University of Groningen

Understanding the evolution of infidelity using the Seychelles warbler system

Raj Pant, Sara

Publication date:
2019

Chapter 1

General introduction

Sara Raj Pant

View from a beach on Cousin, with Praslin (Seychelles) in the distance.

1.1. Sexual reproduction

Sex is the prevailing form of reproduction among vertebrates and about 99% of eukaryotic species engage in it at least occasionally (Otto 2008). Sexual reproduction, however, comes with high costs, including the time and energy spent finding a partner, attracting it and mating with it (Daly 1978), in addition to the risks associated with such activities, such as predation (Wing 1988), disease transmission (Hurst and Sharpe 1995) and physical injury (Parker 1979). The most striking among the costs of sex is probably its so-called 'two-fold cost': while asexual reproduction allows any one individual to generate one offspring (via genome replication), sex requires two individuals to produce one offspring, and each parent will only transmit 50% of their genes to the next generation.

However, without sex and the resulting gene shuffling, populations suffer a reduction in genetic variation produced by different gene combinations (Weismann 1889). Fitness is also reduced over the generations in a ratchet-like manner ('Muller's ratchet'): individuals, most of which carry at least some deleterious mutations, are bound to transfer these to offspring when reproducing asexually; the mutation load therefore increases at each generation (Muller 1964). Recombination can purge deleterious alleles (Fisher 1930; Muller 1964) and gather disparate fit alleles from different individuals and combine them into the next generation (Fisher 1930; Muller 1932), therefore restoring genetic variation and enabling selection (Weismann 1889). Moreover, since individuals live in a changing environment, sex and recombination enable the breakage of gene combinations that are detrimental, or no longer suited to the current conditions, and the creation of new and advantageous gene associations (Otto 2009). Therefore, despite the costs, sex persist because it confers a strong advantage to those engaging in it, i.e. it provides greater scope for adaptation. Whether this is achieved via the ability to evolve novel genotypes for parasite resistance (Hamilton 1980), to cope with spatially and/or temporally varying selection (Otto and Lenormand 2002; Otto 2009), the capacity to fix beneficial alleles (Fisher 1930; Muller 1932) or the ability to purge deleterious mutations (Fisher 1930; Muller 1964), is still unclear and is likely to result from a combination of mechanisms, with variation across taxa.

1.2. Promiscuity

Although necessary for reproduction in sexual species and most likely adaptive, mating is costly. Therefore, it may seem surprising that multiple mating (i.e. engaging in several sexual encounters within the same reproductive cycle) is a common behaviour across taxa. Females often mate at rates higher than those necessary to ensure fertilization, and there is some evidence that this can improve individual fitness (Arnqvist and Nilsson 2000). The mating act itself (Opp and Prokopy 1986) and the presence of viable sperm in the female reproductive tract (Gromko et al. 1984) can increase female fecundity and, consequently, also fertility (Thornhill and Alcock 1983; Choe and Crespi 1997). However, multiple mating may cause polyspermy (i.e. the fertilization of one egg by more than one sperm) and consequent embryo mortality (Eberhard 1996).

Although in some cases individuals re-mate with the same partner during a single reproductive cycle, multiple mating with several partners (promiscuity) is very common across taxa (Tregenza and Wedell 1998; Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Griffith et al. 2002). Why individuals would be promiscuous and thus endure, in addition to the costs involved in multiple mating, those caused by mating with more than one partner, e.g. the larger amount of time, energy and risks involved in finding and copulating with multiple mates (Daly 1978; Parker 1979; Wing 1988; Hurst and Sharpe 1995), is yet to be fully resolved.

