S3a). However, a high percentage of random tests (7 out of 25; Table S1) showed equal or higher AIC support than what we observed in the real data set (Figure S3c). This fact suggests that the relationship that we found in the data may be spurious. Furthermore, there was no single and clear peak in the landscape of 'AIC support' (Figure S3b).

II. Total rainfall

The sliding window analysis for total amount of rainfall indicated a positive quadratic effect on maternal provisioning rate 78 to 63 days before egg laying (AIC support = -10.97; Table S1; Figure S4a). Total rainfall did not affect maternal provisioning rate for most of the range in rainfall values but breeding females seemed to increase their provisioning rate in very wet conditions (Figure S4a). There appeared to be a clear peak in the landscape of AIC support (Figure S4b). However, three random simulations showed higher AIC support than the real data set, representing a probability of 12% of a false positive result (Figure S4c).

C – Helper effects on maternal body condition

We routinely caught and measured body weight (g) and tarsus length (mm) of mothers throughout the study period. We used this information to investigate if maternal body condition before laying a given clutch was associated with the number of helpers present in a previous clutch. We restricted the analysis to those ‘previous’ clutches in which at least one nestling fledged and, therefore, helpers had time to contribute towards post-natal care and, potentially, reduce the costs of maternal post-natal investment. We used weight measurements from mothers up to 45 days before they produced a new clutch (i.e. pre-laying). The data set comprised 45 clutches by 33 mothers in 26 social groups. We calculated scale mass index of body condition following (Peig & Green, 2009) and built a linear mixed model to explain variation in this variable (hereafter ‘maternal pre-laying body condition’). We investigated the effect of female and male helper number in a previous breeding attempt (included as fixed effects) on maternal pre-laying body condition. We also included a fixed effect for the number of previous clutches laid by the mother within breeding years (from 0 to 4 clutches). Mother ID, social group ID and breeding year were included as random effect intercepts. Given the small sample size, random intercept variance for Mother ID and year could not be estimated. Dropping these terms from the initial model did not change the results. We found no evidence suggesting that the number of male or female helpers in a given breeding attempt affected maternal pre-laying body condition in a subsequent breeding attempt. The intercept-only model scored the lowest AIC and no other model passed the nesting rule (Richards, 2008). Female helper number appeared, having a negative effect, in the third model being 1.84 AIC points worse than the intercept-only model.

D – Female helper effects on egg volume do not depend on time since the last breeding attempt

To investigate if maternal variation in egg volume according to helper number could actually reflect load-lightening by helpers in past reproductive events (rather than a pre-emptive response given an ‘expectation’ of receiving post-natal help with the clutch currently being laid), we re-fitted the egg volume model presented in the main text to include ‘time since last breeding attempt’ as an additional fixed effect predictor if such previous breeding attempt had been successful (i.e. nestlings were fledged and, therefore, helpers did have the opportunity to lighten maternal post-natal workload; n = 136 eggs). If mothers lay larger eggs when assisted by more female helpers as a consequence of *past* female helper contributions, we would expect the female helper effect to decrease in magnitude with increasing time since the last breeding attempt (i.e. an interaction between female helper number and time since last breeding attempt). Time since last breeding attempt did not explain variation in egg volume either as a simple predictor or as an interaction with female or male helper number (these latter predictors only appeared in the model set after a ΔAIC value of 5.03 and 4.86 respectively).

Supplementary Figures Chapter 3

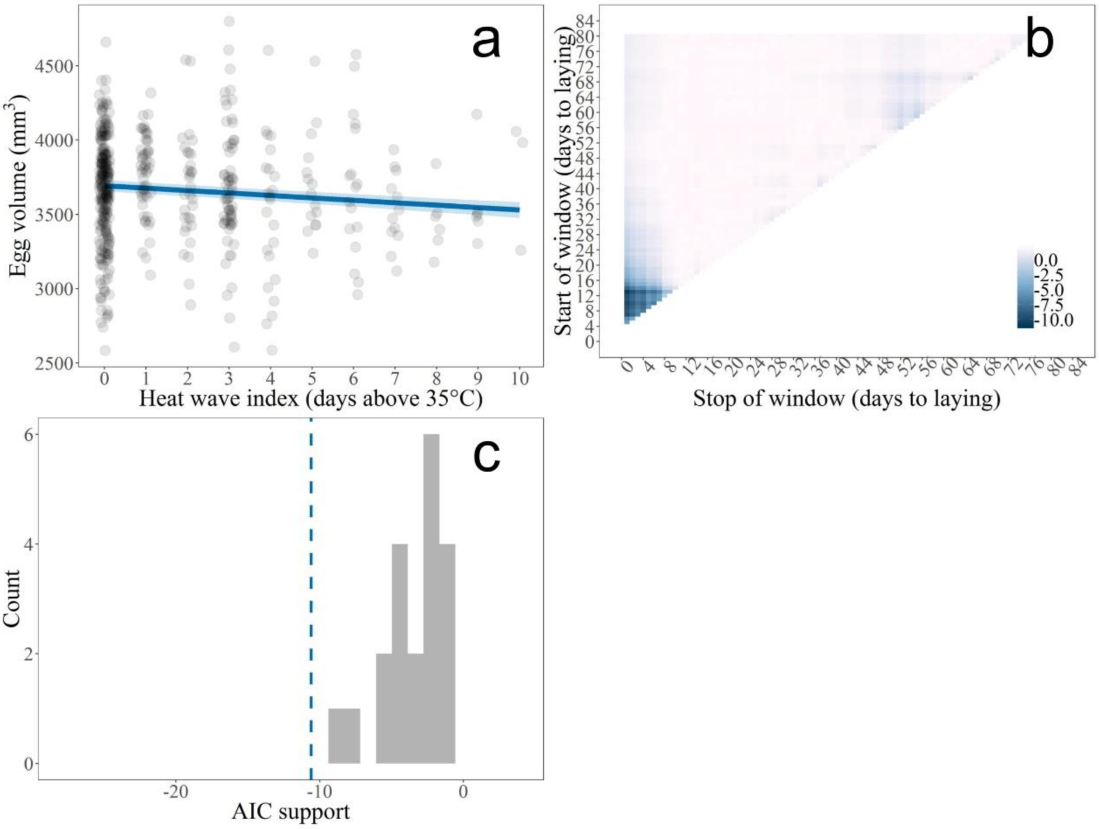

Figure S1. Sliding window analysis for the effect 'heat waves' (days above 35°C) on egg volume. (**a**) Effect of 'heat waves' on egg size. Raw data points in black and regression line (± SE) in blue. (**b**) AIC support (i.e. difference in AIC between a given sliding window model and the baseline model) for sliding windows up to 80 days before egg laying. The darker the colour of the tiles, the stronger the support for a given window. (**c**) Histogram representing the best AIC support for 25 randomised sliding window analyses (e.g. AIC support expected if no relationship exists between egg volume and heat waves). The blue dashed line illustrates the AIC support achieved using the real data set. No randomised test scored better (i.e. lower) AIC support than the real data set, strongly suggesting that the relationship between egg volume and heat waves was very unlike to have been generated by chance and, therefore, indicating that such relationship was a real signal present in the data.

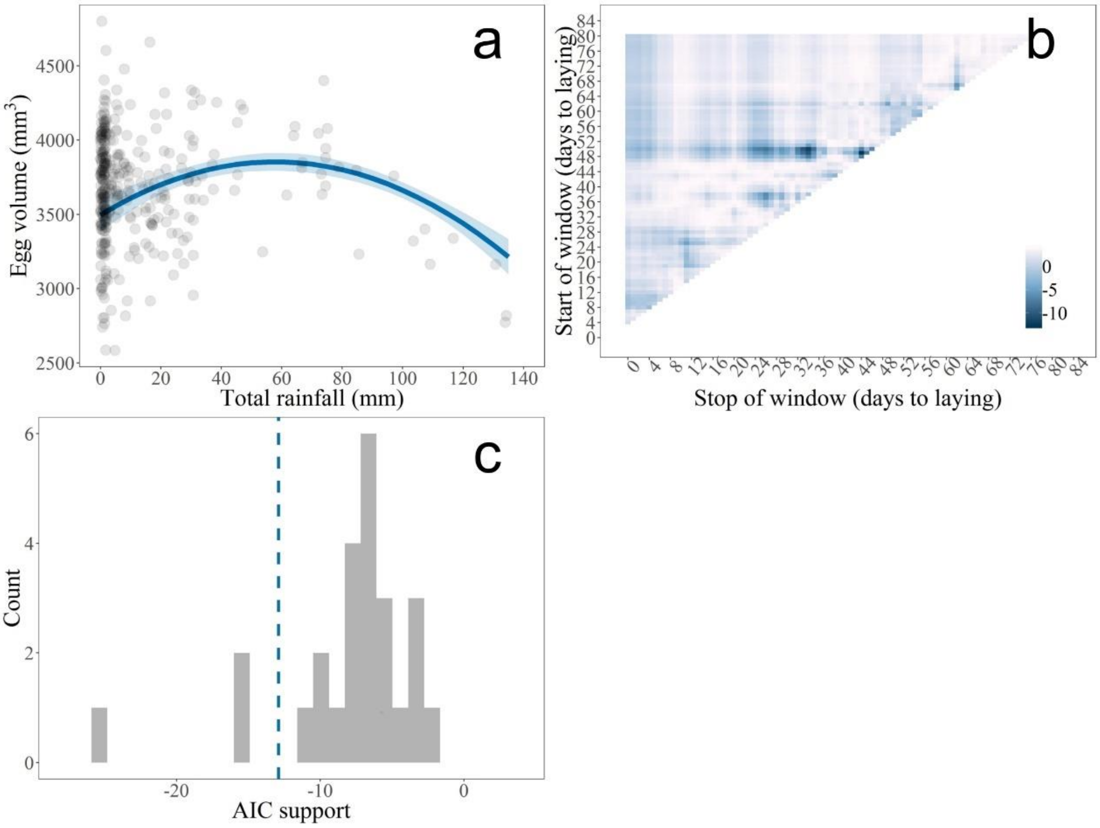

Figure S2. Sliding window analysis for the effect of total rainfall (mm) on egg volume. (**a**) Effect of total rainfall on egg size. Raw data points in black and regression line (± SE) in blue. (**b**) AIC support (i.e. difference in AIC between a given sliding window model and the baseline model) for sliding windows up to 80 days before egg laying. The darker the colour of the tiles, the stronger the support for a given window. (**c**) Histogram representing the best AIC support for 25 randomised sliding window analyses (e.g. AIC support expected if no relationship exists between egg volume and heat waves). The red dashed line illustrates the AIC support achieved using the real data set. Three randomised test scored better (i.e. lower) AIC support than the real data set, suggesting that the relationship found in the data set has a 12% probability of representing a false positive result (i.e. strong AIC support with no real relationship between rainfall and egg volume).

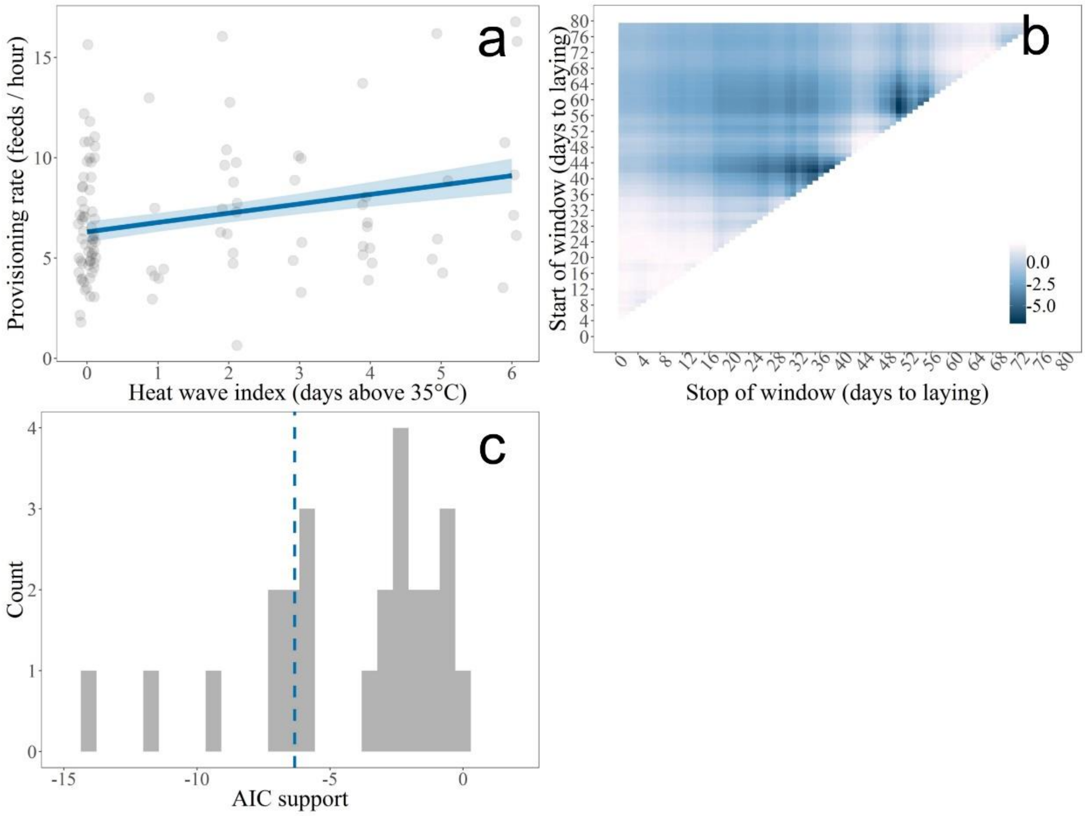

Figure S3. Sliding window analysis for the effect of 'heat waves' (days above 35°C) on maternal provisioning rate. (**a**) Effect of 'heat waves' on maternal provisioning rate (i.e. post-natal investment in reproduction). Raw data points in black and regression line (± SE) in blue. (**b**) AIC support (i.e. difference in AIC between a given sliding window model and the baseline model) for sliding windows up to 80 days before egg laying. The darker the colour of the tiles, the stronger the support for a given window. (**c**) Histogram representing the best AIC support for 25 randomised sliding window analyses (e.g. AIC support expected if no relationship exists between maternal provisioning rate and heat waves). The blue dashed line illustrates the AIC support achieved using the real data set. Seven randomised test scored better (i.e. lower) AIC support than the real data set, suggesting that the relationship found in the data set has a 28% probability of representing a false positive result (i.e. strong AIC support with no real relationship between heat waves and maternal provisioning rate).

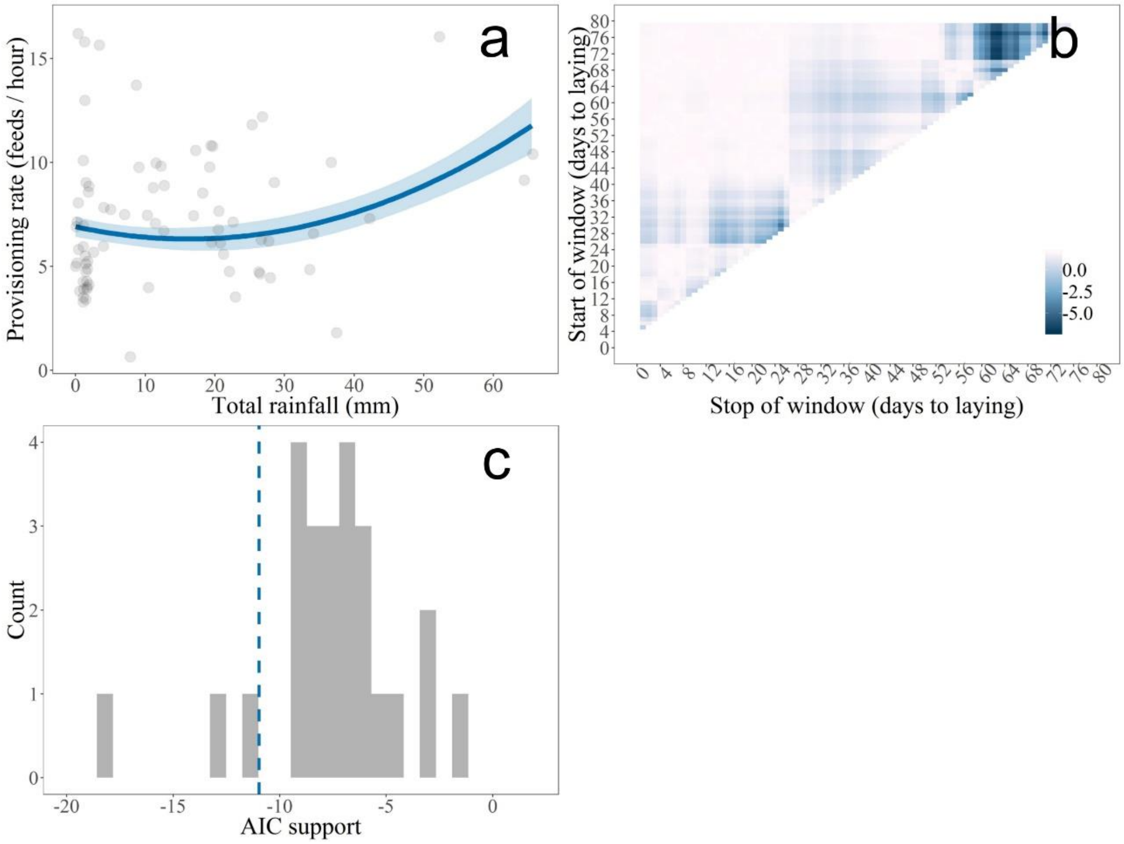

Figure S4. Sliding window analysis for the effect of total rainfall (mm) on maternal provisioning rate. (**a**) Effect of total rainfall on maternal provisioning rate (i.e. post-natal investment in reproduction). Raw data points in black and regression line (± SE) in blue. (**b**) AIC support (i.e. difference in AIC between a given sliding window model and the baseline model) for sliding windows up to 80 days before egg laying. The darker the colour of the tiles, the stronger the support for a given window. (**c**) Histogram representing the best AIC support for 25 randomised sliding window analyses (e.g. AIC support expected if no relationship exists between maternal provisioning rate and rainfall). The blue dashed line illustrates the AIC support achieved using the real data set. Three randomised tests scored better (i.e. lower) AIC support than the real data set, suggesting that the relationship found in the data set has a 12% probability of representing a false positive result (i.e. strong AIC support with no real relationship between rainfall and maternal provisioning rate).

Supplementary Tables Chapter 3

Table S1. Summary of the sliding window analysis for the effect of heat waves and total rainfall on i) egg volume (i.e. pre-natal maternal investment) and ii) maternal feeding rate (i.e. post-natal maternal investment). The start (‘Best Start’) and end (‘Best Stop’) of the top-performing windows (‘0’ = laying date; their value represents days before egg laying) for each environmental index are shown along with their AIC support (i.e. improvement in AIC compared to the baseline model). Randomisation tests (assessing the probability of false positive result) for environmental effects on egg volume suggested a considerable probability of false positive regarding the quadratic rainfall effect, whereas this probability was very low for the heat wave effect. For maternal feeding rates, random tests suggested a considerable probability of false positive results for both the heat wave and the rainfall effects.

Biological response	**Environmental index**	**Best Start (days)**	**Best Stop (days)**	**AIC**	**AIC support**	**Probability of false positive**
i) Egg volume	Heat wave	13	0	918.07	-11.13	0%
	Rainfall^1	10	0	925.31	-3.90	-
	Rainfall^1 + Rainfall^2	49	44	916.36	-12.90	12%
ii) Maternal feeding rate	Heat wave	58	51	282.23	-6.85	28%
	Rainfall^1	77	63	279.77	-7.23	-
	Rainfall^1 + Rainfall^2	78	61	276.02	-10.97	*12%*

Table S2. Model selection table containing every model within ΔAIC < 6 explaining variation in egg volume. Three models passed the nesting rule (‘Retained’, Richards, 2008). Standardised model coefficients are shown along with number of model parameters (‘k’), AIC, ΔAIC and Akaike’s weight (‘w’).

Intercept	**Number female helpers**	**Number male helpers**	**Clutch size**	**Rainfall^1**	**Rainfall^2**	**Egg position**	**Heat waves**	**Clutch size x Number female helpers**	**Clutch size x Number male helpers**	**Egg position X number female helpers**	**Egg position X number male helpers**	**k**	**AIC**	**ΔAIC**	**w**	**Retained**
0.006	0.078			-0.069	-0.107	-0.119	-0.114					11	890.4	0	0.237	✓
0.007	0.069	0.026		-0.068	-0.106	-0.118	-0.117					12	891.8	1.40	0.117	
0.007	0.079			-0.069	-0.107	-0.116	-0.114			0.015		12	892.0	1.59	0.107	
0.006	0.078		0.000	-0.069	-0.107	-0.118	-0.114					12	892.4	2.00	0.087	
0.008	0.070	0.025		-0.068	-0.107	-0.116	-0.116			0.014		13	893.4	3.03	0.052	
0.006	0.069	0.025		-0.068	-0.106	-0.120	-0.117				-0.011	13	893.5	3.16	0.049	
0.007	0.069	0.026	0.003	-0.068	-0.106	-0.119	-0.116					13	893.8	3.39	0.043	
0.011	0.081		0.006	-0.068	-0.108	-0.119	-0.113	0.021				13	893.8	3.47	0.042	
0.007	0.079		0.001	-0.069	-0.107	-0.117	-0.114			0.015		13	894.0	3.59	0.039	
-0.001		0.051		-0.067	-0.105	-0.120	-0.115					11	894.1	3.71	0.037	✓
-0.008				-0.071	-0.104	-0.121	-0.109					10	894.4	4.05	0.031	✓
0.008	0.071	0.024		-0.068	-0.108	-0.118	-0.117			0.021	-0.019	14	894.8	4.44	0.026	
0.012	0.072	0.025	0.009	-0.068	-0.107	-0.119	-0.115	0.020				14	895.3	4.93	0.020	
0.009	0.070	0.026	0.004	-0.068	-0.107	-0.117	-0.116			0.014		14	895.4	5.01	0.019	
0.004	0.070	0.024	-0.004	-0.067	-0.107	-0.118	-0.118		-0.019			14	895.4	5.08	0.019	
0.006	0.069	0.026	0.002	-0.068	-0.106	-0.121	-0.117				-0.011	14	895.5	5.15	0.018	

0.011	0.081		0.006	-0.069	-0.108	-0.117	-0.113	0.017		0.009		14	895.7	5.33	0.017
-0.002		0.051		-0.067	-0.106	-0.122	-0.116				-0.010	12	895.9	5.51	0.015
-0.002		0.050	-0.003	-0.067	-0.105	-0.119	-0.115					12	896.1	5.70	0.014
-0.008			-0.009	-0.071	-0.105	-0.118	-0.109					11	896.3	5.97	0.012

Table S3. Summary of models within a ΔAIC value of six explaining variation in egg volume, partitioning within- (prefix 'Δ') and among-mother (prefix 'µ') variation in the number of helpers. Three models passed the nesting rule ('Retained'; see 'Methods'; Richards, 2008). Standardised model coefficients are shown along with number of model parameters ('k'), AIC, ΔAIC and Akaike's weight ('w').

