Comparing social dynamics and telomere attrition between high promiscuity and low promiscuity flocks of zebra finches (*Taeniopygia guttata*)

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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

(Signature) KLmathison .....

Abstract

Extra-pair paternity (EPP) is now recognised as a widespread phenomenon among socially monogamous avian systems, but the factors driving intraspecific variation in extra-pair mating behaviours are still poorly understood. Here, I quantified EPP in two promiscuity breeding lines of the zebra finch (*Taeniopygia guttata*), which had been selected to have high or low breeding values of male sex drive. I found that the majority of birds were involved in extra-pair mating behaviour, and the prevalence of EPP did not differ between the two breeding lines. I present evidence that males can benefit from an increased reproductive output by engaging in extra-pair mating strategies. However, I found no evidence that males reproduced with a larger number of different mates as compared to the females in the population. I also tested the hypothesis that extra-pair mating behaviour could be stressful due to its potential to compromise social pair bonds. To do so, I conducted a within-individual repeated-measures study of telomere attrition across an experimentally-controlled breeding season. Telomere dynamics have become widely regarded as a long-term indicator of cumulative stress and biological age. I found no conclusive evidence that receiving infidelity or experiencing weaker pair bonds induces sufficient physiological stress in zebra finches for it to affect telomere dynamics. I present evidence that in this species, some individuals experienced telomere lengthening while others experienced shortening, with the longest telomeres shortening the fastest. I demonstrate that individuals with stronger social associations produced more eggs together, both within and outside the social pairs (i.e. through EPP). For this reason, I suggest that future research investigating extra-pair mating behaviours should consider the role that the social environment plays in extra-pair reproduction. This could help us gain a further understanding of how social associations influence extra-pair mate selection and the prevalence of EPP within socially monogamous species.

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Chapter one: General Introduction

Abstract

Despite a breadth of research aiming to understand the causes of extra-pair mating behaviour within monogamous systems, relatively little attention has been paid to the potentially maladaptive consequences of Extra-Pair Paternity (EPP). Social pair bonds play an important role in socially monogamous species, but extra-pair mating behaviour has the potential to compromise these bonds and induce a form of social stress. Exposure to chronic stress can have a detrimental impact on health and lifespan due to its negative impact on telomere dynamics. This project investigates the potential impact of pair bond strength and EPP on proxies of stress physiology and fitness in a well-studied avian system, the zebra finch (*Taeniopygia guttata*).

Introduction

The mating system and social environment in which individuals are embedded can affect multiple aspects of their behaviour and fitness (Wan *et al*., 2013; Maldonado-Chaparro *et al*., 2018). An individual's mating behaviours can differ depending on its social environment, such as the timing of their social mate's breeding cycle (Canal *et al.*, 2012; Girndt *et al*., 2018), its social associations to others in the population (Beck *et al.*, 2020) and the strength of pair bonds (Spoon *et al.,* 2006; Forstmeier *et al.,* 2014).

Mating strategy can differ drastically between species due to a range of factors, such as the requirement of parental care (Ball *et al.,* 2017) and predation risk (Yuta and Koizumi, 2015). Mating strategy and mating behaviours can also vary widely within species, but this intraspecific variation is still poorly understood (Petrie and Kempenaers, 1998; Griffith *et al*., 2002; Olivero *et al*., 2017). Understanding the factors influencing this variation is important because it can have substantial consequences for individual fitness (Griffith *et al.*, 2002; Westneat and Stewart, 2003). The prevalence of particular mating strategies within a species can also have broader impacts on the evolution of sexual characteristics and social mating systems (Møller and Birkhead, 1994; Sheldon and Ellegren, 1999; Møller, 2000; Yuta and Koizumi, 2016).

Social monogamy and Extra-Pair Paternity

The development and application of molecular genetic tools has dramatically changed how we view mating systems, particularly in birds. It was previously thought that monogamy was widespread and common in avian mating systems (Lack, 1968; Westneat and Stewart, 2003). However, a substantial proportion of species thought to be socially as well as genetically monogamous have turned out to be sexually promiscuous; some estimates suggest that true genetic monogamy occurs in only 25% of the socially monogamous species studied (Griffith *et al*., 2002). The phenomenon of individuals copulating outside their social pair is termed "extra-pair copulation" (EPC), and the production of offspring from EPCs is termed "extra-pair paternity" (EPP) (Wan *et al*., 2013). Although many earlier studies provided detailed estimates of promiscuity

through observation, the modern study of extra-pair mating strategies is based almost entirely on quantifying rates of EPP using DNA methods (Griffith *et al.,* 2002; Griffith *et al*., 2010; Wan *et al*., 2013). These methods have revealed that EPP is especially common in small passerine birds (Wan *et al.*, 2013). The increased reliability of these methods has allowed us to further investigate how promiscuity plays a role in mating systems and why these behaviours occur.

There has been much theorising about the evolution and benefits of extra-pair copulations, and the potential benefits are not as clear as they first might seem. Generally, males can benefit from extra-pair copulations by increasing their number of genetic offspring, as they do not have the same energetic limitations on producing offspring as females (Selman and Houston, 1995). The benefit to females of mating outside of the social pair is less clear, as it is unlikely to increase the number of offspring they produce, so the possible extra benefits of each copulation partner are most likely relatively small compared to males (Sheldon, 1993; Wink and Drycz, 1999). Female multiple mating was originally considered to be a rare occurrence. However, since genetic studies have revealed its true prevalence, several authors have attempted to pin down the reasons behind this behaviour (Pizzari and Wedell, 2013; Boulton *et al*., 2018).

Several hypotheses have been put forward to understand the potential extra-pair copulation benefits to females, the most prominent being the “genetic benefits hypothesis”, also known as the “good genes hypothesis”. This hypothesis proposes that by engaging in EPCs with males of higher genetic quality than their social mate, females can gain indirect genetic benefits for their offspring via viability genes or genes for attractiveness (Birkhead and Møller, 1992; Krokene *et al.,* 1998). Support for this hypothesis had been found in a number of species, perhaps most notably in studies on blue tits (*Parus caeruleus*). These studies showed that female extra-pair mating behaviour related to male phenotype, with the males successful in obtaining EPCs being on average older with longer tarsi, corresponding to a larger body size (Kempenaers *et al.*, 1992; Kempenaers *et al*., 1997). It was also reported that within nests, extra-pair young were significantly heavier and more likely to be males, supporting the hypothesis that females engage in EPCs to gain indirect

genetic benefits and obtain good genes for their offspring (Kempenaers *et al*., 1997; Jennions and Petrie, 2000; Mingju *et al*., 2017).

Despite the large numbers of studies discussing the "genetic benefits hypothesis", empirical evidence is mixed, and doubts have been raised on the applicability of this hypothesis to the real world (Wan *et al.,* 2013; Forstmeier *et al*., 2014). For example, another study conducted on blue tits found no association between EPP and either male phenotype or offspring body mass (Kempenaers *et al.,* 1992; Kempenaers *et al.*, 1997; Krokene *et al.,* 1998). This demonstrates that the patterns and functions of EPP are likely to differ across different study populations. The authors of the latter study attributed the patterns of EPP they observed, not to females attempting to obtain good genes for their offspring, but instead to females trying to insure against infertility (Krokene *et al*., 1998). There has been increasing interest in the "fertility insurance hypothesis" (Wetton and Parkin, 1991; Hasson and Stone, 2009; Forstmeier *et al.*, 2014). However, in this scenario all females would have to pay the costs of extra-pair copulations, such as increased risk of sexually transmitted diseases (Sheldon, 1993), while only females paired to social males with low-quality sperm would likely gain a reward by increasing their number of viable offspring (Petrie and Kempenaers, 1998; Jennions and Petrie, 2000; Forstmeier *et al.,* 2014).

Thus far, relatively little attention has been paid to potentially maladaptive consequences of extra-pair mating. For example, successful extra-pair sires are often older, but fertilization rate tends to decrease with age and these males often carry an increased risk of *de novo* mutations in the sperm germ line (Johnson and Gemmell, 2012). There is the potential for these mutations to be passed onto offspring, which would mean that extra-pair copulations with these males would be detrimental to females' fitness (Forstmeier *et al.,* 2014). However, a study on blue-footed boobies (*Sula nebouxii*) showed that females can use certain secondary sexual signals as an indication of mate quality to help them avoid pairing with males with oxidative damage to their germline (Johnson and Gemmell, 2012), so perhaps female mate choice is more important in extra-pair copulations than previously thought.

Females may also engage in polyandry not to gain fitness benefits, but instead to limit the costs imposed upon them by harassing males; this is known as 'convenience polyandry' and is thought to occur when resistance to EPCs is more costly than mating (Thornhill and Alcock, 1983; Boulton *et al*., 2018).

Despite the multitude of theories, it is still not clear in what circumstances extra-pair copulations in birds are most likely to occur and to what extent females might reap fitness benefits. We still have little understanding of many of the factors influencing intra-specific variation in EPP and further research is needed to understand the prevalence, drivers and consequences of EPP among socially monogamous species.

Mating strategies in a 'model' species

The zebra finch (*Taeniopygia guttata*) has been, and is likely to continue to be, one of the most widely used avian model systems in evolutionary biology and animal behaviour (Griffith *et al.,* 2017). It has been a key species in the development of our understanding of areas of research such as sexual selection, reproductive investment and sperm competition; the species has even been described as a 'supermodel species' (Griffith and Buchanan, 2010; Griffith *et al*., 2010).
The zebra finch is a highly social small passerine that forms long-lasting, in many cases lifelong, social pairs (Zann, 1996; Mariette and Griffith, 2012). In the wild, it has very low levels of extra-pair paternity (1.7% offspring in 5% of broods), suggesting that this is one of the most genetically monogamous bird species that has been surveyed so far (Griffith *et al.,* 2010).

In its native Australian habitat, this species has no fixed breeding season and birds breed whenever environmental conditions and food resources are most favourable (Selman and Houston, 1996; Zann, 1996). The same holds true in domesticated populations, where birds will breed continuously when food is abundant. Both males and females contribute to nestling rearing and the synchrony in nest visiting has been found to correlate to reproductive output, including factors such as hatching rate and number of offspring (Zann, 1996; Mariette and Griffith, 2012). However, domesticated zebra finch populations are

often very different to the wild type in that they show much higher levels of promiscuity, with EPP rate ranging from 10-30% (Forstmeier *et al*., 2011; Ihle *et al.,* 2013; Maldonado-Chaparro *et al.,* 2018). The difference in observed EPP rates between wild and domesticated zebra finch populations could be caused by social or environmental factors, as well as the artificial selection pressures imposed by aviculturists on over 100 zebra finch generations since their export from Australia (Griffith *et al.,* 2010; Griffith *et al.,* 2017). Although it is thought that life history strategies have remained the same in wild and domesticated zebra finch populations, it is likely that the domestication process has led to behavioural changes that may also influence reproduction and the social system (Tschirren *et al.,* 2009). Further investigation into the differences between populations of the same species, including factors influencing promiscuity, could help to uncover the root cause of extra-pair mating behaviours and their effects on sociality and fitness (Griffith *et al*., 2010).

The importance of pair bonds

Pair bonds play an important role in reproduction, particularly in species that provide parental care. Variation between pairs in reproductive success is often attributed to compatibility between mates and the strength of pairs bonds (Spoon *et al.,* 2006). In species with long-lasting pair bonds such as the zebra finch, pair bond strength may influence pair coordination in providing parental care, which in turn can influence reproductive success (Mariette and Griffith, 2012). Evidence for the importance of pair bonds for reproductive success is provided by other bird species such as barnacle geese (*Branta leucopsis*), where individuals that maintain long-lasting pair bonds over their lifetime produce more offspring compared to those with shorter-term pair bonds; it is thought that the requirement of female-male cooperation during breeding attempts has selected for long-term pair bonds in this species (Black, 2001). Similarly, several studies on cockatiel pairs (*Nymphicus hollandicus*) showed that mates with higher behavioural combability (e.g. behavioural synchrony) showed better coordination of parental care and higher reproductive success (Millam *et al*., 2004; Spoon *et al.,* 2006). Although pair bond duration did not predict behavioural compatibility, pairs that laid eggs did have significantly longer relationships than those pairs that failed to lay eggs.

In zebra finches, like in other species, pair bond strength is thought to increase with pair bond duration (Ihle *et al.,* 2013). Social pairs are thought to maintain strong pair bonds all year round (Ihle *et al.,* 2013). These bonds can be physically costly to maintain, with behaviours such as vocalizations and grooming common after pairs are temporarily separated (Remage-Healey *et al.,* 2003). Further evidence of the importance of pair bonds in zebra finches is provided by the observation that individuals will remain with their bonded mate, even in situations where it may be more beneficial to break the pair bond and find a new mate, such as in cases of egg hatching failure (Ihle *et al.,* 2013). This may be because separation from the social mate has been shown to be stressful for zebra finches (Remage-Healey *et al*., 2003). The latter study showed that stress hormone levels rose during separation from the social mate, and only returned to baseline levels when the original mates were reunited, not when individuals were placed with a new partner.

It is possible that extra-pair mating behaviour might result from a weakness in the social pair bond. It has been suggested that the high levels of EPP reported in domesticated populations of zebra finches is due to the domestication process selecting for weaker pair bonds (Forstmeier *et al.,* 2014). Divorce in this species is rare and costly, particularly in the wild; environmental conditions are highly unpredictable and remaining with a partner speeds up the initiation of reproduction when suitable breeding conditions occur (Adkins-Regan and Tomaszycki, 2007; Crino *et al.,* 2017). However, captive breeding of domesticated zebra finches, which often involves forced pairing and repairing, may have selected for weaker pair bonds and favoured the ability to divorce, so that individuals are able to quickly re-pair and reproduce when separated from their previous partner (Forstmeier *et al.,* 2014).

Stress and telomere dynamics

When investigating the causes and consequences of EPP, focus has often been on reproductive output and fitness (Forstmeier *et al.*, 2014). Few studies have focussed on the effect of extra-pair mating behaviour on the individuals themselves, particularly the physical effects. It is possible that an individual

engaging in extra-pair copulations could have negative impacts on the fitness and physiology of their social partner. As mentioned above, in highly gregarious species like zebra finches, social bonds play an important role. The occurrence of EPP, where these bonds are compromised, could act as a stress-inducing situation. In addition, it is likely that defending social mates from other potential partners looking for extra-pair copulations is a stressful experience. Pair bond challenges by un-paired challenger males has been shown to increase corticosterone levels in monogamous geese (Hirschenhauser *et al.,* 2000).

While the immediate stress response provides significant benefits in the short-term, the stress response may be detrimental and even fatal if activated for the long-term (Monaghan *et al.,* 2011). Long-term over-secretion of glucocorticoids is referred to as 'chronic stress' (Romero, 2004; Monaghan, 2014), and may result in the shortening of telomeres, the repetitive, noncoding sequences of DNA that occur at the ends of chromosomes. There is a well-established relationship between ageing and telomere attrition, with good evidence that telomeres generally get shorter with age (Monaghan, 2010). However, a number of environmental factors have also been linked to accelerated telomere shortening. For example, it has been suggested that the well-established link between chronic stress and poor health in humans is due to the effect of stress on telomeres (Epel *et al*., 2004). The reason for this relationship is that cortisol causes telomere attrition by reducing telomerase activity, therefore slowing telomere repair (Campisi *et al.,* 2001; Choi *et al*., 2008). Indeed, stress-induced telomere attrition has been observed across a number of taxa and the measurement of telomere attrition has been proposed as a method of assessing the lifetime experience of captive animals (Bateson, 2016). The study of telomere dynamics can thus help provide insight into the effect of environmental factors and particular stressors on animals' fitness and physiology.

The potential impact of pair bond strength and EPP on zebra finch physiology and fitness

The aims of my thesis were to investigate sociality and extra-pair mating behaviour in the zebra finch, and to study the potential effects of pair bond

strength and EPP on telomere dynamics. I conducted a within-individual repeated-measures study of telomeres across an experimentally-controlled breeding season to quantify the influence of infidelity and pair bond strength on telomere attrition. I used zebra finches from two captive populations maintained at the Department of Behavioural Ecology and Evolutionary Genetics, MPIO Seewiesen, Germany. The zebra finches were from two different domesticated selection lines, experimentally selected over three generations for either high or low breeding values of male sex drive (rate of courtship in standardized tests: Mathot *et al.*, 2013; Forstmeier *et al.*, 2011; Forstmeier *et al.,* 2014). These were designated high and low promiscuity breeding lines. The project was conducted on site at the Max Planck Institute of Ornithology in Radolfzell, Germany, in collaboration with researchers from the University of Konstanz. My first data chapter investigates variation in extra-pair mating behaviours and the relationship between pair bond strength and reproductive output. My second data chapter tests how pair bond strength and extra-pair mating behaviour link to telomere dynamics in zebra finches.

Together, my findings provide some novel insights into the factors influencing variation in EPP and telomere attrition within a species. This might contribute to our understanding of telomere dynamics and the role that social environment, in particular pre-breeding social associations, plays in the prevalence of EPP within socially monogamous systems.

Chapter two: Sociality and extra-pair mating behaviour

Abstract

Extra-Pair Paternity (EPP) is a common occurrence among monogamous bird species, yet we still have a limited understanding of the factors that cause variation in an individual's propensity to engage in extra-pair mating behaviours. This study focusses on the social and reproductive behaviour of the socially monogamous zebra finch (*Taeniopygia guttata*), a passerine that forms long-term pair bonds. Wild populations show lower levels of EPP than their derived domesticated populations, and the latter also vary in EPP rates. However, the drivers and consequences of this variation in EPP remain unclear. We examined the variation in promiscuity and reproductive output between captive zebra finches from two domesticated breeding lines, selected for high or low breeding values of male sex drive. Contrary to our predictions, we did not find significant evidence of variation in promiscuity between the two breeding lines. We discuss how the measure of infidelity our study used, rate of EPP, may not provide us with the whole picture of the mating behaviours occurring in a population. Looking at individual-level differences, our findings support previous theory on sex-based variation in mating strategy; males benefitted more from EPP in terms of reproductive output, despite both sexes showing similar levels of promiscuity. The social environment in which an animal is embedded may also influence patterns of reproduction and EPP within socially monogamous populations. Previous research has had difficulty clarifying the influence of pair-bond strength on reproductive output due to confounding factors, such as individual age and bond duration, which are known to influence reproduction. The design of our study system enables us to clearly present further evidence that pair-bond strength, separate from other factors, is a strong predictor of a pair's reproductive output. Examining the influence that pair bond strength can have on reproductive behaviour could help to explain the variation in reproductive success and rates of EPP within other species.