Why individuals may be promiscuous will vary between the sexes. That is, because in multicellular eukaryotes, sex is the fusion of two dissimilar gametes – male and female – unequal in size (anisogamy) and, hence, differing in the amount of energy required for their production. This disparity – sperm being smaller and therefore cheaper to produce compared to eggs – sets in place an evolutionary cascade leading to a difference in mating strategies between the two sexes (Bateman 1948; Arnqvist and Rowe 2005). Males are expected to invest less energy than females in the production of each gamete, which should enable them to generate a greater number of gametes per reproductive cycle and to produce a higher number of offspring by inseminating as many mates as possible (Bateman 1948). Females, on the other hand, produce fewer, larger and more expensive gametes. Moreover, due to their reproductive physiology, females are limited in the number of young they can produce per cycle, regardless of the number of sexual partners (Bateman 1948). Therefore, the sexes are predicted to differ in their mating strategy: females should be interested in copulating with the best male available, rather than with many mates, while males should try and mate with

as many females as possible in order to increase their reproductive success (Bateman 1948). This disparity is expected to cause higher potential reproductive rate (i.e. the maximum reproductive rate when access to mates is unconstrained; Clutton-Brock and Vincent 1991) in males and, consequently, higher competition for mates and higher variance in reproductive success compared to females (Bateman 1948).

1.3. Polyandry

It seems intuitive that promiscuity should be adaptive to males, because mating with more females should allow them to sire a higher number of young. On the other hand, females cannot normally increase offspring production by having more sexual partners and, due to anisogamy, should also incur higher costs of reproduction than males. Moreover, across taxa, studies have shown that females may experience additional costs of mating due to male manipulation, including mechanical injury suffered during copulations (Blanckenhorn et al. 2002) and chemical damage caused by ejaculate toxins promoting sperm success (Wigby and Chapman 2005). Explanations for the occurrence of polyandry (i.e. females mating with multiple males) have therefore been sought since the 1980s, when new molecular techniques allowing parentage assignment helped reveal how widespread this behaviour is, even in socially monogamous species (Griffith et al. 2002). Although various hypotheses for the benefits of polyandry have been formulated, no consensus has been reached to date. Most explanations for the evolution and maintenance of polyandry in nature can be grouped in two broad categories: adaptive vs non-adaptive hypotheses.

1.3.1. Adaptive models

Adaptive explanations assume that polyandry has evolved under direct selection increasing female fitness by either limiting the costs of re-mating (Thornhill and Alcock 1983; Smuts and Smuts 1993) or providing benefits which could be either material (Sheldon 1994; Birkhead 1995; Wedell 1997; Lombardo and Thorpe 2000) or genetic (Hamilton and Zuk 1982; Watson 1991; Zeh and Zeh 1996; Brown 1997).

COST LIMITATION. Female promiscuity could limit the costs associated with sexual conflict over mating rates (Thornhill and Alcock 1983), which are predicted to be higher for males compared to females (Trivers 1972). It has been postulated that, in species where females are

coerced into copulation, the costs of resisting male mating attempts can be higher than those resulting from allowing additional copulations. When a threshold of harassment is reached, female promiscuity could be convenient (Rowe 1992; Lee and Hays 2004). However, studies addressing this 'convenience polyandry' hypothesis (Thornhill and Alcock 1983) have either only provided indirect evidence via observational field data (e.g. Lee and Hays 2004; Wright et al. 2013), or contrasting results in laboratory settings (reviewed in Rowe et al. 1994; Sakurai and Kasuya 2008; Janowitz and Fischer 2012; Boulton et al. 2015). Moreover, most experimental studies did not differentiate between the effects of *mating multiply with one male* (see Hunter et al. 1993 for hypotheses behind this) and *copulating with several different males* (Slatyer et al. 2012).

Another cost limitation hypothesis maintains that polyandry evolved as an infanticide avoidance mechanism, in species where this is a common practice (Smuts and Smuts 1993; Wolff and MacDonald 2004). This model predicts that extra-pair males that have mated with a female will refrain from killing her young, as they might be sires. Such a hypothesis might hold true for certain mammal and bird species, in which multiple males interact socially with females due to home range overlap or multi-male group living (Ebensperger and Blumstein 2007). A review of studies on 133 mammal species highlighted that polyandry occurs in 87% of carnivore species and 62% of primate species where infanticide is common, while only 9% of non-infanticidal primate species were promiscuous (Wolff and MacDonald 2004). Moreover, Wolff and Macdonald (2004) stressed that copulations with multiple males are usually solicited by females, though they did not provide an explicit quantification of this (but see Table 2 in Wolff and MacDonald 2004). A few studies have provided experimental evidence in support of the infanticide avoidance theory, including work on bank voles (*Myodes Glareolus*; Klemme and Ylönen 2010) and on tree swallows (*Tachyneta bicolor*; Robertson and Stutchbury 1988). Such results, coupled with the strong benefit entailed by offspring survival, lend some support to this hypothesis.