Intercept	Δfemale helpers	µfemale helpers	Δmale helpers	µmale helpers	Rainfall^1	Rainfall^2	Egg position	Heat waves	k	AIC	ΔAIC	w	Retained
-0.006	0.061				-0.068	-0.108	-0.119	-0.114	11	890.5	0	0.285	✓
-0.006	0.055		0.019		-0.067	-0.107	-0.118	-0.116	12	892.0	1.45	0.138	
0.001	0.061	0.032			-0.068	-0.107	-0.118	-0.114	12	892.2	1.72	0.121	
-0.002	0.061			0.030	-0.068	-0.107	-0.118	-0.114	12	892.3	1.74	0.120	
0.001	0.055	0.032	0.019		-0.067	-0.107	-0.118	-0.116	13	893.7	3.16	0.059	
-0.002	0.055		0.019	0.030	-0.067	-0.107	-0.118	-0.116	13	893.7	3.18	0.058	
0.001	0.061	0.022		0.019	-0.068	-0.107	-0.118	-0.114	13	894.2	3.64	0.046	
-0.006			0.038		-0.066	-0.106	-0.120	-0.115	11	894.3	3.79	0.043	✓
-0.008					-0.071	-0.104	-0.121	-0.109	10	894.4	3.90	0.041	✓
0.001	0.055	0.022	0.019	0.019	-0.067	-0.107	-0.118	-0.116	14	895.6	5.08	0.022	
0.001		0.031	0.039		-0.067	-0.105	-0.120	-0.115	12	896.0	5.52	0.018	
-0.002			0.039	0.029	-0.067	-0.105	-0.120	-0.115	12	896.1	5.54	0.018	
-0.002		0.024			-0.071	-0.104	-0.121	-0.109	11	896.3	5.74	0.016	
-0.004				0.021	-0.071	-0.104	-0.121	-0.109	11	896.3	5.77	0.016	

Table S4. Models within a ΔAIC of six explaining variation in maternal provisioning rate. Twenty-six models scored a ΔAIC lower than six of which 8 passed the nesting rule (‘Retained’, Richards, 2008). Standardised model coefficients are shown along with number of model parameters (‘k’), AIC, ΔAIC and Akaike’s weight (‘w’).

Intercept	Number female helpers	Number male helpers	Brood size	Rainfall^1	Rainfall^2	Heat waves	Number female helpers X Brood size	Number male helpers X Brood size	k	AIC	ΔAIC	w	Retained
0.004	-0.181		0.461	0.147	0.212	0.211			10	269.8	0	0.172	✓
-0.019	-0.167	-0.091	0.448	0.143	0.217	0.188		-0.121	12	270.8	0.93	0.108	
0.000	-0.176	-0.076	0.453	0.140	0.211	0.196			11	271.0	1.18	0.095	
0.005	-0.180		0.463	0.150	0.211	0.209	0.014		11	271.8	1.97	0.064	
-0.037	-0.172	-0.057	0.456	0.134	0.220	0.200		-0.124	13	272.0	2.19	0.058	
-0.018	-0.163	-0.092	0.458	0.153	0.214	0.183	0.054	-0.134	13	272.4	2.56	0.048	
-0.013	-0.182	-0.049	0.458	0.133	0.213	0.203			12	272.4	2.57	0.048	
-0.019	-0.154	-0.128	0.436	0.192	0.237			-0.128	11	272.9	3.04	0.038	✓
0.000	-0.175		0.461	0.208	0.237				9	272.9	3.07	0.037	✓
0.001	-0.175	-0.076	0.456	0.143	0.210	0.194	0.015		12	273.0	3.15	0.036	
0.013			0.463	0.157	0.213	0.199			9	273.1	3.23	0.034	✓
-0.007	-0.170	-0.111	0.449	0.193	0.233				10	273.2	3.40	0.031	
-0.013		-0.114	0.446	0.158	0.219	0.163		-0.135	11	273.3	3.46	0.031	
-0.037	-0.168	-0.056	0.468	0.144	0.217	0.194	0.061	-0.139	14	273.6	3.71	0.027	

0.009		-0.090	0.453	0.152	0.211	0.179			10	274.0	4.16	0.021	
-0.017	-0.150	-0.127	0.449	0.203	0.232		0.067	-0.146	12	274.3	4.49	0.018	
-0.012	-0.180	-0.049	0.462	0.136	0.212	0.201	0.018		13	274.4	4.53	0.018	
-0.012		-0.147	0.432	0.200	0.237			-0.147	10	274.4	4.58	0.017	✓
-0.028	-0.155	-0.114	0.439	0.186	0.240			-0.129	12	274.7	4.81	0.016	
0.001	-0.172		0.467	0.213	0.235		0.031		10	274.8	4.95	0.014	
0.013	-0.182		0.417	0.156		0.238			9	274.9	5.02	0.014	✓
-0.017	-0.173	-0.096	0.453	0.188	0.236				11	275.0	5.15	0.013	
-0.006	-0.168	-0.110	0.455	0.198	0.231		0.027		11	275.2	5.31	0.012	
0.013			0.461	0.216	0.235				8	275.5	5.61	0.010	✓
0.009		-0.124	0.445	0.201	0.230				9	275.5	5.62	0.010	
0.023	-0.187		0.433			0.300			8	275.8	5.92	0.009	✓

Table S5. Summary of models within a ΔAIC value of six explaining variation in maternal provisioning rate, partitioning within- (prefix ‘Δ’) and among-mother (prefix ‘µ’) variation in the number of helpers. The first three models (i.e. strongest statistical support from the data) suggest a negative effect of within-mother variation in female helper number on maternal provisioning rate (i.e. plasticity to the number of female helpers). There was much lower support for such an effect of the number of male helpers. ‘Retained’ refers to whether a given model passes the nesting rule by Richards (2008). Standardised model coefficients are shown along with number of model parameters (‘k’), AIC, ΔAIC and Akaike’s weight (‘w’).

Intercept	Δ female helpers	µ female helpers	Δ male helpers	µ male helpers	Brood size	Rainfall^{1}	Rainfall^{2}	Heat waves	k	AIC	ΔAIC	w	Retained
0.004	-0.130	-0.117			0.462	0.147	0.212	0.211	11	271.8	0	0.101	✓
0.004	-0.150			-0.112	0.468	0.147	0.215	0.194	11	272.2	0.34	0.086	✓
0.008	-0.135				0.478	0.150	0.214	0.206	10	272.2	0.37	0.084	✓
0.009		-0.122			0.447	0.154	0.210	0.204	10	272.5	0.70	0.071	✓
0.002	-0.142	-0.092		-0.082	0.458	0.146	0.213	0.201	12	272.8	0.99	0.062	
0.013					0.463	0.157	0.213	0.199	9	273.1	1.23	0.055	✓
0.002	-0.124	-0.123	-0.038		0.457	0.143	0.211	0.204	12	273.6	1.79	0.041	
0.011				-0.094	0.454	0.159	0.213	0.185	10	273.7	1.88	0.040	
0.008		-0.105		-0.060	0.444	0.156	0.210	0.195	11	274.0	2.18	0.034	
0.005		-0.132	-0.060		0.442	0.148	0.208	0.193	11	274.0	2.18	0.034	
0.004	-0.151		0.005	-0.113	0.469	0.148	0.215	0.195	12	274.2	2.34	0.032	
0.007	-0.133		-0.014		0.477	0.149	0.214	0.203	11	274.2	2.34	0.031	
0.002	-0.144			-0.131	0.465	0.203	0.238		10	274.5	2.66	0.027	✓

0.001	-0.138	-0.097	-0.021	-0.078	0.456	0.143	0.212	0.198	13	274.8	2.94	0.023	
0.011			-0.036		0.461	0.153	0.212	0.191	10	274.9	3.05	0.022	
0.000	-0.123	-0.116			0.461	0.208	0.237		10	274.9	3.07	0.022	✓
0.006	-0.126				0.476	0.210	0.238		9	275.0	3.19	0.021	✓
0.008		-0.121			0.445	0.214	0.234		9	275.2	3.38	0.019	✓
0.013					0.461	0.216	0.235		8	275.5	3.61	0.017	✓
-0.003	-0.139	-0.084		-0.102	0.457	0.203	0.237		11	275.5	3.64	0.016	
0.011				-0.114	0.449	0.213	0.234		9	275.6	3.76	0.015	
0.011			-0.025	-0.092	0.452	0.156	0.213	0.180	11	275.6	3.79	0.015	
0.005		-0.115	-0.051	-0.051	0.440	0.150	0.209	0.187	12	275.7	3.82	0.015	
0.002		-0.136	-0.090		0.435	0.200	0.229		10	276.1	4.22	0.012	
-0.005	-0.112	-0.129	-0.072		0.452	0.197	0.233		11	276.2	4.33	0.012	
0.008		-0.096		-0.082	0.440	0.212	0.233		10	276.3	4.49	0.011	
0.000	-0.139		-0.030	-0.127	0.462	0.198	0.236		11	276.4	4.54	0.010	
0.004	-0.119		-0.048		0.471	0.203	0.235		10	276.7	4.86	0.009	
0.011			-0.067		0.454	0.206	0.232		9	276.8	4.97	0.008	
0.013	-0.126	-0.122			0.416	0.156		0.238	10	276.9	5.02	0.008	✓
-0.006	-0.129	-0.097	-0.053	-0.091	0.451	0.195	0.234		12	277.1	5.26	0.007	
0.010			-0.054	-0.108	0.444	0.205	0.232		10	277.2	5.34	0.007	
0.017	-0.132				0.432	0.157		0.235	9	277.3	5.42	0.007	✓
0.017		-0.128			0.404	0.166		0.228	9	277.3	5.42	0.007	✓

0.013	-0.147			-0.109	0.422	0.152		0.225	10	277.5	5.61	0.006	
0.003		-0.114	-0.078	-0.067	0.432	0.200	0.229		11	277.5	5.65	0.006	
0.023	-0.134	-0.121			0.433			0.300	9	277.8	5.92	0.005	✓

Table S6. Summary of Gaussian models explaining variation in clutch size. The intercept-only model received the strongest support from the data and was the only one passing the nesting rule ('Retained', Richards, 2008). Standardised model coefficients are shown along with number of model parameters ('k'), AIC, ΔAIC and Akaike's weight ('w').

Intercept	Number female helpers	Number male helpers	Clutch order	k	AIC	ΔAIC	w	Retained
-0.044				5	945.40	0	0.213	✓
-0.039		-0.071		6	946.10	0.68	0.152	
-0.047	-0.071			6	946.10	0.69	0.151	
-0.035			0.061	6	946.20	0.76	0.146	
-0.039	-0.068		0.058	7	947.00	1.56	0.098	
-0.032		-0.065	0.055	7	947.10	1.66	0.093	
-0.042	-0.062	-0.062		7	947.10	1.69	0.092	
-0.035	-0.060	-0.057	0.053	8	948.20	2.74	0.054	

Table S7. Summary of Poisson mixed models explaining variation in the number of clutches laid per year. The model with 'Total rainfall' as a single predictor received the strongest support from the data and was the only one passing the nesting rule ('Retained', Richards, 2008). Removing this term caused a decrease in AIC of 10.12. Standardised model coefficients are shown along with number of model parameters ('k'), AIC, ΔAIC and Akaike's weight ('w').

Intercept	Number female helpers	Number of male helpers	Total rainfall	k	AIC	ΔAIC	w	Retained
0.832			0.159	4	710.4	0	0.468	✓
0.831		0.039	0.158	5	711.7	1.25	0.251	
0.832	0.019		0.155	5	712.3	1.82	0.188	
0.831	0.009	0.037	0.156	6	713.7	3.21	0.094	

Chapter 4

Dispersal and the evolution of sex differences in cooperation

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4.1. Abstract

Sex differences in contributions to cooperation within the natal group are widespread in animal societies but their evolutionary origins remain poorly understood. The philopatry hypothesis proposes that the more philopatric sex may contribute more to natal cooperation as it stands to gain a greater downstream direct fitness payoff from natal cooperation. Here we provide novel support for the philopatry hypothesis by demonstrating that a cooperative bird with female-biased philopatry (a rare reversal of the typical avian pattern of philopatry) shows an unusual female bias in natal cooperative generosity. That this occurs in the absence of a sex difference in relatedness to recipients is consistent with the hypothesised role for direct fitness payoffs in shaping sex differences in cooperation. While it is typically suggested that the more philopatric sex stands to gain a greater direct fitness *benefit* from natal cooperation if it is more likely to breed within the natal group, this mechanism cannot readily account for our findings, as this species shows no sex difference in the probability of inheriting a dominant (breeding) position within the natal group. Our findings point instead to a role for sex differences in the direct *costs* of cooperation: where investment in cooperation trades-off against extra-territorial prospecting, the less philopatric sex may experience a greater direct *cost* of natal cooperation, given its greater need to prospect for dispersal opportunities. Using a large-scale automated radio-tracking study, we demonstrate that the less philopatric sex (males) does indeed prospect at higher rates, both during and outside peak periods of cooperation. Our findings lend new strength to the philopatry hypothesis and highlight the possibility that patterns of philopatry shape sex differences in cooperation via impacts on the sex-specific costs, as well as benefits, of cooperation.

4.2. Introduction

Sex differences in contributions to cooperative behaviour are widespread in social species but their evolutionary origins remain poorly understood (Cockburn, 1998; Clutton-Brock *et al.*, 2002; Hodge, 2007; Williams & Hale, 2007). In many cooperatively breeding societies, for example, offspring of both sexes delay dispersal from their natal groups and help to rear future generations of offspring born to a dominant breeding pair; these male and female helpers often differ in their contributions to this cooperative care (Cockburn, 1998; Koenig & Dickinson, 2016). These sex differences in natal cooperation are interesting as they can occur in the absence of sex differences in relatedness to recipients, suggesting a role for sex differences in direct fitness payoffs in shaping their evolution (Johnstone & Cant, 2008). The philopatry hypothesis proposes that such sex differences in natal cooperation arise because the more philopatric sex stands to gain a greater downstream direct fitness payoff from cooperation (Clutton-Brock *et al.*, 2002; Young *et al.*, 2005). The observation that female helpers frequently contribute more to natal cooperation in cooperative mammals (e.g. Ostermeyer & Elwood, 1984; Owens & Owens, 1984; Clutton-Brock *et al.*, 2002; Zöttl *et al.*, 2016; which typically show female-biased philopatry, Greenwood, 1980) while the reverse is often true in cooperatively breeding birds (Stallcup & Woolfenden, 1978; Curry, 1988; Cockburn, 1998; Legge, 2000; Canestrari *et al.*, 2005; which typically show male-biased philopatry) is broadly consistent with this view. However, the rarity of sex-reversed philopatry within either clade leaves the hypothesis challenging to test (though see Williams & Hale, 2007). It is also unclear whether sex differences in philopatry may shape sex differences in natal cooperation principally via impacts on the sex-specific direct fitness benefits or costs of cooperation (Clutton-Brock *et al.*, 2002; Young *et al.*, 2005; Hodge, 2007; Downing *et al.*, 2018).

It is typically suggested that the more philopatric sex may contribute more to natal cooperation because it stands to gain a greater direct fitness *benefit* from cooperation (Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). The more philopatric sex may stand to gain greater downstream direct fitness benefits from natal cooperation whenever the continued accrual of such benefits is contingent upon remaining within the natal group. For example, the more philopatric sex may be more likely to inherit a breeding position within the natal group, and so could be more likely to be helped back by those individuals that they have helped to rear (Woolfenden & Fitzpatrick, 1978; Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). The more philopatric sex could also stand to gain differential direct fitness benefits from natal cooperation *prior* to dominance (breeding position) acquisition, if, for example, helping increased the size of the natal group and group size enhanced the survival of all residents (e.g. as envisaged under the group augmentation hypothesis for the evolution of helping; Kokko *et al.*, 2001; Kingma *et al.*, 2014). Given the rarity of evidence of a role for direct fitness benefits in shaping animal cooperation, compelling evidence that sex differences in cooperation are a product of sex differences in direct benefits would be of particular interest. While a number of studies have suggested that this is the case (Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018), empirical associations between sex differences in philopatry and cooperation could instead reflect a role for sex differences in the direct *costs* of cooperation (Young *et al.*, 2005; Hodge, 2007; Williams & Hale, 2007).

Helping has the potential to be more costly for the more dispersive (i.e. less philopatric) sex if individuals face a trade-off between investing in helping within the natal group and activities that enhance their dispersal prospects, such as prospecting for dispersal opportunities outside the natal territory (Young *et al.*, 2005). Helpers in many animal societies are known to carry out extra-territorial prospecting movements (Doerr & Doerr, 2005; Young *et al.*, 2005, 2007; Mares *et al.*, 2014). Prospecting can yield direct fitness benefits if it increases the likelihood of successful dispersal to a breeding position outside the natal group (Doerr & Doerr, 2005) and could also yield an immediate fitness payoffs if prospectors also engage in extra-group mating (Young *et al.*, 2007). Prospecting movements are likely to trade off against helping at the natal group, simply because helping and prospecting cannot be carried out at the same time, but also because costs associated with prospecting (e.g. energetic costs and/or exposure to stressors; Young & Monfort, 2009; Kingma *et al.*, 2016) have the potential to reduce the ability of individuals to help upon their return to the natal territory (Young *et al.*, 2005; Ridley *et al.*, 2008; Young & Monfort, 2009; Kingma *et al.*, 2016). Frequent prospectors may also reduce their contributions to cooperation more generally, in order to maintain traits (e.g. competitive body condition) that promote successful prospecting and/or dispersal. There are, however, very few studies of extra-territorial prospecting behaviour, doubtless due in part to the difficulty of studying the cryptic, fast and long distance movements that prospectors make (Doerr & Doerr, 2005; Young *et al.*, 2007; Kingma *et al.*, 2016). As such, whether sex differences in prospecting have the potential to explain sex differences in cooperation via impacts on the sex-specific costs of cooperation remains poorly understood (Young *et al.*, 2005; Williams & Hale, 2007).

The philopatry hypothesis has received comparatively little attention to date due in part to the rarity of species that show sex-reversed philopatry relative to the mammalian and avian norms, with which to test the prediction that such species should also show sex-reversed patterns of cooperation. In meerkats (*Suricata* *suricatta*), for example, female helpers are more philopatric than male helpers and, as predicted by the philopatric hypothesis, female helpers contribute more than male helpers towards cooperative care of offspring (Clutton-Brock *et al.*, 2002). Brown jays (*Cyanocorax morio*) are unusual among birds in that they also show female-biased philopatry, and in this species female helpers also invest more than male helpers in cooperative care of offspring (Williams & Hale, 2007). A comparative study of cooperatively breeding birds has also recently tested the hypothesis that sex differences in the probability of inheriting a breeding position within the natal group drive sex differences in cooperative effort (i.e. one route through which sex differences in philopatry could yield a sex difference in the direct *benefits* of cooperation; Downing *et al.*, 2018). Downing et al. (2018) found that the sex that is more likely to breed in their natal territory invests more in cooperative helping. However, this analysis could be confounded by sex differences in the mean relatedness of helpers to their recipients, as it did not explicitly isolate sex differences in cooperative effort of individuals *within* their natal groups. As such, helpers of the sex that is less likely to inherit a natal breeding position (most likely the more dispersive sex) may be more likely to contain immigrants, who may help less given their likely lower mean relatedness to recipients. It is therefore unclear to what extent the association is attributable to a role for kin selection versus sex differences in the direct benefits of helping (or indeed sex differences in its direct costs, which may be higher for the more dispersive sex; see above).

Here we test the philopatry hypothesis in a cooperatively breeding bird that shows female-biased philopatry; a rare reversal of the typical avian sex bias in philopatry (Greenwood, 1980). Note that we follow a broad definition of this hypothesis, an association between sex differences in natal cooperation and sex differences in philopatry due to sex differences in downstream direct fitness payoffs from cooperation (Young *et al.*, 2005), regardless of whether sex differences in cooperation principally arise from sex-specific direct fitness benefits or costs of cooperation. If the hypothesis holds, female helpers should contribute more to natal cooperation than male helpers, a pattern rarely seen in birds (Cockburn, 1998; Williams & Hale, 2007; Downing *et al.*, 2018). We then investigate the potential for sex differences in the species dispersal behaviour to have selected for sex differences in natal cooperation via impacts on the sex-specific direct fitness *benefits* and *costs* of cooperation. White-browed sparrow-weavers are passerine birds distributed across East and Southern Africa (Collias & Collias, 1978; Lewis, 1982a). This species lives in social groups of 2-12 birds, in which a single dominant male and female monopolise within-group reproduction and offspring of both sexes delay dispersal, forego reproduction and help to feed future generations of nestlings produced by the dominant pair (Lewis, 1982a; Harrison *et al.*, 2013a). Analyses of population genetic structure, coupled with direct observations of dispersal distances between birth and breeding locations, reveal that this species is unusual among birds in showing female-biased philopatry (Harrison *et al.*, 2014). Whether, as the philopatry hypothesis would predict (see definition above), subordinate females do contribute more to the cooperative feeding of offspring within the natal group is unknown. While female subordinates do feed offspring at higher rates on average than male subordinates (Chapter 2), this pattern could reflect a higher incidence of subordinate immigrant males than females in our study population given female-biased philopatry (as immigrants may help less than natal birds, given their likely lower relatedness to recipients; see above). Field observations also suggest that this species conducts extra-territorial prospecting forays (Lewis, 1982b; Harrison *et al.*, 2014), and the rarity of extra-group parentage by subordinates in this species

(Harrison et al. 2013) suggests that the primary function of such forays is to assess dispersal opportunities in the surrounding population. Whether there is a sex difference in prospecting in this species, and whether such prospecting occurs during peak periods of natal cooperation (i.e. nestling feeding periods) is as yet unknown.

Specifically, we first test the key prediction of the philopatry hypothesis: that female-biased philopatry in this cooperative bird should have led to the evolution of female-biased helping within the natal group (i.e. female helpers cooperatively feeding the offspring of breeders at higher rates than male helpers). We then investigate in more detail the nature of the sex difference in dispersal behaviour in this species, with a view to shedding light on the extent to which it could have led to selection for sex differences in natal cooperation via impacts on the sex-specific direct fitness *benefits* or *costs* of cooperation. First, we investigate whether the more philopatric sex (females) (i) is more likely to inherit a breeding position within its natal group, and/or (ii) resides for longer within its natal group (due to either a lower incidence or later mean age of dispersal from the natal group), given the potential for each to impact a helper's accrual of downstream direct fitness *benefits* from natal cooperation (see above). Second, we conduct a unique large-scale automated radio-tracking study to investigate whether the less philopatric sex (males) conducts extra-territorial prospecting forays at higher rates (both during and outside peak periods of cooperation), given the potential for trade-offs between cooperation and prospecting to leave the direct fitness *costs* of cooperation higher in the sex that relies more heavily on prospecting.

4.3. Methods

4.3.1. White-browed sparrow-weaver study population

White-browed sparrow-weavers live in semi-arid regions of South and Southeast Africa. Our study population is located in Tswalu Kalahari Reserve in the Northern Cape Province of South Africa (27°16’S, 22°25’ E). Field work was carried out from September to May between 2007 and the 2017. Approximately 40 reproductive units of white-browed sparrow-weavers were monitored, each one dominating a small territory in an area of approximately 1.5 km^2. Sparrow-weaver reproductive groups were easily monitored and distinguished in the field as all group members foraged together, engaged in weaving and territory defence, and roosted together in individual woven chambers in a single tree or cluster of trees close to the centre of their territory. All birds in our population are fitted with a single metal ring and three colour rings for identification from the beginning of the study period (under SAFRING license 1444).

Every white-browed sparrow-weaver group contains a single dominant (reproductive) pair (Harrison *et al.*, 2013a) and a variable number of subordinate individuals (Chapter 2). Dominant and subordinate individuals are easily identified in the field because they display a distinct set of behaviours. In every group, dominant birds are in very close association and these two birds spend much time foraging and duetting together (Walker *et al.*, 2016). White-browed sparrow-weaver groups were regularly monitored every one or two days to detect new clutches. Once a new clutch was found, nests were checked daily until the clutch had been completed. Then, clutches were checked 8 days after the first egg was laid to confirm successful progress. Following this latter mid-incubation check, clutches were checked daily starting 15 days after the first egg was laid until the fate (hatch or failure) and hatching date of every egg was determined. Nestlings were ringed 13 days after hatching and were last observed in the nests 17 days after hatching. After this day we did not inspect nests anymore to avoid premature fledging. The sex of every individual could be determined after the first six months of life as our study population present beak colour sexual dimorphism (Leitner *et al.*, 2009).

White-browed sparrow-weaver group composition was assessed every week in the early morning throughout every field season. Social groups were observed at least once every week and birds were identified on the basis of their colour-ring combination. In the course of these behavioural observations, we sometimes observed non-resident birds (i.e. prospecting birds) in a given focal group. The colour-ring combination of these prospecting birds was recorded when possible between October 2011 and April 2017 but due to aggression from resident members of the focal group, this information could be rarely collected. Additionally, birds were routinely caught while roosting at night and this information was also used to define group memberships.

