Factors affecting the evolution of plumage colouration, sperm morphology and mating behaviour in parrots of the world

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Table of contents

Summary

Phenotypical differences between sexes in terms of body size or colouration, known as sexual dimorphism, have been mainly attributed to sexual selection. Sexual selection is stronger in species with polygamous mating systems than in species with monogamous mating systems as a consequence of more intense competition for access to mates in the former. Therefore, it is expected that among polygamous species there would be more cases of sexual dimorphism, and this is indeed the case. However, sexual differences in monogamous species are still observed and its incidence seems to be a consequence of sexual selection but at the genetic level. Thanks to the development of molecular tools, we now have evidence suggesting that among the bird species classified as monogamous (approximately 90% of bird species), 76% are actually genetically polyandrous. This indicates that females engage in extra-pair copulations which lead to a competition between males to fertilize their eggs, a phenomenon called sperm competition. Different studies have shown that sperm competition is stronger in species that are sexually dichromatic (i.e. different colouration between the sexes within a species), explaining the incidence of sexual dichromatism in some socially monogamous species. However, there are other mechanisms besides sexual selection that might have driven the evolution of sexual dimorphism, such as environmental factors, social interactions and life-history traits. It is therefore important to consider these mechanisms when investigating the factors that have shaped the evolution of sexual differences in species.

In my PhD, I explored whether sexual selection has shaped the evolution of parrot species, a group of birds considered to be mainly monogamous. The majority of parrots seem to be socially monogamous with some forming lifelong pair bonds; however, few studies have investigated the breeding behaviour of these species in detail. Estimating the level of extra-pair paternity in species is possible when long-term studies are established and paternity

analyses can be carried out over many years along different breeding seasons. This requires long-term funding and relies on pairs breeding in consecutive years, a task that might be difficult for some long-lived parrot species that might not breed every year. Due to these difficulties, we can instead estimate the level of extra-pair paternity through sperm competition indexes. In **Chapter 1**, I used sperm length and coefficients of variation in sperm length to evaluate the varying levels of sperm competition across 62 parrot species, and performed comparative analyses to understand whether some species might have stronger sperm competition. In **Chapter 2**, I then estimated the level of sexual dichromatism across 398 parrot species using bookplates, and investigated what factors could explain the varying levels of sexual dichromatism. Finally, **in Chapter 3**, I assessed whether mating behaviour (i.e. within-pair copulation frequency and duration) was related to sperm competition indexes or other factors in 103 parrot species in a captive population.

The results of **Chapter 1** of my dissertation revealed that there are varying levels of sperm competition in parrot species. I found that sexually dichromatic species and gregarious species have longer sperm, indicating that these species potentially have stronger sperm competition. This finding is in line with previous findings showing that extra-pair paternity rates are higher among sexually dichromatic species. To further understand the factors shaping the evolution of plumage colouration and sexual dichromatism in parrots, in **Chapter 2**, I performed a more detailed estimation of the colour elaboration and sexual dichromatism levels in all extant parrot species. Among many results, I found that males and females of larger parrot species are more colour elaborated but less sexually dichromatic, whilst smaller parrot species are less colour elaborated but more sexually dichromatic. These findings suggest that smaller parrot species might have higher extra-pair paternity rates and/or higher selective pressure for crypsis, and larger parrot species might experience stronger mutual mate choice. Additionally, in **Chapter 2**, I found that parrots follow Gloger's

rule, showing that darker coloured parrots live in wetter environments. Finally, I wanted to explore whether these factors have also affected the mating behaviour of parrots. In **Chapter 3**, I explored whether the within-pair copulation frequency and duration were related to body size, gregariousness, sexual dimorphism, sexual dichromatism, male colour elaboration or sperm length. However, I did not find any significant relationships within this new, vast, dataset. Overall, the results of my dissertation suggested that parrots have varying levels of sperm competition, and that this sexual selection mechanism, together with environmental factors, social interactions and life-history traits, have shaped the evolution of parrot plumage colouration and sexual dichromatism. Although the majority of parrots might be socially monogamous, my dissertation has shown that there are varying levels of sperm competition among the different species, and that this, together with other factors, has driven the evolution of plumage colouration in parrots. Therefore, further studies are still needed to unravel the breeding biology of parrots, which could have important implications for conservation in a group of birds that is highly threatened.