DIRECT MATERTIAL BENEFITS. Polyandry could be selected for if females obtained material (non-genetic) benefits from their sexual partners (reviewed in e.g. Jennions and Petrie 2000; Forstmeier et al. 2014). Such benefits could include adequate sperm supply for fertilization (fertility assurance hypothesis; Sheldon 1994), nutrients (e.g. nuptial gifts) or other substances increasing egg production (Wedell 1997), advantageous sexually transmitted microbes (Lombardo and Thorpe 2000) or additional access to resources or parental care from more than one male (Birkhead 1995).

Empirical studies in reptiles, mammals and birds have provided contrasting evidence for the fertility assurance hypothesis and have often been observational (Uller and Olsson 2008; Hasson and Stone 2009). The only experimental study that, to my knowledge, tested this hypothesis, found no support for it. This study showed that, in captive zebra finches (*Taeniopygia guttata*), females who had experienced hatching failure were not more likely to engage in extra-pair copulations subsequently (Ihle et al. 2013). Theoretical models have shown that females should benefit from fertility assurance via polyandry, but only under specific circumstances, i.e. when they are paired with truly infertile males featuring a low sperm count and/or motility (Hasson and Stone 2009). Given that there is strong selection against true infertility in nature, this trait is likely to be very rare. Therefore, it has been argued that the potential costs associated with infidelity (including polyspermy) are probably not offset for most females (see Forstmeier et al. 2014).

In insects, a meta-analysis (Arnqvist and Nilsson 2000) of 122 experimental studies testing for direct fitness effects of singly vs multiply mated females (under different mating rate treatments) showed a general trend of increased fecundity and fertility in promiscuous subjects. Moreover, female longevity was higher under polyandry, but only for females in taxa with nuptial gifts (i.e. nourishment during/after copulations). Interestingly, Arnqvist and Nilsson (2000) found that female fitness increased up until an optimum copulation rate, beyond which additional mating had detrimental effects, such as decreased lifespan. They interpreted this as the result of sexually antagonistic co-evolution. However, the lack of phylogenetic correction in the analyses may have undermined the robustness of the results. Moreover, this study did not account for potential genetic effects deriving from copulations with males of different genetic composition. Therefore, the detected fertility enhancement in promiscuous females may have been caused by genetic benefits resulting, for instance, from fertilizations by less inbred (more compatible) males, a mechanism known to improve population fitness and to slow down extinction rates in inbred systems (Michalczyk et al. 2011; Lumley et al. 2015).

INDIRECT GENETIC BENEFITS. The indirect genetic benefits theories maintain that polyandry biases paternity towards genotypes that augment offspring fitness via increased genetic quality, thus providing fitness benefits to promiscuous females (Hamilton and Zuk 1982; Zeh and Zeh 1996; Brown 1997; reviews: Andersson 1994; Jennions and Petrie 2000; Forstmeier et al. 2014). Given several ecological constraints, such as low population density or nest site availability, females might mate and, in socially monogamous species, pair up with males that are not necessarily of the highest genetic quality. Polyandry could therefore

be a 'trading up' mechanism allowing females to copulate with preferred males as they become available to them (Jennions and Petrie 2000).

One of the most popular indirect genetic benefits theories is 'the good genes' hypothesis (Hamilton and Zuk 1982), which maintains that polyandry enables the acquisition of high quality paternal gene variants in offspring (i.e. alleles that increase offspring fitness by additive effect, independent of the genome architecture of the parents). This is expected to occur via female choice based on male phenotypic features signalling genetic quality, such as body size, ornaments and/or age (e.g. Westneat 1990; Hasselquist et al. 1996; Ackay and Roughgarden 2007; Cleasby and Nakagawa 2012; Hsu et al. 2015; E et al. 2017).