4.3.2. Provisioning behaviour and weaving behaviour

Natural provisioning behaviour for subordinate individuals was recorded for breeding attempts between 2007 and 2016. We collected provisioning behaviour data using video-recordings between the 6th and 12th day after the first egg of a given clutch hatched. Video recordings were watched using VLC media player and data were extracted from every provisioning event: time of the feed, duration of the feed, feeder sex (based on beak coloration; Leitner *et al.*, 2009) and feeder identity based on a unique vent pattern (see below) and colour-ring combination. For every recorded breeding event, we always followed a standardised protocol at least five days before data collection started, we (i) caught and marked the vent of every bird in the group apart from the dominant female using hair dye (Chapter 2, Walker, 2015) to aid video identification and (ii) deployed tripods in the field to acclimatise the birds to its presence. On recording days, video cameras were set up in the morning at standard times that tracked monthly changes in sunrise (Table S1). Provisioning behaviour was recorded for approximately three hours per brood and day.

We calculated the number of feeds delivered by every individual in a given group. In few cases, we did not get reliable bird identifications from the provisioning videos, adding uncertainty to our observation of the individual feeds. We cleaned the original data set to retain only those days of observation and individuals where there were fewer than five feeds of uncertainty in our calculation (158 observations excluded). We also discarded provisioning data from individuals of unknown sex (one observation excluded) and of unknown social rank at a given time (six observations excluded). The final data set contained 994 observations for the number of feeds delivered by natal (i.e. part of the social unit in which they hatched) subordinate individuals with accurate hatching date and therefore age (largest possible error in individual's age = ± 60 days). Within-nest cameras have confirmed that all nest visits in which the birds are not conspicuously carrying grass entail the visiting bird carrying a single food item to the nest and delivering it to the chicks (Walker, 2015). No visits were non-provisioning visits and in no cases were visiting birds observed to eat the delivered food item themselves (Walker, 2015). Using the same data stream, we were also able to quantify individual contributions towards weaving. Natal subordinates were recorded bringing grass to the nest and weaving

37 times (3.72% of a total of 994 days and individuals observed). With these data, we investigated sex-differences in the probability of weaving.

4.3.3. Prospecting behaviour

4.3.3.1. Tracking of prospecting movements of subordinate individuals using Encounternet

We used Encounternet to investigate prospecting movements of subordinate white-browed sparrow-weavers. This technology allows automated simultaneous tracking of multiple individuals (i.e. tags; Mennill *et al.*, 2012). In short, the Encounternet system is formed by stationary wireless receivers (hereafter 'basenodes') that log tag pulses and record the ID number of the tag, the time and signal strength of the tag pulse (Received Signal Strength Indication – RSSI). We set up Encounternet tags (1.2 grams) to broadcast a digital pulse with their number ID every five seconds. Between March and April 2017, we tagged 32 adult subordinate birds using a figure-eight leg harness made of a stretchable, porous material obtained from common bike baggage straps (Snijders *et al.*, 2014, 2017). Tagged birds were all resident on their natal territory (hereafter referred to as their 'home' territories for the purposes of prospecting analyses) at the time of tagging and remained so for the duration of the Encounternet deployement. Additionally, we built an array of 35 Encounternet basenodes placed in a central tree of 35 contiguous white-browed sparrow-weaver territories (Figure S1). Clock synchronisation across the 35 basenodes was checked regularly with a maximum basenode time error of six seconds but normally between 0 and 1 second (hence logging times across basenodes were comparable). Basenode batteries were changed every 10 days, before they were discharged.

To account for between receiver variation in detection sensitivity, we calibrated all receivers before deployment. We attached two tags to a wooden pole with one of the tag antennas positioned parallel and the other perpendicular relative to the receiver's antenna to account for different antenna angles when the tags were on birds. To calibrate receivers, we held the tags on the pole at a fixed distance of two meters and height of 1.70 meters for two minutes, resulting in 48 pulses with signal strength values for each receiver. We then calculated the mean signal strength logged by each receiver ($\text{mean}_{\text{RECEIVER}}$) and the mean signal strength over all receivers ($\text{mean}_{\text{RSSI}}$). For 10 receivers with $\text{mean}_{\text{RECEIVER}}$ lower or higher than $\text{mean}_{\text{RSSI}} \pm 1$ sd, we adjusted the signal strength value of all logs during analysis by $\Delta|\text{mean}_{\text{RSSI}}-\text{mean}_{\text{RECEIVER}}|$ to avoid an over or underestimation of the distance between tagged birds and respective receivers.

We used RSSI signal strength values logged by receivers to estimate distances between detected tags and receivers (Mennill *et al.*, 2012; Snijders *et al.*, 2014) using a calibration RSSI-distance curve (Figure S2). To determine the relationship between signal strength and distance we placed two tags (one with antenna placed parallel and one perpendicular relative to the receiver antenna) on a wooden pole at different distances along 10 transects throughout the study site. At each distance, we held the pole in position for four minutes, placing tags for two minutes at 1.70 meters of height and two minutes on the ground, simulating birds in different positions and different heights. We chose transects between receivers placed on roosting trees of neighbouring groups and measured signal strength values every 10 meters, starting at two meters from the receiver up to a maximum of 120 meters. For one transect, we started at eight meters from the receiver, measuring every 10 meters up to 108 meters. We then used a RSSI-distance regression including all transect measures to predict the signal strength at 50 m (obtaining a value of -11.48 RSSI). We subsequently used the signal strength at 50 meters to inform the decision of whether or not to treat a bird as present in its home territory (Figure S3). Tags were detected 89% ± 23% of the time (mean ± standard deviation, SD) when within 20 meters and 76% ± 34% of the time (mean ± SD) when within 50 meters of a receiver.

4.3.3.1. Encounternet data processing

Basenode log files were arranged to generate files that contained logs from a given bird only. This produced 32 files, each corresponding to a different tagged bird. These 'bird files' were then processed one at a time. First, we discarded logs in the first day that a given bird carried a tag and we also discarded logs from the day following a catching session at a bird's home territory. Then, logs were explored in moving windows of 15 seconds of total length, in time steps of 5 seconds. For each of these 15-sec windows, a basenode location was assigned following the set of rules described in Figure S3. This algorithm resulted in a location assignment every 5 seconds. Individual birds were assigned known locations (either their 'home' territory or any other territory in the study site with an Encounternet basenode) on average 64% of their total tracked time (ranging between 94% and ~0% per bird; Figure S4). Five birds were excluded from further analyses as they were assigned to known locations only for a low percentage of their total tracked time (< 30% - Figure S4a), due most likely to tag failure (Figure S4b). Excluding these five birds, the average proportion of the time that birds were assigned locations within the study site increased to 73%. Consecutive time intervals assigned to the same location were then combined to represent time intervals of presence in a given territory. Location information was not recorded when birds were not assigned to any basenode. Using this data set, we identified individual forays as time periods when birds were not present at home and were assigned elsewhere. In order to ensure that movements of birds to the boundaries of their home territory were not misclassified as extra-territorial prospecting forays, we defined forays as incidences in which the bird's furthest assigned location was more than 250 meters from their home territory and the foray lasted more than 15 seconds (i.e. three location assignments in a row, at five second intervals). As neighbouring groups within our study site are always within 250 m of each other (Figure S1), all 'forays' constituted movements *beyond* the territories of neighbouring groups. This conservative approach will leave our analyses underestimating the true incidence of local forays by excluding true forays to neighbouring groups, but shared territory boundaries already provide the members of neighbouring groups with ample opportunity to interact without the need to conduct dedicated extra-territorial forays. For each foray, we calculated the time elapsed between the focal bird's first detection outside their home territory to their subsequent first detection back on their home territory ('foray duration') and the linear distance between the bird's home territory and the furthest assigned territory location for the foray ('foray distance').

4.3.4. Statistical methods

4.3.4.1. Modelling individual provisioning rate, feed duration, feed type and individual contributions to weaving

Variation in the number of provisioning events recorded for individual subordinate white-browed sparrow-weavers was analysed using a zero-inflated negative binomial generalised linear mixed model using the R package 'glmmADMB' (Fournier *et al.*, 2012). When there was uncertainty in our observations for individual number of feeds per video session (always < ± 2.5 feeds, see above), we calculated the average between the lowest and the highest possible values (for 754 out of 994 observations of individual number of feeds, we observed the number of individual feeds accurately; there was ± 0.5 feeds of uncertainty for 116 individual observations, ± 1 feed for 56 observations, ± 1.5 feeds for 37 observations, and > ± 2 feeds for 21 individual observations of the number of feeds). We included the sex of the provisioning individual, their age (a 3-level factor: "age < 1 year", "1 year < age < 2 years" and "age > 2 years"), the interaction between sex and age, brood size (1 to 3 nestlings) and brood age (from 6 to 12 days old) as fixed effect predictors. We added the duration of data collection per session as an offset to model variation in provisioning rate (feeds / hour). Social group ID, clutch ID, season and individual ID were included as random intercepts. The model was fitted to 994 observations of the number of individual feeds in 321 video sessions from 243 subordinate individuals for 132 different clutches in 32 different social groups. We found evidence for important zero-inflation (zero-inflation parameter = 0.168 ± 0.052 SE) and over-dispersion (dispersion parameter = 3.047 ± 0.527 SE) accounted by our zero-inflated negative binomial model.

Using the same data set (containing a total of 4,736 feeding events), we analysed feeding duration fitting a linear mixed model and including the same fixed and random effects as for the analysis of feeding rates (see above). Feeding visit duration was 'log+1' transformed to fulfil the assumption of normality in model residuals.

For 1,287 feeding events, the beak of the provisioning bird was visible and we categorised the food item as 'small' or 'large' if it was smaller or larger than the beak of the bird. Using this data set, we analysed the probability of feeding a large food item fitting a (binomial) generalised linear mixed model which contained the same fixed and random effects as for the analysis of individual feeding rates (see above).

Lastly, the probability of weaving was analysed using a binomial generalised linear mixed model including the same fixed and random effects than for the model of individual provisioning rate (as described above).

4.3.4.2. Modelling individual dispersal timing

Sex differences in direct benefits from cooperation may arise if each sex spends a different amount of time in their natal territory. Therefore, we investigated any sex difference in the length of the natal tenure by modelling the age-specific probability of disappearance form the natal territory for birds older than one year (before this age, dispersal from the natal territory is very low). This analysis contained individuals hatched between 2007 and April 2014 but utilised group census data up until April 2016, and so every individual included in the analysis was given at least two years to disperse. Whether or not an individual dispersed in a given twelve month window of their life was evaluated until the point of their disappearance from the group (i.e. individuals were only included in the analysis for those twelve-month windows of their lives for which they present within their natal group at the start and in a subordinate position. Individuals ceased to feature in the data set for any twelve-month windows in their life that either followed their disappearance from the natal group or started beyond April 2016). Then, we modelled the probability of disappearance from the natal group fitting a binomial generalised linear mixed model. The model included age as a categorical variable (with four level: '1-2y',2-3y', 3-4y', '>4y'), sex and the interaction between age and sex as fixed effects. Breeding season of hatching and individual ID were included as random effect intercepts. For

85 individuals with known dispersal territories, we explained variation in dispersal distance using a liner mixed model that included sex as a fixed effect and, breeding season of hatching and natal group as random intercepts.

4.3.4.3. Modelling prospecting behaviour

Following our definition of prospecting forays (see above), we carried out three complementary analyses to explain variation in (i) the daily number of prospecting forays (Poisson trait; number of prospecting forays per day; 'prospecting rate'); (ii) prospecting foray duration (Gaussian trait) and (iii) distance between the home territory and the furthest away visited territory per prospecting foray ('foray maximum distance 'Gaussian trait). We used generalised linear mixed models (GLMM) for the daily rate of prospecting and linear mixed models (LMM) for foray duration and foray maximum distance. Poisson GLMMs were checked for over-dispersion by simulating scaled model residuals 1,000 times in the R package 'DHARMa' (Hartig, 2018) and assessing their uniformity. These models were also checked for zero-inflation in a similar way (i.e. assessing whether the model predicted significantly fewer zeros than observed in the data). We did not find statistical evidence for over-dispersion or zero-inflation in these models.

In the four models outlined above, we included the same set of fixed effect predictors: age of the individual, its sex, breeding stage ('provisioning' yes or no for whether offspring existed in the home territory) as well as the interaction between sex and breeding stage. Bird ID, home territory ID and day of the year were included as random intercept terms. The analysis of prospecting behaviour comprised 27 individuals, 13 males and 14 females, hatched between September 2013 and January 2017; there was no statistical difference between the age of male and female subordinates included in the prospecting analysis (linear model, ‘sex’ (male) effect = 208.4 ± 170.8 days, t = 1.22, p = 0.234).

4.3.4.4. General statistical approach

Unless otherwise stated, we used Akaike’s Information Criterion (AIC, Burnham *et al.*, 2011) to rank models based on their fit to the data. We fully considered models within a ΔAIC value of six (i.e. AIC difference between a given model and the most-supported model, whose ΔAIC equals to zero) and applied the nesting rule suggested by Richards (2008) to simplify the final set of candidate models (Harrison *et al.*, 2018). We then discuss our results based on the statistical evidence (i.e. ΔAIC) from a set of models explaining the data. Statistical analyses were carried out using R 3.6.0. (R Core Team, 2019). Continuous model predictors were mean centered and standardise to one standard deviation to ease the comparison of their effect sizes (Gelman & Hill, 2007).

4.4. Results

4.4.1. Female-biased natal cooperation in the absence of sex differences in relatedness to recipients

Statistical modelling of the cooperative provisioning rates of subordinate males and females within their natal groups revealed strong support for sex, age, brood size and brood age effects (Table S2 & see Table 1 for the effect sizes for the top model). The top model (i.e. that which scored the lowest AIC) contained these four predictors and no interaction terms. Removing any one of these terms from the top model reduced support for the model by 10.86 or more AIC points (Table S2). Subordinate females fed broods at higher rates than subordinate males (Figure 1a). Subordinate provisioning rates increased initially with advancing age and then plateaued (Figure S5). There was no statistical support for an interaction between sex and age, indicating that the magnitude of the sex difference in provisioning rate remained stable with age (Figure S5; Table S2). Brood size and brood age both positively predicted subordinate provisioning rates (Table 1).

We also found strong evidence of a sex difference in the duration of provisioning visits. Subordinate females spent longer than subordinate males in the nest with the brood on each provisioning visit (Figure 1c; Table S3a). The higher provisioning rates of subordinate females cannot be readily attributed to them feeding the offspring with smaller food items than males, as there was no sex difference in the probability that subordinates provisioned offspring with a food item that was large (Figure 1d; Table S3b). These sex differences in the cooperative provisioning behaviour of subordinates within their natal groups occurred in the absence of sex differences in relatedness to recipients (i.e. the dominant breeding pair or the offspring being fed; Figure 1b; Table S4).

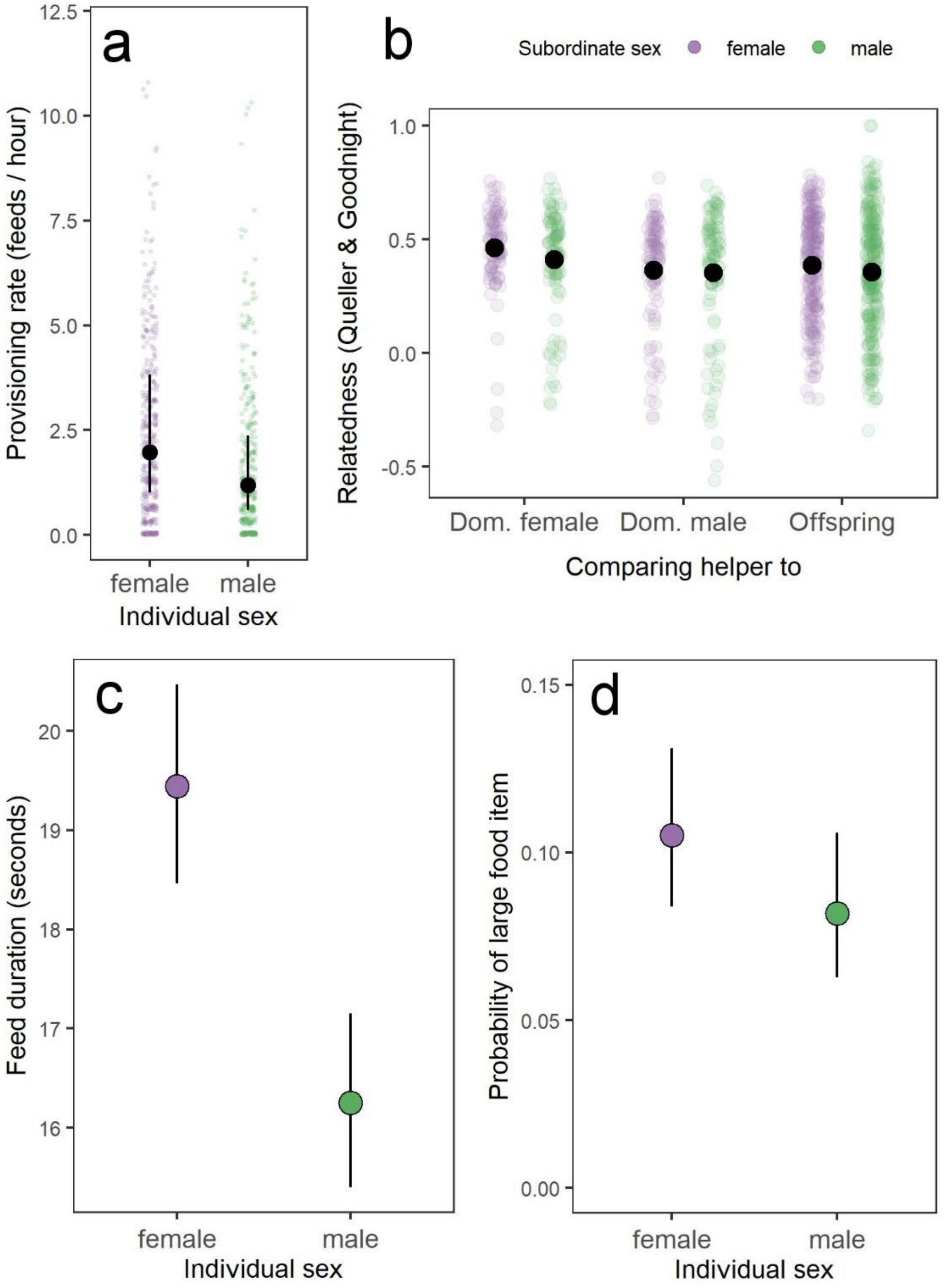

Figure 1. Sex differences in the cooperative contributions to nestling provisioning of subordinates within their natal groups. Analyses are based on 994 observation periods of individual provisioning rates by 243 natal subordinates to 132 broods in 32 social groups. (**a**) Natal subordinate females provisioned offspring at higher rates than natal subordinate males (see Tables 1 & S2). (**b**) These sex differences in helping behaviour occurred in the absence of a sex difference in the genetic relatedness of these natal subordinates to the dominant (breeding) birds ('Dom. female' and 'Dom. male') or the offspring that they were feeding (Table S4;

based on 12 microsatellite markers; for details see Harrison *et al*. (2013a). (**c**) Natal subordinate females also spent more time with nestlings (i.e. time within the nest) than natal subordinate males, during each provisioning visit (Table S3). (**d**) There was no sex difference in the probability of subordinates provisioning the brood with a food item that was large (Table S3). Transparent points represent raw data whereas larger black points and error bars give mean model predictions ± SE (standard errors; in panel b SEs are not visible as they do not extend beyond the marker for the mean estimate).

Table 1. Model coefficients of the best supported negative-binomial generalised linear mixed model explaining variation in the cooperative contributions to offspring provisioning of subordinates within their natal groups. The model response term was the number of provisioning visits by the individual during a given observation period, and the model included the duration of these observation periods as an offset (thereby effectively modelling provisioning rate; feeds / hour). The model accounted for zero-inflation (zero-inflation parameter = 0.16, SE = 0.03) and over-dispersion (negative binomial dispersion parameter = 2.59, SE = 0.40). Model coefficients are shown in the link-function scale ('log').

Fixed Effects

Terms	Estimate	SE	Z Value
Intercept	-1.762	0.454	-3.88
Sex (Male effect)	-0.512	0.135	-3.80
Age (1 y -2 y)	0.499	0.132	-3.79
Age (> 2 y)	0.591	0.171	3.45
Brood age	0.097	0.027	3.58
Brood size	0.535	0.150	3.57

Random Intercepts

	Variance estimate
Individual ID	0.518
Clutch ID	0.398
Group ID	< 0.001
Year	< 0.001

4.4.2. Female-biased philopatry without a female bias in the probability of inheriting the breeding position within the natal group

Sex differences in dispersal incidence and timing

We observed 54 subordinate males and 31 subordinate females dispersing away from their natal group, to another social group within our study population where they then became resident. This pattern suggests a significantly male-biased incidence of dispersal from the natal group (Binomial test against 50:50 distribution; $z = 2.46$, $p = 0.014$). Indeed, statistical modelling of the age-specific probability of dispersal from the natal group revealed robust evidence that subordinate males are more likely to disperse from their natal groups than subordinate females across all age classes (Figure 2a; Table S5; 'sex' appeared in every model within a ΔAIC value of 8.70). There was also strong evidence that dispersal probability initially increased with age before plateauing (Figure 2a; Table S5; 'age' appeared in every model within a ΔAIC value of 47.12). There was no compelling evidence that the pattern of age-related change in age-specific dispersal probability differed between the sexes (Table S5; the best-fitting model containing the sex-by-age interaction scored 4.08 AIC higher [i.e. weaker support] than the top model).

Probability of inheriting the dominant (breeding) position within the natal group

We found no evidence of a sex difference in the probability that subordinates that had survived to adulthood (one year old) within their natal group ultimately attained dominance within their natal group (Figure 2b; the intercept-only model outperformed the model containing the 'sex' predictor by 1.58 AIC points). This pattern may arise despite the evidence above of female-biased philopatry (Harrison *et al.*, 2014; and above) because when dominance turnovers did occur, it was rare in both sexes for an individual born within the focal group to become the new dominant

(7 of 32 (21.86%) male dominance turnovers, 9 of 32 (28.13%) female dominance turnovers; there was also no evidence of a sex difference in this likelihood: Fisher's exact test: $\chi^2 = 0.33$, df = 1, p = 0.564).

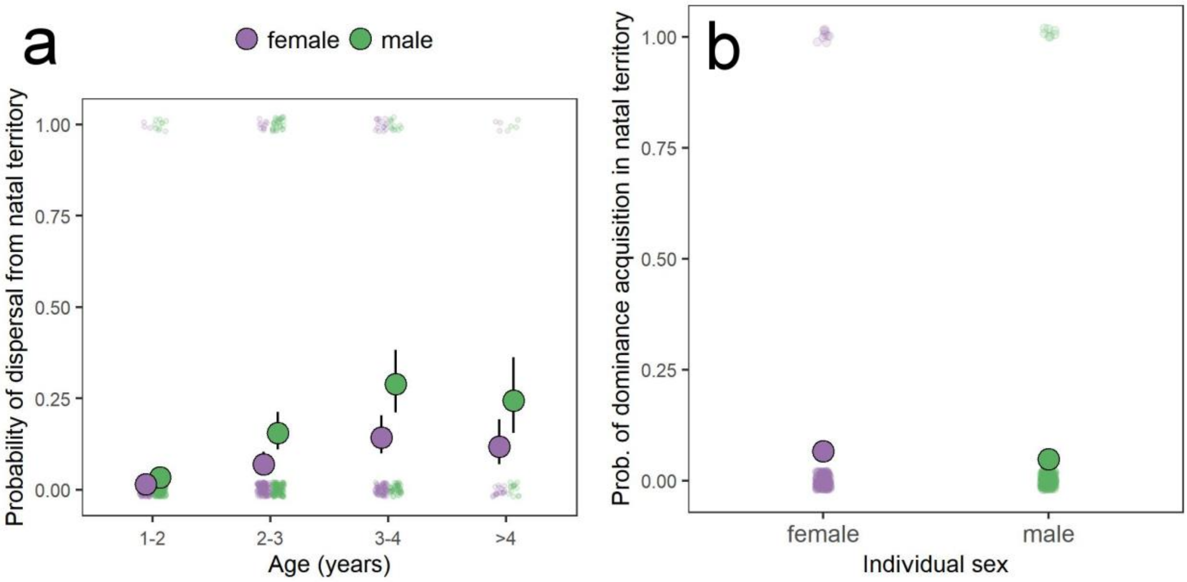

Figure 2. Sex differences in philopatry but not sex difference in probability of dominance acquisition in the natal territory. (**a**) The probability of dispersal from the natal territory after the first year of life was higher for subordinate males than for subordinate females, across all age classes (Table S5). (**b**) However, there was no evidence of a sex difference in the probability of inheriting a dominant (breeding) position within the natal group (see results text). Raw data points along with predicted means ± SE are shown for female and male subordinates (in panel b SEs are not visible as they do not extend beyond the marker for the mean estimate).