Introduction

Mating systems are characterised by the reproductive biology, social behaviour and mating strategies of the individuals within them. In turn, the social environment and mating system an individual is embedded in can have a major influence on its mating behaviour and reproductive output (Maldonado-Chaparro *et al.,* 2018). It was initially thought that in birds, monogamy was the most widespread and common mating system. In a review of avian breeding systems, Lack (1968) declared that more than 90% of the 9000+ species reviewed were monogamous, a viewpoint which remained prominent for several decades (Westneat and Stewart, 2003). Within the framework of socially monogamous systems, several mating strategies exist to try and maximise individual fitness. Although many species reap the benefits of monogamy, mainly in the form of higher reproductive output and faster initiation of reproduction, we now know that genetic monogamy is not as prevalent as it once appeared (Black, 2001; Adkins-Regan and Tomaszycki, 2007). Monogamy imposes constraints on mate choice which can be overcome by engaging in Extra Pair Copulations (EPCs) (Møller, 2000). Genetic studies have revolutionised our understanding of avian mating patterns and it is now apparent that Extra Pair Paternity (EPP), which results from mating outside the social pair, is a widespread phenomenon among monogamous avian systems. Parentage studies have revealed that in more than 70% of bird species, there are offspring sired by a male other than the social father (Griffith *et al.,* 2002; Westneat and Stewart, 2003).

The frequency of EPP varies hugely among species, populations and individuals. A large body of research has been dedicated to understanding what factors drive this variation and the conditions under which extra-pair mating behaviour may be adaptive (Petrie and Kempenaers, 1998; Griffith *et al.,* 2002; Westneat and Stewart, 2003; Yuta and Koizumi, 2016). Variation in extra-pair mating behaviour may arise from variation in the relative costs and benefits of this mating strategy between species (Westneat and Stewart, 2003). The benefits to males of engaging in extra-pair reproductive behaviour seem to be clear; given that sperm is relatively cheap to produce, males can try and

increase their reproductive success through EPCs (Petrie and Kempenaers, 1998). The costs to males appear to vary depending on the social system, but often time spent seeking extra pair copulations could leave female partners unguarded and potentially lead to within-pair paternity loss (Freeland *et al.,* 1995; Petrie and Kempenaers, 1998).

Uncovering the adaptive benefits of EPP in females has been more complicated. Unlike in males, engaging in EPCs does not necessarily increase a female's reproductive success, i.e. her number of offspring (Wink and Drycz, 1999; Forstmeier *et al.,* 2014). The main exception to this is if a female has a social male with low-quality sperm leading to unviable or low-quality within-pair offspring, then she may be able to increase her number of viable offspring through EPP (Petrie and Kempenaers, 1998; Jennions and Petrie, 2000).

In general, females in monogamous mating systems are thought to suffer direct costs of infidelity (Westneat and Stewart, 2003). The main potential cost to females of engaging in EPCs is thought to be the retaliatory withholding of parental care by her social mate (Birkhead and Møller, 1992; Arnqvist and Kirkpatrick, 2005). This idea is supported by the empirical finding that when males are less certain of their paternity, they provide less parental care (Møller and Birkhead, 1993; Perlut *et al.,* 2012). We would expect species where male parental care is important to show lower levels of EPP, as it would be costly for females to potentially jeopardise the parental care given by their partner by engaging in EPCs (Møller, 2000; Arnold and Owens, 2002; Westneat and Stewart, 2003). EPP does appear to be particularly common when the male only plays a small role in parental care and the successful rearing of offspring (Møller, 2000). For example, the importance of biparental care for reproductive success in woodpeckers is thought to be an important reason for their predominant social and genetic monogamy (Pechacek *et al.,* 2005). Similarly, a comparison between three species of penduline tit (Remizidae) showed that the Eurasian penduline tit (*Remiz pendulinus*) displays low levels of male parental care and high levels of EPP; in contrast, the other two species exhibit biparental care and high levels of fidelity (Ball *et al.,* 2017).

It has been suggested that females engaging in EPCs balance the cost of potential loss of parental care through indirect fitness benefits, namely increasing offspring fitness via genetic gains (Jennions and Petrie, 2000). The "good genes hypothesis" suggests that the good genes of an extra-pair mate could be passed onto offspring, increasing their chance to obtain higher survival and reproductive success (Wink and Drycz, 1999). According to this theory, if males in a population vary in genetic quality, females paired to lower quality males will benefit from mating with a higher quality extra-pair male (Birkhead and Møller, 1992; Petrie and Kempenaers, 1998). However, there is no consensus on whether females engaging in this strategy are in reality rewarded with increased offspring fitness, and whether EPP is overall detrimental to females continues to be debated (Griffith and Montgomerie, 2003; Arnqvist and Kirkpatrick, 2005; Wan *et al.,* 2013; Forstmeier *et al.,* 2014).

Several factors have been investigated for their potential role in driving interspecific variation in EPP. One of these factors is the species-specific adult annual mortality rate. Several studies have argued that EPP rate should be higher in species with higher adult mortality (Mauck *et al.,* 1999; Wink and Drycz, 1999; Arnold and Owens, 2002). By compiling data for over 100 avian species, Wink and Drycz (1999) showed that long-lived avian species where both members of a social pair are likely to survive to breed the next year, had significantly lower EPP rates. This has been attributed to the idea that by engaging in EPP, individuals of these species would have an increased risk of retaliation in the form of reduced parental care or divorce in future breeding attempts (Arnold and Owens, 2002). Breeding synchrony is another factor that may lead to high EPP rates, because it may allow females to more easily compare the quality of potential mating partners (Westneat and Stewart, 2003; Ferretti *et al.,* 2019). This hypothesis was tested by comparing several species of swallow in the genus *Tachycineta*, in which the percentage of nests containing extra-pair young varies widely from 13 to 87% between species (Ferretti *et al.,* 2019). However, the study found that this variation in EPP was not explained by differences in breeding synchrony. Indeed, recent work has suggested that several factors previously thought to be important drivers of promiscuous behaviour, such as breeding synchrony and population density, do not affect EPP as consistently or strongly as first proposed (Westneat and

Stewart, 2003; Yuta and Koizumi, 2016; Ferretti, 2019). This emphasises the need for continued investigation into what factors are driving variation in this mating strategy.

In addition to variation in EPP between species, there is also a surprisingly large amount of variation in extra-pair mating behaviour between populations of the same species (Petrie and Kempenaers, 1998). For example, one study found 0% extra-pair offspring in willow warblers (*Phylloscopus trochilus*) (Gyllensten *et al.,* 1990) whilst another study reported that 50% of all broods in a different population contained extra-pair offspring (Petrie and Kempenaers, 1998). Within a socially monogamous species there may be significant variation in mating strategies and reproductive behaviours between individuals (Griffith *et al.,* 2002). As the frequency of EPP is a population-level variable that results from interactions and behaviours at an individual level (e.g. occurrence of EPCs), uncovering why individuals engage in this behaviour is key to understanding population differences (Petrie and Kempenaers, 1998; Westneat and Stewart, 2003).

The social environment in which an animal is embedded may influence extra-pair mating decisions and patterns of EPP within socially monogamous populations (Maldonado-Chaparro *et al.*, 2018). Monogamous mating systems are typically characterised by the presence of stable breeding pairs within the population. These pairs are defined as the association of reproductively mature pairs of adults of opposite sexes, and their relationships are also referred to as pair bonds (Shultz and Dunbar, 2010; Maldonado-Chaparro *et al.,* 2018). Forstmeier *et al.* (2014) suggested that extra-pair mating behaviour might result from a weakness in the pair bond. They attributed the variation in EPP observed in domesticated populations of the socially monogamous zebra finch to a weakening of pair bonds in those populations with higher infidelity rates, potentially caused by captive breeding and selection for weaker pair bonds during the domestication process. Examining the influence of pair bond strength on reproductive behaviour at an individual level could thus help us untangle the influence of social factors on rates of EPP at the population level.

Pair bond strength may also be an important driver of a breeding pair's reproductive output. In most monogamous avian species pair bond strength is thought to increase with pair bond duration (Ihle *et al.,* 2013). Long-term and strong pair bonds have been associated with higher reproductive success across a number of avian species, including barnacle geese (*Branta leucopsis*) (Black, 2001), short-tailed shearwaters (*Puffinus tenuirostris*) (Bradley *et al.,* 1995) and bearded reedling (*Panurus biarmicus*) (Griggio and Hoi, 2011). This increased success has been attributed to a number of aspects, such as faster initiation of breeding and increased male-female coordination of parental care (Fowler, 1995; Black, 2001; Griggio and Hoi, 2011). However, previous observational studies have had difficulties untangling the impact of pair bond strength from other factors such as age (Fowler, 1995). Isolating the effect that pair bonds have on reproductive output could help explain inter-individual variation in reproductive success within a population.

This study focusses on the social and reproductive behaviour of the zebra finch (*Taeniopygia guttata*). This species is a highly gregarious, socially monogamous passerine that forms long-term, often lifelong, pair bonds (Zann, 1996; Mariette and Griffith, 2012). Zebra finches are a good model with which to investigate factors influencing mating behaviour for several reasons: Firstly, the social environment, particularly pair bonds and biparental care, play an important role in their reproductive success and secondly, several studies have reported distinct differences in the frequency of extra-pair mating behaviours between different study populations (Mariette and Griffith, 2012; Ihle *et al.,* 2013; Griffith *et al.,* 2017). Captive zebra finches typically have not been bred with the intention of preserving genetic diversity or maintaining natural behaviour (Griffith *et al.,* 2017). As a result, wild populations differ from captive, domesticated ones in the rate of EPP; in the wild, zebra finches have very low levels of EPP, whereas populations of domesticated zebra finches have much higher levels of promiscuity (Griffith *et al*., 2010; Ihle *et al.,* 2013). Various domesticated populations also show differences in EPP rates, which may be partly due to genetic differences between them (Forstmeier *et al*., 2007a; Griffith *et al.,* 2017).

In this study, we examined the variation in extra-pair mating behaviour and reproductive output between captive zebra finches from two domesticated breeding lines; a 'high promiscuity' line and a 'low promiscuity' line. Birds in these lines were selected for high or low breeding values of male sex drive (Forstmeier *et al.,* 2011). These groups were studied across the course of an experimentally induced breeding season, in an arrangement that enabled us to quantify the potential impact of social, genetic and environmental variables on the birds' reproductive behaviour.

To check that the high and low promiscuity breeding lines showed the expected differences in EPP levels (Forstmeier *et al.,* 2011), we tested whether birds from these two lines differed in their number of social mates versus the number of extra-pair mates contributing genetic material to the eggs (i.e. 'genetic mates'). We predicted that birds from the high promiscuity line would have weaker pair bonds, higher rates of EPP and a larger number of different genetic mates as compared to birds from the low promiscuity line. For the high promiscuity line, we also expected that the number of eggs an individual produced as an extra-pair partner would have a greater impact on their overall reproductive output. We predicted that within breeding lines, individuals with a stronger pair bond to their breeding partner would have higher within-pair reproductive output. Also, we predicted that males would receive a larger benefit to their reproductive output from EPP.

This study used distinct populations of zebra finch to examine whether differing selection on promiscuity translates to variation in extra-pair mating behaviours. We also investigated the effect that social environment, in this case pair bonds, have on mating behaviour and reproductive output in this species. We predicted that the relationship between pair bond strength and EPP rate would be most prominent at the population level, with generally higher EPP rates and weaker pair bonds strengths in the high promiscuity breeding line.
However, as pair bond strength will differ between pairs within a population, we also examined whether there was a similar relationship present at an individual level.

Methods

Study site and test subject

We conducted this study on site at the Max Planck Institute of Ornithology in Radolfzell, Germany, in collaboration with researchers from the University of Konstanz. The project used zebra finches (*Taeniopygia guttata*) from two captive populations maintained at the Department of Behavioural Ecology and Evolutionary Genetics, MPIO Seewiesen, Germany. The zebra finches were from two different domesticated selection lines. Birds from the domesticated lines probably go back to imports from about 1880 (Forstmeier *et al.,* 2007a), corresponding to roughly 100 generations of domestication. In this species the rate of EPP ranges from 2% in the wild to 10-30% in captivity (Forstmeier *et al*., 2011; Ihle *et al.,* 2013; Maldonado-Chaparro *et al.*, 2018). The birds from the domesticated population were experimentally selected over three generations for either high or low breeding values of male sex drive, which differed by approximately two phenotypic standard deviations in courtship rate (rate of courtship in standardized tests: Mathot *et al.,* 2013; Forstmeier *et al*., 2011; Forstmeier *et al.,* 2014). These were designated high and low promiscuity breeding lines. Although the breeding lines were selected on male sex drive, we still investigated the difference in promiscuity between the breeding lines for both sexes. Research on a related population of zebra finch showed that there was a genetic component to extra-pair mating behaviour (Forstmeier *et al.,* 2011). The authors proposed that in a captive environment, selection on males to produce extra-pair offspring will also lead to an increase in extra-pair mating behaviour in females. In accordance with this theory, we predicted that there would be a difference in the number of partners and individual rates of promiscuity between the different breeding lines, with this difference being present for both sexes. Surprisingly, the rates of EPP for these selection lines appear not to have been estimated before.

At the start of the experiment, all test subjects were between 11 and 15 months old and sexually naive. The birds were raised in mixed-sex aviaries until sexual maturity and then kept in unisex aviaries, split by breeding line, until the start of the experiment. Therefore, males and females may have been familiar with

each other before the start of our experiment, but it is unlikely that they would have formed social pair bonds before they were sexually mature. The mass (in grams) of each bird was measured before release into the aviary complex. We separated the birds into four aviaries with 14 of each sex in each aviary. Each of the selection lines were split across two aviaries, one on each side of the complex (Figure 1). Each bird was individually identified with several unique tags. We used a unique combination of coloured leg rings (black, pink, blue, red, white, green, yellow and violet) with one ring on each leg, which could be easily seen at a distance. Each bird also carried a metal numbered leg ring that was used to double-check the bird's ID during handling and collection of blood samples. All birds were also fitted with a small paper 'backpack' that contained a Passive Integrated Transponder (PIT) tag and a QR code (Figure 2) (Alarcón-Nieto *et al*., 2018).

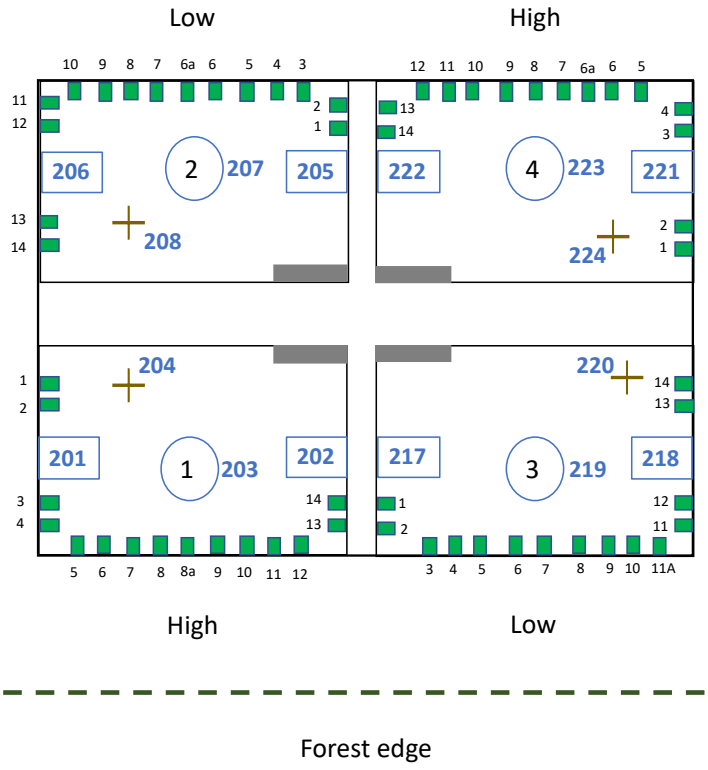

Figure 1: *Diagram of the aviary complex displaying the layout of the aviaries. Birds were housed in large outdoor aviaries (4 x 3 x 2.3 m high), with two per selection line. Aviaries were equipped with 15 nest boxes each, represented here as the small green rectangles). The centre of each aviary contained a feeding table (represented here as circles) where two feeders provided mixed finch seed ad libitum. Social perches were attached on the right and left side of the aviaries, represented by the larger rectangles in the figure. The mating perches are represented by crosses, with one located in the corner of each aviary. Raspberry Pi cameras are represented by numbers ranging from 201 - 224.*

The aviary complex was located on the edge of a forest (Figure 1) in a rural location, with two of the four aviaries adjacent to the forest edge. Each of the four aviaries contained a feeding table with two feeders, two social perches and a mating perch. The social perches were the main area for the birds to sit and interact as a group. Mating perches, designed to replicate a tree with branches, were located in the inner corner of each aviary (Figure 1). This was the place where birds most commonly performed mating dances.

After an adjustment period of three weeks where the birds were left to socialise, 14 nest boxes and an unlimited supply of nesting material in the form of coconut fibres were introduced to each aviary. Two weeks after the initial addition of the nest boxes, another nest box was added to aviaries, bringing the total count of nest boxes up to 15. The reason for this was the observed need for extra nesting space; in two of these aviaries there had been cases of birds attempting to build nests at random places including the floor and the box containing nesting material. The introduction of nest boxes and nesting material signalled the start of the breeding season which lasted three months, from July to September 2017.

Figure 2: *Methods of individual identification. Left: zebra finch fitted with backpack showing unique QR code on top and coloured leg band on the left leg. Centre: Circular RFID antennae fitted to the entrance of a nest box. Right: A social perch.*

Breeding data

Every weekday nest-checks were conducted, in which we quantified the number of coconut fibres in each nest and checked the nest box for eggs. When a new egg was found, it was removed from the nest and replaced with a dummy egg. We labelled each egg and its corresponding dummy egg with the date that it was laid, the aviary and nest box it came from, and what number in the egg

laying sequence of the clutch it was (e.g. first, second). Eggs were removed so that they could be externally incubated for five days to allow them to continue developing in a controlled environment, meaning there would be as much embryonic tissue as possible for DNA and parentage analysis. The dummy eggs were used as a method to reduce the chance that the birds would try to keep producing eggs to maintain the clutch number. Birds of many species will replace eggs that are lost during laying and studies on species including lesser black-backed gulls (*Larus fuscus*) have shown that when an egg is removed from a clutch, the female will lay an extra egg to complete their normal clutch size (Monaghan *et al.,* 1995). The breeding pattern for zebra finches in captivity is to lay an egg every day for 4-5 days. The effort required to obtain the necessary resources for egg laying may be considerable and producing a larger number of eggs than expected could potentially have negative impacts on parental fitness (Monaghan and Nager, 1997).

Use of the dummy eggs also allowed us to monitor the amount of time between eggs being laid and the start of incubation for the clutch (determined by whether the dummy eggs were kept warm to the touch). The dummy eggs were kept in the nests 10 days after the first egg in that clutch was recorded to start being incubated. This corresponded to approximately 5 days of egg laying and 5 days of just incubation. Once these 10 days had passed, all eggs in the clutch were removed and this signalled the experimental end of a clutch. Keeping the dummy eggs in the nest for this time ensured that the clutch had been completed and that there was a designated incubation period between the production of consecutive clutches. Our breeding season lasted three months, during which most pairs laid several clutches.