General introduction

Sexual selection and secondary sexual characters

Sexual selection was first described by Charles Darwin as *'the advantage that certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction'* (Darwin 1871). Sexual selection is generally accepted as the main functional process driving the evolution and maintenance of sexual dimorphism (Darwin 1871; Andersson 1994), with many secondary sexual characters being targets of this evolutionary process (Svenson and Gosden 2007). When the presence of these characters provides one individual an advantage over another of the same sex by improving their competing skills and hence their reproductive success, it is likely that these characters have evolved through sexual selection (Goodenough et al. 2001). Secondary sexual characters are normally ornate, exaggerated traits that are costly to produce and maintain (Moller 1996). Therefore, it has been proposed that the differential cost of secondary sexual characters is a necessary condition for reliable signalling, with low-quality individuals being unable to produce a larger or more elaborated sex trait compared with high-quality individuals because of its high cost of production and maintenance (Zahavi 1975; Iwasa et al. 1991). In other words, if secondary sexual characters are condition dependent, an individual's quality would be associated to the expression of these sex traits. For example, it has been found that antler size was significantly correlated with male breeding success in a red deer (*Cervus elaphus*) population (Kruuk et al. 2002). Males with larger antlers sired more calves across their lifetime than males with shorter antlers, even when corrected for body size.

Sexual selection mechanisms

Sexual selection can take place through two mechanisms which allow individuals of one sex to gain access to mates. These mechanisms are known as intrasexual selection (typically *male-male competition*) and intersexual selection (typically *female choice*) (Darwin 1871; Goodenough et al. 2001). Intrasexual selection has led individuals of one sex (usually males) to evolve a great range of attributes related to intense competition among them, such as the antlers of male red deer and the enormous horns of male rhinoceros beetles (Figure 1A). On the other hand, intersexual selection arises when individuals of one sex (usually females) choose mates according to certain preferred characteristics, and this mechanism has led to the evolution of traits, such as the vocalisations of male singing frogs and the extravagant plumage colouration of males of birds of paradise (Figure 1B) (Darwin 1871; Moller 1996).

Regardless of which of these two sexual selection mechanisms takes place, males are typically competing for access to females and females are typically choosy selecting a mate (Goodenough et al. 2001). These differences in mating strategies between sexes have been mainly explained by sex differences in gamete production or parental care (Trivers 1972; Goodenough et al. 2001). Females produce a limited number of large, energetically expensive eggs, whereas males produce millions of small, less expensive sperm. This difference in the number and size of gametes produced by the sexes could explain why females are normally the choosy sex. As eggs are a limited and expensive resource to produce, males compete for access to it (Goodenough et al. 2001). However, if the difference in gamete investment was the only explanation behind the difference in mating strategy between sexes, we would expect than in all species females were the choosy sex and males would compete to gain access to females, but this is not always the case. There are some species that do not follow this pattern, and in these, females compete to gain access to males; this process is called sex-

role reversal (Jenni 1974). The explanation behind sex-role reversal is related to parental care. It seems that sex differences in parental roles can override anisogamy, and under this scenario the sex that invests more in the care of the offspring becomes the limiting resource for which the sex that invest less competes for (Trivers 1972; Goodenough et al. 2001; Kokko and Jennions 2008).