4.4.3. Male-biased extra-territorial prospecting from the natal group

Direct observations of prospecting behaviour

On 678 occasions between October 2011 and April 2017 unexpected birds were observed visiting groups in which they were not currently resident. While these birds were often rapidly attacked by resident birds, leaving them difficult to identify, we were able to identify the bird in 293 (43.2%) cases. Out of these 293 observed events with identified birds, 185 involved males (63.1%), 107 involved females (36.5%) and 1 (0.3%) involved birds of unknown sex. Although these observations suggest male-biased prospecting behaviour given the approximately equal adult sex ratio in our population, sex differences in the incidence of prospector sightings could reflect sex differences in traits other than the incidence of, or time spent, prospecting (e.g. sex differences in their behaviour when visiting other territories or in how they are treated by resident birds could both generate sex differences in the probability that forays are detected by human observers). We therefore utilised a novel automated radio-tracking technology, Encounternet, to provide continuous high-resolution information on the prospecting movements of subordinate natal males and females for the first time in a cooperatively breeding species.

Automated radio-tracking study of prospecting behaviour

We deployed an Encounternet array of 35 receivers across our study population (Figure S1) and tagged 32 subordinate birds living within their natal groups (but used 27 birds for final analysis of prospecting movements, 13 males and 14 females; Figure S4) with Encounternet radio tags (Mennill *et al.*, 2012). Birds were tracked for an average of 37 days (range = 11-59 days) generating a total of 22,518,022 detection logs (individual base-nodes log all tags that they detect, every 5 seconds). After processing the data (see Methods), we detected a total of 953 extra-territorial prospecting forays. A single foray was defined as a single continuous run (in time) of location estimates for the bird which suggested that the bird was not on its resident (natal) territory, during which the bird's location was *also* estimated to be > 250m away from the centre of its resident territory for > 15 seconds (Figure S1). As the mean (± SE) distance between the centres of two neighbouring territories was 93.7 m (± 4.56 m), this typically meant that forays constituted movements *beyond* the territories of neighbouring groups (Figure S1). This conservative approach will minimise the chance that resident bird's interactions with its neighbouring groups are falsely interpreted as extra-territorial prospecting but is likely to underestimate the true incidence of extra-territorial prospecting by excluding more local forays. The 953 forays detected principally occurred between 06:00h and 19:00h (Figure 3a), with a median duration of 60 seconds (inter-quantile range = 30 - 160 seconds; range 20 – 1,730 seconds (28.8 minutes)). The furthest from the natal group at which we detected the birds on each of these forays showed a median of 359.8 m (inter-quantile range = 269.2 – 396.4 m; range 251.1 – 1,013.1 m). This is likely to be an underestimation of the true maximum prospecting distance for many forays as our ability to detect forays over longer distances is constrained by the spatial scale of our receiver array (see Figure S1).

This unique data set provides clear evidence for male-biased prospecting behaviour in subordinates residing within their natal groups. Subordinate males showed higher daily rates of prospecting than subordinate females (Figure 3b; Table 2) and the magnitude of this sex difference was not affected by whether the bird's social group was provisioning young at the time (Figure 3b; Table 2). There was also some evidence to suggest that prospecting rates were lower on average during provisioning periods, when helping occurs, than at other times (Figure 3b; the ‘provisioning’ term was present in the top model, but was absent from a model 1.24 AIC points below; Tables 2). There was no evidence of an effect of subordinate sex or provisioning periods on either foray duration or foray maximum distance (Table S6); in both cases, the intercept-only model received the strongest support. Variation in subordinate age did not explain variation in any of the three variables tested for prospecting behaviour (Tables 2 & S6).

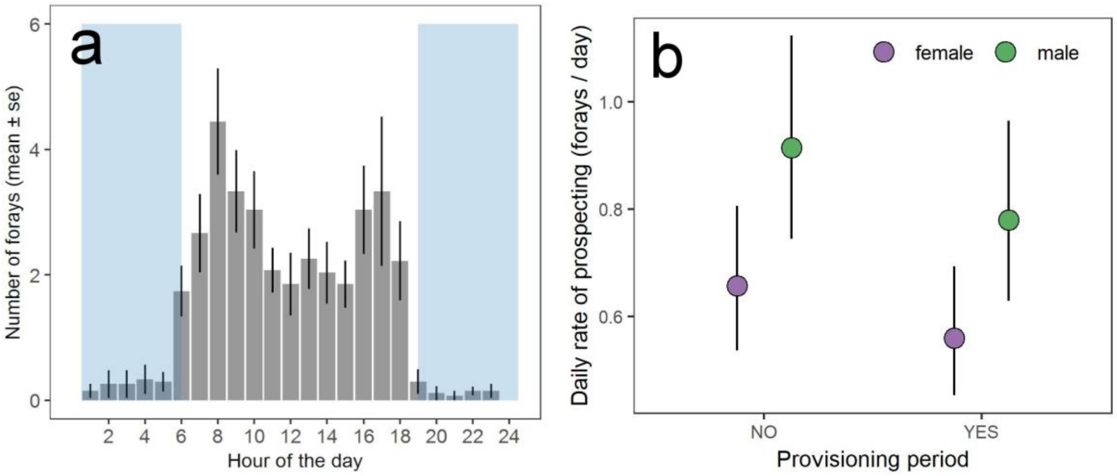

Figure 3. The extra-territorial prospecting behaviour of subordinates within their natal groups. (**a**) Number of forays per hour of the day (mean ± SE) across 27 birds with Encounternet tags. The shaded light blue area illustrates night hours (using as an approximation the civil twilight times for our study site on March 11th 2017 [06:05 and 19:14 hours]; the date in the middle of our 6-week long prospecting study. The few forays appear to occur at night could reflect rare incidences of the focal birds being flushed from their roost chambers by predators. (**b**) The analysis of the daily rate (forays / day) of prospecting forays reveals that subordinate males have a higher daily rate of prospecting than subordinate females, both inside and outside peak periods of cooperative provisioning (i.e. nestling provisioning periods). Mean predictions and standard errors are plotted based on the top models in Table 2.

Table 2. Analysis of daily rate of prospecting movements. The rate of forays per day was most strongly predicted by the sex of the subordinate individual. The interaction between ‘sex’ and ‘provisioning’ appeared in the second-best model with 1.06 ΔAIC points poorer fit than the top model and was not retained after applying the nesting rule. Models within a ΔAIC value of six that passed the nesting rule (Richards, 2008) are shown. Full model sets within a ΔAIC value of six are shown in Table S6a. k = number of estimated model parameters. ‘age’ was included in the analysis but was not retained in any model within a ΔAIC value of six that passed the nesting rule.

Intercept	Sex ('female' effect)	Provisioning ('YES' effect)	Sex ('female' effect) x Provisioning ('YES' effect)	k	AIC	ΔAIC
-0.334	-0.165	-0.080		6	2397.0	0.00
-0.299	-0.166			5	2398.2	1.24
-0.332		-0.079		5	2399.6	2.61
-0.296				4	2400.7	3.74

4.5. Discussion

We combined a long-term data set of cooperative provisioning and a large-scale automated radio-tracking study of white-browed sparrow-weavers to test the predictions of the philopatry hypothesis for the evolution of sex differences in natal cooperation. Our findings provide novel support for the philopatry hypothesis. White-browed sparrow-weavers show a rare sex-reversal of the typical avian pattern of philopatry: females are more philopatric than males (Harrison *et al.*, 2014; and Table 1). As predicted by the philopatry hypothesis, this female-biased philopatry is accompanied by female-biased natal cooperation (itself unusual among birds; Cockburn, 1998; Downing *et al.*, 2018): female helpers cooperatively provisioned offspring at higher rates than male helpers and spent longer in the nest during each provisioning visit. These sex differences in cooperation arise without sex differences in the genetic relatedness of helpers to recipients (the dominant [breeding] birds or their offspring), suggesting that they cannot be readily attributed to a role for kin selection alone. That female helpers provision offspring at higher rates than male helpers is ultimately reflected in apparent sex differences in the impact of their help upon recipients. First, the number of female helpers in a group strongly predicts the overall rate at which broods are provisioned and the probability of nestling survival to fledging in harsh conditions (Chapter 2). Second, dominant females (Chapter 3) and males (Capilla-Lasheras &, Young, unpublished data) both reduce their offspring provisioning rates when aided by more female helpers. Third, dominant females increase their egg investment when assisted by more female helpers (Chapter 3). By contrast, none of these relationships hold for the number of male helpers. Below, we (i) consider the potential for the philopatry hypothesis and alternative hypotheses to explain the evolution of the observed sex difference in natal cooperation, and (ii) utilise our dissection of sex differences in key dispersal traits (dispersal incidence, natal inheritance, and prospecting behaviour) to shed light on whether the observed association between philopatry and cooperation is likely to have arisen via impacts on the sex-specific direct *benefits* or *costs* of cooperation.

To what extent might evolutionary explanations other than sex differences in philopatry account for the observed sex difference in natal cooperation? Kin selection (Hamilton, 1964) cannot readily explain the observed sex difference in cooperation because these natal subordinates of both sexes were equally related to the recipients of their cooperative care (i.e. dominant individuals and their offspring). The heterogamety hypothesis for the evolution of sex differences in cooperation (Whitney, 1976) notes that, while sons and daughters may be equally related to the recipients of their help in their natal territory from the perspective of autosomal genes, the same need not be true for genes on sex chromosomes (Whitney, 1976). However, this hypothesis cannot explain the observed sex difference as it predicts lower rates of cooperative investment in the homogametic sex (females in birds), whereas our findings reflect the reverse. The paternity hypothesis for the evolution of sex differences in cooperation (Charnov, 1978) proposes that when there is extra-pair paternity (and therefore paternity uncertainty) the expected fitness returns from breeding compared to helping will be devalued for males, increasing the relative net fitness payoffs of helping versus breeding for males (Charnov, 1978). This hypothesis thus predicts male-biased cooperation wherever extra-pair paternity occurs (Charnov, 1978). As such, this too cannot explain the observed female-biased cooperation in white-browed sparrow-weavers (despite this species showing 12-18% extra-group paternity; Harrison *et al.*, 2013b).

It has also been suggested that the sex that shows higher variance in lifetime reproductive success may invest more in helping as their chance of ultimately securing direct fitness returns may be lower (Koenig *et al.*, 1983). It seems unlikely that this hypothesis can explain our findings as in white-browed sparrow-weavers (i) both sexes only breed as dominants (Harrison *et al.*, 2013a), (ii) there is no apparent sex difference in the probability of acquiring dominance during the lifetime (in the natal group or anywhere else; Capilla-Lasheras & Young, unpublished data), and (iii) while there is scope for the modest amount of extra-group paternity in this species (Harrison *et al.*, 2013b) to enhance variance in reproductive success among males relative to females, this would leave the hypothesis predicting male-biased cooperation in this species. Our results also cannot be readily attributed to a sex difference in the division of different types of cooperative labour between the helper sexes (e.g. in meerkats, male helpers contribute more to cooperative anti-predator behaviour while female helpers feed offspring at higher rates; Clutton-Brock *et al.*, 2002), as female white-browed sparrow-weavers contribute just as much as males to all other forms of cooperative behaviour in this species: cooperative territorial defence (York *et al.*, 2019), sentinelling (Walker *et al.*, 2016) and weaving (Table S7).

Our analyses reveal that white-browed sparrow-weavers show sex-reversed patterns of both philopatry and cooperation relative to those generally observed in birds (Greenwood, 1980; Cockburn, 1998). As both of these phenomena are rare in isolation (Greenwood, 1980; Cockburn, 1998; Williams & Hale, 2007) it seems unlikely that their association in this species is coincidental. While only a small proportion of female and male helpers ultimately acquire a dominant (breeding) position within their natal group (Figure 2b), multiple lines of evidence indicate female-biased philopatry in this species: (i) the age-specific probability of dispersal from the natal group is higher for subordinate males than females across all age classes (Figure 2a), (ii) males conduct extra-territorial prospecting forays at higher rates than females (Figure 3b), and (iii) analyses of both population genetic structure and direct observations suggest that birth to breeding distances are also higher for males than females in this species (Harrison *et al.*, 2014). While some of these findings are based on the subset of dispersal events that were possible to observe directly (as they were between groups within our study population), several lines of evidence suggest that the sex differences observed are unlikely to have arisen as an artefact of undetected dispersal events beyond the boundaries of our study population. First, dispersal is extremely local in this species even within the spatial scale of our study population (Harrison *et al.*, 2014). Second, there is a low overall rate of arrival of birds dispersing in from outside the population (suggesting that the rate of birds leaving our population may also be low) and those that do disperse in show a clear male bias, suggesting that if anything undetected long-distance dispersals are more likely to have occurred among males (Harrison *et al.*, 2014). Overall, this sex difference in philopatry could therefore explain the evolution of the observed sex difference in natal cooperation, through potential impacts on the sex-specific net direct fitness payoff from natal cooperation (see Introduction; Kokko *et al.*, 2001; Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018).

It is typically suggested that the more philopatric sex may help more because they stand to gain a greater downstream direct fitness *benefit* from cooperation given their greater likelihood of ultimately becoming a breeder within the natal group and thus benefiting themselves from the future presence and/or cooperative behaviour of the offspring that they are helping to rear (Woolfenden & Fitzpatrick, 1978; Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). However, this alone cannot readily account for the sex difference in cooperation observed here as, while females are more philopatric than males they are not ultimately more likely to secure a dominant breeding position within their natal group. This result appears to have arisen because inheritance of the natal group is generally rare in this species, with newly dominant individuals in both sexes typically having dispersed in from other groups. Accordingly, the sex difference previously documented in birth-to-breeding distance in this species appears to be attributable to a sex difference in the *distance* dispersed between birth and breeding, rather than the *incidence* of dispersal between birth and breeding (Harrison *et al.*, 2014). While subordinate females are no more likely to become dominant within their natal group, it is conceivable that they stand to gain greater direct fitness benefits from cooperation via benefits that accrue during their time as natal subordinates (given that the lower age-specific dispersal probabilities of females are likely to leave females living in their natal group for longer on average than males). Previous work on this species suggests that the payment of rent (Gaston, 1978) and social prestige (Clarke, 1989; Zahavi, 1995) mechanisms for the accrual of direct benefits from cooperation are unlikely to apply in this species (Walker, 2015). Arguably the most widely applicable mechanism by which helping could yield direct benefits is group augmentation, in which helpers are envisaged to benefit directly from cooperation if it increases group size and doing so is beneficial (Kokko *et al.*, 2001; Kingma *et al.*, 2014). However, the group augmentation hypothesis, at least as traditionally formulated, cannot be readily applied to white-browed sparrow-weavers, as our work to date suggests that helping does not increase offspring survival on average (Chapter 2) or reproductive rates (Chapter 3) and so may not increase group size. Positive effects of helping on the survival of the dominants are conceivable (given that helping appears to lighten their workloads; see above), but whether prolonging dominant survival would yield a net direct benefit to helpers is unclear, particularly given the potential (albeit limited) for a helper to inherit dominance when their same-sex breeder dies. As such, while it is conceivable that sex differences in the direct *benefits* of cooperation have yielded the sex difference in cooperation observed in this species (as invoked in the original formulation of the philopatry hypothesis in Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018), our findings do suggest a need to attend to the possibility of a role for sex differences in the direct costs of cooperation.

Sex differences in the direct costs of cooperation could arise whenever investment in cooperation trades off against alternative routes to fitness in a sex-specific manner (Young *et al.*, 2005; Hodge, 2007). The less philopatric sex specifically (males here), could suffer a greater direct cost of cooperation if investment in cooperation trades off against activities that promote successful dispersal to independent breeding positions (Ridley *et al.*, 2008; Bonte *et al.*, 2012; Kingma *et al.*, 2016, 2017). One such activity is extra-territorial prospecting, which numerous social species use to assess dispersal opportunities in the surrounding population prior to committing to a permanent transfer (Waser, 1996; Young, 2003; Doerr & Doerr, 2005). While few studies have investigated prospecting behaviour given the difficulty of tracking forays, there is evidence to suggest that prospecting can entail significant costs (Young *et al.*, 2005; Ridley *et al.*, 2008; Young & Monfort, 2009; Kingma *et al.*, 2016), which may leave frequent prospectors contributing less to cooperation (Young *et al.*, 2005). Our automated radio-tracking study revealed that subordinate male sparrow-weavers (the less philopatric sex) do indeed conduct extra-territorial prospecting forays at higher rates than subordinate females, both during and outside provisioning periods. It seems unlikely that this sex difference in prospecting rate is itself a product of the observed sex difference in cooperation (i.e. a reversal of the causal direction suggested above) as subordinate males still prospected at higher rates than females outside provisioning periods. Our findings also revealed some evidence to suggest that prospecting rates are lower during provisioning periods than at other times, consistent with the expectation of a trade-off between cooperation and prospecting.

Our findings do, however, suggest that prospecting forays (at least those beyond neighbouring groups, which were the focus of our analyses; see results) are typically short in duration (median duration = 60 seconds) and are only conducted on average about once a day, even among males outside provisioning periods. These findings render it unlikely that a simple trade-off between energy expenditure during cooperation and prospecting can explain the observed sex difference in cooperation, as the energetic costs of forays of this duration and rate seem unlikely to be substantial. Instead, prospecting is likely to entail significant risks, as prospectors are typically attacked when detected by members of other groups (Harrison *et al.*, 2014; Young et al. unpublished data) and could also face elevated predation risk while moving alone. As such, regular prospectors (i.e. males) may stand to benefit more than others from maintaining a competitive phenotype (e.g. good body condition), to both mitigate the risk of injury and maximise the chance of success while prospecting. Experimental work does suggest that cooperative provisioning entails body mass costs in this species (Cram *et al.*, 2015), lending strength to the suggestion that investment in cooperation may indeed trade off against the maintenance of optimal prospecting condition.

It is increasingly appreciated that the accrual of direct fitness returns of helping could play an important role in the evolution and maintenance of cooperation (Clutton-Brock, 2002; Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). The observation that the philopatric sex contributes to cooperation more than the dispersive sex in animal societies has been interpreted as an indication of a role for direct fitness benefits in shaping animal societies (Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). Our findings, however, suggest that female-biased cooperation in white-browed sparrow-weavers accompanies female-biased philopatry in the absence of an evident mechanisms through which females could gain greater direct benefits of cooperation than males. Instead, our results indicate that a sex difference in direct costs associated with a sex difference in philopatry is a more credible explanation for the observed sex-reversed pattern of cooperation in white-browed sparrow-weavers. Given the potential for patterns of philopatry to impact the sex-specific costs of cooperation in other species too, our findings highlight a need for caution when interpreting associations between sex differences in philopatry and cooperation as evidence of a role for direct fitness benefits only in shaping animal cooperation.

Chapter 4: Supplementary Material

Supplementary Figures Chapter 4

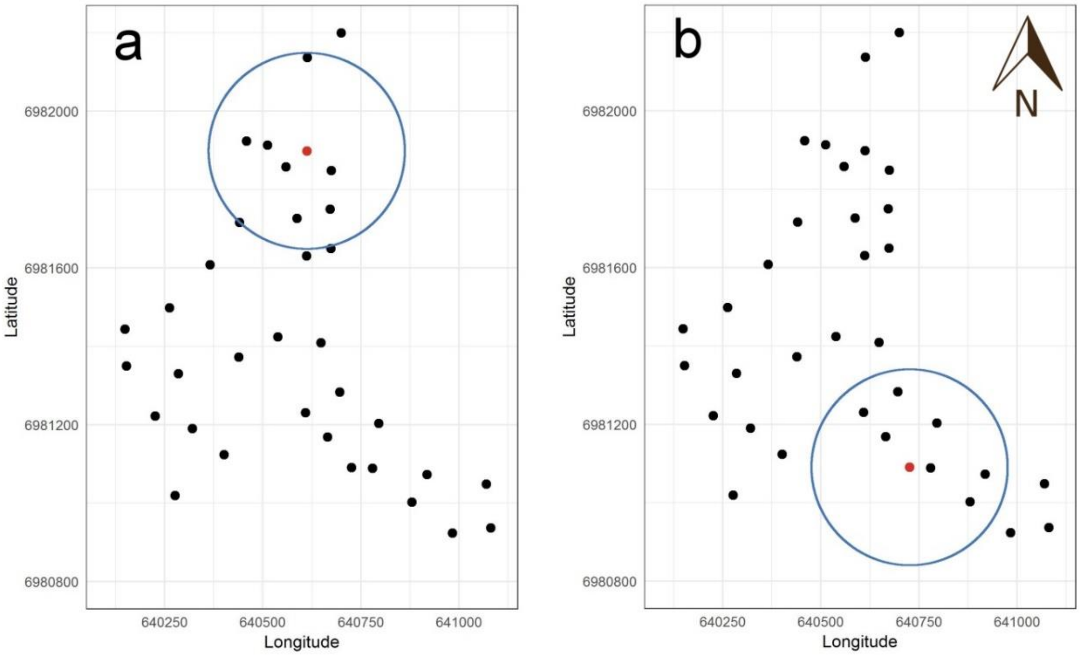

Figure S1. Encounternet receiver array (i.e. basenodes) and detection of forays. Panel **a** and **b** show two simplified maps of the study site with the location of the 35 receivers (i.e. Encounternet basenodes – black and red dots – most of which have been placed in the centre of distinct sparrow-weaver territories; see methods). In each panel, a circle of 250 meters radius around a focal receiver (red dot) is illustrated with a blue line. Movements of a bird from a focal territory on which they were resident (e.g. red dots) were considered to be prospecting forays if the bird visited a territory outside the blue circle and if the foray lasted for longer than 15 seconds.