Once the eggs had been externally incubated for the required time, they were removed from the incubator on the fifth day before the embryos reached the size and stage of development that classifies them as living animals (Animals (Scientific Procedures) Act 1986, 1990). The next stage was to extract the embryos. Embryos were removed by dissecting the egg and separating the tissue from other egg components such as yolk. The embryo tissue was stored in an Eppendorf with 0.5ml of alcohol for later parentage analysis.

Pair identification

Throughout the breeding season there was a two-step approach to identifying the breeding pairs. Radio-frequency identification (RFID) antennae were fitted to the entrance of the nest boxes, set to record the PIT tags of the birds entering and leaving the nest boxes (RFID logger board, Priority 1, Australia; Figure 2). The antennae were calibrated so that they would only register PIT tags moving through the hoop surrounding the nest box entrance, rather than tags just in the general vicinity of the nest boxes. There was an unforeseen limitation on the number of antennae available for the project, so there were not enough to fit one to each nest box. To compensate for this, the antennae were rotated between nest boxes on a daily basis to provide a good coverage of nest box visitation during the breeding period.

These data were supplemented by daily “live” observations of aviaries by the experimenters. Observations aimed to identify which pairs were occupying given nest boxes. They initially provided confirmation that the RFID system was working correctly and that it was picking up the individuals that we would expect based on which individuals we had seen at certain nest boxes. Daily observations across the breeding season also acted as a backup for the occasions where certain nest boxes were not being monitored by the RFID antennas due to the antenna shortage. Every time a bird left or returned to a nest box, we recorded its identity via notation of the bird’s sex and leg band colours. Additional observations of the mating perches helped us to identify social pairs and gave insight into potential extra-pair copulations. We observed each aviary daily from Monday to Friday for 15 minutes per aviary between 8 – 9.30 AM. We observed each aviary for 30 min each on Saturdays and Sundays, as on these days no nest checks were conducted and the birds were less disturbed by experimenter activity. Using these methods, we were able to record the identity of the individuals in each pair. We also allocated ‘social parents’ to each clutch of eggs, defined as the breeding pair which were occupying the nest box when each clutch was laid.

Pair bond strength

To quantify social pair bond strength, each aviary contained four Raspberry Pi Cameras in standardised locations: one above each of the two social perches, one above the two feeders and one above the mating perch (Figure 1). Each Raspberry Pi camera took a photograph of the designated section of the aviary every two seconds during daylight hours (i.e. when light levels were sufficient). These photographs captured the QR codes on the backpacks of each bird (Figure 2). The photos were converted into videos and an automatic detection system, custom-written by Jacob M Graving and made using PinPoint library in Python, was used to analyse the videos and register the QR codes present in each frame (Alarcón-Nieto *et al.,* 2018; Graving *et al.*, 2019). From this system we could extract information on the identity of individuals and their proximity to others in their aviary. We determined which birds were clumped together by using data on the identity of each bird, its position (x,y coordinates) and its orientation in the video frame. We defined clumping behaviour, where two birds perch in body contact, as being when two birds were detected at a distance of <8 pixels. These QR code data were used to quantify social metrics at the pair level during the pre-breeding time period (i.e. the 3 weeks before nesting materials were introduced) when the pair bonds were forming. Using a simple association index we then calculated the association strength between each pair of individuals as the probability of observing that pair clumping together at any given time, given that either one was detected in frame (Farine and Whitehead, 2015). These data were used to quantify the pre-breeding pair bond strength for each pair: a measure of the propensity of a pair of individuals to synchronise their clumping behaviour.

Parentage and measuring EPP

The genetic parents for each egg were determined through DNA parentage analysis. This analysis was conducted at the MPIO in Seewiesen using 15 microsatellite markers (Forstmeier *et al*., 2007b). This was combined with the observational data on pair identification to create measures of extra-pair paternity for each individual. In cases where the genetic parents of an egg differed from the social parents, this indicates infidelity and extra-pair paternity.

The measure of promiscuity used in the analyses was the amount of infidelity received by an individual. In females this was defined as the number of eggs fathered by a female's social male in a different female's clutch. In other words, the eggs shared the father but not the mother with the rest of the clutch. For males it was defined as the number of eggs in a male's clutch that were produced by his social female but were from another genetic father.

Data analysis

Statistical analyses were run using R Studio/R version 3.5.2 and packages *lme4* to run models, *sna*, *igraph* and *asnipe* for social network analysis and *ggplot2* to produce graphics.

In order to determine whether birds from the high-promiscuity breeding line had a larger number of genetic and/or social mates as compared to birds from the low-promiscuity breeding line, we ran two separate generalised linear mixed-effect models (GLMMs) (Poisson family). For the first, the response variable was number of genetic mates, defined for each bird as the number of individuals with whom they produced at least one egg. Fixed effects were breeding line and sex, including the interaction between these two factors. Mass was also included as a fixed effect, as mass is often an indication of size and thus potentially individual quality, which in turn may affect mate choice or reproductive output.

The aviary that each bird was located in was included as a random effect, to account for the separation of the birds into four separate breeding populations. We also included forest proximity as another random effect, to account for the potential influence that aviary location could have had on reproductive output (Figure 1); it is possible that the two aviaries located closest to the forest edge may have been shielded by the forest, as they appeared to be slightly cooler in temperature. The birds in these aviaries also appeared to have a stronger negative reaction to human presence, possibly because they were less exposed to everyday human noise (personal observations), or because they were generally more vigilant due to the potential presence of predators in the forest.

For the second GLMM (Poisson family), the response variable was number of social mates, to assess whether birds from the high-promiscuity breeding line

switched social mates more often throughout the breeding season as compared to birds from low-promiscuity line. This model had the same fixed and random effects as the first.

Another GLMM (Poisson family) was used to explore if birds from the high-promiscuity breeding line received more infidelity than birds from the low-promiscuity breeding line. The response variable was “received infidelity”. For females this was defined as the number of eggs fathered by a female’s social male in a different female’s clutch. For males it was defined as the number of eggs in a male’s clutches that were produced by his social female but were from another genetic father. In this model the fixed effects were promiscuity line, sex and mass. Aviary and forest proximity were included as random effects.

To investigate how performing infidelity affected reproductive success, we assessed the relative contribution that both breeding within the social pair and breeding outside the social pair (i.e. EPP) made to an individual’s overall reproductive output. A GLMM (Poisson family) was created in which the response variable was the number of genetic eggs an individual produced. Fixed effects were the number of social eggs associated with that individual (i.e. eggs that were located in nests which that bird occupied but to which the bird was not necessarily genetically related) and “infidelity made” (the number of extra-pair eggs an individual produced). The interaction between sex and infidelity made was included as a fixed effect to test for potential sex-based differences in mating strategy and reproductive benefits of EPPs. Promiscuity line was also included as a fixed effect, with aviary and forest proximity as random effects.
The data were then split according to sex, and the same model was run for females and males separately with the fixed effect of sex omitted. This was done to further investigate potential sex-based differences in the influence of fixed effects, particularly regarding potential sex-based differences in the reproductive benefits of EPPs.

We examined whether a breeding pair’s pre-breeding bond strength affected the number of eggs they produced together over the breeding season, by using a GLMM (Poisson family). The model included data on each pair that had

produced eggs together at any time during the breeding season and only included females to avoid data point duplication (as the two birds in each pair had the same pair bond strength). For each female, we included her bond strength to every male that she bred with (i.e. produced genetic eggs with) and the number of eggs they produced together. So, a female could be in the analysis twice if she produced eggs with two males, once with the eggs produced with her social mate and their bond strength, but also another time with the eggs produced with an extra-pair male and her bond strength to that extra-pair male.

The number of eggs produced within the pair was used as the response variable. Pair bond strength and mass were tested as fixed effects, with forest proximity and aviary as random effects. Visualisation of the data identified a potential outlier for pair bond strength (>0.3) which appeared to be considerably higher than the rest of the dataset (values <0.2). To identify influential points in the dataset, we calculated Cook's Distance for each observation in the dataset and then plotted these distances to see which observations were larger than the traditional threshold of 4/n: The single value of pair bond strength >0.3 was identified as a potential outlier (Cook's Distance = 0.53) and the model was run for a second time with the potential outlier removed to compare the results.

For each of the models, important factors were isolated through model reduction and simplification. We started with fitting the maximal model which included all factors, interactions and variables of interest. Then we inspected parameter estimates and did stepwise removal of least significant terms, starting with high order interaction terms, then low order interaction terms and finally main explanatory effects. This was done until a minimum adequate model (MAM) for significant fixed effects ($p<0.05$) was reached.

Ethical note

All experimental procedures were approved by University of Exeter Biosciences Ethics Committee.

Results

Reproductive output

Over the course of the breeding season, the zebra finches in our study laid a total of 514 eggs. Each aviary had several individuals whose genetic and social egg counts were zero. This indicates that both promiscuity lines contained individuals who did not engage in reproduction during the breeding season. The high promiscuity line had higher means for all of our measures of reproductive output (Table 1). However, it is important to note that these means, particularly for the number of genetic and social eggs, have large standard deviations. For both promiscuity lines, the mean number of genetic eggs and genetic clutches were higher than the mean number of social eggs and social clutches, indicating the occurrence of EPP (Table 1).

Table 1: *Reproductive output in our study populations of zebra finches, split by promiscuity breeding line and sex. For each promiscuity line we present the mean, SD and range for; number of genetic eggs (i.e. overall number of eggs produced), number of social eggs (i.e. eggs within social clutches), number of genetic clutches (i.e. number of clutches containing genetic eggs) and number of social clutches (i.e. only clutches produced within social pairs).*

Variable	***High promiscuity line***				***Low promiscuity line***			
	Male		*Female*		*Male*		*Female*	
	μ ± SD	*Range*	*μ ± SD*	*Range*	*μ ± SD*	*Range*	*μ ± SD*	*Range*
Number of genetic eggs	10.1 ± 8.3	0 - 27	10.1 ± 4.9	0 - 18	8.2 ± 7.7	0 - 29	8.5 ± 4.0	0 -16
Number of social eggs	10.1 ± 8.1	0 - 27	7.9 ± 6.3	0 - 18	7.9 ± 7.6	0 - 28	8.6 ± 5.6	0 - 20
Number of genetic clutches	3.9 ± 3.3	0 - 12	3.3 ± 1.4	0 - 5	3.5 ± 3.1	0 - 12	3.3 ± 1.4	0 - 5
Number of social clutches	3.2 ± 2.3	0 - 7	3.0 ± 1.5	0 - 5	2.5 ± 2.3	0 - 8	2.7 ± 1.3	0 - 4

Do birds from different breeding lines have a different number of social or genetic mates?

In contrast to our predictions, individuals from the high promiscuity breeding lines (n = 56) and low promiscuity breeding lines (n = 55) appeared not to differ in the number of genetic mates they produced eggs with: number of genetic mates was not predicted by the interaction between promiscuity line and sex (slope estimate ± s.e. = 0.201 ± 0.283, z = 0.712, p = 0.477, Figure 3), promiscuity line (slope estimate ± s.e. = -0.090 ± 0.146, z = -0.615, p = 0.539), sex (slope estimate ± s.e. = 0.134 ± 0.141, z = 0.949, p = 0.343), nor mass (slope estimate ± s.e. = 0.068 ± 0.050, z = 1.365, p = 0.172).

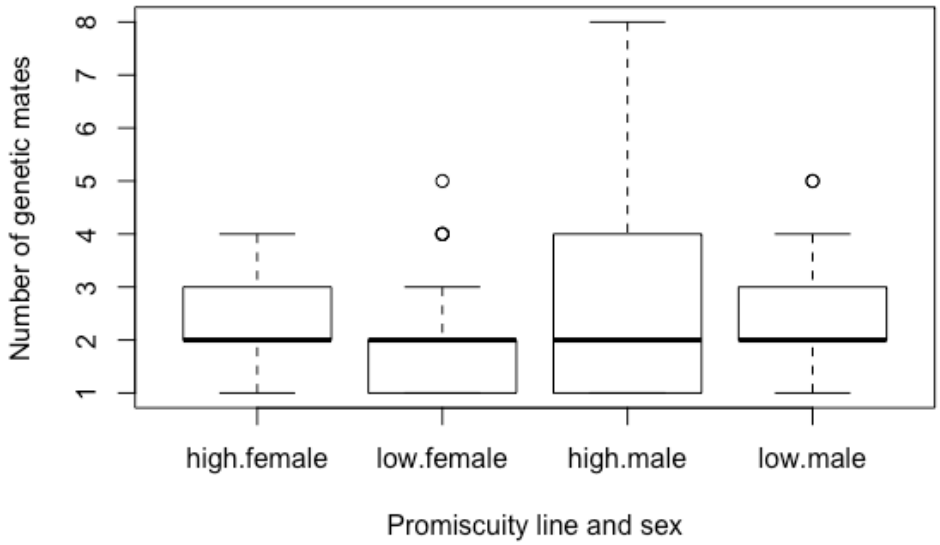

Figure 3: *The number of individuals that birds of different sexes and promiscuity lines produced eggs with across the breeding season. Boxplots show the median, upper (Q3) and lower quartiles (Q1) and the interquartile range (IQR = Q3 − Q1). Whiskers represent maximum and minimum values, excluding outliers. Outliers are represented as circles.*

Similarly, individuals from the high and low promiscuity lines did not differ in the number of social mates: the interaction between promiscuity line and sex was not a significant predictor (slope estimate ± s.e. = 0.128 ± 0.335, z = 0.381, p = 0.703, n = 111) and neither was promiscuity line (slope estimate ± s.e. = -0.090

± 0.146, z = -0.615, p = 0.539). Both sex (slope estimate ± s.e. = 0.134 ± 0.141, z = 0.949, p = 0.343) and mass were also insignificant (slope estimate ± s.e. = 0.068 ± 0.050, z = 1.365, p = 0.172).

Do birds from different breeding lines receive different levels of infidelity?

There was no significant difference in received infidelity (i.e. lost parentage of eggs) between birds from the high promiscuity line (n = 49) and those from the low promiscuity line (n = 46) (slope estimate ± s.e. = 0.462 ± 0.560, z = 0.825, p = 0.409). We found that individuals with higher mass received more infidelity (slope estimate ± s.e. = 0.231 ± 0.079, z = 2.941, p = 0.003, Figure 4). There was also no difference depending on sex (slope estimate ± s.e. = -0.110 ± 0.231, z = -0.475, p = 0.635).

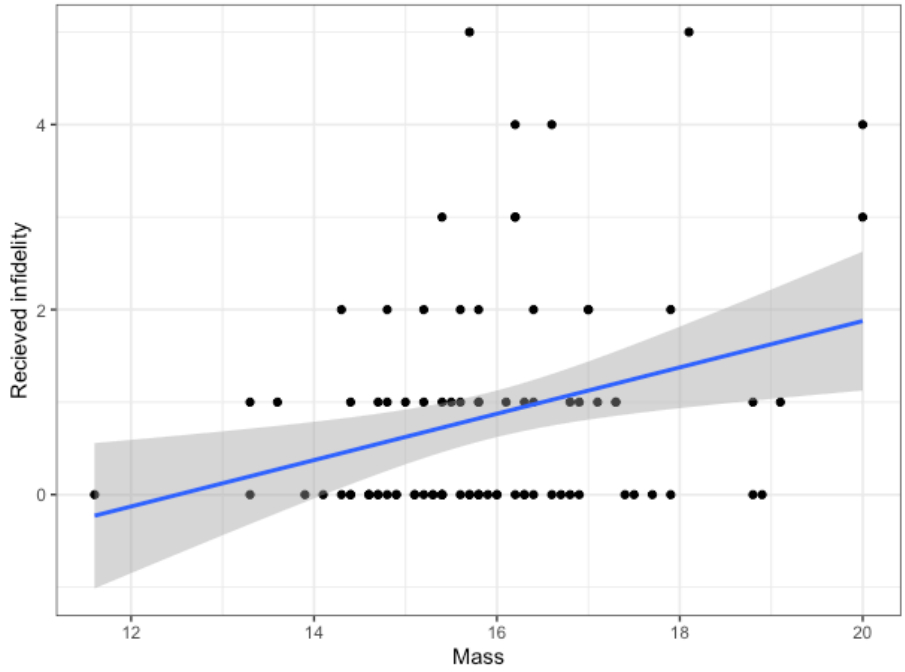

Figure 4: *Cases of received infidelity (i.e. lost parentage of eggs) according to that individual's mass (grams). Each dot represents an individual bird, the straight line indicates the linear regression line as estimated by the model, and the grey ribbon indicates the 95% confidence interval.*

How does performing infidelity affect reproductive success?

By themselves, neither performed infidelity nor sex had a significant effect on the number of genetic eggs an individual produced (males: n = 56, females: n = 55) (performed infidelity, slope estimate ± s.e. = -0.038 ± 0.042, z = -0.899, p = 0.369; sex, slope estimate ± s.e. = -0.136 ± 0.084, z = -1.612, p = 0.107). However, the interaction between performed infidelity and sex had a significant effect on reproductive output (slope estimate ± s.e. = 0.119 ± 0.047, z = 2.518, p = 0.012, Figure 5). The number of social eggs produced within a social pair also had a strong positive effect on the overall number of genetic eggs an individual produced, meaning that a large proportion of eggs were produced within social pairs rather than by EPCs (slope estimate ± s.e. = 0.075 ± 0.005, z = 14.554, p <0.001). The number of genetic eggs produced was not affected by promiscuity line (slope estimate ± s.e. = -0.029 ± 0.066, z = -0.437, p = 0.662).

To investigate the interaction between performed infidelity and sex on reproductive output, the model was then run separately for each sex.
Males with a higher number of EPP eggs produced more genetic eggs overall (slope estimate ± s.e. = 0.084 ± 0.023, z = 3.713, p<0.001, Figure 5), We found qualitatively the same results for both other factors when the model was run for just males (number of social eggs: slope estimate ± s.e. = 0.075 ± 0.006, z = 12.763, p<0.001; promiscuity line: slope estimate ± s.e. = 0.051± 0.090, z = 0.566, p = 0.571),
For females, performed infidelity did not have a significant effect on the number of genetic eggs produced (slope estimate ± s.e. = -0.034 ± 0.044, z = -0.784, p = 0.433). We found qualitatively the same results with the other factors when the model was run for just females (number of social eggs: slope estimate ± s.e. = 0.074 ± 0.011, z = 7.024, p<0.001; promiscuity line: slope estimate ± s.e. = -0.128 ± 0.097, z = -1.324, p = 0.185).