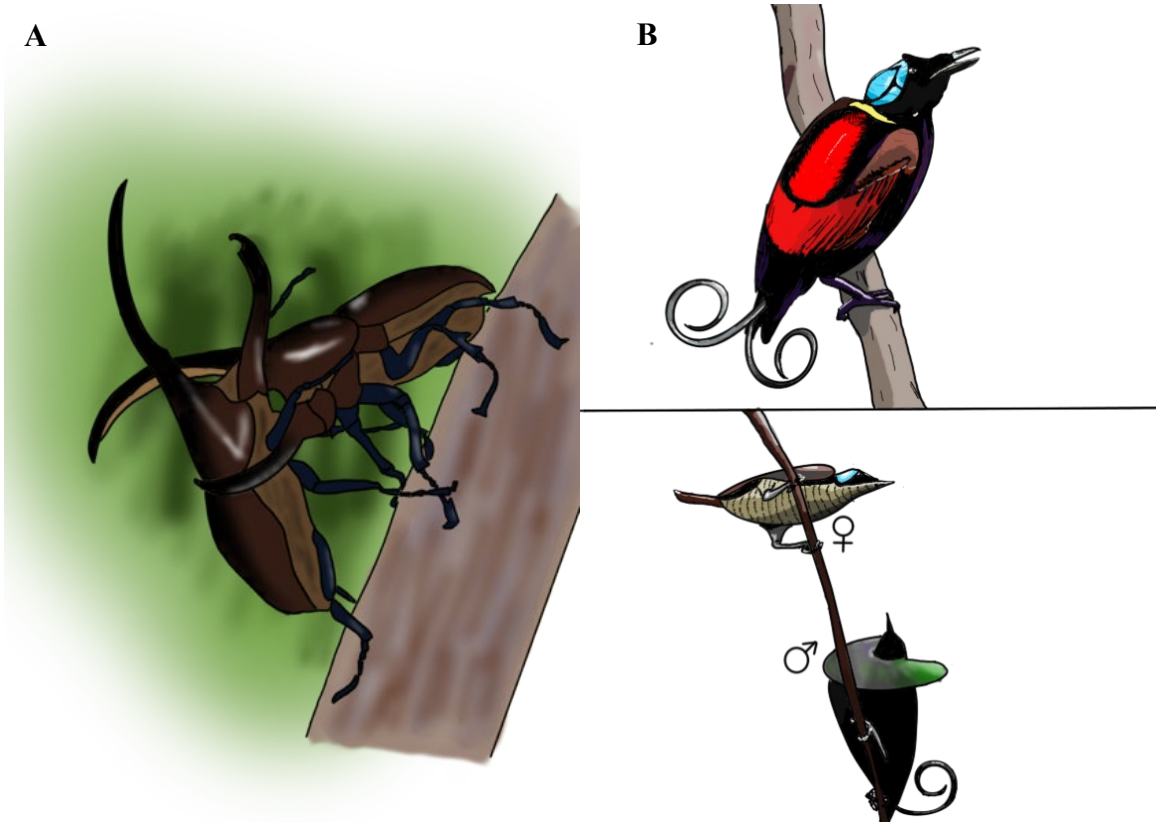

Figure 1. Secondary sexual characters evolved via sexual selection. (A) Males of rhinoceros beetle have large horns in their heads which are used as weapons during male-male competition. (B) Males of the Wilson's bird-of-paradise exhibit the most colourful plumage of all the species within the family. During the courtship display, males will show their striking breast shield while dancing and vocalising to the female. Drawings by Dr Laurie O'Neill.

Other factors affecting sexual dimorphism

There are other factors besides sexual selection that might have driven the evolution of sexual dimorphism (West-Eberhard 2014; Dale et al. 2015). Males and females can be differently affected by environmental factors, such as foraging patterns and predation risk, or by social interactions, and these differences may have influenced the evolution of phenotypical differences between the sexes (Goodenough et al. 2001; Dale et al. 2015). Sexual dimorphism in the trophic apparatus (e.g. mouthparts) may have risen as a consequence of differences between males and females in the rate of feeding or the type of food consumed (Shine 1989). For example, females in many insect species feed on blood to be able to produce eggs by using their piercing mouthparts to penetrate the skin of an animal, whereas males feed on plant juices or nectar and their mouthparts are less strong (Shine 1989).

The most striking examples of sexual dimorphism are the differences in plumage colouration between males and females (i.e. sexual dichromatism) of many bird species. The evolution of sexual dichromatism in birds, particularly male-biased secondary sexual characters, has mainly been attributed to sexual selection (Darwin 1871). However, a large comparative analysis exploring the evolutionary drivers of both male and female ornamentation of passerine birds found that females have more elaborated colours in cooperatively breeding species, where female-female competition (social competition) for ecological resources may be high (Dale et al. 2015). Additionally, this study showed that in species where sexual selection is stronger and male colour elaboration increases, females show a reduction in their plumage colouration. This finding illustrates the power of both sexual and natural selection; in species with strong-male biased sexual selection the parental care is normally carried out by the female only. Thus, while sexual selection is driving an increase in male colour elaboration, natural selection is intensifying selection for crypsis by making females less

colourful, which is possibly related to the increase in predation risk females experience while caring for their offspring.

Mating systems

Two important factors in the evolution of mating systems are the need for parental care and the spatiotemporal distribution of females (Emlen and Oring 1977). Depending on these two factors species are classified as monogamous, polygynous, polyandryous or promiscuous (Emlen and Oring 1977; Goodenough et al. 2001). These categories are defined according to the number of individuals with which one individual mates and forms pair-bonds.

The mating system favoured in a given species would depend on whether parental care from both parents is required for successful rearing of young (Emlen and Oring 1977). When males and females can increase their reproductive success by sharing parental care duties, monogamy is favoured (Goodenough et al. 2001). This is the case for the large majority of birds where approximately 90% of species are socially monogamous (Lack 1968; Goodenough et al. 2001; Bennett and Owens 2002). The spatiotemporal distribution of females also plays a role in defining the mating system. If females are distributed close in space, males are able to mate with more females, and under this scenario polygyny would be favoured (Emlen and Oring 1977; Goodenough et al. 2001). However, the time in which females become sexually receptive would also determine the number of mates a male can monopolize (Emlen and Oring 1977). A male would have less chances to increase the number of mates monopolized when females show synchrony in their sexual receptivity and when females are sexually active for a short period, as occurs in temporally defined breeding seasons.

Monogamy is then defined as the mating system where both males and females mate with only one partner per breeding season (some pair-bonds can last beyond one season) and show biparental care (Lack 1968; Goodenough et al. 2001; Bennett and Owens 2002). When one sex is freed from parental care duties, polygamous mating systems prevail. Polygyny refers to the mating system where males gain access to multiple females and polyandry when females monopolized multiple males (Emlen and Oring 1977; Goodenough et al. 2001). When there is no association whatsoever between the sexes and males and females meet exclusively for mating, the mating system is called promiscuity.