The 'compatible genes' hypothesis (Zeh and Zeh 1996; Brown 1997), on the other hand, maintains that female preference (pre- or post-copulation) is based on the level of genetic compatibility between maternal and paternal genomes – 'compatible alleles' being those that increase fitness via either epistasis, dominance or over-dominance. According to this hypothesis females are under selective pressure to avoid males carrying genetic elements which would cause intra-genomic conflict in the embryo (Zeh and Zeh 1996; Tregenza and Wedell 2000; Zeh and Zeh 2001). Additionally, females are expected to avoid inbreeding, as this may lower offspring fitness by increasing the expression of deleterious recessive alleles and by decreasing heterozygosity (Thornhill 1993; Brown 1997; Kempenaers 2007). For this reason, females are also predicted to seek fertilizations from males that are genetically dissimilar to them or that share an intermediate amount of similarity, in order to maximise, or optimise, offspring heterozygosity (Brown 1997; Milinski 2006). Moreover, polyandrous females may increase offspring heterozygosity by choosing males that are heterozygous at many loci or a few loci in key genomic areas, e.g. at the major histocompatibility complex (MHC; Brown 1997).

Studies evaluating indirect genetic hypotheses have mainly focused on socially monogamous species (particularly birds) and have provided mixed evidence so far. In particular, research relating variation in extra-pair paternity (EPP) to variation in specific male traits linked to quality (a common approach in assessing the good genes hypothesis) has provided no clear evidence. While some studies found a correlation between EPP and traits signalling genetic quality, including ornamentation, song structure, body size and immune response (Hasselquist et al. 1996; Forstmeier et al. 2002; E et al. 2017), other studies did not (e.g. Krokene 1998; Charmantier et al. 2004; Dietrich et al. 2004a). Meta-analyses (Ackay and Roughgarden 2007; Cleasby and Nakagawa 2012; Hsu et al. 2015) also failed to detect evidence for the good genes hypothesis. This could result from the lack of indirect genetic

benefits to promiscuous females, but also from the meta-analytic approach itself, which homogenizes results across taxa, when in fact male quality could be signalled differently depending on the species. However, one consistent result that has been often been picked out in studies on individual species (e.g. Wagner et al. 1996; Richardson and Burke 1999) as well as meta-analyses (Ackay and Roughgarden 2007; Cleasby and Nakagawa 2012; Hsu et al. 2015) is the positive relationship between EPP acquisition and male age. Old age could indicate a higher probability that an individual is able to overcome disease, predation and other selection pressures in its environment, yet it is still debated whether age truly signals an individual's genetic quality (see e.g. Kokko 1998; Johnson and Gemmell 2012).

Recent studies have shown that polyandry is an effective means of improving population fitness and of slowing down extinction rate in inbred populations (Michalczyk et al. 2011; Lumley et al. 2015), which suggests that promiscuity could evolve as an inbreeding avoidance mechanism. Research addressing a potential correlation between within-pair relatedness/ genetic similarity and EPP in socially monogamous species – the most commonly used way to assess the genetic compatibility hypothesis (via inbreeding avoidance, Ackay & Roughgarden 2007) – has provided mixed evidence (e.g. Blomqvist et al. 2002; Eimes et al. 2005; Schmoll, Quellmalz, et al. 2005; Edly-Wright et al. 2007). Studies comparing the pairwise genetic similarity of females to their social males vs the extra-pair sires (that cuckolded the social males) provided differing results (e.g. Foerster et al. 2003; but see e.g. Freeman-Gallant et al. 2006). Akcay and Roughgarden (2007) investigated such relationship in their meta-analysis and found no support for it across bird species. However, a more recent meta-analysis (Arct et al. 2015) found that within-pair relatedness predicted EPP in birds. Despite this, several authors have criticized this study and have urged caution in interpreting such results as evidence for inbreeding avoidance via extra-pair copulations. One of the criticisms to Arct et al. (2015)'s meta-analysis was that this study found a significant positive relationship between pairwise genetic relatedness and EPP only when including studies based on microsatellite markers (many of which relied on few such markers; Reid 2015). Heterozygosity and relatedness can feature sampling bias when estimated from a few microsatellite markers (Reid 2015), especially when these markers are also used to assign paternity (Wetzel and Westneat 2009) and when samples contains inbred or related individuals (Wang 2014). Another methodological critique (Griffith 2015) to Arct et al. (2015)'s meta-analysis was the inclusion of a species that is not socially monogamous so EPP could not take place by definition.