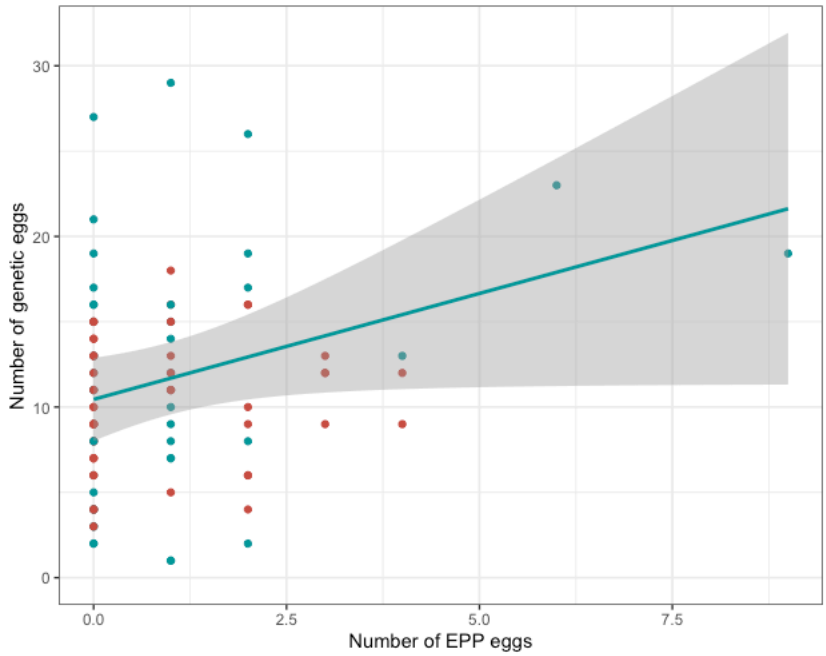

Figure 5: *Comparison between sexes in the influence the number of EPP eggs an individual produced had on their overall number of genetic eggs (reproductive output). Red points represent females, blue points represent males. The line represents the linear regression line for males as estimated by the statistical model and the grey ribbon indicates the 95% confidence interval.*

Does a pair's bond strength affect how many eggs they have together?

For breeding pairs (n = 107) there was a strong positive relationship between how strong their pair bond was and how many genetic eggs they produced together (slope estimate ± s.e. = 4.978 ± 0.707, z = 7.047, p < 0.001, Figure 6). We also found that pairs in the high promiscuity line produced more genetic eggs together than those in the low promiscuity line (slope estimate ± s.e. = -0.307 ± 0.105, z = -2.927, p = 0.003. Female mass had no significant effect on number of genetic eggs produced (slope estimate ± s.e. = -0.028 ± 0.038, z = -0.734, p = 0.463). Cook's distance was calculated for all observations and a single value of pair bond strength (x = 0.325) was identified as a potential outlier (Cook's Distance = 0.53). We found qualitatively the same results when we excluded this potential outlier for pair bond strength (>0.3: pair bond strength: slope estimate ± s.e. = 6.788 ± 0.870, z = 7.801, p < 0.001, promiscuity line:

slope estimate ± s.e. = -0.325 ± 0.097, z = -3.366, p < 0.001; mass: slope estimate ± s.e. = 0.008 ± 0.038, z = 0.213, p = 0.831).

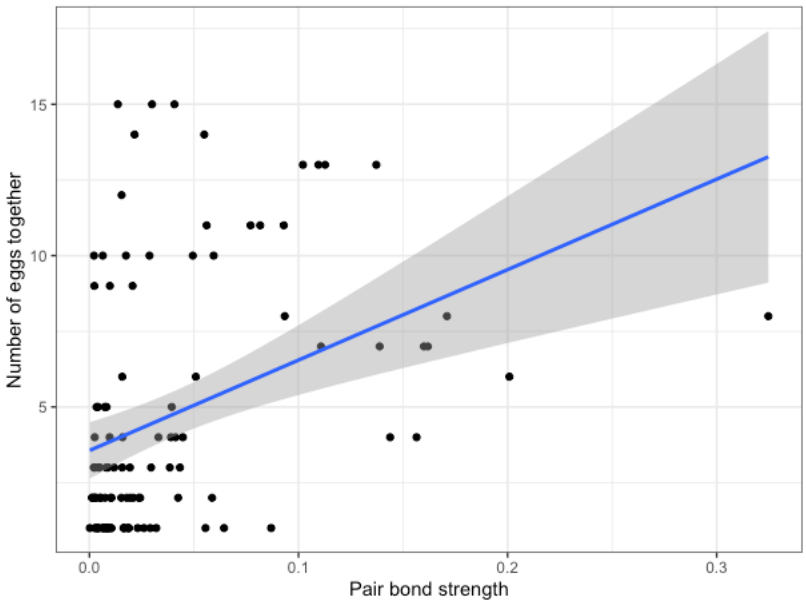

Figure 6: *The number of eggs each female produced within a breeding pair against the strength of the pair's bond. Each dot represents an individual female, the straight line indicates the linear regression line as estimated by the model, and the grey ribbon indicates the 95% confidence interval. The single value of pair bond strength >0.3 was identified as a potential outlier, so the corresponding GLMM was run with and without this point for comparison.*

Discussion

This study aimed to uncover drivers of both individual and population-level variation in extra-pair mating behaviour. We did not find a difference in number of mates or occurrence of EPP between zebra finches from two promiscuity breeding lines selected to have differing levels of male promiscuity; EPP was present across both breeding lines in all four of our study populations. Although a large proportion of birds bred with more than one partner, there was no significant difference between sexes in the number of mates they reproduced with. Our results support the prediction that males receive a larger benefit, in terms of their reproductive success, from engaging in extra-pair mating behaviour. We also investigated the influence pair bond strength has on reproductive output; we found that the strength of a pair's pre-breeding bond was a strong indicator of the number of eggs they would produce together.

Birds from different breeding lines had a similar number of social and genetic mates. One reason why most birds bred with multiple mates can be seen by looking at the patterns of social mating. Almost all of the birds in our study changed social mate at least once over the course of the breeding season; a very small proportion of pairs stayed socially and genetically monogamous. This is surprising, considering how this species is known for its strong and long-term pair bonds (Zann, 1996; Mariette and Griffith, 2012). This deviation from expected mating behaviour may be due to the domestication and selection process that these birds have been through. Captive breeding of domesticated zebra finch, which often involves forced pairing and repairing, may have selected for weaker pair bonds and the ability to divorce so that individuals are able to quickly re-pair and reproduce when separated from their previous partner (Forstmeier *et al.,* 2014).

By investigating the level of infidelity each individual received from their social mates, we determined that breeding lines did not appear to differ in the amount of parentage lost to extra-pair mates. We also found that neither sex received more infidelity than the other. Perhaps surprisingly, we found that having a higher mass correlated with receiving more infidelity. Previous studies have suggested that heavier individuals, who might have better body condition and

thus be of higher quality, might show an increased rate of EPP (Bjørnstad and Lifjeld, 1997), but not an increased rate of being cheated on. Plaza *et al*. (2019) found that females with higher mass during the egg laying period had a higher level of EPP in pied flycatchers (*Ficedula hypoleuca*). They suggested that this could be because a higher body mass equates to higher wing loading, meaning females are less able to escape from unwanted copulations with extra-pair mates. The authors also offered an alternative explanation: having a higher body mass at the egg-laying stage might signify a better body condition and increase a female's ability to evade mate guarding and search for extra-pair mates. Likewise, for males, being heavier could equate to being of higher quality, which could lead to them being able to obtain more EPCs (Mingju *et al.,* 2017). In zebra finches, Burley *et al*. (1994) found that females were more likely to engage in unforced EPCs if they had attractive bills or were mated to males with unattractive bills. In contrast, our study found no effect of individuals' mass on either the number of genetic or social mates they had, providing only evidence that heavier individuals were being cheated on more often.

Our findings thus suggest that an individual's infidelity might depend on their social mate's mass. Possessing physical characteristics that correspond to being of lower quality or relatively unattractive, such as having a lower mass or smaller body size, leads to an increased risk of receiving infidelity in various avian species (Bjørnstad and Lifjeld, 1997; Plaza *et al.,* 2019), but this is not always the case (Hsu *et al.,* 2015). Our results seem to show the opposite trend and it may be that the relationship between mass and extra-pair mating behaviour is more complex than initially thought. In the blue tit (*Parus caeruleus*), for example, males successful in obtaining EPCs had longer tarsi, corresponding to a larger body size (Kempenaers *et al.,* 1997). However, the same study found that these successful males also weighed less relative to their size and that the males they cuckolded usually weighed more and were in better condition. It is possible that in this case, heavier males were losing paternity as they were actually lower quality; males that lost paternity were less likely to survive and it could be that these low-quality males have to insure against uncertain foraging success by carrying larger fat reserves (Kempenaers *et al.,* 1997).

Another potential explanation for lighter male blue tits obtaining more EPCs, and potentially being of higher quality, is related to the timing of the body mass measurements; these were taken during the offspring nestling stage, at a time when successful (and high quality) extra-pair males may have been investing more in feeding offspring, performing mating displays and in sperm production (Kempenaers *et al.,* 1997). Our study findings are limited by the fact that mass was only measured once at the start of the breeding season. Single assessments of a physical characteristic that is likely to change over time could lead to studies generating different results, an issue also noted in a previous study investigating the influence of bill colouration on EPP in zebra finches (Burley *et al.,* 1994). We refrained from measuring mass multiple times to avoid the stress induced by frequent capture of the free-flying individuals in our aviaries. Assessing physical characteristics regularly throughout the study period could help future studies to better understand the impact that characteristics such as mass have on extra-pair mating behaviours.

The breeding lines used in our study were selected for high or low breeding values of male sex drive. This was measured via male courtship rate i.e. the rate they solicited copulations. Our study appears to suggest that selection on this factor does not necessarily translate to a difference in EPP. This lack of difference could be because many of the trade-offs involved in the relative costs and benefits of engaging in a particular mating strategy, in this case infidelity, would be the same across populations (Tschirren *et al.,* 2009).

Another key factor to note is that the measure of infidelity we used, variation in the frequency of EPP, is a result of both the variation in the frequency of EPCs and the variation in the success of these copulations (Petrie and Kempenaers, 1998). The correlation between EPCs and EPP is often weak, due to the fact that copulations are not necessarily followed by fertilisations (Birkhead and Møller, 1995; Wink and Drycz, 1999). This can be seen in the northern fulmar (*Fulmarus glacialis*), where EPCs occur but they do not lead to fertilizations and EPP (Hunter *et al.,* 1992). This lack of alignment between the rate of EPCs and EPP has been largely attributed to the influence of sperm competition and the ability of the female to manipulate this process (Wink and Drycz, 1999; Girndt *et al.,* 2018). The lack of alignment has also led to questioning of the true

relationship between extra-pair mating behaviour and factors which have traditionally been attributed to its variation, such as age. It has been well established that in socially monogamous birds, older males are more likely to sire extra pair offspring (Hsu *et al.,* 2015; Girndt *et al.,* 2018, Hsu *et al.,* 2017). When studying the mechanisms behind this link using captive house sparrows (*Passer domesticus*), Girndt *et al*. (2018) discovered that despite there being a strong relationship between male age and EPP, with middle-aged males gaining the most EPP, male age was not related directly to extra-pair mating behaviour as predicted. As the number of EPCs were not associated with male age, the relationship between male age and EPP could be the result of post-copulatory mechanisms that favoured fertilisation by older males (Laskemoen *et al*., 2008). This demonstrates that measuring just the EPP rate does not provide us with the whole picture of the mating behaviours occurring in that population. It is also possible that any genetic components linked to sex-drive and promiscuous behaviour could be confounded by strong individual-level variation in behaviour. This variation can be due to a wide range of factors: levels of mate guarding, an individual's relative attractiveness as an extra-pair mate and individual mating effort (Forstmeier *et al.,* 2011; Canal *et al*., 2012). It is also possible that our birds may have shown more variation in mating strategy under different conditions. We investigated EPP across one breeding season, in a system where the breeding cycle was stopped at the incubation stage. However, there could be variation in mating strategy across a full breeding cycle or between cycles that we could not observe. Mating strategy and the propensity to engage in EPCs is thought to vary between related species depending on the requirement of parental care (Ball *et al*., 2017). However, it has been shown that male EPP can also vary within a species according to both the stage of breeding cycle and the requirement of paternal care at that time; male pied flycatchers (*F.hypoleuca*) invested in EPP mainly in the period after their social female's peak fertility and before their social nestlings hatched (Canal *et al.,* 2012). More research is needed that directly compares individual performance of EPCs and EPP across breeding cycles to fully understand the selection pressures and individual-level factors influencing EPP in a population.

We also investigated how performing infidelity affected reproductive success. As predicted, the number of eggs an individual produced as an extra-pair mate

had a greater impact on overall reproductive output for males than for females. We found that males with a higher number of EPP eggs fathered more genetic eggs overall. Males therefore received a larger benefit, in terms of their reproductive output, from engaging in extra-pair mating behaviour. This finding supports previous research on the sex-specific benefits of EPP, which proposes males can benefit more than females by performing EPCs as they can increase their reproductive success due to the low costs of sperm, whereas females are more limited by the number of eggs they can lay (Petrie and Kempenaers, 1998; Wink and Drycz, 1999). We also found that the number of eggs an individual produced within its social pair was a strong predictor of how many eggs it would produce overall. This indicates that despite the widespread occurrence of EPP, for most individuals the majority of their eggs were still produced with their social mate. It would be interesting to see whether the relative contribution of within-pair eggs versus extra-pair eggs to overall reproductive output changes over time if investigated over a longer study period. In another socially monogamous and genetically polygamous species, the house sparrow (*P.domesticus*), it appears that males partition their reproductive effort differently between within-pair and extra-pair paternity depending on their age (Hsu *et al.,* 2017). As they aged, a greater proportion of their annual paternity success was achieved by EPP, while their within-pair paternity declined. Given this finding, it is possible that we might see within-pair eggs become a less significant predictor for male reproductive success as they age, with extra-pair eggs instead being the main predictor until reproductive senescence.

In several socially monogamous bird species, reproductive output has been shown to increase with pair bond strength and/or duration (Bradley *et al.,* 1995; Black 2001; Griggio and Hoi, 2011). In the zebra finches we studied, we identified a strong positive relationship between the strength of a breeding pair's bond and the number of eggs they produced together. As all the birds in our study were of a similar age, sexually naive and all moved into mixed-sex aviaries at the same time, our findings are not confounded by issues facing studies in the wild, such as the fact that individuals in prolonged pair bonds with the same mate often have a higher reproductive output due to experience and age (Bradley *et al.,* 1995; Fowler, 1995). Our measure of pair bond strength

was created from association data recorded during the pre-breeding period. Our findings indicate that the social connections made during this important socialising period last for a prolonged period, even if the pair do not breed together initially. It is important to remember that in this analysis we included for every female each male she bred with (i.e. produced genetic eggs with) and her bond strength to that individual; therefore, including both social-pair mates and extra-pair mates. Although we found no difference between the promiscuity breeding lines in measures of EPP or individual reproductive output, it appears that there was variation in pair-level reproductive output. We found that pairs in the high promiscuity line produced more eggs together than those in the low promiscuity line. This could be a by-product of genetic differentiation between the lines, or possibly an indication that there was a slight difference in mating strategy which was different to what was anticipated. This result may be a consequence of our analysis into pair-level reproductive output including both within-pair breeding and extra-pair breeding. Even though there was not a significant difference in EPP level or number of mates between the two breeding lines, it is possible that there may have been a trend towards birds from the high promiscuity line producing more eggs within their extra-pair relationships but that it was non-significant at this time. Our measurement of EPP made it difficult to quantify how many eggs were produced within a particular extra-pair relationship, so we were unable to look at whether pair bond strength influenced reproductive output differently between within-pair paternity and EPP. Most of the research looking at the effect of pair bond strength on reproduction naturally focusses on social pairs, rather than social associations with extra-pair mates (Beck *et al.,* 2020). Expanding future research to include these associations could help to highlight potential connections between social associations and later extra-pair mate selection (Maldonado-Chaparro *et al.,* 2018).

In conclusion, we did not find significant evidence of variation in extra-pair mating behaviour between zebra finches from two distinct breeding lines. Future research into variation in EPP between populations and species would benefit from empirically quantifying the link between EPCs and EPP. Although the females and males in our study showed generally similar levels of promiscuity, males benefitted more from EPP in terms of it generating a larger proportion of

the genetic eggs they fathered. Our findings therefore support previous findings on sex-based variation in mating strategy (Wink and Drycz, 1999; Westneat and Stewart, 2003). This study also provides further evidence that pair bond strength, separate from individual age or bond duration, is a strong predictor of a pair's reproductive output.

Chapter three: Investigating the effect of pair bond strength and EPP on telomere dynamics

Abstract

Telomeres, non-coding repetitive sequences of DNA located at the end of eukaryotic chromosomes, play an important role in preserving genomic integrity and promoting chromosomal stability. They have become regarded as important biomarkers of biological age and also indicators of reproductive output and survival. Understanding the factors influencing telomere dynamics is key to uncovering some of the drivers of variation in health, fitness and lifespan. Exposure to chronic stress can have a range of impacts on physiology, behaviour and telomere attrition. One area of research that needs further investigation is the impact of stress from the social environment. It is possible that the occurrence of Extra-Pair Paternity (EPP) within monogamous systems could act as a form of social stress, due to its potential to compromise social-pair bonds. This study aimed to examine the relationships between EPP, social-pair bonds, reproduction and telomere dynamics in a well-known socially monogamous species: zebra finch (*Taeniopygia guttata*). We conducted a within-individual repeated-measures study of telomere attrition across an experimentally-controlled breeding season. Contrary to our predictions, we found no evidence of a relationship between telomere length and fitness, providing no support for the idea that telomere length could be used as a quality indicator in this species. However, we did find that for both sexes, longer telomeres experienced more shortening. Our results also provide only weak evidence that receiving infidelity or experiencing weaker pair bonds induces sufficient physiological stress in zebra finches for it to affect telomere dynamics. Further work is needed to investigate the effects of the social environment on telomere dynamics. In particular, conducting experimental manipulations of pair bonds could help future studies uncover the potential consequences of extra-pair mating behaviour.

Introduction

Reacting appropriately to stressors is critical for survival and fitness. Difficult conditions and events can be potent stressors that elicit a stress response, especially ones that are unpredictable or uncontrollable such as food scarcity, predation and agonistic behavioural interactions (Creel, 2001; Haussmann and Marchetto, 2010). The vertebrate stress response is a suite of physiological, hormonal and behavioural response mechanisms that allow organisms to cope with stressors (Romero, 2004; Haussmann and Marchetto, 2010). When an animal is confronted by a stressor, the hypothalamic-pituitary-adrenal (HPA) axis is activated, resulting in the release of glucocorticoids (GCs). Fish and most mammals generally release cortisol, whereas birds, reptiles, amphibians, and most rodents generally release corticosterone (Romero, 2004). GCs mediate changes that maximise immediate chances of survival; they act to mobilise energy stores to facilitate the "fight or flight" response, but in order to conserve energy they inhibit other physiological processes such as reproduction, immune function and growth (Haussmann and Marchetto, 2010; Monaghan, 2014). Most stressors last a short period of time and after they end, or the animal has removed itself from the stressful conditions, the level of GCs should return to baseline. However, if stressors continue for a longer period of time, this can lead to a potentially problematic state, chronic stress: the long-term secretion of GCs (Romero, 2004; Monaghan, 2014).