The strength of sexual selection varies according to the mating systems. Sexual selection is relatively lower in monogamous mating systems, and it is more intense among polygamous species (Emlen and Oring 1977).

Sperm competition

The development of molecular techniques provided new information regarding parentage in birds, showing that mating can take place outside the pair bond (Burke and Bruford 1987). Thanks to these new genetic tools it has been found that 76% of species which have been previously considered socially monogamous are actually genetically polyandrous, meaning that socially monogamous species show varying levels of extra-pair paternity, with the social male partner not always being the sire (Brouwer and Griffith 2019). In these socially monogamous species in which genetic polyandry has been found, an average of 19% of offspring was sired by an extra-pair male (Brouwer and Griffith 2019). Social monogamy does not always mean genetic monogamy, and it has actually been suggested that genetic monogamy is rare across bird species (Brouwer and Griffith 2019).

When females engage in extra-pair copulations the sperm of the different males enter a competition to fertilize the ova of a particular female, and this process is called sperm competition (Parker 1970). Sperm competition is an important component of sexual selection as it results in differential reproductive success among males, and it is a selective force that has shaped the evolution of behaviours and morphology associated with reproduction (Birkhead 1987). Before parentage analyses were available, the occurrence of sexual dimorphism among socially monogamous species was puzzling as the intensity of sexual selection was expected to be low among these species. There is now evidence showing that bird species with high levels of extra-pair paternity have higher levels of sexual dichromatism (Møller and Birkhead 1994; Owens and Hartley 1998).

Mating systems and sperm competition in parrots

The majority of parrots appear to be socially monogamous with lifelong pair bonds (Toft and Wright 2015), although few species have been studied in detail. From the few studies carried out in the wild, it has been documented that pairs of the Major Mitchell's cockatoo (*Cacatua leadbeateri*) and the white-tailed black-cockatoo (*Calyptorhynchus funereus*) stay together until one of the partners dies (Saunders 1982; Rowley and Chapman 1991). It was observed that females that had lost their mate deserted the nest, and males that had lost their mate failed to rear the chicks. Failing to rear a brood when one of the members of a pair has died is understandable in species such as parrots that have biparental care (Toft and Wright 2015). It is a general pattern across parrots that females incubate fulltime and males provide food for themselves, their females and their chicks, once these have hatched. Additionally, as parrots are long lived species (Wasser and Sherman 2010), the higher parental investment required to

rear a brood by one member of the pair alone in the current breeding opportunity does not pay off when there are more future breeding opportunities.

These lifelong pair bonds are often used as evidence by some researchers to suggest that parrots seem to be of the few species achieving true monogamy (Toft and Wright 2015). Furthermore, some studies have shown that parrots are genetically monogamous as well as socially monogamous (Masello et al. 2002; Caparroz et al. 2011; Eastwood et al. 2018), in other words, it appears that some parrots do not engage in extra-pair paternity. Nevertheless, there are also some parrot species that show varying levels of extra-pair paternity, with the green-rumped parrotlet (*Forpus passerines*), the monk parakeet (*Myiopsitta monachus*) and the swift parrot (*Lathamus discolour*) showing 14%, 40% and 50.5%, respectively, of nests with extra-pair paternity (Martínez et al. 2013; Waltman and Beissinger 1992; Heinsohn et al. 2019). With so few studies exploring extra-pair paternity in parrots, and such potential variation in results, it seems necessary to carry out more studies to further understand the breeding biology and mating systems of parrots.

Not all parrots are socially monogamous and there are a few fascinating mating systems present in the order. The Kākāpō (*Strigops habroptila*), a native of New Zealand, is the largest parrot species and also the only flightless one (Toft and Wright 2015). They have a lek polygynous mating system in which males gather to display in a fixed arena, and females come to meet them when they are ready to mate (Toft and Wright 2015). This brief encounter to copulate is the only relationship males and females have. Another interesting mating system found in parrots is the one shown by the greater vasa parrot (*Coracopsis vasa*). They are polygynandrous, which means that both males and females mate and maintain social bonds with multiple individuals (Toft and Wright 2015). Observations in the wild found that the greater vasa parrot females mate with an average of five or six males, and these attend the