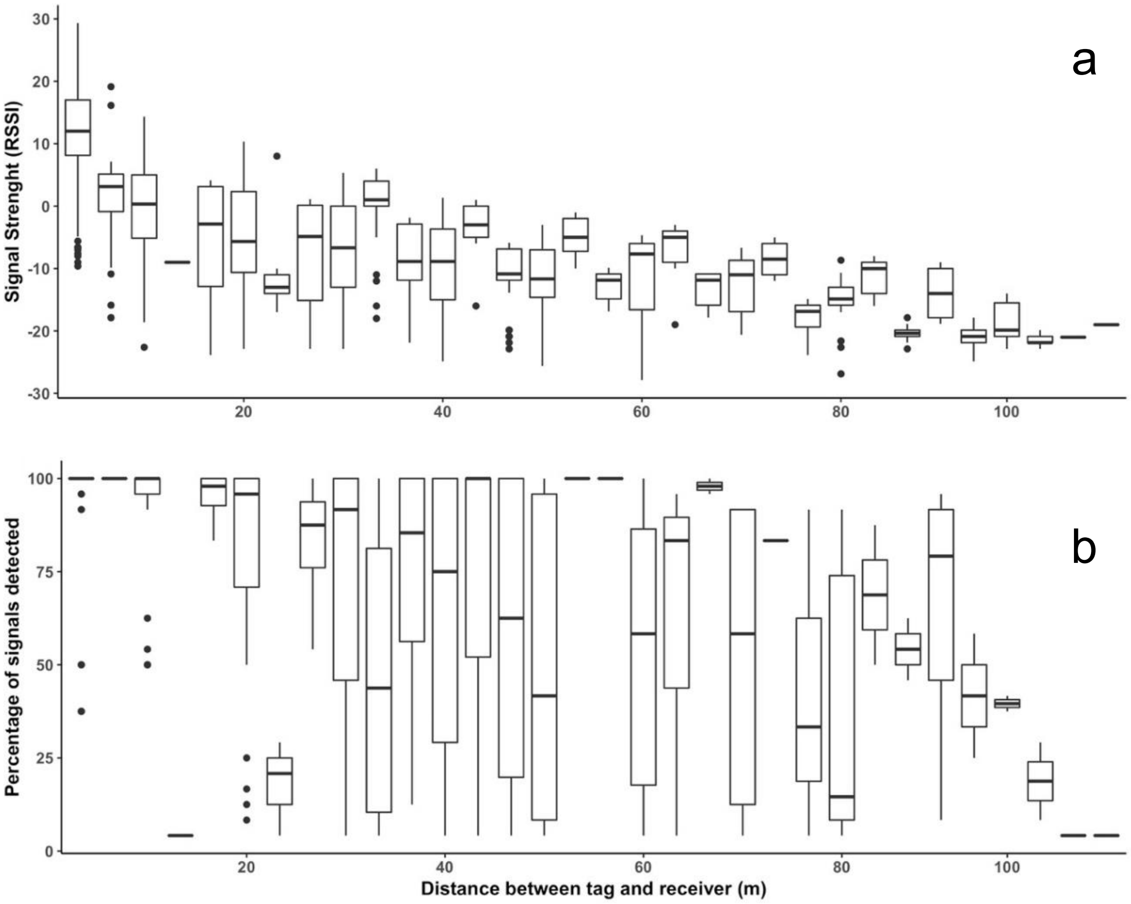

Figure S2. Signal strength (**a**) and percentage of detected signals (**b**) decreased with the distance between tags and receiver. The number of measurements varied between different distances. Tags were detected by receivers up to 120 meters but detectability highly varied for distances larger than 20 meters.

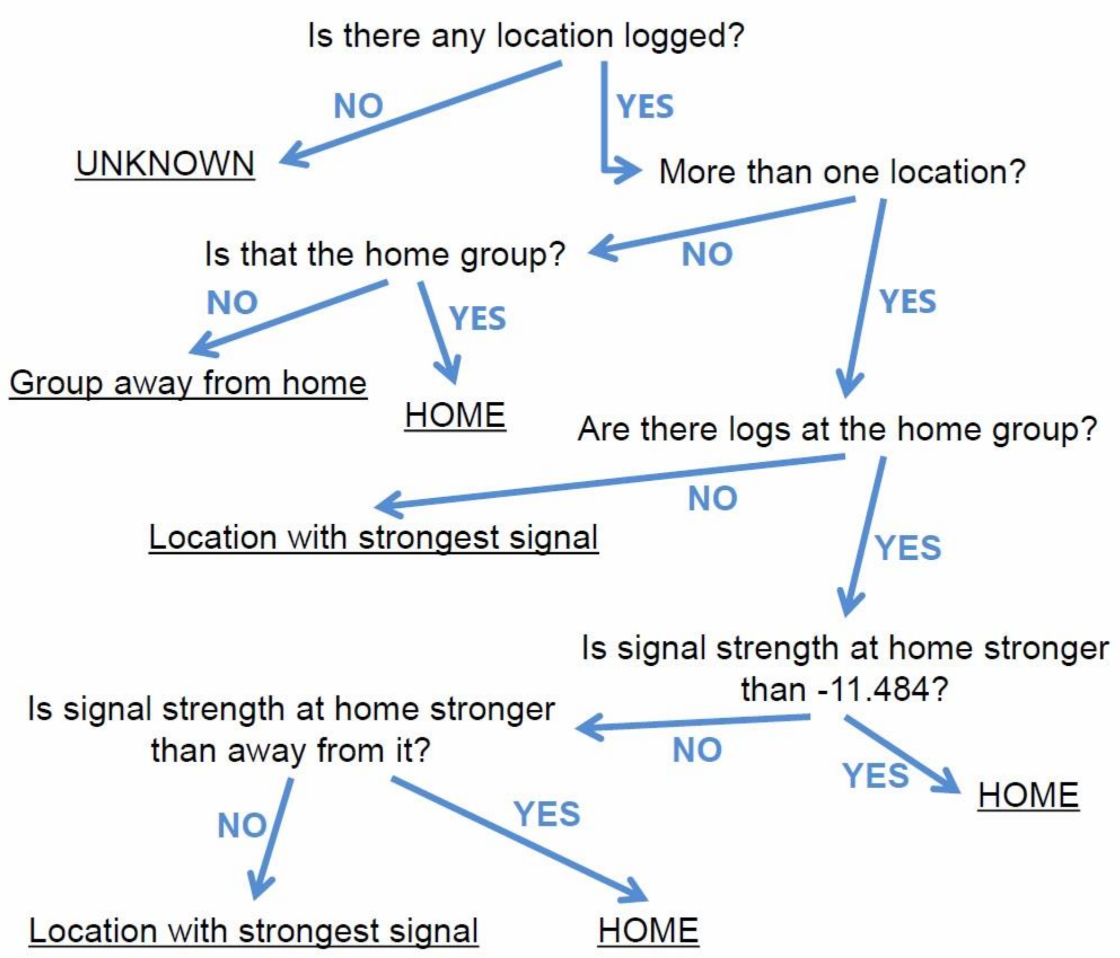

Figure S3. Decision tree applied to assign locations to logs from Encounternet receivers in 15-second moving time windows. A signal strength value of -11.484 refers to the mean signal strength at 50m from a given basenode, based on our field calibrations (see main text).

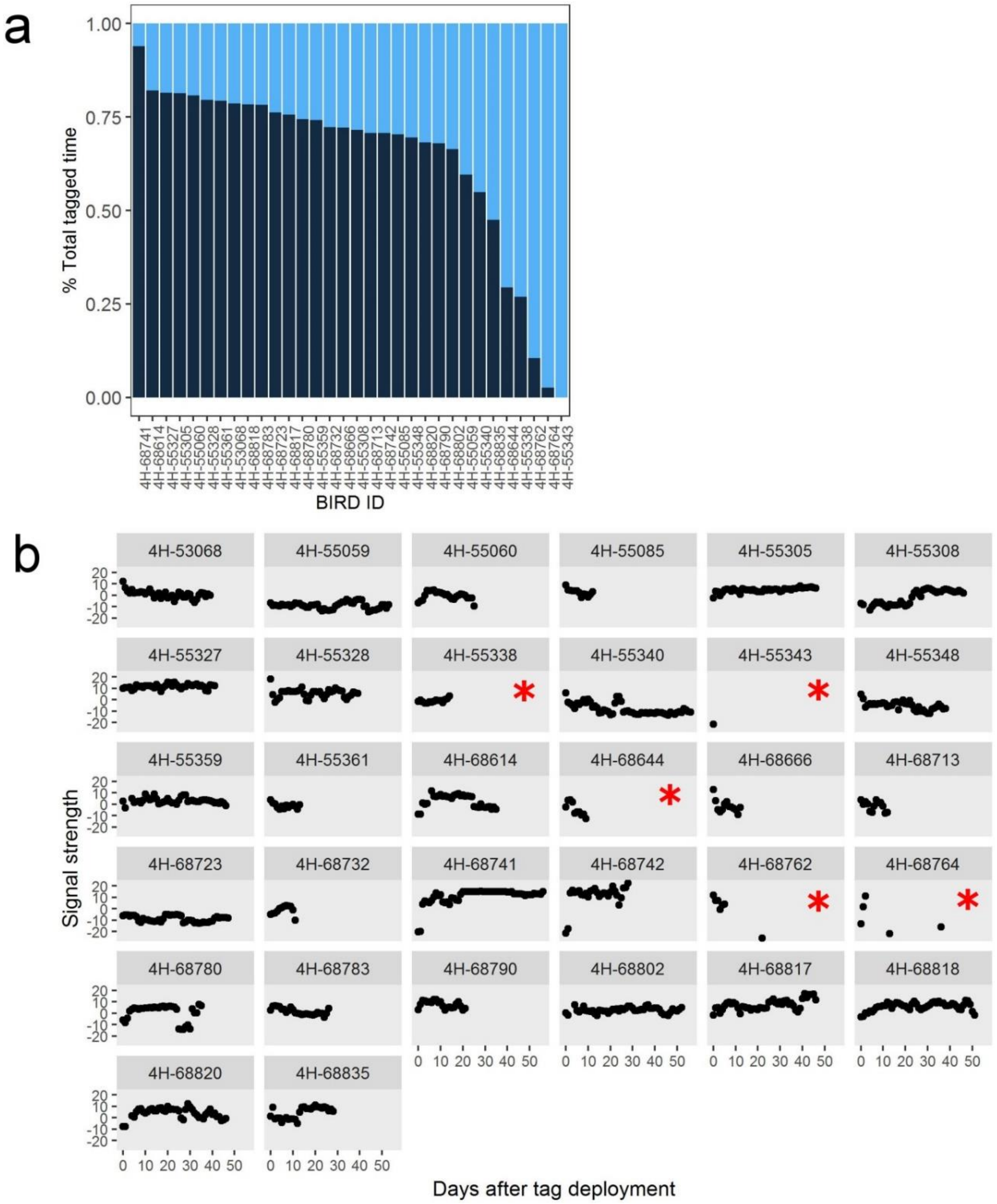

Figure S4. (**a**) Proportion of time that a tagged bird (x axis) was located within the study site (dark blue) out of the total time that the bird was tagged for (dark blue plus light blue). (**b**) Encounternet signal strength (RSSI) at the 'home' territory for all 32 tagged subordinate birds against the number of days after tag deployment. Stars signify the five birds that were removed from the analysis due to low percentage of time located within the study site.

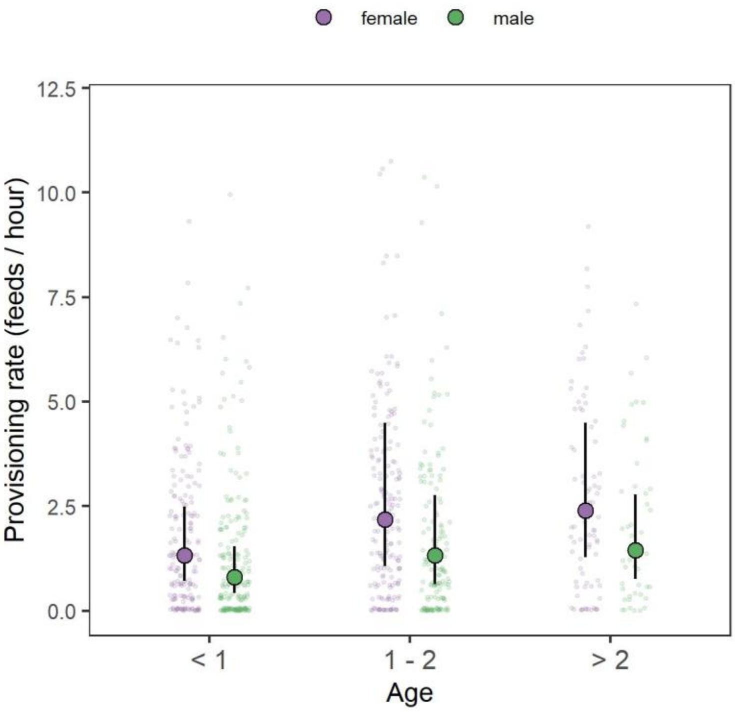

Figure S5. Age effects on natal subordinate cooperative care. Subordinate natal birds increased their provisioning rates with age and subordinate females consistently showed higher provisioning rates than subordinate males. Model predictions based on the top model in Table S2. Transparent points represent raw data whereas larger points and error bars give mean model predictions ± SE.

Supplementary Tables Chapter 4

Table S1. Monthly calendar of morning start times for provisioning data collection. Provisioning data collection started within the placement window specified for every month and lasted for approximately three hours.

Month	Placement window
September	07:00-07:30
October	06:45-07:15
November	06:30-07:00
December	06:15-06:45
January	06:30-07:00
February	06:45-07:15
March	07:00-07:30
April	07:15-07:45

Table S2. Model selection table for the analysis of provisioning rates for natal subordinate individuals. [A] terms absent from the focal model relative to the top model (i.e. that with the lower AIC score); presented for ease of comparison. [B] added variable in comparison with the top model.

Terms in model	**Terms absent[A]**	**AIC**	**ΔAIC**
age + sex + brood age + brood size + intercept		4843.2	0.00
age x sex[B] + age + sex + brood age + brood size + intercept		4843.4	0.24
age + sex + brood size + intercept	brood age	4854.1	10.86
age + sex + brood age + intercept	brood size	4854.5	11.26
sex + brood age + brood size + intercept	age	4857.3	14.14
age + brood age + brood size + intercept	sex	4857.4	14.18
Intercept	age + sex + brood age + brood size	4889.5	46.32

Table S3. Model selection for the analysis of (**a**) provisioning visit duration and (**b**) probability of provisioning a large food item. (**a**) When provisioning offspring, subordinate females spent more time in the nest than subordinate males. Provisioning birds reduced the length of their feeding visits with offspring age. (**b**) For the probability of a large food item being delivered, the intercept only model received the strongest support from the data and was the only model that passed the nesting rule (Richards, 2008). The interaction between sex and age was also considered as a predictor in both the feed duration and food item size models but did not appear in any models within a ΔAIC value of six. Models within a ΔAIC of size are presented. Model coefficients are standardised to ease comparison of effect sizes across models.

(a) Provisioning visit duration (‘log+1’ transformed)

Intercept	Sex ('female' effect)	Brood age	Brood size	Age	k	AIC	ΔAIC
0.050	0.142	-0.087			7	12471.9	0
0.040	0.141	-0.089	-0.053		8	12476.7	4.88

(b) Probability that a provisioning subordinate delivers a large food item

Intercept	Sex ('female' effect)	Brood age	Brood size	Age	k	AIC	ΔAIC
-2.256					5	931.8	0
-2.257		-0.118			6	932.7	0.94
-2.280	0.139				6	932.8	1.01
-2.252			0.022		6	933.8	1.98
-2.280	0.132	-0.112			7	933.8	2.04
-2.257		-0.118	0.002		7	934.7	2.94
-2.275	0.14		0.028		7	934.8	2.98
-2.174				+	7	934.8	2.99
-2.279	0.132	-0.111	0.009		8	935.8	4.04
-2.204	0.129			+	8	935.9	4.15
-2.188		-0.101		+	8	936.0	4.22
-2.172			0.012	+	8	936.8	4.99
-2.216	0.124	-0.098		+	9	937.2	5.43

Table S4. Microsatellite relatedness of subordinate birds, dominant birds and offspring. This table shows the results of linear models explaining variation in microsatellite relatedness (Queller & Goodnight, 1989) between subordinate individuals (males and females) and (**a**) the dominant male, (**b**) the dominant female and (**c**) the offspring that they are helping to rear. In every model 'sex' was included as a fixed effect predictor. For details of microsatellite genotyping, see Harrison *et al.*, 2013a.

		Estimate	**SE**	**t value (df)**	**p**
(a) Relatedness to dominant male (n = 191)					
	Intercept	0.364	0.028		
	Subordinate sex ('males')	-0.011	0.039	-0.285 (189)	0.776
(b) Relatedness to dominant female (n = 184)					
	Intercept	0.464	0.021		
	Subordinate sex ('males')	-0.053	0.029	-1.801 (182)	0.074
(c) Relatedness to offspring (n = 478)					
	Intercept	0.386	0.016		
	Subordinate sex ('males')	-0.030	0.022	-1.33 (476)	0.184

Table S5. Analysis of age-specific dispersal probability from the natal group. [A] Model estimates for the 4-level factor ‘age’ are given as follow: ‘age 2-3y / age 3-4y / age >4y’, with ‘age 1-2y’ being the reference level. k = number of estimated model parameters.

Intercept	**Sex ('male' effect)**	**Age[A]**	**Age[A] x sex ('male' effect)**	**k**	**AIC**	**ΔAIC**
-4.239	0.888	1.651 / 2.382 / 2.549		7	480.7	0
-4.396	1.120	1.601 / 2.812 / 2.668	0.095 / -0.669 / -0.739	10	484.8	4.08
-3.638		1.609 / 2.281 / 2.157		6	489.4	8.70
-2.707	0.6504			4	527.8	47.12
-2.321				3	532.5	51.82

Table S6. Analysis of (**a**) daily rate of prospecting movements, (**b**) (log-transformed) foray duration and (**c**) foray maximum distance. Models within a ΔAIC value of six are shown. k = number of estimated model parameters. Model coefficients are standardised to ease comparison of effect sizes across models.

(a)	intercept	Sex ('female' effect)	Provisioning ('YES' effect)	Sex ('female' effect) x Provisioning ('YES' effect)	Age	k	AIC	ΔAIC
	-0.334	-0.165	0.080			6	2397.0	0
	-0.327	-0.155	-0.074	0.040		7	2398.0	1.06
	-0.299	-0.166				5	2398.2	1.24
	-0.339	-0.148	-0.080		0.055	7	2398.4	1.44
	-0.331	-0.139	-0.075	0.038	0.052	8	2399.5	2.57
	-0.332		-0.079			5	2399.6	2.61
	-0.304	-0.148			0.056	6	2399.6	2.67
	-0.340		-0.079		0.099	6	2400.0	3.09
	-0.296					4	2400.7	3.74
	-0.305				0.100	5	2401.2	4.22

(b)	intercept	Sex ('female' effect)	Provisioning ('YES' effect)	Sex ('female' effect) x Provisioning ('YES' effect)	age	k	AIC	ΔAIC
	0.133					5	2535.5	0
	0.129				-0.033	6	2536.9	1.34
	0.142		0.022			6	2537.2	1.66
	0.132	0.006				6	2537.5	1.96
	0.139		0.022		-0.033	7	2538.6	3.02

	0.129	-0.005			-0.034	7	2538.9	3.33
	0.142	0.005	0.022			7	2539.2	3.64
	0.138	-0.002	0.017	-0.031		8	2540.5	4.92
	0.139	-0.007	0.022		-0.035	8	2540.5	5.00

(c)	**Intercept**	**Sex ('female' effect)**	**Provisioning ('YES' effect)**	**Sex ('female' effect) x Provisioning ('YES' effect)**	**Age**	**k**	**AIC**	**ΔAIC**
	-0.003					5	2501.2	0
	-0.009		-0.013			6	2503.1	1.89
	-0.005				-0.020	6	2503.1	1.89
	-0.002	0.015				6	2503.2	1.95
	-0.011		-0.013		-0.020	7	2505.0	3.78
	-0.008	0.015	-0.013			7	2505.0	3.83
	-0.004	0.009			-0.018	7	2505.1	3.87
	-0.013	0.004	-0.019	-0.037		8	2506.0	4.83
	-0.010	0.009	-0.013		-0.018	8	2507.0	5.76

Table S7. Model selection summary for binomial generalised linear mixed models for the individual probability of weaving. Models within a ΔAIC value of six and passing the nesting rule are shown. We found little support for a statistical sex difference in weaving. The top model contains the ‘sex’ predictor but there is relatively similar support for a second model without this effect and for the intercept-only model. Model coefficients are standardised to ease comparison of effect sizes across models.

Intercept	Sex ('female'effect)	Brood age	k	AIC	ΔAIC
-5.018	0.445	-0.329	7	310.8	0
-6.388		-0.399	6	311.2	0.32
-4.842	0.427		6	311.4	0.53
-5.368			5	311.8	1.00

Chapter 5

Heritable variation in cooperative generosity in a wild social bird

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5.1. Abstract

Consistent individual differences in cooperative behaviour have now been documented in a wide range of organisms, but few studies have investigated the relative importance of genetic and environmental variation in generating these differences. Indeed, given the substantial work to date on the evolution of cooperation, it is notable that we still lack compelling empirical evidence of additive genetic variation in cooperative generosity. Here we investigate the existence and determinants of consistent individual differences in cooperative breeding behaviour (helping to rear the offspring of others) in a wild population of white-browed sparrow-weavers, *Plocepasser mahali*. First, using assessments of helping behaviour by 256 individuals observed provisioning offspring in 160 broods, we find strong evidence of consistent variation among non-breeding helpers in how much food they provide to offspring. Second, using high-resolution genomic information (>38,000 SNPs) and animal models that account for shared environmental effects, we detect moderate additive genetic variation (i.e. heritability) for cooperative provisioning rates ($h^2 = 0.117$). This heritability estimate for cooperative food provisioning by non-breeding helpers is similar in magnitude to the few existing heritability estimates for parental provisioning rates in non-cooperative species. Our estimates of the among-individual and additive genetic variance in helper provisioning rates were similar in magnitude, suggesting that the consistent individual differences detected in cooperative generosity are primarily attributable to additive genetic effects rather than other mechanisms. The fitness consequences of cooperative helping in white-browed sparrow-weaver societies are known to vary with environmental conditions, likely yielding spatial and temporal variation in selection on cooperative generosity. Our findings suggest that the heritable variation required for an evolutionary response to such pressures is present in this species.

5.2. Introduction

In cooperatively breeding species, non-breeding helpers typically vary in their levels of cooperation (Komdeur, 2006). These cooperative individuals can adjust their cooperative behaviour according to changes in their physical (Wiley & Ridley, 2016) and / or social environment (Hatchwell & Russell, 1996; Hatchwell, 1999; Clutton-Brock *et al.*, 2002, 2003; Russell *et al.*, 2003b; Johnstone, 2011; Adams *et al.*, 2015). While many extrinsic factors have the potential to affect individual levels of cooperation, consistent individual differences in cooperative behaviours have been found in a wide number of species (reviewed in Bergmüller *et al.*, 2010). For example, in meerkats (*Suricata suricatta*), subordinate individuals show consistent differences in their cooperative contributions towards babysitting and pup provisioning (English *et al.*, 2010). Similarly, consistent individual differences in babysitting and juvenile care have also been found in banded mongooses (*Mungos mungo*, Sanderson *et al.*, 2015). Moreover, consistent individual differences have also been found in other cooperative activities, such as group hunting (Gazda *et al.*, 2005). Theoretical modelling highlighted the potential adaptive value of consistent individual differences in cooperative activities (Wolf *et al.*, 2007; Biro & Stamps, 2008; McNamara *et al.*, 2009; Dall *et al.*, 2012). The empirical evidence for the existence of consistent individual differences in cooperation and its theoretical adaptive value poses the question of whether this trait is heritable and, therefore, can respond to selection, but we lack compelling empirical evidence of additive genetic variation in cooperative generosity.

Few studies have investigated the genetic basis of cooperative behaviours. Additive genetic variation for the propensity to help at the nest has been quantified in western bluebirds (*Sialia mexicana*, Charmantier *et al.*, 2007) and Tibetan ground tits (*Pseudopodoces humilis*, Wang & Lu, 2018). In both species, whether or not a given individual ever became a helper was found to be heritable (Charmantier *et al.*, 2007; Wang & Lu, 2018). However, estimates of additive genetic variation in Wang and Lu (2018) have been questioned (Engelhardt *et al.*, 2018; Gilbert, 2018) and may indeed have been confounded by the propensity to delay dispersal (as the act of help is dependent on delaying dispersal in Tibetan ground tits and the authors did not uncouple these two processes; Griesser *et al.*, 2017; Gilbert, 2018). In contrast to studies on bird species, a laboratory study on a cooperatively breeding fish found that additive genetic variance in cooperative behaviour was negligible, despite being conducted in controlled laboratory conditions (Kasper *et al.*, 2017a). Indeed, this work led the authors to conclude that heritable genetic variation plays only a minor role in generating the consistent individual differences in cooperative behaviour observed in their study species (Kasper *et al.*, 2017a). Compelling evidence that heritable variation contributes appreciably to inter-individual variation in cooperative generosity is therefore lacking.