Comparisons of fitness between within- and extra-pair young have provided a perhaps more direct approach to assess both the genetic compatibility and the good genes hypotheses. Once again, results are contrasting (e.g. Sheldon et al. 1997; Kempenaers et al. 1999; Foerster et al. 2003; but see e.g. Krokene 1998; Whittingham et al. 2001; Kleven et al. 2006). Akcay and Roughgarden (2007)'s meta-analysis found no significant relationship between offspring viability and EPP in birds. A more recent meta-analysis (Slatyer et al. 2012) of studies conducted in vertebrate and invertebrate taxa also failed to detect any significant difference in the performance of offspring of monoandrous vs polyandrous females. However, many studies comparing fitness components of within- and extra-pair offspring fitness have done so by assessing hatching success or fledging success and ignoring viability (and/or reproduction) in later life stages, such as survival to breeding age (but see e.g. Foerster et al. 2003; Edly-Wright et al. 2007; Hsu et al. 2014) and life-span/lifetime reproductive success (but see e.g. Schmoll et al. 2009; Annavi 2012; Hsu et al. 2014). However, an individual's fitness may be confounded by other factors (e.g. environmental, social) and may be condition-dependent (Schmoll, Dietrich, et al. 2005) or sex-specific (Annavi 2012). Moreover, fitness comparisons of within- and extra-pair offspring do not strictly test whether extra-pair offspring are fitter than the within-pair offspring that that a female would have produced had she only copulated with the pair male (i.e. the true assumption of indirect genetic benefit models). To my knowledge, only one study has performed such a comparison, availing itself of an extensive pedigree from a natural population of song sparrows (*Melospiza melodia*; Reid and Sardell 2012). This study estimated the additive genetic value for recruitment (i.e. the sum of the average additive effect of an individual's alleles on recruitment) of the extra-pair offspring and their hypothetical within-pair siblings. Interestingly, Reid and Sardell (2012) found that extra-pair offspring had lower additive genetic value for recruitment and suggested that there may be a (weak) indirect selection against female extra-pair reproduction in the song sparrow. More studies like this are needed if we are to better understand whether EPP confers indirect (additive) genetic benefits to promiscuous females.

Another explanation for polyandry within the indirect genetic benefits framework is the genetic diversity hypothesis (Ridley 1993; Schmid-Hempel 1994; Keller 1995; Sherman et al. 1998; Aguirre and Marshall 2012). This hypothesis predicts that polyandry provides indirect benefits by increasing the amount of genetic diversity within a female's entire brood, which leads to increased mean offspring fitness. This mechanism has been hypothesised to evolve more easily in systems where half-siblings remain in contact after birth, so that genetic diversity can alleviate sibling competition, e.g. in parasitoid wasps (Ridley 1993; Aguirre and Marshall 2012), and/or reduce disease and parasite spread in colonial species

(Schmid-Hempel 1994; Aguirre and Marshall 2012). Most evidence in support of this hypothesis comes from research on eusocial insects (Jennions and Petrie 2000; McLeod and Marshall 2009). Such studies showed that, in addition to being more resistant to infection/ parasites, genetically diverse colonies are also more productive (Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999). Moreover, while multi-queen colonies feature monogamous queens, single-queen colonies are governed by promiscuous queens (Keller 1995). Although quite compelling, evidence is indirect and mainly limited to social insects. Experimental studies across a range of taxa are therefore needed to assess the validity of the genetic diversity model.