female and provide food for her and her chicks during the entire breeding season (Ekstrom et al. 2007; Toft and Wright 2015). During the incubation and rearing of the chicks, the greater vasa parrot female loses all of her head feathers showing her bright yellow skin, which possibly attracts males to continue providing for her (Toft and Wright 2015). A final extraordinary characteristic of this species is that both sexes display an enlarged cloacal protrusion, which is even larger in males. This large, erect, cloacal protrusion forms a hemipenis that facilitates a copulatory tie that can last for up to an hour (Ekstrom et al. 2007; Toft and Wright 2015). A final example is the eclectus parrot (*Eclectus roratus*). They are also grouped as polygynandrous, but this species exhibits a reverse sexual dichromatism, with females displaying bright red and blue plumage and males showing an inconspicuous green colour (Heinsohn and Legge 2003; Toft and Wright 2015). Males and females are so contrastingly different that it was previously thought they were a different species. Reverse sexual dichromatism is normally associated with sex-role reversal, however, this is not the case in the eclectus parrot because, as is typical for parrots, males provide food to the incubating female and later on also to the nestlings (Heinsohn and Legge 2003). The striking sexual dichromatism in this species has been attributed to differences in life-style between the sexes. Females need to be conspicuous to display nest-hollow ownership that they compete for with other females, and males need to be cryptic when foraging, as they spend most of their time looking for food to feed themselves, their females and their chicks (Heinsohn et al. 2005).

Research goal and thesis outline

The overall aim of my PhD thesis was to understand whether sexual selection has had an effect on the evolution of behaviours and morphology related to reproduction across the order

Psittaciformes. As environmental and social factors can also affect phenotypical and behavioural traits associated with reproduction (West-Eberhard 2014; Dale et al. 2015), I also investigated the possible effect of these traits in the evolution of parrots. I used comparative analyses to explore the combined effect of sexual selection, environmental factors, life-history and social interaction on sperm morphology (**Chapter 1**), plumage colouration (**Chapter 2**) and mating behaviour (**Chapter 3**) in parrots.

Sperm competition, as a sexual selection mechanism, has driven the evolution of sperm morphology (Briskie et al. 1997; Immler et al. 2008; Kleven et al. 2008; Lüpold et al. 2009; Lifjeld et al. 2010). In birds, different studies have shown that when sperm competition is stronger (i.e. levels of extrapair paternity are higher and/or males have larger testes) males of a given species have longer sperms and lower coefficients of variation in sperm length, both within and between males. Studies exploring extrapair paternity in parrots are limited, therefore in **Chapter 1** I measured sperm morphology traits (i.e. mean sperm length and within-male and between-male coefficients of variation in sperm length) across 62 species to understand whether parrots experience sperm competition.

To further understand whether parrot species might experience different levels of sexual selection, in **Chapter 2** I estimated sexual dichromatism, colour elaboration and colour diversity indexes across 398 species. Parrots show some of the most outstanding plumage colours across birds, in fact parrots are more colourful than expected for their species richness (Delhey 2015), and in many species both males and females are highly ornamented (Berg and Bennett 2010). The plumage colouration in parrots has been attributed to their unique pigments, called psittacofulvins, which they can synthesize and deposit in their feathers to produce yellow to red colours (Stradi et al. 2001; McGraw and Nogare 2004). However, the evolutionary forces behind the highly elaborated colours parrots display

is yet to be understood (Berg and Bennett 2010). For this reason, I explored whether sexual selection indicators, social interactions, or life-history traits affect plumage colouration in parrots.

Sexual selection via sperm competition can affect copulation behaviour in birds (Birkhead and Moller 1992). Males risk losing their paternity when females engage in extra-pair paternity, thus to reduce this risk males can copulate frequently or for longer with their females (Mougeot 2004; Wysocki and Halupka 2004). Therefore, in **Chapter 3** I investigated whether the variations in copulation frequency and duration across 103 parrot species were related to sperm competition indicators or to other aspects of the species' social environment and life-history.

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Chapter 1

Sperm morphology and evidence for sperm competition among parrots

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Sperm morphology and evidence for sperm competition among parrots

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Abstract

Sperm competition is an important component of post-copulatory sexual selection that has shaped the evolution of sperm morphology. Previous studies have reported that sperm competition has a concurrently directional and stabilizing effect on sperm size. For example, bird species that show higher levels of extrapair paternity and larger testes (proxies for the intensity of sperm competition) have longer sperm and lower coefficients of variation in sperm length, both within and between males. For this reason, these sperm traits have been proposed as indexes to estimate the level of sperm competition in species for which other measures are not available. The relationship between sperm competition and sperm morphology has been explored mostly for bird species that breed in temperate zones, with the main focus on passerine birds. We measured sperm morphology in 62 parrot species that breed mainly in the tropics and related variation in sperm length to life-history traits potentially indicative of the level of sperm competition. We showed that sperm length negatively correlated with the within-male coefficient of variation in sperm length and positively with testes mass. We also showed that sperm is longer in sexually dichromatic and in gregarious species. Our results support the general validity of the hypothesis that sperm competition drives variation in sperm morphology. Our analyses suggest that post-copulatory sexual selection is also important in tropical species, with more intense sperm competition among sexually dichromatic species and among species that breed at higher densities.