Chronic stress or repeated exposure to stressors can change the functioning of the HPA axis in several ways. It may induce significant and lifelong acclimation, where the animal no longer responds in the same manner to repeated or prolonged stressors (Romero and Wikelski, 2002; Romero, 2004). For example, a moderate stressor in chicks, such as reduced parental provisioning rates due to living in a degraded habitat, could conceivably result in decreased GC responses when those chicks become adults (Romero, 2004). On the other hand, in some cases the acclimation process can instead prime the animal to have an enhanced response to different stressors, a process called facilitation (Romero, 2004). Therefore, exposure to stress early in life, and/or for long periods of time, can have lifelong effects on behaviour and physiology (Spencer *et al*., 2003; Farine *et al.,* 2015; Zito *et al.,* 2017).

There are also clear detrimental consequences of chronic stress due to the link between long-term elevated GC levels and poor health. In free-living baboons (*Papio anubis*), for example, having a chronically elevated GC response as a result of subordinate status is linked to cardiovascular problems (Sapolsky and Share, 1994). Chronic stress is well known to cause and exacerbate disease in humans (Romero, 2004), and exposure to high levels of stress and increased levels of stress hormones has been linked to a reduction in life expectancy (Monaghan *et al.,* 2011).

It has been suggested that the well-established link between chronic stress and poor health in humans is due to the effect of stress on telomeres (Epel *et al.,* 2004). Telomeres are non-coding repetitive sequences of DNA that are located at the end of eukaryotic chromosomes (Monaghan, 2010; Reichert *et al.,* 2013). During cell division they enable cells to distinguish between a chromosome end and a double-strand break (Campisi *et al.,* 2001; Haussmann and Marchetto, 2010). This prevents chromosome degradation and hence telomeres play an important role in preserving genomic integrity and promoting chromosomal stability (Campisi *et al.,* 2001; Monaghan and Haussmann, 2006). As telomeres progressively shorten at each cell division, it was originally thought that telomere length could be used as a form of 'mitotic clock' to measure the chronological age of individuals (Haussmann and Vleck, 2002). Telomere length generally decreases with age in a large variety of taxa with only a few exceptions (Gao and Munch, 2015). This phenomenon is particularly commonplace amongst birds and has been demonstrated in a number of avian species, in both domesticated (Haussmann and Vleck, 2002) and natural populations (Pauliny *et al.,* 2006; Barrett *et al*., 2013). However, there is often a large amount of variation in telomere length between individuals of the same age and telomere dynamics have been shown to differ with body condition and individual quality (Kotrschal *et al.,* 2007; Barrett *et al.,* 2013). Due to this variation, rather than being a reliable indicator of chronological age, telomeres have become regarded as important biomarkers of biological age and also indicators of longevity and survival (Monaghan, 2010; Plot *et al.,* 2012; Reichert *et al.,* 2013).

The reason for the relationship between telomere length, longevity and chronic stress is that GCs cause telomere attrition by reducing the activity of telomerase, the enzyme responsible for the maintenance of telomere length, consequently slowing telomere repair (Campisi *et al.,* 2001; Choi *et al.,* 2008). Telomeres get shorter each time a cell divides in a process known as telomere attrition and if telomere repair is diminished, the rate of shortening is increased (Haussmann and Marchetto, 2010; Reichert *et al.,* 2013). Unchecked telomere degradation and attrition can affect DNA repair systems, meaning that chromosomes are at risk of degradation, recombination or random fusion. Most normal cells respond to short telomeres by undergoing cellular senescence, which irreversibly arrests cell growth, causes changes in cell functions and can lead to cell death (Campisi *et al.,* 2001). The accumulation of senescent cells can contribute to the decline of tissue function and integrity, which is associated with ageing (Campisi *et al.,* 2001**;** Monaghan and Haussmann, 2006), as such tissue has a reduced capability to respond to damage, predisposing it to cancer and other diseases (Campisi *et al.,* 2001; Monaghan and Haussmann, 2006; Haussmann and Marchetto, 2010). The possession of longer telomeres on the other hand is an advantage that increases the probability of survival (Haussmann *et al.,* 2005). However, it appears not to be the absolute length of telomeres that explains differences in lifespan, but the rate of telomere shortening (Monaghan and Haussmann, 2006). Faster shortening of telomeres has been directly linked to shorter lifespan, on both an individual level (Salomons *et al.,* 2009) and interspecific level (Haussmann *et al.,* 2003).

By measuring the change in an animal's telomere length over a designated period of time, we can thus create a measurement of how an individual has biologically aged over that time period and then relate this to potential stress-causing factors. For example, Epel *et al*. (2004) compared rates of telomere shortening and biological ageing between 'control mothers' and caregiving mothers who were looking after chronically ill children. The authors found that the more years of caregiving, even after controlling for chronological age, the lower the telomerase activity and the shorter the mother's telomere length. It was estimated that the telomere shortening experienced by those mothers who had higher levels of perceived stress was equivalent to their lymphocytes ageing an additional 9-17 years. This demonstrates *in vivo* that chronic

activation of the stress response through chronic exposure to stressors correlates with stress-induced premature senescence through telomere attrition.

A key life experience that may cause telomere shortening is reproductive investment (Reichert *et al*., 2014; Sudyka *et al.,* 2014). For most species, reproduction is physically costly in some way, whether that is from building nests or using resources to lay eggs (Reichert *et al.,* 2014), allocating energy to gestation and lactation (Gittleman and Thompson, 1988), or providing parental care (Hamel *et al.,* 2010). Higher costs of reproduction, for example if an individual is breeding more frequently, generating more offspring or investing more in those offspring, could result in shorter telomeres. In the leatherback turtle (*Dermochelys coriacea*), for example, telomeres were found to be shorter in females that were breeding after a two-year migration compared to those breeding after three years, which may mean that there is a higher cost of reproduction in those breeding more frequently (Plot *et al.,* 2012). Across a range of taxa, higher reproductive output has been linked to both shorter lifespans (mammals: Hamel *et al.,* 2010; insects: Flatt, 2011) and shorter telomeres (birds: Sudyka *et al.,* 2014; fish: Gao and Munch, 2015), indicating the widespread occurrence of a trade-off between reproduction and survival.

In addition to telomere attrition potentially indicating past exposure to demanding conditions such as reproductive investment, measuring telomere length could also provide a way to estimate future reproductive output. Telomere attrition can be an indicator of the stress and conditions experienced by an animal, but telomere length itself may be predictive of the animal's behaviour and physiology. For example, Pauliny *et al*. (2006) investigated whether telomere length, corrected for chronological age, correlated with various fitness components. They showed that in natural populations of sand martins (*Riparia riparia*) and dunlins (*Calidris alpine*) telomere length predicted individual fitness. In dunlins, lifetime reproductive success was higher in males with longer than expected telomeres for their age than in males with shorter telomeres. Also, males that produced at least one recruit had significantly longer telomeres than those that failed. The authors proposed that the relationship they observed was due to fact that the 'higher quality' individuals, i.e. those with longer than expected telomeres for their age, were better able to

resist potential telomere-shortening stressors (Pauliny *et al.,* 2006). A similar pattern was reported in the king penguin (*Aptenodytes patagonicus*), where the chicks of birds with longer telomeres survived longer resulting in parents with longer telomere tending to have higher breeding success than those with shorter telomeres (Le Vaillant *et al.,* 2015). This pattern suggests that telomere length might be used as a general indicator of individual quality.

One type of environmental stressor that may have a range of impacts on individuals' behaviour, stress physiology and telomere dynamics is social stress. Behavioural interactions within a social group, particularly aggressive or agonistic ones, can be very potent stressors that increase GC secretion and impact behaviour (Creel, 2001; Muller and Wrangham, 2004). For a long time, it was thought that stress played a major role in dominance hierarchies, with the mechanism behind reproductive suppression being increased GC levels in subordinates due to aggressive interactions with dominants (Creel, 2001). Although there is evidence for this in certain species such as the alpine marmot (*Marmota marmota*), it now appears that this is not the case for most dominance hierarchies (Louch and Higginbotham, 1967; Hackländer *et al.,* 2003). A review of studies of cooperative breeders, for example, has shown that the GC levels of dominant individuals were most often higher or the same as in the subordinates of the same species (Creel, 2001). This is also the case in wild chimpanzees (*Pan troglodytes schweinfurthii*), where dominants actually have higher levels of urinary cortisol; this is likely due to the energetic demands of certain behaviours such as displays of aggression, rather than psychological stress (Muller and Wrangham, 2004). In this species, the levels of social stress experienced by those in different ranks shift depending on the stability of the dominance hierarchy; dominants are more likely to have lower GC levels than subordinates in periods of stability as they benefit from control and predictability, whereas periods of instability when their positions are threatened may lead to dominants showing increased aggression and having a heightened stress response (Sapolsky, 1992; Muller and Wrangham, 2004). This demonstrates the range of impacts that behavioural interactions can have on stress levels.

We hypothesised that extra-pair mating behaviour could potentially be a form of social stressor and investigated this idea by assessing the impact of infidelity on telomere dynamics in domesticated populations of zebra finch (*Taeniopygia guttata*). Although this species is well known for being a socially monogamous bird that forms long-term pair bonds, it is thought that the strong selection pressures from domestication have led to an increase in the rate of promiscuity and "extra-pair paternity" (EPP) present in many current captive populations (Griffith *et al*., 2010; Griffith *et al*., 2017). Zebra finches show remarkably low levels of EPP in the wild (2%), yet studies on domesticated populations report EPP rates of 10-30% (Forstmeier *et al.,* 2011; Ihle *et al.,* 2013; Maldonado-Chaparro *et al*., 2018). This increase in extra-pair mating behaviour in domesticated zebra finches has been attributed to a weakness in pair bonds; pairs with stronger pair bonds may be less likely to suffer from infidelity (Forstmeier *et al.,* 2014). Divorce is rare and costly in this species as remaining with a partner speeds up the initiation of reproduction, an important factor in a short-lived species from the Australian outback where the environmental conditions suitable for breeding are highly unpredictable and brief (Adkins-Regan and Tomaszycki, 2007). As the cost of separation is high, extra-pair mating behaviour could induce social stress in this socially monogamous mating system.

This study addresses the hypothesis that an individual engaging in EPP could have negative behavioural and physiological effects on its social mate. In particular, we focussed on how telomere attrition is affected by pair bond strength, infidelity and reproductive output. Four groups of domesticated, sexually naive zebra finches were monitored over the course of their first breeding season, in a captive arrangement that allowed measurement of their reproductive output, individual level of infidelity (i.e. contributing genetic material to eggs outside the pair bond), pair bond strengths, and change in the length of their telomeres.

We predicted that if telomere length acts as a quality indicator in this species, those with longer pre-breeding telomere lengths would have a higher reproductive output, measured in the number of eggs they were the genetic parent of, than those who had shorter lengths. We envisioned that due to the physical costs of reproduction, telomere length should decrease with number of

eggs laid. We also predicted that birds with weaker pair bonds to their partner, or those whose partner performed higher rates of infidelity, would suffer increased rates of telomere shortening (while controlling for reproductive output) across the period of observation. We anticipated that females would experience more telomere attrition than males, as they would be expected to suffer higher costs of reproduction and receive more infidelity. Due to this, we also predicted that when potential factors influencing change in telomere length were investigated for each sex separately, reproductive output and infidelity received would be a more significant predictor of telomere length change in females than in males.

Variation in telomere length among individuals of the same age can be used to address how life history traits may impact the rate of biological ageing and hence provide a way to measure the relative cost of life events and trade-offs (Haussmann & Vleck 2002; Nakagawa *et al*., 2004; Pauliny *et al*., 2006). Through studying a population of birds that were all of similar age and experienced their first reproductive attempt, this study provides insight into the relationship between reproductive behaviour, sociality and biological ageing during this important life history stage.

Methods

Study site and test subjects

The zebra finch (*T.guttata*) used in this study came from two captive domesticated populations held at the Department of Behavioural Ecology and Evolutionary Genetics, MPIO Seewiesen, Germany. These populations came from breeding lines corresponding to approximately 100 generations of domestication and probably go back to imports from about 1880 (Forstmeier *et al.,* 2007a). At the start of our experiment, the birds were aged between 11 and 15 months old and all were sexually naive. Birds were raised in mixed-sex aviaries until they reached sexual maturity and then they were kept in unisex aviaries until the start of the experiment. This means that birds may have been familiar with each other before the start of the experiment, but it is unlikely that they would have formed social pair bonds.

This project was conducted in collaboration with researchers from the University of Konstanz and run on site at the Max Planck Institute of Ornithology in Radolfzell, Germany. Birds were separated into four aviaries with 14 of each sex in each aviary (Figure 7). The aviary complex was located in a rural area on the edge of a forest, with two of the four aviaries adjacent to the forest edge. Each aviary contained a feeding table with two feeders, a mating perch and two social perches (Figure 7). Mating perches were located in the inner corner of each aviary. These were designed to replicate a tree with branches and was the place where birds most commonly performed mating dances. The social perches were designed to be the main area for the birds to sit and interact as a group.

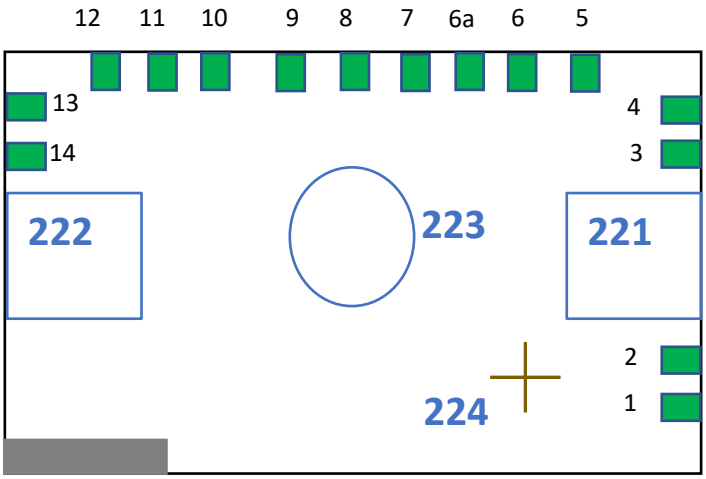

Figure 7: *Diagram of the layout of an aviary. Birds were housed in large outdoor aviaries (4 x 3 x 2.3 m high). Each was equipped with 15 nest boxes (represented here as the small green rectangles), which consisted of the planned 14 boxes and another box added two weeks into the breeding season (additional nest box pictured here was 6a). Raspberry Pi cameras are represented by numbers ranging from 221 - 224. The centre of each aviary contained a feeding table (represented here as a circle) where two feeders provided mixed finch seed ad libitum. Social perches were attached on the right and left side of the aviaries, represented by the larger rectangles in the figure. The mating perch is represented by a cross, with one located in the corner of each aviary.*

Each bird was individually identified with several unique tags. These consisted of a unique combination of coloured leg rings (i.e. black, pink, blue, red, white, green, yellow or violet) with one ring on each leg, which could be easily seen at a distance. Also, each bird carried a metal numbered leg ring to double-check the bird's ID during handling and collection of blood samples. The final method of identification was a small paper 'backpack' that contained a Passive Integrated Transponder (PIT) tag and a QR code (Figure 8) (Alarcón-Nieto *et al.,* 2018). The mass (in grams) of each bird was measured before release into the aviaries.

The birds were introduced to the aviaries without nest boxes and left to socialise for an adjustment period of three weeks. After this, we introduced the 14 nest boxes and an unlimited supply of nesting material in the form of coconut fibres

to each aviary. The introduction of nest boxes and nesting material signalled the start of the 3-month breeding season, which lasted from July to September 2017 (Table 2).

We observed birds in two of the aviaries attempting to build nests at several random places, including the box containing the nesting material. To prevent this, two weeks after the initial boxes were placed, we added an additional one to provide more nesting space, bringing the total number of nest boxes to 15 (Figure 7).

Pair identification

We took a two-step approach to identifying the breeding pairs throughout the breeding season. On the entrance of the nest boxes were fitted Radio-frequency identification (RFID) antennae, set to record the PIT tags of the birds entering and leaving the nest boxes (RFID logger board, Priority 1, Australia). These antennae were calibrated so that, rather than tags just in the general vicinity of the nest boxes, they would only register PIT tags moving through the hoop surrounding the nest box entrance. Unfortunately, there were not enough antennae available for this project for us to fit one to each nest box. We compensated for this by rotating the antennae between nest boxes on a daily basis to provide a good coverage of nest box visitation during the breeding period.

These RFID data were supplemented by daily "live" observations of the aviaries by the experimenters, aimed to identify which pairs were occupying given nest boxes. Every time a bird left or returned to a nest box, its identity was recorded via notation of the bird's sex and leg band colours. We conducted additional observations of the mating perches to help identification of social pairs and gain insight into potential extra-pair copulations. During the breeding season, we conducted observations of each aviary daily from Monday to Friday for 15 minutes per aviary between 8 – 9.30 AM. On Saturdays and Sundays, we observed each aviary for 30 minutes each, as on these days no nest checks were conducted and the birds were less disturbed by experimenter activity. Initially these observations provided confirmation that the RFID system was

working correctly by comparing the individuals it recorded at each nest box to the record of the individuals we would expect to be picked up based on our observations. Our observations also functioned as a backup for the occasions where certain nest boxes were not being monitored by the RFID antennas due to the antenna shortage.

Pair bond strength

In order to measure the social network and quantify social pair bond strength, each aviary contained four Raspberry Pi Cameras in standardised locations: one above each of the two social perches, one above the mating perch and one above each of the two feeders (Figure 7).
Each Raspberry Pi camera took a photograph of the designated section of the aviary every two seconds during daylight hours (i.e. when light levels were sufficient) to record the QR codes of the birds present in the photo frame at that time (Figure 8). Photos were converted into videos and processed using an automated detection system, custom-written by Jacob M Graving using PinPoint library in Python (Alarcón-Nieto *et al.,* 2018; Graving *et al.,* 2019). This system registered the QR codes present in each frame of the video. From this we extracted information on the identity of each bird, its position (x,y coordinates) and its orientation in frame. This information on individuals' positions relative to others in the aviary allowed us to infer clumping behaviour. This behaviour, where two birds perch in body contact, was defined in our photos as occurring when two birds were detected at a distance of <8 pixels. Measurement of this behaviour was used to create social networks to characterize the social structure for each aviary. The strength of association between each dyad of individuals was measured by a simple association index: the probability of observing that dyad clumping together at any given time, given that either one was detected in frame (Farine and Whitehead, 2015). The time period for which it was particularly important to quantify social metrics was the pre-breeding period (i.e. the 3 weeks before nesting materials were introduced), as this was when pair bonds were forming (Table 2). This pre-breeding social association data was used to create measures of pre-breeding pair bond strength for each breeding pair, for each of the aviaries.