The bet-hedging theory is an additional, and controversial, genetic benefits hypothesis for the evolution of polyandry (Watson 1991; Yasui 1998; Yasui 2001; Fox and Rauter 2003; Sarhan and Kokko 2007; Garcia-Gonzalez et al. 2015; Holman 2015). This theory was first brought forward as a risk-spreading strategy in economics, where goods are divided for their protection in a risky environment (Bernoulli 1954). The bet-hedging theory was first conceptualised into an evolutionary framework by Gillespie (1974). Since then, it has been invoked by evolutionary biologists as an explanation for the evolution of many life-history traits, including polyandry. Bet-hedging explanations can be considered as hypotheses gathering elements from other genetic benefits theories. In particular, the 'genetic bet-hedging hypothesis' maintains that polyandry should evolve in a stable environment when females are incapable of selecting mates that carry good and/or compatible genes (Garcia-Gonzalez et al. 2015). In this case, polyandry would improve female fitness by reducing the risk that eggs are fertilised by males with low quality and/or incompatible genes (Yasui 1998; Fox and Rauter 2003). The 'genetic diversity bet-hedging hypothesis' posits that in a fluctuating/ unpredictable environment polyandry lowers the risk that all ova are fertilised by males who are not adapted to current environmental conditions (Yasui 1998). There is some evidence for these models in invertebrates but, owing to their difficulty, studies testing such hypotheses are too scarce to validate the underlying assumptions (Garcia-Gonzalez et al. 2015). Moreover, a recent meta-analysis of bet-hedging studies (Holmes 2015) did not support this theory. Holman (2015) quantified the selective advantage of polyandry vs monoandry via diminished variance of offspring fitness within promiscuous systems. He found that any advantage in offspring fitness was probably too low to contribute to the evolution of polyandry through bet-hedging.

Finally, the kin-selection hypothesis – based on Hamilton's (1964) theory – posits that polyandry may be promoted by selection on inclusive fitness benefits gained by females

mating with extra-pair relatives (regardless of the within-pair level of relatedness) when such benefits outweigh inbreeding costs (Kokko and Ots 2006). Studies testing this hypothesis are limited in number and in the amount of taxa targeted (e.g. only 20 out of all cooperative avian species; Wang and Lu 2011). Moreover, to my knowledge, only two empirical studies so far have provided evidence in support of this hypothesis, in barn swallows (*Hirundo rustica*; Kleven et al. 2005) and in Tibetan ground tits (*Pseudopoces humilis*; Wang and Lu 2011). More research is needed to assess this hypothesis.

1.3.2. Non-adaptive models

Non-adaptive hypotheses for the evolution and maintenance of polyandry assume that polyandry is not adaptive, or indeed may be maladaptive, to females but maintained as a by-product of positive selection on traits in the same or opposite sex (Halliday and Arnold 1987; Arnqvist and Kirkpatrick 2005; Forstmeier et al. 2011; Forstmeier et al. 2014). These hypotheses have been given little attention yet and very few studies to date have assessed their validity.

It has been postulated that polyandry evolved under sexually antagonistic selection with traits enhancing male reproductive competitiveness (Jennions and Petrie 2000; Arnqvist and Kirkpatrick 2005). An influential hypothesis posits that the evolution and maintenance of promiscuity in females is favoured by genetic covariance between polyandry and paternity success, in a system where male-male competition occurs. Such covariance could arise via linkage disequilibrium deriving from assortative mating between promiscuous females and successful sires (Keller and Reeve 1995). A recent study (Reid, Arcese, and Losdat 2014) on the socially monogamous song sparrow showed no genetic/phenotypic trade-off between male within-pair and extra-pair reproductive success and estimated a positive genetic covariance between these two reproductive components. This is expected to promote polygyny and may contribute to the evolution of polyandry via indirect selection. Reid et al. (2014) tested this idea and found a positive genetic covariance between female propensity for extra-pair copulations and male within-pair paternity success, but this was not significant. This result is perhaps not surprising, as assortative mating is unlikely to be complete in socially monogamous species, where many within-pair offspring are produced (Forstmeier et al. 2011; Forstmeier et al. 2014).