Here, we investigate the existence of consistent individual differences in cooperative helping effort (non-breeding helpers feeding the offspring of breeders) and its genetic basis in a cooperatively breeding bird, the white-browed sparrow-weaver (*Plocepasser mahali*; Collias & Collias, 1978; Lewis, 1982a). White-browed sparrow-weavers live in family groups in which a dominant breeding pair monopolises reproduction (Harrison *et al.*, 2013a) and 0-10 non-breeding subordinate birds of both sexes (hereafter termed ‘helpers’) help to feed their young (Lewis, 1982a; Harrison *et al.*, 2013a). Helpers are typically offspring from previous breeding attempts of the dominant pair, having delayed dispersal from their natal group (Harrison *et al.*, 2013a). The presence of female helpers increases the total rate at which offspring are fed, which appears to increase offspring survival in dry conditions (Chapter 2). Furthermore, breeding females appear to respond to the presence of female helpers by increasing their pre-natal investment in the egg and reducing their post-natal investment in nestling feeding (Chapter 3). In this chapter, we use long-term data from a wild population (501 hours of observations of helper provisioning behaviour covering 256 helpers feeding 160 broods in 32 social groups) to investigate whether there is evidence of consistent among-individual differences in cooperative generosity (specifically, the rate at which non-breeding helpers provision young). We then use detailed genomic data to carry out an animal model analysis of 212 helpers and investigate the extent to which the detected consistent individual differences in cooperative generosity are attributable to additive genetic variation in cooperative generosity.

5.3. Methods

5.3.1. Field monitoring

Our study population of white-browed sparrow-weavers is located in Tswalu Kalahari Reserve in the Northern Cape Province of South Africa (27°16’S, 22°25’ E). Data was collected from the field from September to May (the Southern summer, when the birds breed) between 2007 and 2017. During this period, 32 social groups of white-browed sparrow-weavers were monitored. Each group occupied a distinct territory in an area of approximately 1.5 km^2 and groups were easily distinguished in the field as all group members forage together, engage in roost weaving and territorial defence, and roost in a single tree or cluster of trees close to the centre of their territory (Collias & Collias, 1978; Lewis, 1982a). All birds in our population are fitted with a uniquely numbered metal ring and three colour rings for identification (under SAFRING license 1444). Social group composition was assessed at least once a week via behavioural observations during the study period. Individual birds were identified on the basis of their colour ring combination. Group composition was also informed by targeted catching sessions in which birds were caught at night from their roosting chambers.

Every white-browed sparrow-weaver group contains a single dominant (reproductive) female (that lays every egg in the group) and a dominant (reproductive) male (Harrison *et al.*, 2013a). Dominant birds are easily identified in the field because they display a distinct set of behaviours. In every group, the dominant female and male are in very close association, spending much time foraging and performing vocal duets together (Walker *et al.*, 2016). Only dominant females have been observed incubating eggs or entering the nest during the incubation phase. Furthermore, genetic analysis confirmed that, in our study population, nests always contain eggs from a single female and that this individual could be readily identified using behavioural observations (Harrison *et al.*, 2013a). Previous work also detected a low percentage of extra-group paternity (*circa* 15% of broods contained extra-group nestlings (Harrison *et al.*, 2013a) but most broods contained offspring from a single male and this individual, again, could be readily identified as the dominant male from behavioural observations (Harrison *et al.*, 2013a). Non-breeding helpers are typically offspring of the dominant pair that delay dispersal and help feed subsequent broods of the dominant pair (Harrison *et al.*, 2013a). Beak coloration was used to determine the sex of individual birds, as this is sexually distinct in our study subspecies (*P. mahali mahali,* Leitner *et al.*, 2009).

5.3.2. Provisioning behaviour

The protocol to collect provisioning behaviour data has been described elsewhere; for details, see Chapters 2, 3 and 4. In brief, we collected provisioning behaviour data using video recordings at nests between the 6th and 14th day after the first egg of a given clutch hatched (for details about the monitoring of breeding activity, see Chapters 2 and 3). Previous research on this study population has confirmed that individuals visiting the nest during our focal recording periods always deliver a food item to the nestlings, unless they are conspicuously carrying a long piece of grass which they then weave in to the nest structure (Walker, 2015). Such rare grass-delivery events were removed from the provisioning visit data set prior to analysis. From these video recordings, we extracted information for every feeding event: feeder sex (based on beak coloration) and feeder identity (using a unique vent pattern placed on the birds during the incubation phase; Walker, 2015). Video cameras were set up in the morning at standard times that tracked monthly changes in sunrise (Table S1). Provisioning behaviour was recorded for approximately three hours for each brood and day. Using this data stream, we calculated the number of feeds delivered by every subordinate individual during every day of recording. In a small proportion of feeding events, individual birds could not be identified, adding uncertainty to the number of feeds delivered by each individual. We thus cleaned the original data set to retain only those days of observation for a focal helper where there were less than five feeds of uncertainty in the total number of feeds carried out by the focal individual per day of recording. As environmental conditions were likely correlated between consecutive provisioning days for a given brood (which could artefactually generate data suggestive of consistent individual differences in cooperative behaviour), we calculated the mean number of feeds delivered per individual, per brood (i.e. averaging over successive days of video recording). In our analysis of provisioning rates, we only include data for natal subordinate individuals, who are most often highly related to the dominant pair and their offspring (Chapter 4).

5.3.3. Restriction site associated DNA (RAD) sequencing

Animal models estimate the amount of phenotypic variation explained by the heritable genetic differences between individuals (Kruuk, 2004). Traditionally, animal models include a pedigree from which to estimate the genetic relatedness (i.e. similarity) between individuals (Kruuk, 2004; Wilson *et al.*, 2010). However, animal models can also be fitted based on genomic relatedness data (Yang *et al.*, 2010). We therefore used restriction site associated DNA (RAD) sequencing to genotype individuals, calculate their genomic relatedness and build a genetic relationship matrix (GRM, Yang *et al.*, 2010). We then used this GRM to estimate additive genetic variance of provisioning rate among non-breeding helping individuals.

Between 2007 and 2017, blood samples from white-browed sparrow-weavers were routinely collected (stored > 96% ethanol; Walker, 2015; Wood, 2017). DNA extracted from these blood samples was used to carry out single-end RAD sequencing following the original protocol (Baird *et al.*, 2008), using SbfI as the restriction enzyme. DNA was extracted using a DNAeasy Blood & Tissue QIAGEN kit. DNA concentrations were measured using a Qubit dsDNA HS Kit (ThermoFisher) and DNA fragmentation was assessed by electrophoresis on a 1.0% agarose gel. Library preparation and RAD Sequencing was carried out by Floragenex Inc. on an Ilumina HiSeq instrument. DNA concentrations and quality passed Floragenex criteria (> 50 µg of high molecular weight DNA at a concentration of 20 ng/µl) for optimal sequencing results. We carried out two RAD sequencing runs to sequence a total of 238 DNA samples from 212 individual white-browed sparrow-weavers. This set of 212 individuals contained every breeding bird in our study population between 2007 and 2016. Library preparation and sequencing was performed independently for these two runs. In the first run, 48 samples were prepared together, barcoded and sequenced on a single HiSeq lane. They included single samples from 38 individuals and five pairs of replicate samples (these replicates consisted of three replicates coming from different blood samples of the same bird and two replicates representing DNA from the same blood sample extracted at different times). The second run contained 190 samples, each from a different individual, which were prepared across two 95 sample libraries. Each library was then sequenced across two HiSeq lanes. This run contained 21 samples (each from a different individual) that also appeared in the first run, to facilitate the resolution of any among-run differences in sequencing performance should they arise (these 21 replicated samples originated from two independent DNA extractions of the same blood sample).

5.3.4. Bioinformatics processing of RAD sequencing data

We used Stacks 2.4 (Catchen *et al.*, 2013) to recover genotypes from the RAD sequencing data. First, we de-multiplexed raw data using *process_radtags*, including the –*c* and –*q* flags to filter out low quality reads. For each of the three DNA libraries (one library for the first run and two for the second run), 90.6% (358,044,906 reads), 75.5% (295,884,741 reads) and 71.7% (240,934,241 reads) of the total raw reads were retained and assigned to individual barcodes by *process_radtags*. Data from the three libraries were analysed together and we assessed the consistency of the results of the Stacks pipeline (number of RAD tags assembled, number of polymorphic RAD tags and number of single nucleotide polymorphisms) to varying its main parameters: *–m*, *–M* and *–bound_high* in *ustacks*, and *–n* in *cstacks*. For this first assessment we closely followed published guidelines for the analysis of RAD sequencing data (Mastretta-Yanes *et al.*, 2015; Paris *et al.*, 2016) (Figure S1 & Figure S2).

We performed this initial assessment to understand how changing the values of the main parameters in the Stacks pipeline affected the recovery of RAD tags, polymorphic tags and SNPs following Paris et al. (2016). We ran the *de novo* script in Stacks (version 2.41; Catchen *et al.*, 2011) varying the values of *–m* and *–M* (from 2 to 9 in both cases) in *ustacks*, *--bound_high* also in *ustacks* (testing values: 0.001, 0.01, 0.05, 0.10, 0.15, 0.25, 0.50, 0.75) and *–n* in *cstacks* (from 0 to 9). Each parameter was tested independently from the others and when a given parameter was tested, the other parameters were kept at fixed values as follows: *–m* = 3, *–M* = 2, --*bound_high* = 0.05 and *–n* = 2. As expected given previous assessments of Stacks (Mastretta-Yanes *et al.*, 2015; Paris *et al.*, 2016), we found a decreasing number of total RAD tags built, polymorphic tags and SNPs recovered with increasing values of *–m* (Figure S1). Changing values of *–M* and *–n* had little effect on the performance of the pipeline (Figure S1 & Figure S2). Low values of *–bound_high* caused the pipeline to recover more polymorphic tags and SNPs; after a value of 0.05 the number of RAD tags, polymorphic tags and SNPs levelled off (Figure S2). Given this initial assessment, we chose *–m* = 3, *–M* = 2, *–bound_high* = 0.05 and *–n* = 2 as parameter values to carry on the analysis. Based on this initial assessment, we chose values for Stacks parameters that yielded consistent results as advised by Paris *et al.* (2016): *–m* = 3, *–M* = 2, *–bound_high* = 0.05 and *–n* = 2 (Figure S1 & Figure S2).

Second, given the potential for differences in sequencing performance across runs to generate noise in our relatedness estimates, we sought to identify SNP filtering parameters that maximised the congruence of the outputs from the two runs for the 21 replicate samples. We thus used the 21 replicated samples (i.e. that were sequenced in both the first and second runs) to assess the effect of different SNP filtering strategies on the within-replicate similarity for several genome-wide metrics: differences in genome-wide heterozygosity, percentage of mismatching genotypes and number of shared loci. We ran the *populations* function in Stacks setting –R to 0.8, *--min-maf* to 0.05 and selecting loci only if they had been built in both runs and in more than 80% of samples within each run. Then, we filtered genotypes based on different minimum read depths and calculated, for each replicate pair separately, the number of loci shared across the replicate pair, the heterozygosity for each member of the replicate pair and the proportion of mismatching genotypes across the replicate pair (Figure S3). We recovered highly consistent metrics for these 21 replicates across the two runs when genotype read depth was set to be equal to or higher than 40x (Figure S3). We therefore applied this genotype read depth threshold to the full data set of 212 samples to generate a table of high quality SNPs per sample (Table S1). The final parameter values used in Stacks to produce genotypes can be found in Table S1. We genotyped 63,815 SNPs at 39,668 variant loci with, on average, 90.71% of the 212 samples genotyped per locus. Filtering to retain genotypes with read depths > 40x, as well as those loci in Hardy-Weinberg equilibrium (Exact Test, $p < 0.01$; Wigginton *et al.*, 2005), yielded a final data set of 60,422 SNPs at 38,166 variant loci, 34.58% of samples were genotyped per locus. This data set was then used to calculate the genetic relationship matrix (GRM) following the method described in (Yang *et al.*, 2010) and implemented in PLINK 1.9 (F3, Chang *et al.*, 2015) and GCTA (Yang *et al.*, 2011).

5.3.5. Creating the Genetic Relationship Matrix (GRM)

In animal models, additive genetic variation for a trait of interest can be estimated using pedigree-based assignments of parental links (established via social or genetic information; Kruuk, 2004; Wilson *et al.*, 2010) or directly with high quality genomic data (Yang *et al.*, 2010). Here we used a combination of both given (i) the availability of high accurate genomic data for all breeders in our study population and (ii) the ability to extend the GRM to include most of the helpers in our behavioural data set that did not themselves become breeders (as most of these helpers are known to be the offspring of genotyped breeders (see below for details).

We directly calculated the genetic relationship matrix (GRM) based on 38,166 variant loci recovered from RAD sequencing (see methods above) for 212 individuals. Out of these 212 individuals, 94 individuals appeared in the data set for the analysis of provisioning effort. A further 162 individuals appearing in the data set for provisioning behaviour were not sequenced and, therefore, they did not initially appear in the GRM. However, we were able to show that using parental links between dominant (breeding) individuals and helpers (based on microsatellite markers), we could include most of these non-sequenced birds in the relatedness matrix (118 out of 162 individuals; Figure S5).

Every reproductive female and male between 2007 and 2016 were RAD sequenced. Additionally, most individuals (regardless of whether or not they ever reproduced) in the study population were genotyped using 13 microsatellite loci (for details see

Harrison *et al.*, 2013a). Therefore, we use microsatellite data to assess genetic paternity and maternity for individuals that had not been RAD sequenced (i.e. 'the focal bird'). We assumed that the dominant female and male in a given group were the genetic parents of a focal individual when the parental genotypes could generate the focal individual's genotype with fewer than two mismatching loci. Previous analyses in our study population have revealed that dominant females are always the genetic mothers of nestlings hatched in their territory, and extra-group paternity is rare (*circa* 15% of broods contain extra-group sires, Harrison *et al.*, 2013a). Once parental links were established (which was done successfully for 118 out of 162 individuals that appeared in our data set for provisioning effort but had not been directly RAD sequenced), we included focal individuals in the genetic relationship matrix (GRM) by adding half of the relatedness of their father and mother to every individual already present in the GRM. Using a subset of individuals that had been RAD sequenced and whose parents were also present in the GRM, we were able to validate this method to incorporate new individuals in our GRM (Figure S5). Thus, in total, our GRM contained 212 individuals for which data on provisioning effort was available.

5.3.6. Statistical analysis

In order to investigate the existence of among-individual variation in cooperative generosity (and subsequently evidence of additive genetic variation in this trait), we built a series of Bayesian Poisson generalised linear mixed models (with a 'log' link function), using the R package 'MCMCglmm' (version 2.29) in R version 3.4.1. (Hadfield, 2010; R Core Team, 2019). Evidence for consistent individual differences in cooperation can be assessed by calculating the repeatability of this behaviour (i.e. the proportion of the phenotypic variation explained by among-individual variation).

To calculate repeatability in generalised linear models, model estimates in the link scale need to be back transformed to the original data scale. We performed this back transformation using the R package 'QGglmm' (de Villemereuil *et al.*, 2016) and thus calculated among-individual repeatability. A similar problem applies when calculating heritability for non-Gaussian traits (de Villemereuil *et al.*, 2016); therefore, we used 'QGglmm' to convert our estimates of additive genetic variance from the link scale to the observed data scale before calculating the proportion of phenotypic variance explained by additive genetic variation (i.e. heritability, h^2). Transformations from the link scale to the original data scale were performed by averaging across fixed effects (de Villemereuil *et al.*, 2016).

A detailed study of individual contributions to cooperative care in white-browed sparrow-weavers (Chapter 4) revealed that age (as a 3-level factor: '< 1 year', 'between 1-2 years' or '> 2 years'), brood size (as a continuous predictor, mean-centered and scaled to one standard deviation; Gelman & Hill, 2007) and sex were important predictors of individual provisioning rates. Therefore, we also include them here as fixed effects in our models. In order to model provisioning rate within a Poisson framework (i.e. number of observed feeds corrected for the duration of the observation period), the duration of the provisioning observation period was fitted as a fixed effect in our models with a fixed slope of one. Investigating the effects of these fixed effect predictors was not the focus of this study (see Chapter 5 for full details) but we included them in our mixed models to control for other sources of phenotypic variation in helper provisioning rates. We interpreted median values of posterior distributions of model coefficients as their best estimates and assessed their statistical importance by inspecting their 95% credible intervals. Default priors were used for fixed effects (Normal distribution, $\mu = 0$, $\sigma^2 = 10^8$) and residual variances (inverse Wishart, V = 1, nu = 1). Parameter expanded priors were used for random variances with V = 1, nu = 1, $\mu_\alpha = 0$ and $\sigma^2_\alpha = 10^3$.

5.3.6.1. Assessing evidence for among-individual variation in helping effort

Using repeated behavioural observations of the provisioning effort of helpers, we first investigated the existence of consistent among-individual variation in this trait. We fitted a Bayesian Poisson generalised linear mixed model to partition phenotypic variation in provisioning rate into among individual, among brood, among territory and residual variation (Expression 1):

$$V_P = V_I + V_B + V_T + V_R$$

Expression 1. Consistent among-individual variation in helper provisioning rates. Partitioning of phenotypic variation (V_P – conditional upon fixed effects structure) in the number of feeds delivered by helpers between among individual variation (V_I), among brood variation (V_B; i.e. this random effect refers to the identity of the brood that receives cooperative care), among territory variation (V_T) and residual variation (V_R).

The model was fitted three times, running three independent Markov chain Monte Carlo (MCMC) and assessing their convergence to similar results (visually and computing the Gelman and Rubin's convergence diagnostic; Gelman & Hill, 2007). Each chain was run for a total of 5,000,000 iterations with a burn-in of 1,000,000 iterations. Chains were sampled every 1,000 iterations, yielding a total of 4,000 sampled iterations used to estimate posterior probabilities.

5.3.6.2. Additive genetic variation in helping effort

We expanded the model above (Expression 1) to disentangle additive genetic (V_A) and non-additive-genetic (i.e. permanent individual environment, V_{PE}) sources of among-individual variation in helper provisioning rates (Expression 2) using an (generalised) animal model.

$$V_P \ = \ V_A + V_{PE} + V_B + V_T + V_R$$

Expression 2. Animal model to explain individual variation in repeated measures of helper provisioning rates. Partitioning of phenotypic variation (V_P – conditional upon fixed effects structure) in the number of feeds delivered by helpers between additive genetic variation (V_A), permanent environment variation (V_{PE}), among brood variation (V_B; i.e. this random effect refers to the identity of the brood that receives cooperative care), among territory variation (V_T) and residual variation (V_R).

However, fitting a generalised animal model to our data following Expression 2 revealed analytical problems disentangling V_A and V_{PE} (Figure S4). We therefore calculated an overall mean number of feeds (rounded to the closest integer) and mean duration of provisioning watches for every individual in the data set (by averaging across all of the broods for which it was assessed) and then fitted a generalised animal model without repeated measures per individual, partitioning variation in provisioning rates as:

$$V_P \ = \ V_A + V_T + V_R$$

Expression 3. Animal model to explain individual variation in mean helper provisioning rates. Partitioning of phenotypic variation (V_P – conditional upon fixed effects structure) in the number of feeds delivered by helpers between additive genetic variation (V_A), among territory variation (V_T) and residual variation (V_R). For this model, we used averaged provisioning rates per individual, ending up with one single observation per individual.

This model structure resulted in appropriate MCMC convergence with posterior distributions for model coefficients not bounded near zero and approximately distributed following a Gaussian curve. Fixed effect predictors for the latter model were sex, and averaged age and brood size across all of the provisioning events for each individual. Following the model description above, the GRM was employed to fit a generalised animal model using the R package 'MCMCglmm' (Hadfield, 2010). The GRM was inversed in R using *ginv()* ('MASS' R Package) and we computed its nearest positive definite matrix using the *nearPD()* function ('nadiv' R Package; Wolak, 2012). Then, the inversed GRM was included in a Poisson mixed model to capture additive genetic variation in provisioning rates of helping individuals. This model was fitted three times, running three independent MCMCs and assessing their convergence to similar results. Each chain was run for a total of 1,750,000 iterations with 50,000 initial burn-in iterations. Chains were sampled every 1,000 iterations, yielding a total of 1,700 sampled iterations used to estimate posterior probabilities.

5.4. Results

5.4.1. Among-individual variation in helping effort

Among-individual variation in provisioning effort by individual helpers was calculated using a data set containing 5,742 feeding events by 256 individuals (134 females and 122 males) at 160 broods (mean [range] of 1.7 [1-7] broods observed per helper; 117 helpers observed feeding one brood, 63 feeding two broods and 32 feeding three or more broods), across 32 territories (i.e. social groups).

We found significant among-individual variation in provisioning effort, with a non-zero estimate for V_I on the observed data scale (V_I = 11.11, 95% confidence interval (CI) = 3.39 - 29.24; Figure 1a; Expression 1). Variation among individuals in provisioning effort accounted for 9.45% of the observed phenotypic variation in this trait conditional on fixed effects (95%CI = 3.09% - 18.40%; Figure 1b). We also found non-zero among-brood variation (Figure 1). In contrast, our models revealed among-territory variation close to zero (Figure 1; variation among territories was likely captured by among-brood variation). As expected given previous studies (Chapter 4), we found important effects of brood size, sex of the individual and age on provisioning rates (Table 1). Individual variation in provisioning rates was similar for male and female helpers (see Table S2 for details).

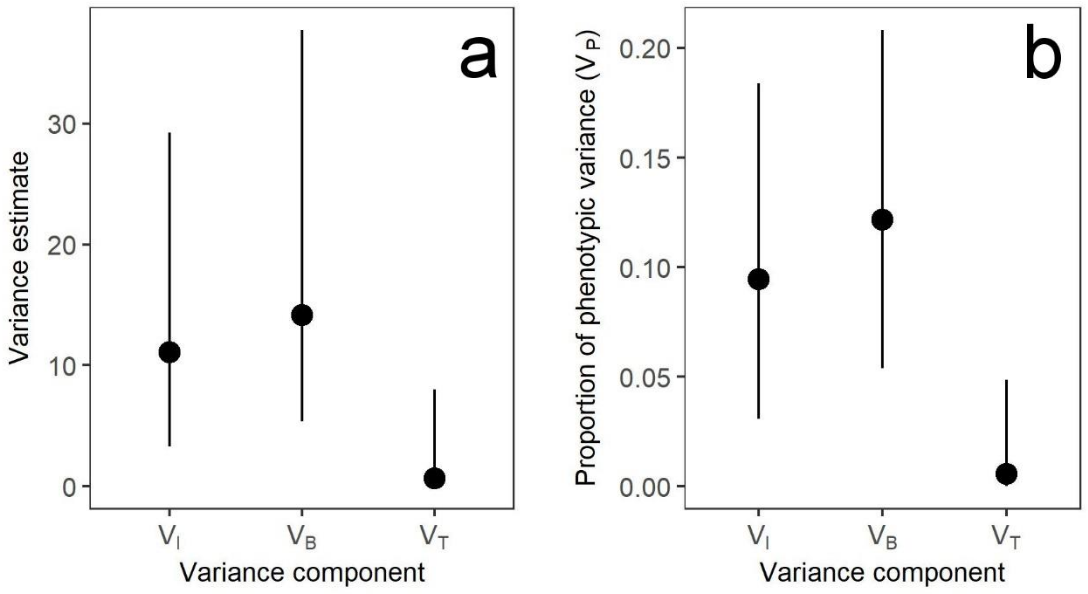

Figure 1. **Partitioning of phenotypic variation in helper provisioning rates** (see Expression 1). (**a**) Combined posterior medians and 95% credible intervals ('CI') of three independent MCMCs for each variance component in the observed data scale. Phenotypic variation conditional on fixed effects, $V_P = 116.74$; residual variation, $V_R = 90.82$. (**b**) Proportion of the phenotypic variance explained by each variance component (median and 95%CI) in the observed data scale. Residual variance accounted for 77.80% of the phenotypic variance conditional on fixed effects. Variance estimates are shown on the observed data scale (i.e. back transforming from the latent scale of the data – a 'log' scale in the case of the employed Poisson GLMMs).