Figure 8: *A snapshot of the automated detection of individual QR codes from the photographs taken by the Raspberry Pi cameras. Associations between these QR codes over time (distance between birds and time spent in close proximity to one another) was used to create measures of pair bond strength between individuals.*

Breeding data

We conducted nest-checks every weekday during the breeding season, which involved quantifying the number of coconut fibres in each nest and checking the nest box for eggs. Each time a new egg was found, it was replaced with a dummy egg. We removed eggs so that they could be externally incubated for five days to allow them to continue developing in a controlled environment. This provided us with as much embryonic tissue as possible for DNA and parentage analysis. Each egg and its corresponding dummy egg were labelled with the date that it was laid, the nest box and aviary that it came from, and what number in the egg laying sequence of the clutch it was (e.g. first, second). Dummy eggs were used to reduce the chance that the birds would try to keep producing eggs to maintain the clutch number. Birds of many species will replace eggs that are lost during laying; this has been shown in lesser black-backed gulls (*Larus fuscus*), where removal of an egg from a clutch caused the female to lay an extra egg to complete their normal clutch size (Monaghan *et*

al., 1995). In captivity, the breeding pattern for zebra finches is to lay an egg every day for 4-5 days. Obtaining the necessary resources for egg laying may require considerable effort and producing a larger number of eggs than expected could have potential negative impacts on parental fitness (Monaghan and Nager, 1997). Use of the dummy eggs also enabled us to monitor the amount of time between eggs being laid and the start of incubation for the clutch (determined by whether the dummy eggs were kept warm to the touch). Once the first egg in a clutch was recorded as being incubated, the dummy eggs were kept in the nests for a further 10 days, corresponding to approximately 5 days of egg laying and 5 days of just incubation. Once these 10 days had passed, all the (dummy) eggs in the clutch were removed which signalled the experimental end of a clutch. By keeping the dummy eggs in the nest for this time, we ensured that the clutch had been completed and that there was a designated incubation period between the production of consecutive clutches. The experimental breeding season lasted three months, during which most pairs laid several clutches.

Once the eggs had been in the incubator for five days, they were removed to stop the embryos from reaching the size and stage of development that classifies them as living animals (Animals (Scientific Procedures) Act 1986, 1990). We removed embryos by dissecting the egg and separating the tissue from other egg components such as yolk. The embryo tissue was stored in an Eppendorf with 0.5ml of alcohol for later parentage analysis.

Parentage data

DNA parentage analysis was conducted on all of the eggs to determine the genetic parents for each egg. This analysis was conducted at the MPIO in Seewiesen using 15 microsatellite markers (Forstmeier *et al*., 2007b). Using the methods of pair identification through observations and RFID data as described above, we had assigned ‘social parents’ to each clutch, defined as the pair present at the nest box when each clutch was laid. By comparing the social parents of each egg to its genetic parents, we were able to estimate the rate of extra-pair fertilisations for each bird.

Blood collection and telomere measurement

Blood samples were obtained from each bird, once before and once after the breeding season (Table 2), by a certified technician of the Max Planck Institute for Ornithology in Radolfzell. Up to 0.5ml of blood was taken from the ulnar vein of each bird on each occasion. Relative telomere length from red blood cells can be used as a proxy for the telomere length in other somatic tissues in zebra finches (Reichert *et al.,* 2013). On the first occasion samples were taken several weeks before the initial release of birds into the aviaries and before the start of the breeding season, while the second sampling was after the removal of the birds from the aviaries four months later, three weeks after the end of the breeding season (Table 2).

Blood samples were frozen to -80°c until the time of DNA extraction. All blood samples were analysed at the same time regardless of the date they were obtained. Genomic DNA was extracted directly from red blood cells by standard procedure (Cawthon 2002; Reichert *et al.,* 2013). The concentration of DNA was assessed using a Nanodrop-10000 Spectrophotometer and sample concentrations were standardized by using double-labelled water. Each PCR plate contained three samples per DNA sample, Gapdh samples, 6 standards (each containing different know concentrations of telomeric sequences) and an internal control (pool of 20 random samples taken from the DNA samples) for determining the reliability of the method across plates. The same primers were used as in Reichert *et al*. (2013).

The measure of telomere length was attained from the T/S ratio given by the qPCR analysis (Cawthon, 2002; Criscuolo *et al*., 2009). Rather than being an absolute measure of telomere length in base pairs, the T/S ratio is the ratio of telomere repeat copy number (T) to the control single gene copy number (S) (Criscuolo *et al*., 2009), i.e. a relative measure of the abundance of certain telomeric sequences within the genome (Nettle et al, 2015). The 6 standards per plate allow better estimations of DNA sample starting amounts. C_t number, the number of replication cycles required for the abundance of DNA sequences to reach a designated threshold, was used to estimate the original starting quantity of DNA in a sample (SQ) (Criscuolo *et al*., 2009). The lower the C_t

number, the higher the abundance of telomeric sequences and the longer the telomere. We used the 6 standards as a reference as the quantity of telomeric sequences they contained was known, then compared these to the quantity of sequences measured in our samples. This allowed us to create a numerical estimation of telomere length for our samples. The telomere and Gapdh thermal profile was 5 minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 35 seconds at 62°C, 30 seconds at 74°C. Assays were followed by melt curve analysis, 30 seconds at 95°C to 30 seconds at 72°C. For a similar procedure, see Reichert *et al*. (2013).

Table 2: ***Timeline of Experimental Procedures.*** *Work conducted by project collaborators is credited below.*

Stage	*Time Period*	*Actions and Data collected*
Arrival	May 2017	Study subjects arrive at Institute and are kept in single-sex cages.
First bleeding	June 2017	First blood samples collected for pre-breeding measurements of telomere length. Mass measurements taken. Birds released into mixed-sex aviaries.
Socializing	Three-weeks: June to July 2017	Birds habituating to aviaries. Live observations to identify social pairs (conducted by author). Data collected on pre-breeding social bond strength (QR code photography).
Breeding season	July to September 2017	Nest boxes introduced to aviaries with RFID monitoring. Recording of reproductive effort with egg and embryo collection. Use of dummy eggs to maintain clutch sizes and standardise incubation periods. (Monitoring of breeding season conducted by author).
Second bleeding	October 2017	Birds removed from aviaries. Second set of blood samples collected for post-breeding measurements of telomere length (assisted by author).
Blood sample analysis	January 2018	DNA extraction from red blood cells. qPCR analysis to obtain T/S ratio measurement of telomere length for pre- and post-breeding samples for each bird (Analyses conducted by Carmen Martin-Ruiz, Newcastle Ageing Research Laboratories).
Parentage analysis	February 2018	DNA extraction from embryos. Sex and genetic parents determined for each egg (Analyses conducted by Wolfgang Forstmeier, Max Planck Institute for Ornithology in Seewiesen).
Social Network analysis	April 2018	Automatic detection of QR codes from photography. Analysis of association data to create estimates of social bond strength between birds (Analyses conducted by Damien Farine, Max Planck Institute for Ornithology in Radolfzell).

Data analysis

Statistical analyses were run using R Studio/R version 3.5.2. We used packages *lme4* to run models, *sna*, *igraph* and *asnipe* for social network analysis and *ggplot2* to produce graphics.

To investigate whether pre-breeding telomere length might be a 'quality indicator' and was correlated with the number of genetic eggs produced by each individual, a generalised linear mixed-effects model (GLMM) (Poisson family) was created for each sex. The response variable was the number of genetic eggs laid (i.e. produced for females versus fathered by males). The main predictor was pre-breeding telomere length, which was the T/S ratio measurement from the blood samples taken before the start of the breeding season. Mass was also included as a fixed effect; as weight is often an indication of size and individual quality, it was included to account for potential effects on mate choice or reproductive output. A random effect of aviary was included in the models, to account for the separation of the birds into four separate breeding populations. We also included forest proximity as another random effect, to account for the potential influence that aviary location could have had on reproductive output (Figure 8). Aviary proximity to the forest edge potentially influenced aviary temperature, noise and possibly predator-perception.

To investigate which reproductive and social factors contributed most to telomere shortening across the breeding season, we used a linear mixed effects model. The response variable was the change between the pre-breeding and post-breeding estimates of telomere length. The model was first run with both sexes included, to test for sex difference in the change in telomere length. The model contained the following fixed effects: number of genetic eggs produced (to account for reproductive output), sex, initial telomere length (pre-breeding) and mass. To determine whether having weaker pair bonds to a social mate was stressful, we included pair bond strength to first social mate as a fixed effect in the model. We used bond strength to just the first social mate because the measurements of pair bond strength we used were from association data taken shortly before the start of the breeding season, so the

relationship to the first social mate was most relevant. To investigate whether infidelity was a social stressor like we hypothesised, we included “received infidelity” as a fixed effect in the model. For females this variable was defined as the number of eggs fathered by her social male in a different female’s clutch. For males, it was defined as the number of eggs in his clutches that were produced by his social female but were fertilised by another male. We also included “Infidelity made” as a fixed effect, this was a count of the number of extra-pair eggs an individual produced and was included to account for potential energetic and physical costs of engaging in EPP. Aviary and forest-proximity were included as random effects.

The data was then split according to sex and the same model was run for females and males separately with the fixed effect of sex omitted.
This was done to account for the fact that bond strength will have the same value for dyads of birds and also to highlight any sex-based differences in the influence of fixed effects.
Visualisation of the data identified a potential outlier for pair bond strength (>0.3) which appeared to be considerably higher than the rest of the dataset (values <0.2). Cook’s distance was calculated for each observation in the dataset to see which observations were larger than the traditional threshold of 4/n: The single value of pair bond strength >0.3 was identified as the only observation over this threshold and was therefore a potential outlier (Cook’s Distance = 0.53). The model was run for a second time with the potential outlier removed to compare the results.

For each of the models, important factors were isolated through model reduction. This was achieved by stepwise removal of least significant terms until a minimum adequate model (MAM) for significant fixed effects ($p<0.05$) was reached. This began with fitting a maximal model which included all factors, interactions and covariates of interest. Parameter estimates were inspected and in turn non-significant interaction terms were removed, followed by the removal of non-significant explanatory variables.

Ethical note

All experimental procedures were approved by University of Exeter Biosciences Ethics Committee.

Results

Inter-individual variation in telomere length

In the pre-breeding measurements of telomere length, mean T/S ratio for males was 1.562 (SD 1.537) and for females was 1.064 (SD 0.949). In males, T/S ratios ranged between 0.02 and 6.52. In females, T/S ratios ranged between 0.01 and 4.09.
For the post-breeding measurements of telomere length, mean T/S ratio for males was 1.445 (SD 1.408) and for females was 1.098 (SD 1.113). In males, T/S ratios ranged between 0.11 and 6.3. For females, T/S ratios ranged between 0.08 and 5.6.
For both the pre-breeding and post-breeding measurements of telomere length, males as a group had slightly higher mean T/S ratios than females. However, it is important to note that there are large standard deviations and ranges for all these means, which may indicate large amounts of inter-individual variation in telomere length in the populations. In both sexes, both before and after the breeding season, there were cases of individuals with T/S ratio's which were over three standard deviations from the mean T/S ratio for their sex.

Does pre-breeding telomere length correlate with the number of genetic eggs laid?

The analyses in this section are based on the subset of 95 birds whom participated in the breeding season by producing eggs.
For females (n = 51), pre-breeding telomere length did not predict reproductive output (slope estimate ± s.e. = 0.037 ± 0.040, $z = 0.908$, $p = 0.364$, Figure 9) and neither did mass (slope estimate ± s.e. = 0.025 ± 0.034, $z = 0.745$, $p = 0.456$).

Similarly, for males (n = 44), pre-breeding telomere length did not have a significant effect on number of eggs fertilised (slope estimate ± s.e. = 0.044 ± 0.039, $z = 1.133$, $p = 0.257$, Figure 9), and neither did mass (slope estimate ± s.e. = 0.013 ± 0.032, $z = 0.395$, $p = 0.693$).

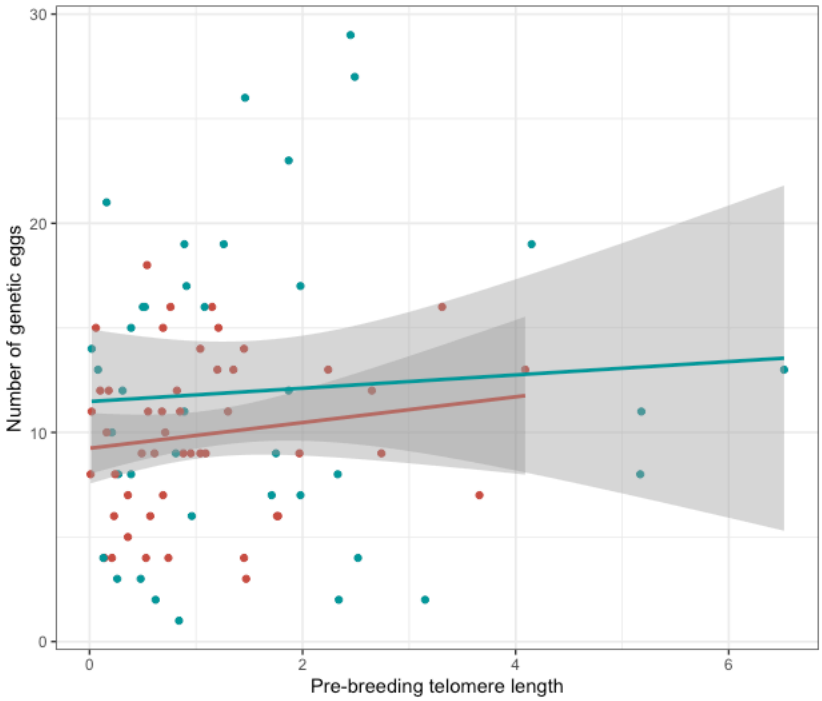

Figure 9*: Pre-breeding telomere length (T/S ratio) for each breeding bird against the number of genetic eggs produced by that individual across the breeding season. Females are represented by red points and males are represented by blue points. Each point represents an individual bird and the straight lines indicate the linear regression line as estimated by the statistical model, and the grey ribbons indicate the 95% confidence intervals.*

Is telomere attrition across the breeding season correlated with infidelity and pair-bond strength?

Surprisingly, we found that a large number of the zebra finch in our study experienced telomere lengthening, where their telomere lengths after the breeding season were longer than the measurements before.
In females, 50% of birds experienced some degree of telomere lengthening and 50% experienced a degree of telomere shortening. In males, 43.6% experienced some degree of lengthening and 56.4% experienced a degree of shortening (Figure 10).

Overall, there was no significant difference in the change of telomere length between males and females (estimate ± s.e. = 0.131 ± 0.285, d.f. = 75.054, t = 0.459, p = 0.648, Figure 10).

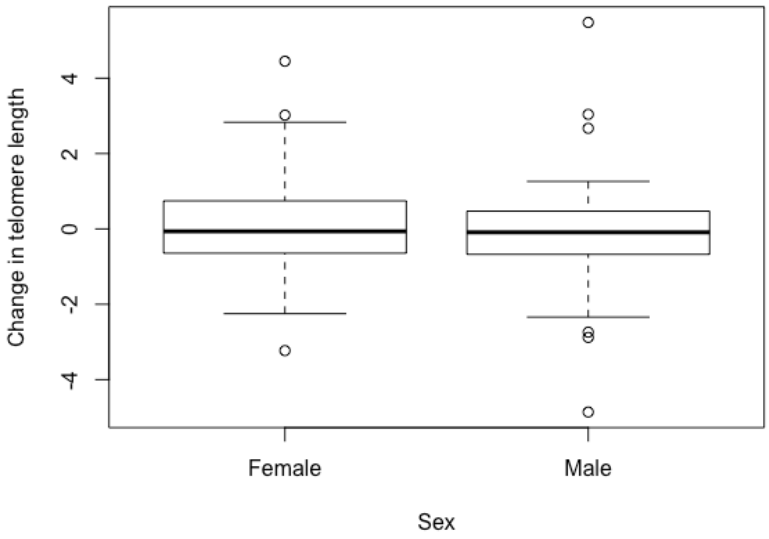

Figure 10*: The change in telomere length for each bird who participated in breeding during the breeding season, split by sex. This change was calculated by comparing pre-breeding and post-breeding measures of telomere length for each individual, attained from T/S ratios. Boxplots show the median, upper (Q3) and lower quartiles (Q1) and the interquartile range (IQR = Q3 − Q1). Whiskers represent maximum and minimum values, excluding outliers. Outliers are represented as circles.*

When both sexes were modelled together, we found that an individuals' pre-breeding telomere length was a strong predictor of their change in telomere length across the breeding season: the longer the pre-breeding telomere length, the higher rate of telomere shortening (estimate ± s.e. = -0.697 ± 0.112, d.f. = 84.202, t = -6.248, p <0.001, Figure 11).

When the model was run separately for only females (n = 51), pre-breeding telomere length still had a strong negative effect on the change in telomere length (estimate ± s.e. = -0.667 ± 0.140, d.f. = 44.718, t = -4.773, p <0.001, Figure 11).

This was also the case when the model included just males (n = 44): pre-breeding telomere length had a significant effect on the change in telomere

length (estimate ± s.e. = -0.732 ± 0.176, d.f. = 35.269, t = -4.152, p <0.001, Figure 11).

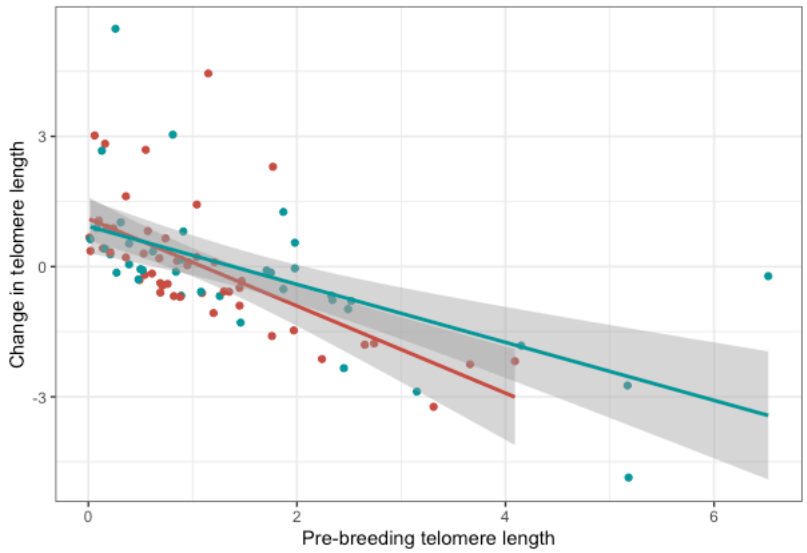

Figure 11: *The change in telomere length for each breeding bird, according to their pre-breeding telomere length (T/S ratio). This change was calculated by comparing each individual's pre-breeding and post-breeding measures of telomere length (T/S ratios). Females are represented by red points and males are represented by blue points. Each point represents an individual bird, the straight line indicates the linear regression line as estimated by the statistical model, and the grey ribbon indicates the 95% confidence interval.*

However, the change in telomere length across the breeding season was not influenced by either the amount of infidelity an individual received (estimate ± s.e. = 0.139 ± 0.114, d.f. = 78.659 , t = 1.211, p = 0.229), or infidelity made (estimate ± s.e. = 0.111 ± 0.099, d.f. = 79.511, t = 1.119, p = 0.267).
These factors were still non-significant when the model was run separately for females (infidelity received: estimate ± s.e. = 0.184 ± 0.140, d.f. = 43.266, t = 1.317, p = 0.195, infidelity made: estimate ± s.e. = 0.220 ± 0.144, d.f. = 41.579, t = 1.532, p = 0.133) and males (infidelity received: estimate ± s.e. = 0.011 ± 0.214, d.f. = 28.617, t = 0.052, p = 0.959, infidelity made: estimate ± s.e. = 0.075 ± 0.135, d.f. = 32.552, t = 0.554, p = 0.583).