An alternative hypothesis maintains that the possible genetic covariance between polyandry and male paternity success is due to pleiotropic effects. In this case, alleles promoting

polyandry could be responsible for male traits enhancing paternity acquisition (Halliday and Arnold 1987; Forstmeier et al. 2011; Forstmeier et al. 2014). This hypothesis seems plausible for socially monogamous species, as sexes usually share a similar behavioural repertoire and therefore the same genetic machinery possibly underlies pair bonding and the propensity for extra-pair copulations (Forstmeier et al. 2014).This theory received support from a study on captive zebra finches, which showed high positive between-sex genetic correlation for the propensity for extra-pair copulations (Forstmeier et al. 2011). However, in a natural population of the song sparrow, female propensity for extra-pair reproduction showed a near-zero genetic correlation with male lifetime reproductive success (Reid and Wolak 2018). Moreover, a study in humans failed to find a cross-sex correlation in extra-pair mating, suggesting that the predisposition of women for polyandry was unlikely to result from selection on men (Zietsch et al. 2015).

Another explanation for the evolution and maintenance of polyandry is that this behaviour is genetically linked to female traits under positive selection. In fact, a few recent studies have suggested a link between promiscuity and specific female personality traits, such as aggression (shown in the lizard *Egernia whitii*; Geoffrey M While et al. 2009) and exploratory behaviour (suggested in great tits, *Parus maior*; Patrick et al. 2012). In their work on captive zebra finches, Forstmeier et al. (2011) tested whether polyandry had pleiotropic effects on responsiveness to the social male, a trait that enhances female reproductive success. However, these authors (2011) failed to find such genetic correlation and were unable to validate this hypothesis. Moreover, a study by Reid (2012) also failed to find a genetic correlation between polyandry and two female fitness components in a natural population of the song sparrow.

So, about four decades after the question was posed, the evolution of promiscuity remains an enigma. Work assessing non-adaptive explanations of polyandry has been very scarce and more research is needed to provide any clear evidence of such hypotheses. Despite their high number, studies addressing adaptive explanations have produced contrasting results and this calls for improved work testing these hypotheses. One of the main issues with most of the past studies is their short time-frame. Promiscuity can vary across years due to changing environmental conditions, such as habitat quality (Westneat 1994), and socio-demographic factors, including breeding density (Alexander 1974), breeding synchrony (Birkhead and Biggins 1987; Stutchbury and Morton 1995) and operational sex ratio (Kokko and Rankin 2006). Short-term studies are therefore unlikely to detect any real trends in the occurrence and frequency of this behaviour. Another problem with the majority of past studies is that they work on open systems, where individual movement and dispersal make it impossible

to sample most individuals within a study population. This leads to a significant lack of paternity and survival data, which are crucial in shedding light on the evolution of mating strategies. Long-term studies of isolated populations are therefore needed to accurately address evolutionary hypotheses on promiscuity.

1.4. The Seychelles Warbler – a model system

The Seychelles warbler (*Acrocephalus sechellensis*) is a small insectivorous passerine endemic to the Seychelles archipelago (Fig. 1.1). The population on Cousin Island (29 ha, 04°20′S, 55°40′E) has been monitored since 1985, as part of a long-term study (Komdeur et al. 2004; Wright et al. 2015). The vast majority of birds (nearly 97% since 1997) are ringed with unique colour ring combinations (Richardson et al. 2001), allowing recognition, tracking and sampling of individuals each year, from birth till death. Given that inter-island migration is extremely rare (<0.1%; Komdeur et al. 2004; Komdeur et al. 2016) and individual re-sighting probability per season on Cousin is very high (*ca* 92–98%; Brouwer et al. 2010), individuals that are not found over two consecutive field seasons can be accurately assumed dead. The study system therefore enables accurate estimation of individual survival, reproductive output and parentage, all essential in uncovering evolutionary questions.

Figure 1.1. Adult Seychelles warbler (*Acrocephalus sechellensis*). Photo by Martijn Hammers.