KEYWORDS
parrots, post-copulatory sexual selection, sperm competition, sperm morphology

1 | INTRODUCTION

When females copulate promiscuously, a competition arises among the sperm of different males to fertilize the same egg (Parker, 1970). This contest is a form of post-copulatory sexual selection referred to as sperm competition. Sperm competition plays an important role in the evolution of sperm morphology, having both a directional and a stabilizing effect on sperm length (Briskie, Montgomerie, & Birkhead, 1997; Calhim, Immler, & Birkhead, 2007; Immler, Calhim, & Birkhead, 2008; Kleven et al., 2009; Kleven, Laskemoen, Fossøy, Robertson, & Lifjeld, 2008; Lifjeld, Laskemoen, Kleven, Albrecht, & Robertson, 2010; Lüpold, Linz, Rivers, Westneat, & Birkhead, 2009). The directional effect has been shown in fish (Balshine, Leach, Neat, Werner, & Montgomerie, 2001), mammals (Gomendio & Roldan,

1991), insects (Morrow & Gage, 2000), anurans (Byrne, Simmons, & Roberts, 2003; Liao et al., 2018), reptiles (Tourmente, Gomendio, Roldan, Giojalas, & Chiaraviglio, 2009) and birds (Kleven et al., 2009; Lifjeld et al., 2010; Lüpold, Calhim, Immler, & Birkhead, 2009), whereby species exposed to higher sperm competition levels tend to have longer sperm. The stabilizing effect on sperm length, that is reduced variation in sperm length—both at the within- and between-male levels—with increasing levels of sperm competition, has been shown in passerine birds (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010).

Different hypotheses have been proposed to explain selection for longer sperm in birds. First, the positive relationship between sperm length and speed (Briskie & Montgomerie, 1992; Lüpold, Calhim, et al., 2009) suggests that longer sperm might have evolved as a consequence of selection on speed, as longer sperm will then outcompete shorter sperm in the race to the ova. Second, the positive relationship between sperm length and the length of the sperm storage tubules (SSTs) in the females' utero-vaginal junction suggests that longer sperm might have evolved to fill the space within the SSTs (Briskie & Montgomerie, 1992; Briskie et al., 1997).

Regarding the stabilizing selection, species with higher levels of sperm competition show reduced variation in sperm length, both within and between males (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010). This suggests that there is an "optimal" sperm morphology, probably achieved by selection that reduces errors during sperm production (Calhim et al., 2007; Hunter & Birkhead, 2002). It has been shown that sperm traits are highly heritable (Birkhead, Pellatt, Brekke, Yeates, & Castillo-Juarez, 2005) and less condition dependent (Birkhead & Fletcher, 1995; Birkhead, Fletcher, & Pellatt, 1999; but see Immler, Pryke, Birkhead, & Griffith, 2010; Lüpold, Birkhead, & Westneat, 2012). These sperm properties could explain the reduction of variation in sperm length under higher post-copulatory sexual selection (Immler et al., 2008).

Given the compelling evidence that sperm competition acts concurrently on sperm morphology in a directional and stabilizing manner, recent studies have used mean sperm length and the coefficients of variation of sperm length, both within and between males, as proxies of the intensity of sperm competition (Omotoriogun, Laskemoen, et al., 2016; Sardell & DuVal, 2014). These studies have been taxonomically restricted to passerine birds (Albrecht et al., 2012; Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010; Lüpold Linz & Birkhead, 2009; Lüpold, Linz, Rivers, et al., 2009; Omotoriogun, Albrecht, et al., 2016; Omotoriogun, Laskemoen, et al., 2016; Sardell & DuVal, 2014), with the exception of one study on shorebirds (Johnson & Briskie, 1999) and one on pheasants (Immler et al., 2007), though the latter found no effect of sperm competition on sperm morphology. Most studies focused on temperate zone species (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010; but see Albrecht et al., 2012; Omotoriogun, Albrecht, et al., 2016; Omotoriogun, Laskemoen, et al., 2016). The temperate zone bias might be due to the general assumption of low levels of sperm competition in tropical birds (Stutchbury & Morton, 1995), possibly associated with the different life-history traits between the species of these two regions (Ricklefs & Wikelski, 2002). For example, species with a shorter lifespan might tolerate higher levels of extrapair paternity (EPP, a proxy of the intensity of sperm competition) because they have fewer breeding opportunities throughout their lives (Arnold & Owens, 2002; Mauck, Marschall, & Parker, 1999). Accordingly, species that have high rates of adult mortality tend to show higher levels of EPP (Arnold & Owens, 2002). Tropical species are characterized by long lifespans (Ricklefs & Wikelski, 2002); hence, lower levels of sperm competition are predicted. However, empirical support for this theoretical prediction is limited (Macedo, Karubian, & Webst, 2008).