Opposite to what we predicted, reproductive output did not have a significant effect on telomere length when both sexes were modelled together (estimate ± s.e. = -0.027 ± 0.024, d.f. = 80.941, t = -1.123, p = 0.265), or for females exclusively (estimate ± s.e. = -0.014 ± 0.045, d.f. = 39.968, t = -0.302, p = 0.764), or males exclusively (estimate ± s.e. = -0.036 ± 0.029, d.f. = 33.347, t = -1.234, p = 0.226).

We also found that there was no significant effect of individual mass either in the combined model (estimate ± s.e. = -0.149 ± 0.100 , d.f. = 82.989, t = -1.484, p = 0.141), or for only females (estimate ± s.e. = -0.222 ± 0.129, d.f. = 42.814 , t = -1.721, p = 0.093), or only males (estimate ± s.e. = -0.124 ± 0.161, d.f. = 31.658, t = -0.771, p = 0.446).

Social bond strength to first mate also did not have a significant effect on telomere attrition when both sexes were modelled together (estimate ± s.e. = 1.698 ± 2.249, d.f. = 74.042, t= 0.755, p = 0.453).
Interestingly, this factor did become significant when the model was run just for females. Females with a stronger pair bond strength to their first mate were more likely to show positive changes in telomere length across the breeding season, corresponding to either a maintenance or increase in length (estimate ± s.e. = 6.727 ± 2.726, d.f. = 41.853, t = 2.468, p = 0.018, Figure 12). This initially suggested that there may have been an interaction between sex and pair bond strength on the change in telomere length.
However, when we excluded a potential outlier for pair bond strength (x = 0.325, Cook's Distance = 0.53), pair bond strength was no longer a significant predictor of change in telomere length in females (>0.3: pair bond strength: estimate ± s.e. = 6.499 ± 3.428, d.f. = 42.981, t = 1.896, p = 0.065). We found qualitatively the same non-significant results with all other factors when the model was run with the outlier removed (pre-breeding telomere length: estimate ± s.e. = -0.621 ± 0.147, d.f. = 44.503, t = -4.215, p <0.001, infidelity made: estimate ± s.e. = 0.239 ± 0.150, d.f. = 41.485, t = 1.590, p = 0.120, infidelity received: estimate ± s.e. = 0.234 ± 0.161, d.f. = 42.670 , t = 1.456, p = 0.153, mass: estimate ± s.e. = -0.221 ± 0.129, d.f. = 40.069, t = -1.704, p = 0.096,

reproductive output: estimate ± s.e. = -0.021 ± 0.046, d.f. = 39.044, t = -0.455, p = 0.651).

Unlike females, pair bond strength to first mate was not a significant predictor of change in telomere length across the breeding season for males (estimate ± s.e. = -2.253 ± 3.414, d.f. = 33.914, t = -0.660, p = 0.514, Figure 12).
We found qualitatively the same results when we excluded the potential outlier for pair bond strength (>0.3: pair bond strength, estimate ± s.e. = -1.824 ± 4.527, d.f. = 30.757, t = -0.403, p = 0.690, pre-breeding telomere length: estimate ± s.e. = -0.776 ± 0.192, d.f. = 33.736, t = -4.040, p <0.001, reproductive output: estimate ± s.e. = -0.034 ± 0.030, d.f. = 31.642, t = -1.134, p = 0.265, mass: estimate ± s.e. = -0.120 ± 0.159, d.f. = 32.955, t = -0.751, p = 0.458, infidelity made: estimate ± s.e. = 0.099 ± 0.142, d.f. = 31.633, t = 0.694, p = 0.493, infidelity received: estimate ± s.e. = 0.018 ± 0.217, d.f. = 27.118, t = 0.082, p = 0.935).

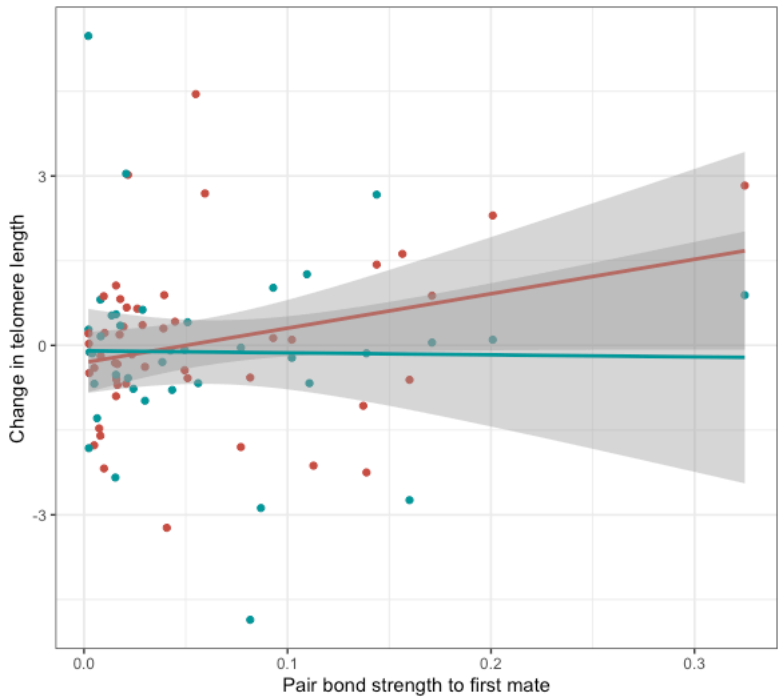

Figure 12*: The effect of pair bond strength to first mate on the change in telomere length across the breeding season, calculated by comparing each individual's pre-breeding and post-breeding measures of telomere length (T/S ratios). Females are represented by red points and males are represented by blue points. Each point represents an individual bird, the straight line indicates the linear regression line as estimated by the statistical model, and the grey ribbon indicates the 95% confidence interval.*

Discussion

Telomere length and rate of attrition have become widely regarded as indicators of both an individual's past life experiences and their future fitness (Monaghan and Haussmann 2006; Pauliny *et al*., 2006; Monaghan, 2010; Angelier *et al.,* 2019). Contrary to our predictions, neither pre-breeding telomere length nor mass predicted reproductive output for either sex in our captive zebra finch populations. We found that for both sexes, pre-breeding telomere length had a strong negative effect on telomere length change, with longer telomeres experiencing more shortening. There was no significant difference in telomere attrition between males and females. Our results provide no support for the hypothesis that an individual engaging in infidelity could increase physiological stress levels in its social mate that may increase telomere shortening, as change in telomere length was not influenced by the amount of infidelity an individual received.

Our study found no evidence that pre-breeding telomere length predicted reproductive output, and therefore no support for the suggestion that it could be used by researchers as an indicator of individual quality (Pauliny *et al*., 2006; Plot *et al.*, 2012). Many ecological studies have tried to identify phenotypic traits and behaviours that are associated with individual quality (Angelier *et al.,* 2019). Several traits have been proposed as quality indicators in zebra finches because of their association with individual variation in reproductive success, including male song signals (Houtman, 1992; Spencer *et al*., 2003) and bill colouration (Burley *et al.,* 1994). However, these traits are not always consistent and reliable indicators of individual quality, especially if they are not regularly assessed throughout the study period (Angelier *et al.,* 2019). This was demonstrated in Forstmeier's (2007) research on EPP in zebra finches. In contrast to previous findings (Houtman, 1992; Burley *et al.,* 1994), this study found that females did not preferentially copulate with males who had a redder beak or sang at a higher rate; bringing into question whether these traits consistently influence reproductive output in zebra finches (Forstmeier, 2007).

Research has attempted to uncover the link between physiological traits, telomere length and reproductive success; a study on the king penguin

(*A.patagonicus)* found a link between longer telomere length, higher natural antibody levels which are indicative of higher quality and higher breeding success (Le Vaillant *et al.,* 2015). Parolini *et al*. (2017) went one step further and demonstrated for the first time a case where telomere length is related to a trait influencing mate choice, and consequently reproductive success in both sexes. They reported that in the barn swallow (*Hirundo rustica*) telomere length is reliably signalled by sexually dimorphic colouration and correlates with reproductive output (Parolini *et al.,* 2017). The authors concluded that this indicates individuals of both sexes may be able to use colouration to choose high quality mates possessing long telomeres. In our study we found no relationship between pre-breeding telomere length and reproductive output, this may mean that in zebra finches telomere length does not correlate with any observable traits that reliably signal telomere length and influence mate choice. Further research which tests whether in this species telomere length relates to physiological traits associated with variation in reproductive output, such as song rate or bill colouration, would provide clarification on whether telomere length can be used by researchers as an indicator of individual quality. Although telomere length did not predict reproductive output in our study system, it could still potentially be an indicator of other life-history traits in this species, such as survival probability and lifespan (Haussmann *et al.,* 2005; Monaghan and Haussmann, 2006), but these traits could not be determined in our study.

It is possible that the lack of relationship between the change in telomere length and reproductive output could be due to low among-individual variation in telomere length in our study. Compared to many other *in vivo* population studies of telomere dynamics which often include subjects with a range of ages (Pauliny *et al*., 2006; Salomons *et al*., 2009; Le Vaillant *et al.,* 2015; Parolini *et al.,* 2017), the subjects in our study were all similar in age, i.e. between 11 and 15 months old. This makes it difficult to directly compare the amount of individual variation in our study populations to other study systems, as it would be expected that populations with a larger age range would have higher variation due to fact that telomere length is a trait that often changes with age (Haussmann and Vleck, 2002).

When investigating how individuals' telomere lengths changed across the course of a breeding season, we found no difference between sexes. We predicted that females would experience more telomere attrition than males for two reasons: female zebra finches are thought to have higher costs of reproduction than males due to the higher investment required to produce eggs as compared to sperm (Monaghan and Nager, 1997; Reichert *et al*., 2014), and they would be expected to receive more infidelity as males generally benefit more from engaging in EPCs (Petrie and Kempenaers, 1998; Wink and Drycz, 1999). The lack of difference in telomere attrition we observed between the sexes could be because neither reproductive output nor received infidelity influenced the telomere length change in either sex.

We predicted that a higher reproductive output would lead to increased telomere attrition (Sudyka *et al.,* 2014). This pattern was reported in another study on zebra finches, in which experimentally increasing brood size led to a reduction in both parents' telomere lengths (Reichert *et al*., 2014). We did not find evidence of a negative correlation between telomere shortening and reproductive investment in either sex (Gao and Munch, 2015). This might be because we investigated telomere length change across one breeding season, in a system where the breeding cycle was stopped at the incubation stage. As zebra finches provide parental care, partaking in a full breeding cycle where parents provision chicks for a month incurs higher reproductive costs (Mariette and Griffith, 2012). Measuring changes in telomere length after a full breeding cycle would provide a more comprehensive evaluation of the full costs of reproduction and assess the impact of these costs on telomere attrition.

There are various ways in which a mate's behaviour and life experiences can have an effect on their partner. For example, there is evidence that in zebra finches, an individual's exposure to stress hormones can have a negative effect on both their own and their partners longevity (Monaghan *et al.,* 2011). We hypothesized that extra-pair mating behaviour could be stressful due to its potential to compromise social pair bonds in a species where divorce or separation is stress-inducing and costly (Remage-Healey *et al.,* 2003; Crino *et al.,* 2017). However, we did not find any evidence that an individual receiving infidelity as a result of their social partner gaining EPP had a negative effect on

any traits we measured. There was no relationship between infidelity received and change in telomere length. This suggests that extra-pair mating behaviour does not act as a form of social stress to the extent that it induces telomere attrition. We also did not find an effect of performing infidelity on telomere length change, indicating that it does not appear to induce sufficient physiological stress for it to affect telomere dynamics.

We investigated the hypothesis that having a weaker pair bond to a social mate is stressful, by testing the relationship between pair bond strength and telomere attrition. Our results initially indicated that females with a stronger pair bond to their first mate were more likely to show positive changes in telomere length across the breeding season, corresponding to either a maintenance or increase in telomere length. However, when we excluded a potential outlier for pair bond strength, pair bond strength was no longer a significant predictor. This means that we are unable to conclude that pair bond strength had an influence on telomere length change. If a relationship between pair bond strength and telomere length does exist in females and not males, it may be because they suffer lower costs of reproduction and lower costs from infidelity when they have stronger pair bonds with their social mates. We had originally predicted that females would suffer higher costs from having weaker pair bonds and from being cuckolded. As a result, these higher costs would cause reproductive output and infidelity received to be more significant predictors of telomere length change in females than in males. However, our study did not provide evidence in either sex of a negative correlation between telomere shortening and either reproductive investment or infidelity received. This meant we cannot conclude that individuals suffered clear costs, in terms of increased telomere attrition, from receiving infidelity or their reproductive output. If birds did not incur the predicted costs over the course of our study, this may explain why there was also not a conclusive relationship between telomere length change and having strong pair bonds, which was a factor we predicted would play a key role in reducing these costs.

Our proposal of extra-pair mating behaviour as a stress-inducing factor that could increase telomere attrition, is based on the idea that this behaviour has the potential to compromise pair bonds. We could have addressed this

hypothesis more effectively by experientially manipulating pair bonds and examining the effect of this on telomere dynamics. Other studies have examined pair bonding behaviour in zebra finches by experimentally manipulating pair bonds through pair separation, followed by reuniting or repairing (Remage-Healey *et al.,* 2003; Svec *et al.,* 2009). Mate separation has been shown to cause changes in behaviour and stress hormone levels, supporting the idea that it is a stressful event for zebra finches (Remage-Healey *et al.,* 2003). Manipulating pair bonds in this way could have allowed us to see if the stress of mate separation and compromised pair bonds was sufficiently strong to increase telomere attrition.

One factor that is often not considered fully in studies investigating potentially stress-inducing factors is how animals of that species perceive that particular stressor. The psychological perception of the stress is important. Epel *et al.* (2004) were the first to demonstrate in humans *in vivo* that perceived and chronic stress correlated with oxidative stress and shorter telomere length, through measuring the stress of caregiving. They determined that it was the level of psychological stress experienced as a result of caregiving, rather than the act of being a caregiver, which was related to telomere length. The psychological perception of stress could be more important to telomere attrition than the actual severity of the stressor. Factors that we, as human observers, think of as stressful might not actually be enough to activate the HPA axis (Creel, 2001). The zebra finch in our study may have not perceived infidelity as stressfully in the way that we imagined, especially if it did not interfere with pair bond-related behaviours such as allopreening and clumping (Zann, 1996; Svec *et al.,* 2009). Alternatively, there may have been variation in the level of reaction individuals had to the behaviours which weakened the relationship between infidelity received and telomere length change in our analysis.

There are various factors which could cause different members of a population to react differently to the same level of stressor. Early-life factors which can have a strong effect on telomere length and stress responses are especially relevant. There is substantial evidence that telomere loss is much faster and more prevalent early in life, where factors such as early-life conditions and growth rate have a large effect (Hall *et al*., 2004; Monaghan and Haussmann,

2006; Haussmann and Marchetto, 2010; Angelier *et al.,* 2018). We should also consider parental effects that can influence offspring. Stressful situations encountered by parents can influence the phenotype and stress levels of their offspring, meaning that we might not see the full impact of a social stressor unless we also study the next generation (Schweitzer *et al.,* 2014; Crino *et al.,* 2017). An individual's telomere length is thought to be partly determined by the telomere length of their parents' germ cells; in tree swallows (*Tachycineta bicolor*), heritability of telomere length prior to fledging is estimated to be around 87% (Haussmann *et al.,* 2005). Atema *et al*. (2015) estimated a high heritability of telomere length in zebra finches, but the analysis had insufficient power to separate paternal effects from permanent environmental effects. Considering the influence of factors such as early-life conditions, parental environment and inheritance of telomere length could provide us with a better understanding of why individuals have the telomere lengths that they do, and how they may respond to potentially stressful situations.

Furthermore, an individual's initial telomere length could affect their response to the stressors they encounter throughout life. It has been suggested that people with longer telomeres could be psychologically more resistant to stressors than those with shorter telomeres (Epel *et al*., 2004). Those who resist potential telomere-shortening stress factors and therefore have longer telomeres for their chronological age have therefore often been considered to be of 'higher quality' (Pauliny *et al*., 2006). It is also why telomeres have frequently been proposed as a biomarker for an individual's ability to cope with stressful situations (Kotrschal *et al.,* 2007). Our results do not support the idea that telomere length is a quality indicator, as we found no relationship between pre-breeding telomere length and reproductive output. We found a strong relationship between initial telomere length and telomere attrition, but in fact found that longer telomeres shortened more across the breeding season. This relationship has been recorded in several other avian species. For example, a study on the European shag (*Phalacrocorax aristotelis*) showed that birds with the longest telomeres as chicks had greater telomere shortening (Hall *et al.,* 2004), and work on western jackdaws (*Corvus monedula*) found that longer telomeres shortened faster in both adults and nestlings (Salomons *et al.,* 2009). The latter study proposed that their results may be due to shorter telomeres being better

protected from attrition. Telomere maintenance mechanisms may preferably protect the shortest telomeres from further degradation, to prevent them shortening to a critical length and inducing cellular senescence (Haussmann and Marchetto 2010; Monaghan, 2010). This pattern of longer telomeres shortening faster is an interesting discovery, because it could suggest that any advantage from having longer telomeres, such as increased probability of survival, might diminish with age (Haussmann *et al.,* 2005; Verhulst *et al.,* 2013).

However, an important reason why an increasing number of longitudinal studies have reported accelerated telomere attrition when initial telomere length is longer, might be a statistical artefact known as “regression to the mean” (RTM) (Johnson and George, 1991; Barnett *et al.,* 2005; Verhulst *et al.,* 2013). This effect occurs because comparisons between measurements of pre-breeding telomere length and the rate of telomere attrition are not independent, because the pre-breeding data contribute to both measurements. This means that any measurement error in pre-breeding telomere length affects both measurements, generating the RTM effect (Verhulst *et al.,* 2013). RTM can also occur in the absence of measurement error, particularly when unusually large or small measurements tend to be followed by measurements of the same individual that are closer to the mean (Johnson and George, 1991; Barnett *et al.,* 2005; Skinner *et al.,* 2015). Verhulst *et al.* (2013) were the first to look at the relationship between initial telomere length and telomere attrition rate before and after correcting for this phenomenon, in humans. They initially found a strong relationship between these two factors, but after correcting for the RTM effect in their analysis, the slope of the relationship was more than halved. This demonstrated that the RTM effect was in part responsible for the strong relationship, despite the measurements of telomere length being highly consistent. However, they also found that even after correcting for the RTM effect, there remained a statistically significant relationship, indicating that longer baseline telomere length is associated with faster attrition (Verhulst *et al.,* 2013). The RTM effect is thus important to consider when investigating telomere attrition, because it has the potential to make natural variation in repeated data look like real change and therefore could lead to incorrect conclusions about the relationship between initial telomere length and telomere

attrition (Barnett *et al.,* 2005; Verhulst *et al.,* 2013). Our results may indicate a real link between longer pre-breeding telomere length and faster attrition, but we must be cautious in their interpretation as the strength of this relationship could have been amplified by the RTM effect.