Table 1. Results of a generalised (Poisson) mixed model explaining variation in helper provisioning rates. Model estimates are shown in the link scale ('log'). Phenotypic variance conditional on the fixed effect structure was partitioned as detailed in Expression 1. Posterior median and 95% credible intervals ('CI') of three independent MCMCs are displayed.

Fixed effects	Median	95%CI	
Intercept	-0.127	-0.402	0.131
Brood size	0.279	0.132	0.428
Sex('male' effect)	-0.519	-0.778	-0.265
Age (1 - 2y)	0.385	0.109	0.665
Age (> 2y)	0.447	0.119	0.776

Variance components	Median	95%CI	
Among individual (V_I)	0.302	0.108	0.551
Among brood (V_B)	0.370	0.179	0.641
Among territory (V_T)	0.021	0.000	0.188
Residuals (V_R)	0.567	0.362	0.850

5.4.2. Additive genetic variation in helping effort

The additive genetic variance in mean provisioning effort by individual helpers was calculated using a data set containing 355 feeding events by 212 individuals (116 females and 96 males) helping to feed 146 broods (mean [range] of 1.68 [1-7] broods observed per helper). The sample size for this analysis differs from the previous one as we are now only including individuals that could be connected in the genetic relationship matrix (GRM).

The estimate of additive genetic variance in helper feeding effort (in the observed data scale) was distinct from zero (V_A = 6.73; 95%CI = 1.73 – 28.38; Figure 2a). The narrow-sense heritability on the observed data scale was estimated as 0.117 (95%CI = 0.046 - 0.234; Figure 2b). Among-territory variation also explained a non-negligible proportion of the variance in helper provisioning rates ($\frac{V_T}{V_P}$ = 8.30%; 95%CI = 3.58% – 17.55%; Figure 2b). The estimates for residual variation and fixed effects in the animal model were similar to those of the individual-level model (Table 2).

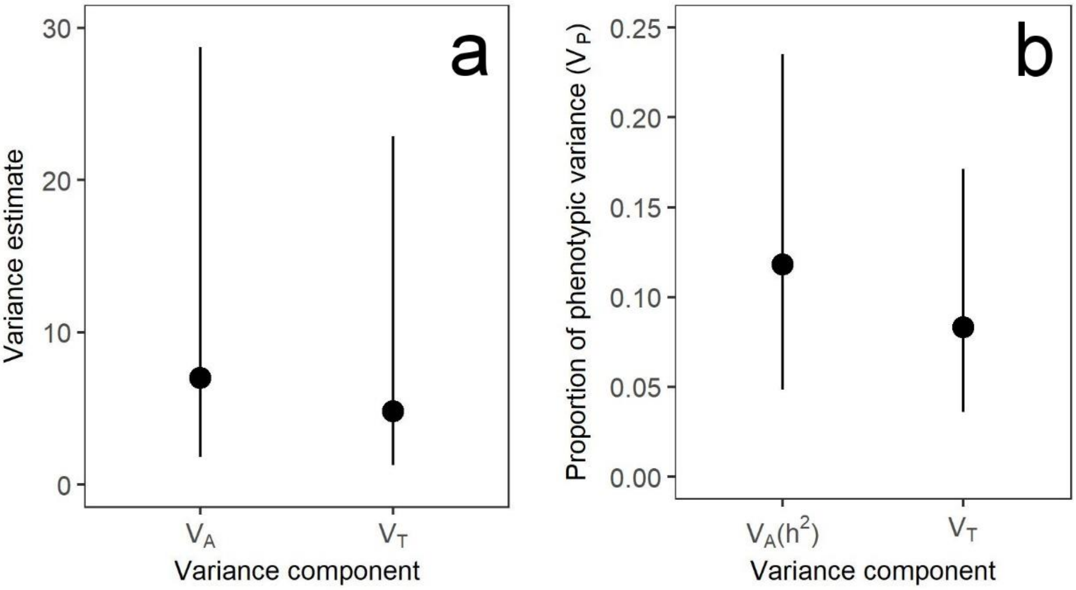

Figure 2. Partitioning of phenotypic variance in helper provisioning rates, including additive genetic variance (see Expression 3). (**a**) Combined posterior medians and 95% credible intervals ('CI') of three independent MCMCs for each variance component. Phenotypic variation dependent on fixed effects, V_P = 58.78; residual variation, V_R = 46.95. (**b**) Proportion of the phenotypic variance explained by each variance component (median and 95%CI). Residual variance accounted for 79.88% of the total phenotypic variance. As provisioning rates were averaged per individual, this second model could not estimate among-brood variation (V_B) in helper provisioning rates. Variance estimates are shown in the observed data scale (i.e. back transforming from the latent scale of the data – a 'log' scale in the case of the employed Poisson GLMMs).

Table 2. Results of a generalised animal model explaining variation in helper provisioning rates. Model estimates are shown in the link scale (‘log’). Phenotypic variance conditional on the fixed effect structure was partitioned as detailed in Expression 3. Posterior median and 95% credible intervals (‘CI’) of three independent MCMCs are displayed.

Fixed effects	Median	95%CI	
Intercept	-0.358	-0.997	0.263
Brood size	0.268	0.120	0.416
Sex ('male' effect)	-0.433	-0.716	-0.149
Age	0.336	0.336	0.099

Variance components	Median	95%CI	
Additive genetic (V_A)	0.247	0.101	0.567
Among territory (V_T)	0.161	0.074	0.373
Residuals (V_R)	0.439	0.439	0.211

5.5. Discussion

We combined behavioural observations and genomic information from a long-term study of a cooperatively breeding bird species to investigate the existence of consistent among-individual differences in cooperative generosity and whether they are explained by heritable variation. Our results provide strong evidence for the existence of consistent among-individual variation in cooperative generosity, measured as the rates at which helpers provision the offspring of breeders, which translated into an adjusted repeatability of ~ 0.10 on the observed scale. Despite the evidence suggesting the existence of consistent individual differences in cooperative behaviours (Bergmüller *et al.*, 2010), only a few studies have attempted to identify the relative importance of genetic factors generating such differences. Here we report unique evidence from a wild animal population with appreciable additive genetic variation in the cooperative provisioning rates of helpers. We discuss these findings and their implications for the evolution of cooperation in more detail below.

Among-individual differences in cooperative behaviour could potentially arise from territory- or brood-level differences in environmental conditions that themselves affect cooperative behaviour. However, these confounds are unlikely to explain our results. First, consistent among-brood variation cannot explain our results of consistent individual differences simply because our analyses did not contain repeated individual provisioning rates within broods. Second, our models estimated consistent individual differences in provisioning effort after controlling for among-territory variation in provisioning effort, accounting for correlated environmental conditions among territories. Additionally, correlated environmental conditions throughout the life of individual helpers could also have generated the apparent consistent among-individual differences in helper provisioning effort observed here. The likelihood of the this explanation generating consistent among-individual differences will decline as within-individual observations span longer periods. Two sources of evidence suggest that this latter explanation does not confound our results of consistent individual differences in cooperation. First, environmental conditions in our study site are temporally highly variable (Chapter 2) and are very likely to differ between repeated individual provisioning events. Second, a complementary analysis in this species has revealed a positive correlation between the provisioning rates of individuals when they were helpers and when they were breeders (Capilla-Lasheras & Young, data unpublished), events which are often separated by multiple years and typically occur on different territories (Harrison *et al.*, 2013a, 2014).

Overall, it would seem that consistent differences in environmental conditions among territories or breeding attempts, or environmental correlations throughout individuals' lives, cannot readily explain our finding of consistent among-individual differences in cooperative provisioning effort.

Consistent individual differences in cooperative behaviours could also be generated through formative developmental responses to early-life environmental conditions (English *et al.*, 2015; Taborsky, 2017). For example, enhanced social conditions during early life have been found to have positive transgenerational effects on parental care, social interactions and brain development in rodents and fish (Curley *et al.*, 2009; Fischer *et al.*, 2015). Early life conditions have also been found to impact the likelihood of becoming a helper in banded mongooses: pups that received more help during early life had a lower probability of becoming helpers than breeders (Vitikainen *et al.*, 2019). Similarly, the physiological state of breeding females in meerkats has been suggested to have transgenerational effects on the cooperative behaviours of offspring (Dantzer *et al.*, 2019). It is possible that formative early-life environmental effects on cooperative behaviour contribute to the consistent among-individual variation in helper provisioning rates observed here. However, it is worth noting that the magnitude of the additive genetic variance estimated in the animal model (Figure 2) was similar to the amount of among-individual variance in provisioning rates calculated by our initial (non-genetic) model (Figure 1). Although comparison across these two models may be only approximate, this indicates that environmental sources of variation may contribute little to differences in individual provisioning rates.

Individuals may show consistency in their provisioning behaviour if genetic variation underpins the expression of the trait (Kasper *et al.*, 2017b). Here, integration of genomic data and behavioural observations in an animal model (Kruuk, 2004; Wilson *et al.*, 2010), indeed, provides evidence for additive genetic variation in helper provisioning behaviour in white-browed sparrow-weavers. We estimated narrow-sense heritability for helper provisioning effort to be 0.117 (95%CI = [0.046 - 0.234]), a value in the low range for behavioural traits as suggested by a recent meta-analysis (Dochtermann *et al.*, 2019). Indeed, our heritability estimation for helper provisioning rate is much lower than the previously reported result for the heritability of the propensity to help in western Bluebirds (*h^2* ± standard error =0.76 ± 0.25: *Sialia mexicana*, Charmantier *et al.*, 2007) and Tibetan ground tits (*h^2* = 0.47, 95%CI = 0.16 – 0.75: *Pseudopodoces humilis*, Wang and Lu 2018). This contrast may be due to a difference in the nature of the traits under investigation. Charmantier *et al.,* (2007) and Wang and Lu (2018) analysed helping as a binary trait, thereby, investigating the probability of helping, whereas our analysis considers continuous variation in helper provisioning rates. Furthermore, Wang and Lu (2018) could not separate helping from delayed dispersal and, therefore, their heritability estimate may indicate heritable variation in delaying dispersal rather than in helping *per se* (Gilbert, 2018). Despite the difference in heritability estimates across species, heritability values can be difficult to compare and interpret across studies given their dependency on the fixed effects used to control for additional sources of variation (Wilson, 2018). Interestingly, one other study that analysed provisioning rates of breeders (not helpers) in a cooperatively breeding bird found a similar proportion of the total phenotypic variation explained by additive genetic effects (approximately 10%; Adams *et al.*, 2015). The extent to which feeding behaviour in breeders and helpers is determined by similar genetic components is unknown to date and deserves attention in its own right.

Besides additive genetic effects, permanent environmental effects can generate consistent individual differences (Kasper *et al.*, 2017b; a). Under certain scenarios, early-life environmental effects shared among individuals can bias heritability estimations upwards (Kruuk & Hadfield, 2007). This problem is expected to be of particular importance when lineages always occupy the same environments (e.g. territories) such that additive genetic effects and environmental effects are strongly confounded. In our study system, individuals express their cooperative behaviour in the territory where they hatched (as we are analysing the provisioning rate of *natal* individuals). However, inheritance of the breeding position in a given territory to a natal individual is rare in both sexes in white-browed sparrow-weavers (Chapter 4). Helpers need to disperse from the natal territory to become dominant individuals and breed, eventually producing helpers in a new territory. This process ensures that close relatives are observed in multiple territories, aiding analytical disentanglement of additive genetic and environmental effects.

In animal societies, helpers often respond to the presence of other helpers by modulating their provisioning effort (Zöttl *et al.*, 2013a; Brouwer *et al.*, 2014; but see also Liebl *et al.*, 2016). Given that provisioning is heritable, an individual's behavioural phenotype may thus be influenced by genes expressed in other group members (i.e. subject to indirect genetic effects, 'IGEs', Wolf *et al.*, 1998; Wilson *et al.*, 2009, 2011; Bailey *et al.*, 2018). IGEs have been shown to be important in determining the evolutionary potential of populations (Wilson, 2014; Bailey *et al.*, 2018), particularly in situations where inter-individual interactions have antagonistic effects on phenotypic expression (Bijma, 2010). Preliminary analyses suggest that the provisioning effort of helpers does not change with the presence of other helpers in white-browed sparrow-weavers (Capilla-Lasheras & Young unpublished data); however, the extent to which helper provisioning behaviour is sensitive to IGEs in this species is unknown.

Overall, our results indicate that white-browed sparrow-weaver helpers show consistent individual differences in cooperative generosity and that this variation arises in part from direct additive genetic variation. To our knowledge this is the only evidence of appreciable heritable variation in cooperative generosity in a wild animal population. The fitness consequences of cooperative helping in white-browed sparrow-weaver societies have been suggested to vary with environmental conditions (Chapter 2). The findings of the present study suggest that the heritable variation required for an evolutionary response to selection is present in this species.

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Chapter 5: Supplementary Material

Supplementary Figures Chapter 5

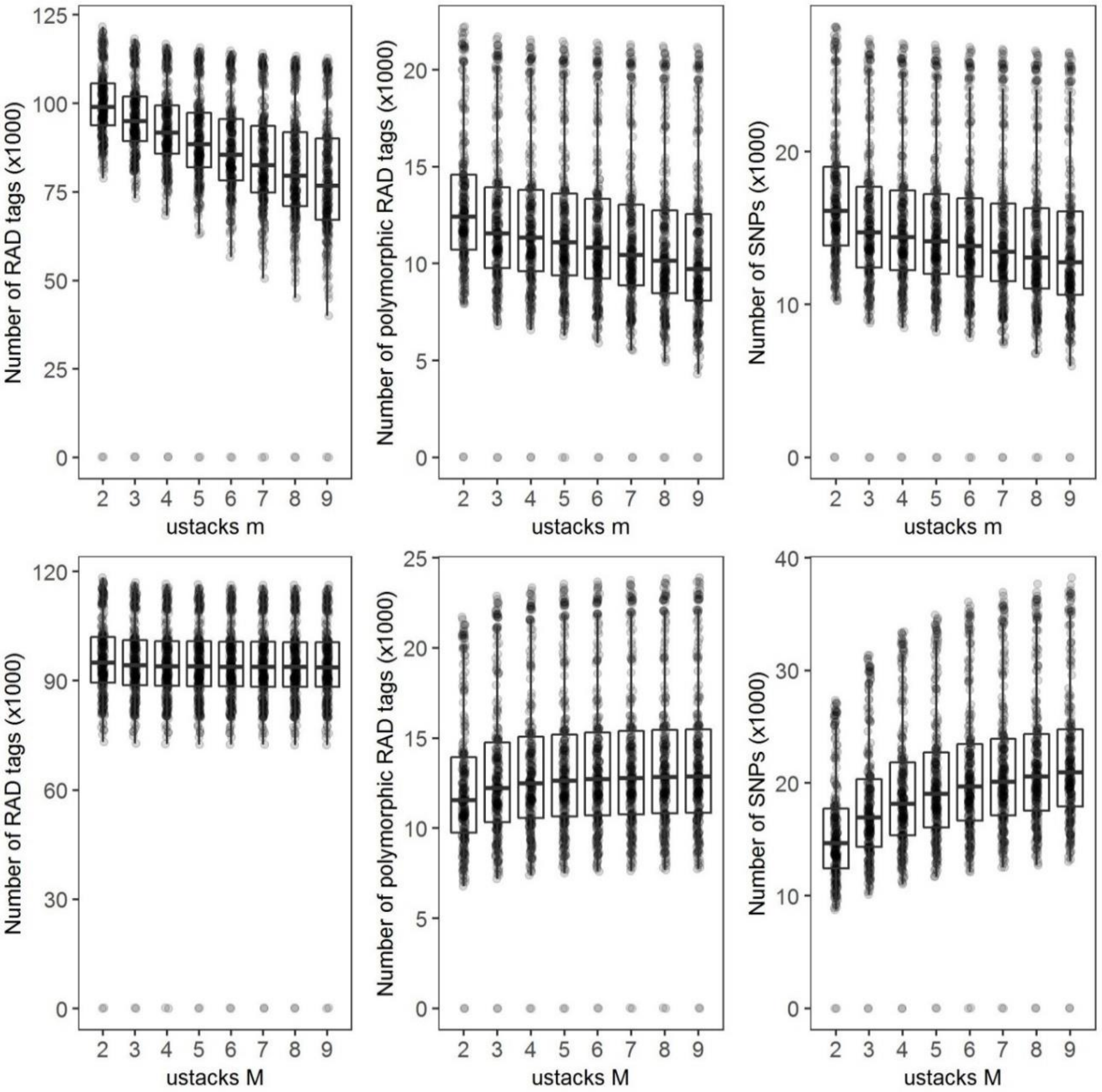

Figure S1. Initial assessment of the Stacks pipeline, –m and –M. Number of RAD tags built (left-hand side column), number of polymorphic RAD tags (central column) and number of SNPs (right-hand side column) recovered by Stacks for different values of –m (upper row) and –M (lower row) (both parameters from ustacks). Raw data are represented by grey dots whereas boxes illustrate median (horizontal thick black line), quartiles (ends of the box) and 1.5 times the inter-quartile range of the raw data distribution.

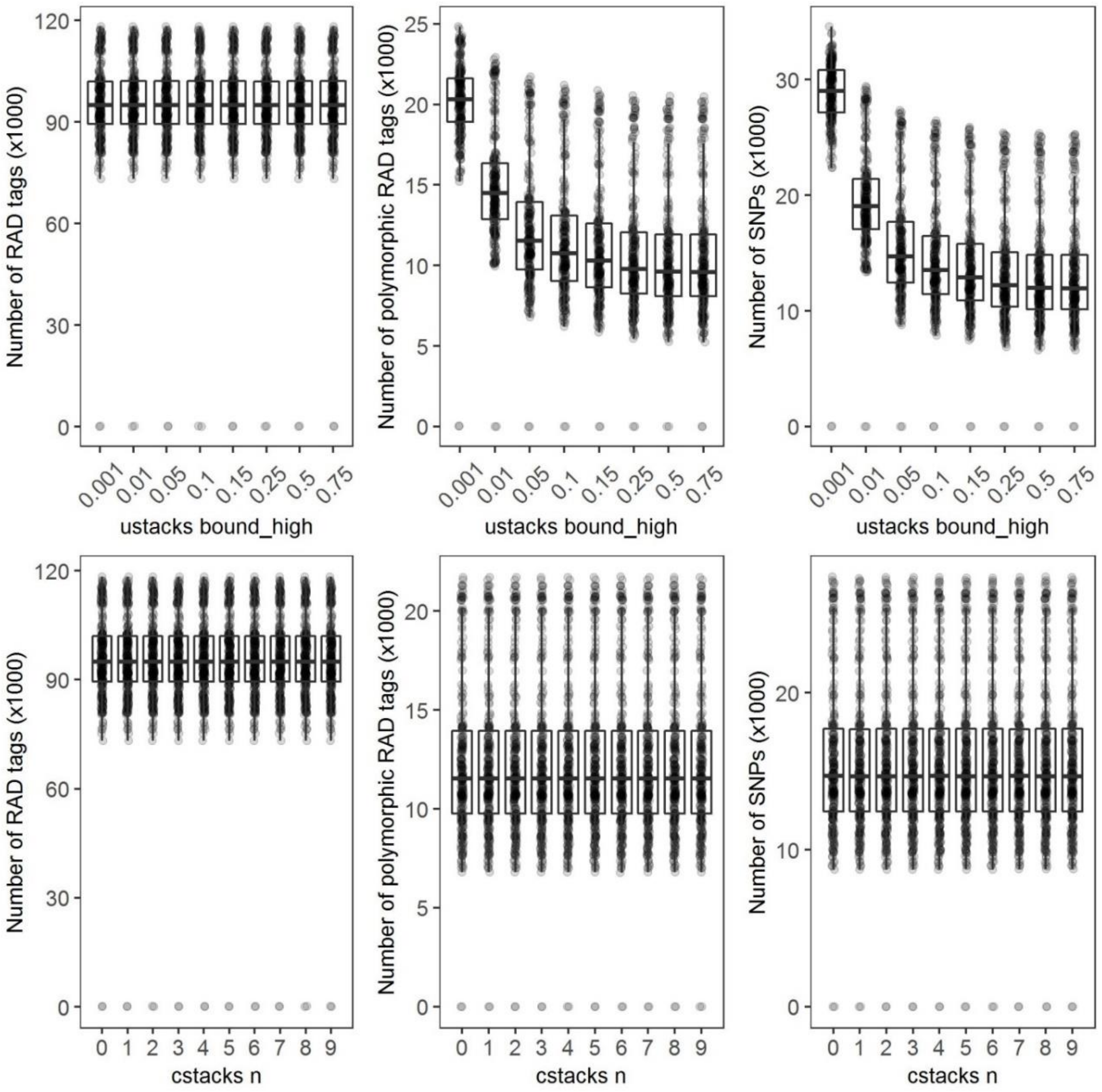

Figure S2. Initial assessment of the Stacks pipeline, –bound_high and –n. Number of RAD tags built (left-hand side column), number of polymorphic RAD tags (central column) and number of SNPs (right-hand side column) recovered by Stacks for different values of –bound_high (upper row; ustacks) and –n (lower row; cstacks). Raw data are represented by grey dots whereas boxes illustrate median (horizontal thick black line), quartiles (ends of the box) and 1.5 times the inter-quartile range of the raw data distribution.

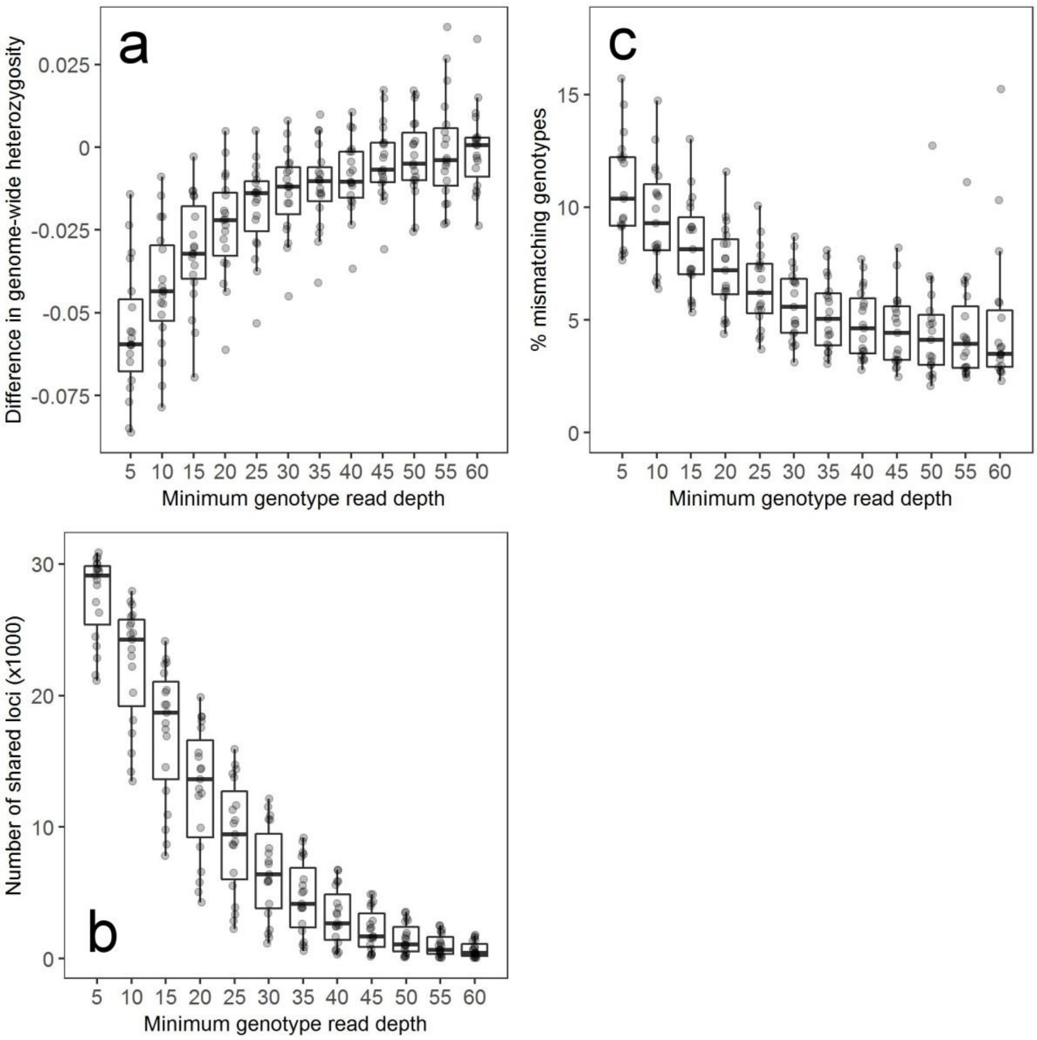

Figure S3. Assessment of consistency in genomic metrics across 21 paired (i.e. replicated) samples using different thresholds for minimum genotype read depth. After running the Stacks pipeline and selecting only RAD tags that were present in at least 80% of samples across and within libraries, we assessed, for every pair of replicate samples, (**a**) the difference in genome-wide heterozygosity, (**b**) the percentage of mismatching genotypes (i.e. SNPs with mismatching genotypes) and (**c**) the number of shared genotypes between paired samples. These three metrics plateaued after a minimum genotype read depth of 40x and we applied this threshold when selecting genotypes for further analyses. For a value of minimum genotype read depth of 40x, genome-wide heterozygosity within replicate pairs was highly correlated, with a regression slope close to one (β ± SE = 0.97 ± 0.36). Raw data are represented by grey dots whereas boxes and whiskers illustrate median (horizontal thick black line), quartiles (ends of the box) and 1.5 times the inter-quartile range of the raw data distribution.