In a comparative analysis that included 99 passerine species from the temperate zone and 31 from the tropical zone, no difference was found in indicators of the level of sperm competition between these two groups of birds (Albrecht et al., 2012). For this reason and given that most birds live and breed in the tropics (Gaston, 2000; Hawkins, Porter, & Diniz-filho, 2003; Valcu, Dale, & Kempenaers, 2012), it is important to explore variation in the level of sperm competition, directly or through its proxies, in nonpasserine tropical species to be able to formulate general rules of how sexual selection operates among birds.

We explored variation in sperm morphology in 62 parrot species (~15% of all Psittaciformes; 30 genera, five families), breeding mainly in the tropics. The general aim of our study was to investigate whether findings from passerine birds can be generalized. Specifically, we tested whether mean sperm length and the within-male and between-male coefficients of variation in sperm length correlated with each other and with other known indicators of the intensity of sperm competition. Parrots are long-lived animals: the average lifespan is 26 years and ranges from 8.5 to 100 years (Wasser & Sherman, 2010). This group of birds is also characterized by social monogamy with lifelong pair bonds, even though there are exceptions (Toft & Wright, 2015). These traits suggest a low intensity of sexual selection, but few studies have used genetic markers to confirm genetic monogamy (Eastwood et al., 2018; Masello, Sramkova, Quillfeldt, Epplen, & Lubjuhn, 2002). On the other hand, Bublat et al. (2017) showed that socially monogamous macaws (species from the genera *Ara*, *Diopsittaca* and *Primolius*) had much lower sperm density compared with polygynandrous Eclectus parrots (*Eclectus roratus*), which might be an adaptation to intense sperm competition in the latter. Despite these life-history traits, parrots show striking coloration and up to 30% of the species are sexually dichromatic (estimated from del Hoyo, Elliott, Sargatal, Christie, & Kirwan, 2017). Parrots also exhibit high levels of cognitive capacities (Van Horik, Clayton, & Emery, 2012; Lambert, Jacobs, Osvath, & von Bayern, 2019) and problem-solving skills (Auersperg, von Bayern, Gajdon, Huber, & Kacelnik, 2011; Auersperg, Kacelnik, & von Bayern, 2013; O'Neill, Picaud, Maehner, Gahr, & von Bayern, 2019), and females may choose males based on these skills (Chen, Zou, Sun, & Cate, 2019). Hence, in parrots, ornamental colours and high cognitive abilities might be consequences of sexual selection. Studying indicators of

sperm competition intensity will allow us to explore variation in the genetic mating system of parrots and to understand the effect of post-copulatory sexual selection in this group.

Using a comparative approach, we tested whether sperm measures (CV and sperm length) were predicted by (a) relative testes mass, a proximate indicator of the intensity of sperm competition (Møller & Briskie, 1995; Pitcher, Dunn, & Whittingham, 2005), (b) sexual size dimorphism and dichromatism, traits considered to be sexually selected (Berry & Shine, 1980; Dale, Dey, Delhey, Kempenaers, & Valcu, 2015; Darwin, 1871; Owens & Hartley, 1998), and (c) gregariousness (proximity to other breeding pairs), a trait facilitating sexual selection (Shuster & Wade, 2003).

2 | MATERIAL AND METHODS

2.1 | Collection of sperm samples

We explored the variation in sperm morphology within and between males in 62 parrot species belonging to 30 genera and five families. In their natural habitat, these species primarily breed in the tropics, some of them extend their breeding range into the subtropical zone and a few into the temperate zone of the southern hemisphere (del Hoyo et al., 2017). We collected one sperm sample per male (total *N* = 138) from birds that were born in captivity and held in the breeding facility of the Loro Parque Fundación (LPF), Tenerife, Spain. Samples were collected between June 2012 and June 2013, and between April and May 2018. In February 2019, sperm samples from two Kākāpō (*Strigops habroptila*) were collected on Codfish Island, New Zealand, in collaboration with the Kākāpō Recovery Team. To collect the samples, we used the electro-stimulation technique (Lierz, Reinschmidt, Müller, Wink, & Neumann, 2013) with three probe sizes (length × diameter [mm]: 25 × 3, 35 × 4 and 50 × 5), depending on the size of the bird sampled. The electric current and the number of electric impulses were adapted to each species, as described by Bublat et al. (2017). Kākāpō samples were collected using this technique or by cloacal massage. Sperm samples were taken directly from the cloaca using scaled glass capillaries (Wiretrol II, 1–5 µl; Drummond Scientific Company). From the samples collected in 2012–2013 and the Kākāpō samples (the latter were previously diluted, one with NaCl and the other with the semen extender Blanco, Schneider et al., 2017), smears were made onto microscope slides, stained with Eosin B2% and covered with a mounting medium (Entellan New, 107961; Merck KGaA), whereas the ones from 2018 were fixed in 50–100 µl of 5% formalin solution. From the samples fixed in formalin, we pipetted a 10 µl aliquot onto a microscope slide, spread it with the side of the pipette tip and allowed it to air-dry. The different methods used did not have an effect on the sperm measurements. We inspected all samples at 200x magnification using a Zeiss Axio Imager.M2 microscope with bright field optics and took between 4 and 25 photographs per slide at 400× magnification with an Axiocam 506 colour camera.