1.4.1. *Infidelity in Seychelles warblers: current knowledge*

Seychelles warblers constitute an interesting system for the investigation of mating patterns and infidelity. Individuals are territorial and facultatively cooperative – one pair of dominant breeding birds occupies each available territory, while sexually mature individuals lacking their own independent breeding opportunity sometimes become subordinates in occupied territories (Komdeur 1992; Richardson et al. 2002; Richardson et al. 2007). This results in approximately 30% (1997-1999) or 50% (2003-2014) of territories on the island being cooperative (Komdeur 1992; Richardson et al. 2002; Richardson et al. 2007; Kingma et al. 2016). In the Seychelles warbler, social mate choice is considered highly constrained by limited habitat availability (resulting from habitat saturation), lifelong social monogamy and long lifespan (Komdeur 1992; Richardson et al. 2005; Wright et al. 2015). A significant portion of young (*ca* 44%) in the population result from fertilisation of females by males other than their social male (Richardson et al. 2001; Hadfield et al. 2006). Clutch size is typically one, but 20% of nests contain one or two extra eggs, usually laid by subordinate females, who are responsible for ca 15% of offspring in the population (Richardson et al. 2001; Hadfield et al. 2006). Almost all paternity is gained by dominant males, with only *ca* 2% of offspring being sired by subordinate males (Richardson et al. 2001; Hadfield et al. 2006), usually those transitioning towards dominant status (H.L. Dugdale, unpublished data). Hence, EPP in this species is almost completely extra-group paternity (EGP), i.e. the result of fertilizations by males outside the group.

Seychelles warbler reproduction is limited seasonally and is energetically expensive with both sexes feeding young for an average of three months after hatching (Komdeur 1991). Therefore, fitness costs resulting from cuckoldry are considerable (Richardson et al. 2001; Hadfield et al. 2006). Males closely mate-guard their social female(s) during the fertile period to reduce the number of extra-pair fertilizations (Komdeur et al. 2007). Given the high energetic costs involved, males adjust their mate-guarding rate to match paternity risk (i.e. the density of neighbouring breeding males; Komdeur 2001). Males are also known to adjust their sperm storage capabilities (via enlarged cloacal protuberance) in relation to EGP opportunities (i.e. neighbouring fertile female density; van de Crommenacker et al. 2004). Three linked studies (Richardson et al. 2004; Richardson et al. 2005; Brouwer et al. 2010) have investigated the evolution of polyandry in Seychelles warblers by addressing the possibility of indirect genetic benefits to females. These studies did not find evidence for infidelity as a mechanism ensuring inbreeding avoidance (Richardson et al. 2004), MHC-disassortative mating or preference for males with an intermediate level of band-sharing (Richardson et al. 2005). However, this research found evidence for genetic benefits in the form of heterozygosity

1

at MHC loci in the paternal genome (MHC diversity; Richardson et al. 2005). This study demonstrated that females were more likely to produce extra-pair offspring when paired with a social male whose MHC diversity was lower than the population average, and that the cuckolding male had higher MHC diversity than the social male. As a result, the extra-group offspring had higher MHC diversity than they would have if they had been sired by the pair male (Richardson et al. 2005).This work indicates that polyandry allows females to acquire more diverse immune genes for their offspring. Following up on this work, Brouwer et al. (2010) confirmed that juvenile (but not adult) survival was positively associated with MHC diversity. This indicates that extra-pair fertilisations conferred an indirect fitness advantage to females paired with low MHC diversity males. However, it is important to note there is no evidence of active MHC-based mate choice by females (Richardson et al. 2005), even in the absence of constraints imposed by restricted territory quality and availability (Wright et al. 2015), so it is still unclear whether the genetic benefits of extra-pair fertilisations resulted from active female choice or post-copulation processes (Richardson et al. 2005; Brouwer et al. 2010; Wright et al. 2015).

1.5. Thesis aims and outline

In this thesis, I aim to investigate several potential drivers of infidelity in the Seychelles warbler. First, I will assess the influence of social, demographic and environmental (socio-ecological) factors on female infidelity (chapter 2). Subsequently, I will address the effect of age, an individual trait which has been linked to patterns of male EPP success across taxa, on both male and female infidelity (chapter 3). Third, I will estimate the heritability of female infidelity to understand whether this trait could have evolved under selection for indirect additive genetic benefits (chapter 4). Finally, I will address a consequence of infidelity in Seychelles warblers, i.e. I will quantify the contribution of EGP to the variance in reproductive success among males. This will allow me to assess whether infidelity increases this variance (and thus the opportunity for sexual selection in the system) beyond that arising from the social mating system (chapter 5).