Another surprising result of our investigation into telomere dynamics was the finding that a similar number of individuals experienced some level of telomere lengthening as the number that experienced telomere attrition. For females, this ratio was 50/50 increase versus decrease in length. Other studies on captive zebra finches have shown that telomere length decreases with age and correlates with maximum lifespan (Haussmann and Vleck, 2002; Haussmann *et al.,* 2003). Telomere lengthening has been reported *in vivo*: Leach's storm-petrels (*Oceanodroma leucorhoa)* are extremely long-lived birds that experience telomere lengthening with age rather than attrition, but this trend is seen for the species overall rather than just in a few individuals (Haussmann *et al.,* 2003). It is unclear whether the results of the study reporting this pattern were actually an indication of telomere elongation within individuals. As it was a cross-sectional study, the positive relationships between age and telomere length could instead be due to differential survival of individuals with very long telomeres (Monaghan and Haussmann, 2006).

Longitudinal studies such as ours which measure telomere length change in the same individuals over time are better able to distinguish the effects of individual variation and population composition from the factors influencing change in telomere length (Hall *et al.,* 2004; Monaghan and Haussmann, 2006; Verhulst *et al*., 2013). Longitudinal studies on humans (Martin-Ruiz *et al*., 2005) and wild-caught house mice (*Mus musculus*) (Kotrschal *et al.,* 2007) also found telomere lengthening for some individuals. After observing telomere lengthening in female mice, Kotrschal *et al.* (2007) suggested that when stress is minimized, telomeres might increase through a form of restoration. Several telomere restoration methods have been discovered but these have mainly been investigated at a cellular level and explain how telomere length is maintained, not increased (Monaghan, 2010). It is clear that more research is needed to fully understand the cellular mechanisms behind telomere lengthening. Combining this with more longitudinal studies to try and measure how prevalent this phenomenon is in different species could help us understand why some

individuals appear to go against the well-documented pattern of telomere attrition over time.

It may be that using a different method of telomere measurement would have given us different results, potentially more similar to what we would have expected given the results of other research on telomere dynamics in zebra finches (Haussmann and Vleck, 2002; Lai *et al.,* 2018). In our study we used qPCR, a widespread method of determining relative telomere length (Aubert *et al.,* 2012). However, there are advantages and disadvantages to different methods that should be considered when choosing a technique. qPCR has become a popular choice in recent research, particularly when dealing with large sample sizes; it is relatively easy to conduct, is much quicker than other methods and doesn't require a large amount of DNA (Aubert *et al.,* 2012; Lai *et al.,* 2018). However, there can be large variability in results among different laboratories and samples (Cawthon, 2002; Aubert *et al.,* 2012). We mitigated against the variability of this technique by including controls on every PCR plate, making comparisons between plates and experiments more reliable (Aubert *et al.,* 2012). Another disadvantage is that qPCR only provides a relative measure of telomere length, meaning that our conclusions are limited as even statistically significant results are only correlative (Lai *et al.,* 2018).

The other main method that starts with genomic DNA is telomere restriction fragment (TRF) length analysis. This technique is still commonly regarded as the 'gold standard' for telomere length measurement and can generally determine an average telomere length per sample by measuring the intensity of telomere smears (Kimura *et al.,* 2010; Aubert *et al.*, 2012). TRF provides the most information on average telomere length and generally has a relatively small amount of error compared to qPCR methods (Aubert *et al.,* 2012; Lai *et al.,* 2018). This technique has its own set of limitations; it requires large amounts of starting DNA, takes a relatively long time compared to qPCR and the measurement of telomere length can vary widely depending on the restriction enzymes used (Cawthon, 2002; Kimura *et al.,* 2010; Lai *et al.,* 2018). It is possible that we might have found a stronger pattern of telomere attrition with larger sample sizes; qPCR can be used to assess general trends between population groups, but this is thought to be best when there is a large number of

samples (Aubert *et al.,* 2012). On the other hand, TRF lends itself better to analysis of smaller populations, up to around 130 samples, where smaller error is needed (Kimura *et al.,* 2010). Our analysis could have been improved if we had either used TRF as the telomere measurement technique or used larger sample sizes to help mitigate the comparative inaccuracy of qPCR (Aubert *et al.,* 2012).

In conclusion, we found no conclusive evidence that receiving infidelity or experiencing weaker pair bonds induces sufficient physiological stress in zebra finches for it to affect telomere dynamics. The weak evidence of a potential interaction between sex and pair bond strength on the change in telomere length suggests that further research is needed to address why this relationship could exist in one sex and not the other. We suggest that future studies investigating the effect of the social environment on telomere dynamics should consider using experimental manipulation of social pairs, this may allow them to come to a clearer conclusion on the effect of EPP and pair bond strength on telomere attrition. Our results indicate that within a population, some individuals may experience telomere lengthening while others experience shortening, with the longest telomeres shortening the fastest. We found no evidence that telomere length can be used as an indicator of individual quality in zebra finches, but further research could clarify this by testing whether telomere length relates to physiological traits associated with variation in reproductive output in this species, such as song rate or bill colouration. Future studies need to fully consider the psychological impact of potential stressors and be cautious that factors such as initial telomere length may cause variation in how individuals in a population react to a potential stressor.

Chapter four: General Discussion

Extra-pair paternity (EPP) is now recognised to be a widespread phenomenon among socially monogamous avian systems (Griffith *et al.,* 2002; Westneat and Stewart, 2003). There is a large amount of intraspecific variation in extra-pair mating behaviour, and discovering the factors driving this variation is key to understanding population differences (Petrie and Kempenaers, 1998; Griffith *et al.,* 2002; Westneat and Stewart, 2003). Variation in EPP between domesticated populations of zebra finches (*Taeniopygia guttata*) has been well-documented but remains poorly understood (Forstmeier *et al*., 2007a; Griffith *et al.,* 2017). The aim of my thesis was to investigate potential links between pair bond strength and extra-pair mating behaviour, and potential effects on telomere attrition, in zebra finches.

In Chapter two I tested whether differing selection on promiscuity translated to variation in extra-pair mating behaviours. I also examined whether pair bond strength was correlated with extra-pair mating behaviour and reproductive output in this species. I determined that birds from two promiscuity breeding lines, which had been selected to have high or low breeding values of male sex drive (Forstmeier *et al.,* 2011), did not differ in the number of mates or occurrence of EPP. As found in other domesticated populations of this species (Forstmeier *et al*., 2011; Ihle *et al*., 2013), the birds in each of the four breeding populations showed a high level of EPP, regardless of breeding line. Consistent with what would be expected in a socially monogamous species, I found that the number of eggs an individual produced within their social pair was a strong predictor of their total reproductive output. I report another interesting pattern: engaging in infidelity had a significant positive effect on reproductive output in males, but not in females. My research therefore provides evidence to support the idea that males generally benefit more than females from engaging in EPCs, in terms of an increased reproductive output (Petrie and Kempenaers, 1998; Wink and Drycz, 1999). Considering this finding, I might have expected to see that males were more inclined to copulate with a larger number of different mates, but I found no evidence of this; males and females had a similar number of both social and genetic mates. However, as my quantification of genetic mates arose from paternity analysis, it only encompassed successful

fertilizations, so I was unable to determine whether the rate of EPCs differed between the sexes. This highlights the necessity of measuring both EPP and EPCs, as measuring just the EPP rate does not provide us with the whole picture of the mating behaviours and strategies occurring in the population (Petrie and Kempenaers, 1998; Forstmeier, 2007).

In Chapter three I investigated how pair bond strength and extra-pair mating behaviour may affect telomere dynamics within our zebra finch populations. Telomeres have become regarded as important biomarkers of biological age and individual quality (Monaghan and Haussmann 2006; Monaghan, 2010; Angelier *et al.,* 2019). The measurement of telomeres has become a valuable tool that can give us insight into an individual's past life experiences (Epel *et al.,* 2004; Kotrschal *et al.,* 2007), and potentially their future reproductive success (Pauliny *et al.,* 2006) and lifespan (Haussmann *et al*., 2005; Salomons *et al.,* 2009). I conducted a within-individual repeated-measures study of telomeres across an experimentally-controlled breeding season to quantify the influence of infidelity and pair bond strength on telomere attrition.

I found a strong relationship between initial telomere length and telomere attrition, with longer telomeres shortening more across the breeding season. This relationship has been reported in several other avian species such as European shags (*Phalacrocorax aristotelis*) (Hall *et al.,* 2004) and western jackdaws (*Corvus monedula)* (Salomons *et al*., 2009). Telomere length is thought to reflect individual quality, as supported by studies reporting links between the possession of longer telomeres and quality indicators such as increased reproductive success, larger body size and higher antibody levels (Wilson and Nussey, 2010; Le Vaillant *et al.,* 2015; Parolini *et al*., 2017; Angelier *et al.,* 2019). This has led to an expectation that those higher-quality individuals with longer telomeres will be better able to resist potential telomere-shortening stress factors and therefore have slower rates of telomere attrition (Epel *et al.,* 2004; Pauliny *et al.,* 2006; Kotrschal *et al*., 2007). However, as my results indicate, this is not always the case. It is possible that shorter telomeres are better protected from attrition by telomere maintenance mechanisms to prevent them from further degradation (Salomons *et al.,* 2009; Haussmann and Marchetto 2010; Monaghan, 2010). It is also feasible that behaviours

associated with being a higher-quality individual could lead to increased telomere shortening. Le Vaillant *et al.* (2015) proposed that an individual having longer telomeres, and thus being of higher quality, could lead to obtaining higher reproductive success, which in turn could lead to higher rates of telomere loss due to the costs of reproduction. This seems to be the case in other species such as common terns (*Sterna hirundo)* (Bauch *et al.,* 2013). I am unable to conclude that this is the reason for the patterns of telomere attrition I observed, as I found no evidence that individuals with longer telomeres pre-breeding had a higher reproductive success, or that reproductive output in terms of number of eggs produced, predicted change in telomere length.

The phenomenon of longer telomeres shortening faster is interesting, because it could mean that any advantage associated with having longer telomeres, such as increased survival probability and increased reproductive success, would gradually diminish with age (Haussmann *et al.,* 2005; Pauliny *et al.,* 2006; Verhulst *et al.,* 2013). This pattern is particularly important, because it now appears that it is not the length of telomeres that explains differences in lifespan, but instead the rate of telomere shortening (Monaghan and Haussmann, 2006). Faster shortening of telomeres has been directly linked to shorter lifespan (Haussmann *et al.,* 2003; Salomons *et al.,* 2009). This may mean that having longer telomeres initially does not equate to having a life-long advantage, especially regarding lifespan. However, the strength of the relationship I present between pre-breeding telomere length and attrition rate could have been amplified by a statistical artefact known as “regression to the mean” (RTM) (Johnson and George, 1991; Barnett *et al.,* 2005; Verhulst *et al.,* 2013), and should thus be interpreted with caution.

As previous research on captive zebra finches has shown that telomeres shorten significantly with reproductive effort across several reproductive cycles (Heidinger *et al.,* 2012), I expected to observe a similar decline in telomere length in my birds across the course of the breeding season. However, instead I observed both telomere shortening and telomere lengthening occurring in my populations. The deviance from other descriptions of zebra finch telomere dynamics might have been due to my chosen method of determining telomere length. I used qPCR analysis; this is a popular choice when dealing with large

sample sizes, but is often considered to be less accurate than other methods such as telomere restriction fragment (TRF) length analysis (Haussmann and Vleck, 2002; Criscuolo *et al.,* 2009; Aubert *et al.,* 2012; Lai *et al.,* 2018). However, if the telomere lengthening I observed was a reflection of real telomere dynamics occurring in my populations, there are several telomere restoration methods which could have mediated it, although more research is needed to clarify how they occur *in vivo* (Chan and Blackburn, 2004; Cesare and Reddel, 2010; Monaghan, 2010).

There is evidence of telomere lengthening in a number of species, including wild-caught house mice (*Mus musculus)* (Kotrschal *et al.,* 2007), Leach's storm-petrels (*Oceanodroma leucorhoa*) (Haussmann *et al.,* 2003) and humans (Martin-Ruiz *et al.,* 2005). Reichert *et al*. (2014) also observed telomere lengthening in zebra finches whilst investigating the effect that experimentally increasing brood size has on telomere dynamics and survival. They found that parents of enlarged broods had reduced telomere length, supporting previous findings that increased reproductive investment links to shorter telomeres (Sudyka *et al.,* 2014; Gao and Munch, 2015). However, Reichert *et al*. (2014) also discovered that in the control groups, where the numbers of chicks were unchanged, and in the reduced groups in which 2 chicks were removed, parents did not appear to incur any costs of reproduction in terms of telomere loss. Instead, these parents experienced slight telomere lengthening. It has been suggested that in periods of minimised stress, telomeres might increase through methods of telomere maintenance or restoration (Kotrschal *et al.,* 2007; Monaghan, 2010). It is also possible that when experiencing optimal conditions, individuals might be able to adjust their reproductive effort accordingly by the activation of telomere maintenance mechanisms (Reichert *et al.,* 2014). It could be argued that the birds in my study were in optimal conditions: food, water and nesting material were supplied *ad libitum*, with an adequate number of nest boxes for all social pairs. In addition to this, the breeding cycle was stopped at the incubation stage, so it is likely that parents experienced lower reproductive costs than they would have during a full breeding cycle, as zebra finches provision chicks for a month (Heidinger *et al.,* 2012; Mariette and Griffith, 2012). The lengthening I observed could therefore be attributed to individuals adjusting their reproductive effort according to their conditions and laying the 'optimal'

number of eggs. However, as they then did not have to raise the chicks, they did not suffer the expected costs of chick provisioning, so the overall change in telomere length was positive. It would be interesting to see how common this occurrence is among different avian species and whether the pattern is maintained long-term. There is increasing evidence that the effect of reproductive investment on telomere length varies according to factors such as individual quality (Hamel *et al.,* 2009; Hamel *et al.,* 2010; Le Vaillant *et al.,* 2015). If this is the case, then the link between costly experiences and telomere attrition may not be as straightforward as it first appears.

When studying the occurrence and potential impact of EPP in my populations, I found no significant effect of receiving infidelity on the change in telomere length. There is thus no support for my hypothesis that extra-pair mating behaviour induces sufficient physiological stress in zebra finches for it to affect telomere dynamics. There was also no difference between sexes in the amount of infidelity they received from their social mate. Perhaps surprisingly, I found that having a higher mass correlated with receiving more infidelity, but my findings are limited by my singular measurement of mass that was taken before the start of the breeding season.

I then considered whether having weaker pair bonds to a social mate is stressful, by testing the relationship between pair bond strength and telomere attrition. I found no conclusive relationship between these two factors. I conclude that experimentally manipulating pair bonds may have more effectively addressed the hypothesis of whether experiencing compromised pair bonds is sufficiently stress-inducing to cause telomere attrition (Remage-Healey *et al.,* 2003). It is also plausible that these factors might need to be examined over several breeding cycles in order to see an effect of pair bond strength on telomere dynamics. As mentioned above, full breeding cycles in this species involve provisioning chicks for a month. The total cost of parental care, and thus pressures on the pair bond, could accumulate and induce a sufficient level of physiological stress to increase telomere attrition. This could be particularly prominent in the presence of EPP, as a female's engagement in EPCs could decrease the amount of parental care provided by her social mate, increasing

her investment and thus cost of reproduction and jeopardising her pair bond (Møller, 2000; Arnqvist and Kirkpatrick, 2005; Mariette and Griffith, 2012). Although I found no evidence that weaker pair bonds were stress-inducing, I did find that stronger pair bonds were correlated with increased reproductive success. This finding supports previous research in other avian species suggesting that stronger pair bonds are related to higher reproductive success, such as in short-tailed shearwaters (*Puffinus tenuirostris*) (Bradley *et al.,* 1995), barnacle geese (*Branta leucopsis*) (Black, 2001) and bearded reedling (*Panurus biarmicus*) (Griggio and Hoi, 2011).

Previous research has highlighted that pre-breeding associations and pair bond strength play a key role in social-pair formation and social-pair reproductive output, but the role of the social environment in EPP has often been overlooked (Bradley *et al.,* 1995; Beck *et al.,* 2020). My study highlights that pre-breeding social associations can have a significant impact for a prolonged period, influencing both social-pair and extra-pair reproduction. My findings indicate that pair bond strength could be a significant predictor of both within-pair and extra-pair reproductive output. For this reason, the expansion of future research to include the role of the social environment when investigating extra-pair mating behaviours could help to determine how social associations influence later extra-pair mate selection and the prevalence of EPP (Maldonado-Chaparro *et al.,* 2018; Beck *et al.,* 2020).

Conclusion

The zebra finch is considered to be a socially monogamous species that displays very low levels of EPP in the wild (Griffith *et al.,* 2010). I have demonstrated that in two domesticated breeding lines of this species, almost all birds had more than one social mate and the majority were involved in extra-pair mating behaviour. This research provides evidence to support previous findings that levels of EPP within domesticated zebra finch populations are higher than those reported in the wild (Ihle *et al.,* 2013; Griffith *et al.,* 2017). This reiterates the importance of understanding the variation in reproductive behaviour and mating strategies within a species. This is particularly imperative in a species such as the zebra finch, which has had great significance in

evolutionary and behavioural studies (Griffith *et al.,* 2017). I have demonstrated that males can benefit from an increased reproductive output by engaging in extra-pair mating strategies, but that this does not necessarily result in them reproducing with significantly more mates than the number that females in a population reproduce with. I have highlighted that social associations play a role in reproductive behaviour, by providing evidence that a breeding pair's bond strength correlates with their reproductive output. I found no conclusive evidence that receiving infidelity or experiencing weaker pair bonds induces sufficient physiological stress in zebra finches for it to affect telomere dynamics. I also found no evidence that telomere length can be used as an indicator of quality or as a predictor of reproductive output in this species. However, I found that some individuals experienced telomere lengthening while others experienced shortening, with the longest telomeres shortening the fastest.

On a broader scale, understanding the factors influencing extra-pair mating behaviour is important because variation in EPP has substantial consequences for individual fitness and the evolution of social mating systems (Møller, 2000; Yuta and Koizumi, 2016). EPP can be an important source of sexual selection on male secondary sexual characters, particularly plumage pattern and brightness (Møller and Birkhead, 1994; Sheldon and Ellegren, 1999). It has even been proposed that the selective pressure of attempting to gain EPCs while still maintaining a social-pair bond is one of the factors contributing to the evolution of large brain size in pair-bonded birds (West, 2014). Despite the breadth of research into extra-pair mating behaviour, it is clear that many of its components remain poorly understood. Further research into the factors influencing extra-pair mating behaviour at both a population and individual level is needed to understand its prevalence, drivers and consequences among socially monogamous species.

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