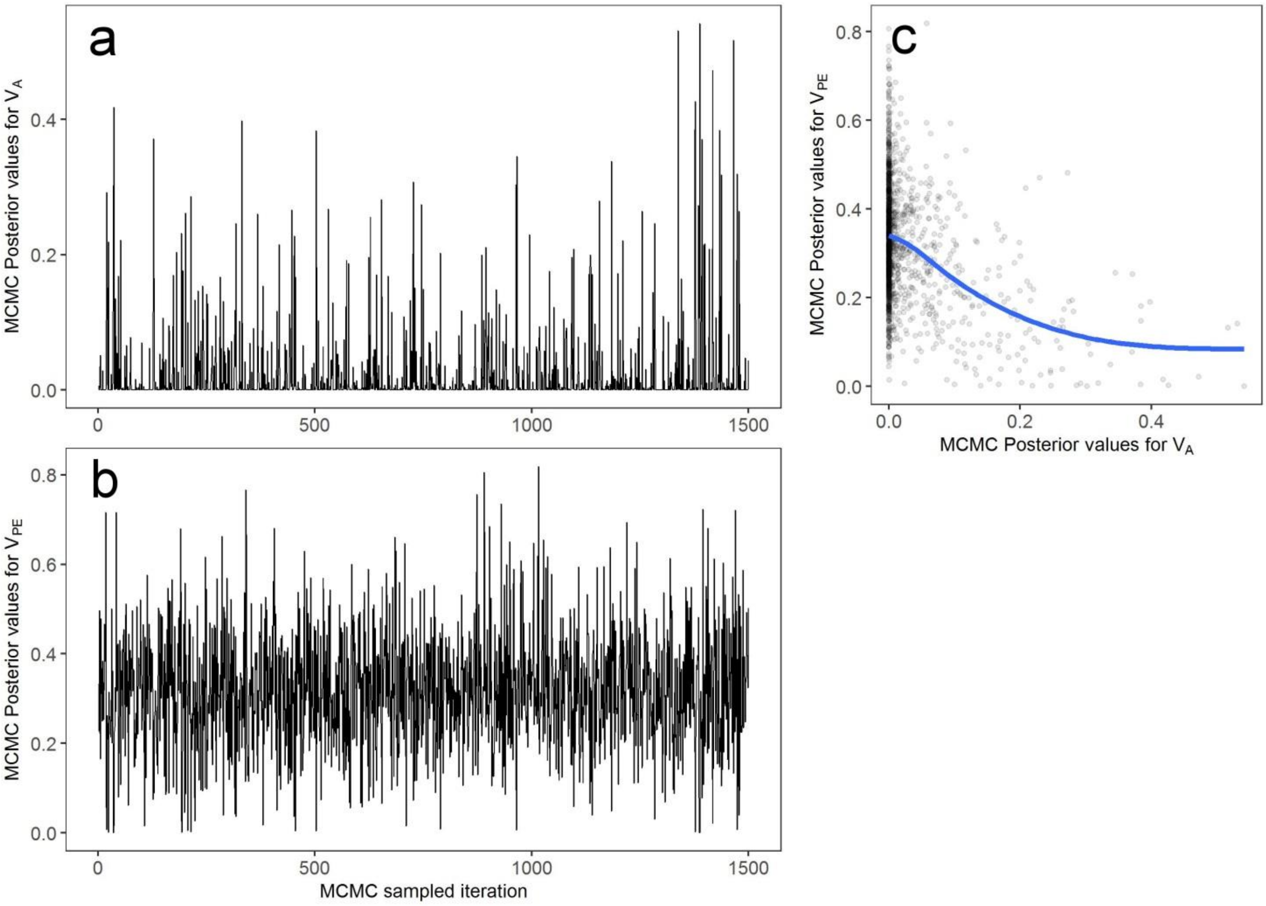

Figure S4. Repeated measures animal model for helper feeding rates. MCMC traces for (**a**) additive genetic variance (V_A) and (**b**) permanent environment variance (V_{PE}) (1,500 final iterations in both cases). (**c**) Values of V_A and V_{PE} at every MCMC iteration (dark dots) showed a negative relationship, suggesting that, given the data structure, the model is not able to appropriately split phenotypic variation between V_A and V_{PE}. Blue line represents the fit of a cubic regression spline.

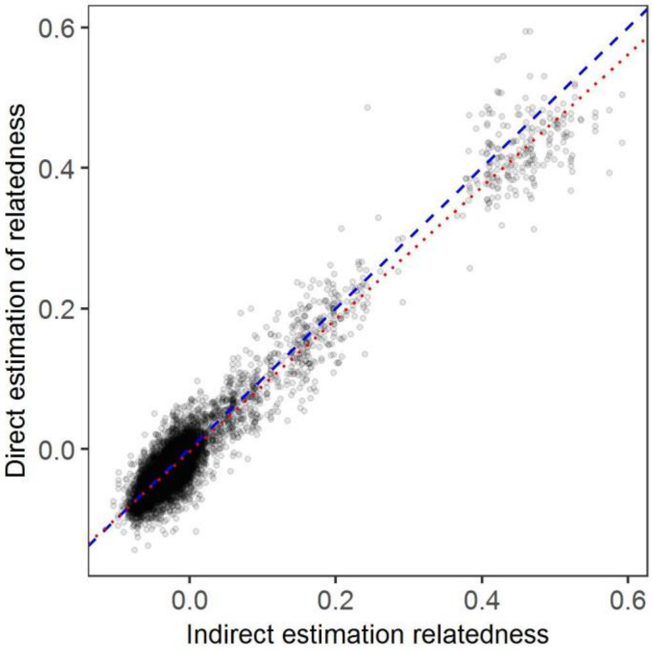

Figure S5. Validation of the indirect method to include individuals in the genetic relationship matrix (GRM). Using individuals that had been RAD sequenced and whose parents had also been RAD sequenced, we estimated their relatedness to every bird in the GRM using the indirect method described in Supplementary information (x axis - i.e. the sum of half the relatedness between their father and mother to every bird in the matrix). This indirect estimation of relatedness strongly predicted relatedness estimated directly from genomic data ($r^2 = 0.93$). Dashed blue line represents a line with slope of one and intercept of zero; red dotted line illustrates the estimated regression line between the indirect and direct estimation of relatedness (slope ± SE = 0.944 ± 0.003; intercept ± SE = -0.005 ± 0.0003).

Supplementary Tables Chapter 5

Table S1. Summary of the bioinformatics pipeline used to genotype 212 individual RAD-sequenced samples. 'Samples per locus' refers to the mean number of samples genotyped per locus (in percentage to the total number of samples).

Software	Parameters	Values	Variant loci	Variant sites	Samples per locus
Stacks; ustacks	--high_bound	0.05	39,668	63,815	90.7%
Stacks; ustacks	-m	3			
Stacks; ustacks	-M	2			
Stacks; cstacks	-n	2			
Stacks; populations	Genotype call rate total (-R)	0.8			
Stacks; populations	Minimum allele frequency (--min-maf)	0.05			
vcftools	Minimum genotype read depth	40	38,166	60,422	34.6%
vcftools	Hardy-Weinberg Equilibrium (p-value)[A]	0.01			

[A] Exact Test (Wigginton *et al.*, 2005)

Table S2. Bivariate generalised mixed model explaining variation in male and female helper provisioning rates. The initial model for provisioning rates was expanded into a bivariate model that explained variation for provisioning rates of male and female helpers. For each of those responses, fixed effects and variance partitioning was applied as outlined in the main text. Posterior median and 95% credible intervals ('CI') of three independent MCMCs are displayed (estimates in the link scale).

Fixed effects	**Helper sex**	**Median**	**95%CI**	
Intercept	males	-0.717	-1.084	-0.364
	females	-0.100	-0.423	0.189
Brood size	males	0.335	0.130	0.546
	females	0.240	0.038	0.434
Age (1 - 2y)	males	0.452	0.016	0.893
	females	0.343	-0.026	0.721
Age (> 2y)	males	0.426	-0.126	0.977
	females	0.549	0.121	0.978

Variance components		**Median**	**95%CI**	
Among individual (V_I)	males	0.428	0.068	0.994
	females	0.288	0.041	0.618
Among territory (V_T)	males	0.052	0.000	0.428
	females	0.016	0.000	0.185
Among brood (V_B)	males	0.325	0.007	0.846
	females	0.452	0.162	0.856
Residuals (V_R)	males	0.731	0.343	1.317
	females	0.428	0.216	0.798

Chapter 6

General Discussion

Understanding the evolutionary origin and maintenance of cooperation in nature is a long-standing question in evolutionary biology (Skutch, 1935; Boland & Cockburn, 2002). New theoretical formulations (Kennedy *et al.*, 2018), recent analyses of the global patterns of occurrence of cooperative species (Cornwallis *et al.*, 2017; Griesser *et al.*, 2017) and the appreciation that both direct and indirect fitness payoffs of cooperation are important to understand its evolution (Clutton-Brock *et al.*, 2002; Young *et al.*, 2005) provide new avenues to investigate and better understand this long-standing evolutionary puzzle. To that end, in this thesis I have investigated the routes through which helpers may acquire indirect fitness benefits, the role of sex differences in direct fitness payoffs from helping in generating sex differences in cooperation, and the genetic basis of cooperative generosity in a wild cooperatively breeding bird. In Chapter 2, I found evidence of (female) helpers reducing the variance in reproductive success of related breeders as envisaged by the altruistic bet-hedging hypothesis (Kennedy *et al.*, 2018). Furthermore, this reproductive variance compression appears to arise because helpers specifically reduce unpredictable *rainfall*-induced variation in reproductive success, just as hypothesised by global comparative studies of the evolution of cooperative breeding in birds (Griesser *et al.*, 2017). In Chapter 3, I then investigated the effect of helpers on the pre- and post-natal reproductive investment of female breeders ('mothers'). I found compelling evidence that mothers plastically adjusting their pre-natal reproductive investment in the egg according to the number of (female) helpers in their group. In Chapter 4, I provided novel support for the idea that sex differences in the direct fitness payoffs from cooperation, arising from sex differences in philopatry, can explain sex differences in natal cooperation. These findings also highlighted the need to consider the potential for sex differences in both the direct fitness benefits and costs of cooperation when trying to understand the evolutionary origin of sex differences in cooperation (or, more broadly, among-individual variation in cooperative behaviour). Lastly, in Chapter 5 I provided evidence of consistent individual differences in cooperative generosity and rare evidence that these arise in large part from additive genetic variance in cooperative generosity, suggesting that this trait could respond to selection. The latter result may be of particular importance as my analyses in Chapter 2 suggest that both the sign and magnitude of the effects of cooperation on breeder reproductive success (a key component of selection on cooperation) depend upon the prevailing environmental conditions, leading to the expectation of temporal and spatial variation in selection on cooperation in this species. Below, I briefly discuss the wider implications of these results for our understanding of the evolution of cooperation and highlight the potential wider utility for the field of the analytical approaches used throughout this thesis to identify helper effects.

6.1. Altruistic bet-hedging and the global distribution of cooperative breeders

Cooperatively breeding species are, in many taxa, globally associated with unpredictable environments (Jetz & Rubenstein, 2011; Sheehan *et al.*, 2015; Griesser *et al.*, 2017). Variation in environmental productivity and variability in rainfall conditions have been suggested as the main environmental drivers of this association (Jetz & Rubenstein, 2011; Rubenstein, 2011; Griesser *et al.*, 2017). This observation has led to the hypothesis that cooperative breeding reduces variance in reproductive success of breeding individuals (Rubenstein, 2011; Starrfelt & Kokko, 2012). Indeed, a recent theoretical formulation, altruistic bet-hedging (Kennedy *et al.*, 2018), predicts that helping acts as a bet-hedging strategy that reduces variance in reproductive success of the recipients of help (Starrfelt & Kokko, 2012; Kennedy *et al.*, 2018). The formulation of altruistic bet-hedging theory also highlights that inclusive fitness benefits arising from helpers reducing variation in reproductive success of related recipients are expected to increase with the extent on unpredictable environmental variation (Kennedy *et al.*, 2018).

In Chapter 2, I show that altruistic helping in white-browed sparrow-weavers fulfills the properties of an altruistic bet-hedging strategy, in that it reduces variance in the reproductive success of related breeders. Furthermore, helping appears to do so in part by reducing unpredictable rainfall-induced variation in reproductive success. Furthermore, my finding that helper effects on the mean and variance in reproductive success of breeders will depend on environmental conditions (Chapter 2) leads to a prediction about the nature of selection for cooperation in different environments. As suggested in Chapter 2, whether altruistic helping will be positively selected at all, and the extent to which selection for it will arise from reproductive variance reduction or increases in the mean reproductive success of breeders, will depend on the relative frequency of different environmental conditions (Chapter 2). My findings suggest that in dry environments cooperation may be selected for principally on the basis of its net positive effects on the reproductive success of related breeders. While in environments in which dry and wet conditions occur at similar frequencies (such as our study site), selection for cooperation may arise more from its reproductive variance reduction properties. As the relative frequency of different environmental conditions varies across time and space, selection for cooperation is predicted to vary with future effects of climate change (i.e. in time) and across the current distribution range of a given cooperative species (i.e. in space). Investigations of the effects of helping on reproductive success of breeders (mean and variance) along an environmental gradient for a given species (e.g. its distribution range) would help confirm or reject this prediction.

6.2. The importance of direct fitness payoffs in the evolution of cooperation

When cooperative individuals do not differ in their genetic relatedness to the recipients of help within a society, indirect fitness returns from cooperation (i.e. kin selection) alone cannot readily explain among-individual variation in cooperation (Clutton-brock, 2002; Griffin & West, 2002). Sex differences in cooperation are common within animal societies (Stallcup & Woolfenden, 1978; Owens & Owens, 1984; Curry, 1988; Cockburn, 1998; Clutton-Brock *et al.*, 2002; Dickinson & Hatchwell, 2004; Canestrari *et al.*, 2005; Koenig & Dickinson, 2016; Zöttl *et al.*, 2016). In the absence of sex differences in indirect fitness returns, sex differences in the direct fitness benefits and costs of cooperation have been hypothesized to play a key role in generating sex differences in cooperation (Clutton-Brock *et al.*, 2002; Young *et al.*, 2005).

The philopatry hypothesis for the evolution of sex differences in cooperation predicts that the more philopatric sex will invest more in natal cooperation (Clutton-Brock *et al.*, 2002). There are two potential mechanisms through which a sex difference in philopatry could generate a sex difference in natal cooperation. Firstly, the more philopatric sex may stand to gain a greater direct fitness benefit from cooperating within the natal group than the less philopatric sex. This would be the case when, for example, the more philopatric sex is more likely to inherit a breeding position within their natal group, and hence be helped back by those individuals that they have helped to rear (Woolfenden & Fitzpatrick, 1978; Clutton-Brock *et al.*, 2002;

Downing *et al.*, 2018). If cooperation increased the size of the natal group and group size enhanced the survival of all residents (Ekman *et al.*, 2000; Kokko *et al.*, 2001; Kingma *et al.*, 2014; Nelson-flower & Ridley, 2016), then the more philopatric sex could also stand to gain differential direct fitness benefits from natal cooperation prior to dominance acquisition. Alternatively, the less philopatric sex may be faced with higher costs of cooperation, arising from trades-off between cooperation and traits that promote dispersal success (Young *et al.*, 2005; Williams & Hale, 2007).

Sex differences in direct fitness benefits of cooperation have been emphasized as the key mechanism creating sex differences in cooperation (Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). However, the role of sex differences in the direct fitness costs of cooperation driving sex differences in cooperative contributions remains largely unknown (Young *et al.*, 2005; Hodge, 2007; Williams & Hale, 2007). In my analyses in Chapter 4, I investigated the potential for sex differences in both the direct fitness benefits and costs of cooperation to explain the sex difference in natal cooperation in white-browed sparrow-weavers. My findings highlighted that sex differences in the direct fitness costs of cooperation may be more important than previously assumed in generating sex differences in natal cooperation. Indeed, a role for sex differences in direct fitness costs provides a credible alternative explanation for the role of direct fitness benefits implicated in previous studies (Clutton-Brock *et al.*, 2002; Downing *et al.*, 2018). The last idea is particularly relevant given the comparatively little compelling evidence of a role for direct fitness benefits in shaping patterns of helping in cooperatively breeding societies (Zöttl *et al.*, 2013b).

6.3. Complications in the study of the evolution of helping

Studies on cooperatively breeding species typically investigate the effects of helping by examining relationships between helper number and performance. However, there are two key problems with this approach. First, in many cooperative societies, helper number strongly correlates with group size, leaving it unclear whether any apparent effects of helpers are attributable instead to simple effects of group size, which could arise independent of helping *per se*. Second, variation in territory quality (or in the quality of the breeders on them) may yield among-territory associations between helper number and reproductive performance that confounds any causal association between helper numbers and reproductive performance. Throughout this thesis, I adopted two novel analytical approaches that allowed me to address both challenges, increasing confidence that the evidence of helper effects, while typically not experimentally determined, is unlikely to be attributable to either of the mentioned confounds.

6.2.1. The importance of isolating the effects of helping *per se*

Cooperative breeding has likely evolved in a two-step process. First, selection needs to favour the delayed dispersal (and parental toleration) of offspring, leading to their retention in the natal group (Koenig *et al.*, 1992). Several ecological explanations have been proposed for the evolution of delay dispersal, amongst them habitat saturation (Zack, 1990; Komdeur, 1992), inheriting a high quality territory (Emlen, 1984; Ekman *et al.*, 2001) and the benefits of group living (Ekman *et al.*, 2000; Kokko *et al.*, 2001; Kokko & Ekman, 2002; Kingma *et al.*, 2014). Secondly, the most common explanation for the subsequent evolution of helping behavior proposes that helping is then selected for due to the indirect fitness benefits that individuals can accrue by helping kin (Hamilton, 1964), while awaiting a breeding opportunity of their own

(Khan & Walters, 2002; Russell *et al.*, 2003b; a; Dickinson & Hatchwell, 2004; Heinsohn, 2004; Koenig & Dickinson, 2016). Therefore, studies trying to understand the evolution of *helping* in cooperative breeders need to, in theory, isolate the effects of helping *per se* from the benefits of groups living (e.g. 'group augmentation'; Kokko *et al.*, 2001; Kingma *et al.*, 2014). In practice, uncoupling these two effects in cooperative breeders is difficult because helpers cooperate and are part of the social group, thereby contributing to group augmentation benefits, at the same time (for an exception see Hatchwell, 2016).

I utilised the sex difference in the helping contributions of subordinate females and males to pin helper effects on cooperation *per se* rather than correlated effects of group size that might arise independent of helping. It is conceivable that such a female helper number effect arises instead from some differential effect of subordinate females relative to subordinate males that arises independent of their sex difference in helping. However, there is no evidence to suggest that that is the case in white-browed sparrow-weavers as subordinates of both sexes contribute equally to territorial defence (York *et al.*, 2019), sentinelling (Walker *et al.*, 2016) and weaving (Chapter 4).

6.2.2. Avoiding the confounding effect of variation in quality among territories

Territory quality is likely to positively affect the size of the group that occupies it by offering more resources for successful upbringing of offspring or by reducing competition and, thus, allowing more individuals to delay dispersal (Cockburn, 1998; Ekman *et al.*, 2001). In this situation, a spurious positive relationship has the potential to arise between group size and reproductive success: large groups are associated with increased reproductive success not because there is an actual relationship between them but because territory quality is positively affecting both at the same time (Cockburn, 1998). The same logic applies to the study of helper effects on any other trait when territory quality may affect the presence of helpers (or their cooperative contributions) and the expression of the trait of interest. This is a common pitfall in studies of cooperative breeders, which can be circumvented by experimental approaches (e.g. Brouwer *et al.*, 2005; Liebl *et al.*, 2016) or by applying particular analytical techniques (van de Pol & Wright, 2009), which has been rarely done (Lejeune *et al.*, 2016). In this thesis, I avoid this potential pitfall by carrying out an experimental manipulation (Chapter 2) and controlling for variation in quality among territories and breeding individuals (Chapter 2 & 3) by applying within-territory and within-individual centering techniques (van de Pol & Wright, 2009; Lejeune *et al.*, 2016).

In particular, investigating the effects of within-individual changes in helper number on pre-natal maternal investment has revealed the first evidence to my knowledge that breeding females respond plastically by changing the size of their eggs when assisted by more (female) helpers (Chapter 3). Indeed, the direction of the relationship (an increase in egg size with increasing female helper numbers) also contrasts with the general pattern found in other cooperative breeders, where egg investment typically negatively correlates with the presence of helpers (Russell *et al.*, 2007a; Dixit *et al.*, 2017; but see Valencia *et al.*, 2017). However, very few studies in this body of literature have controlled for the potential confounding effect of variation in quality among territories or individuals. Indeed, the only study that did so found that the correlation between helper number and egg size actually arose from helper effects on *among*-mother variation in egg size rather than from helper effects on *within*-mother variation (Lejeune *et al.*, 2016). Therefore, the real extent to which our results in Chapter 3 contrast with the general pattern of maternal plasticity in pre-natal investment in cooperative breeders in reality will only be elucidated once more empirical studies either apply experimental approaches (e.g. Taborsky *et al.*, 2007) or separate the effects of variation in territory and/or breeder quality from the effects of helping.

6.4. Final summary

Throughout this thesis, I have investigated the evolution of helping behavior in a wild cooperatively breeding bird. I have shown how indirect fitness benefits may arise from helper effects on both offspring survival (via mean and variance effects on the reproductive success of breeders; Chapter 2) and, potentially, the post-natal workloads of mothers (Chapter 3). In Chapter 4, I provided evidence for the philopatry hypothesis for the evolution of sex differences in cooperation and highlighted the importance of investigating direct fitness benefits and costs of cooperation to understand sex differences in cooperation. Finally, in Chapter 5 I have provided rare evidence for the existence of heritable variation in cooperative generosity, suggesting that this trait can potentially respond to selection.

7. References

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