2.2 | Sperm morphometry

We measured sperm morphometry (head and flagellum length) from the photographs using the software ZEN 2, blue edition (Carl Zeiss Microscopy GmbH), including only normal-looking spermatozoa (total *N* = 1,996). To minimize observer error, all measurements were taken by one person (K.T.). We calculated total sperm length as the sum of the two measurements. We did not measure mid-piece length separately, because in most samples it could not easily be distinguished. When the mid-piece was visible, we included it in the measure of flagellum length, assuming that the nonvisible mid-pieces would most likely blend into the tail. The average number of spermatozoa measured per male and species was 14.9 (range: 3–62). The repeatability of sperm measurements per male was 0.261 (95% CI: 0.17–0.36) and 0.419 (95% CI: 0.29–0.55) per species; these were obtained through 1,000 parametric bootstrap iterations (Stoffel, Nakagawa, & Schielzeth, 2017). We calculated coefficients of variation (CV = [*SD*/mean]) both within and between males, and adjusted them to correct for variation in sample size ($CV_{adj} = [1 + 1/(4n)]$*CV; Sokal & Rohlf, 1981). The adjusted coefficient of variation within males was denoted as CV_{wm} and for between males as CV_{bm}.

We collected sperm samples from one to twelve males of each species (median per species = 2). For six species (the Yellow-crowned amazon *Amazona ochrocephala*, the Yellow-headed amazon *Amazona oratrix*, the Sulphur-crested cockatoo *Cacatua galerita*, the Yellow-crested cockatoo *Cacatua sulphurea*, the Eclectus parrot and the Red-breasted parakeet *Psittacula alexandri*), we obtained samples from two or more subspecies, but these were averaged to obtain species-specific values for the analyses. We used the CV_{wm} of each individual to calculate a mean CV_{wm} for each species. To calculate CV_{bm}, we used the mean and standard deviation (*SD*) of sperm length for each of the males of a given species. Given the small number of males sampled per species (often only one, median 2), we only use CV_{wm} for further analyses. However, we note that even with the limited sampling, the total sperm CV_{wm} correlated positively with the CV_{bm} (Pearson's $r = 0.496$, $N = 29$ species).

Because our sperm samples came from males bred in captivity, we expect higher levels of inbreeding compared with males from wild populations. Studies exploring the effect of inbreeding on sperm characteristics of mammals and birds have shown that the proportion of abnormal sperm is higher, and sperm velocity lower in inbred compared with outbred males (Gomendio, Cassinello, & Roldan, 2000; Heber et al., 2012; Opatová et al., 2016). However, there is no evidence for inbreeding depression on the morphology (e.g., length, CV) of normal-looking sperm of fish, fruit flies and birds (Ala-Honkola et al., 2013; Mehlis, Frommen, Rahn, & Bakker, 2012; Opatová et al., 2016). Specifically, in zebra finches (*Taeniopygia guttata*) inbreeding depression seems to have no more than a modest effect on the length (Cohen's $d = -0.55$) and a small effect on the CV ($d = 0.24$) of normal-looking sperm (Opatová et al., 2016). We therefore assume that our results reflect the variation in sperm morphology observed in wild parrots.

2.3 | Explanatory variables: Data collection and analysis

We considered six explanatory variables potentially explaining variation in sperm morphology. These predictor variables were collected before the sperm morphology data were collected. At the time of performing the measurements, the person measuring the sperm was unaware of the predictor variables.

2.3.1 | Testes mass

Testes mass has been used as an indicator of the intensity of sperm competition, because species with higher levels of EPP show relatively larger testes (Møller & Briskie, 1995). Data on testes mass was obtained from the literature (Calhim & Birkhead, 2007; Krishnaprasadan, Kotak, Sharp, Schmedemann, & Haase, 1988; Wilkinson & Birkhead, 1995). We only found data for 10 of the 62 species studied here. For analysis, we log10-transformed this variable to improve normality. We added body mass in all the analyses that included testes mass to control for a possible allometric effect, as it has been reported that testes mass relates to body mass in birds and other taxa (Birkhead, 1998; Morrow & Gage, 2000).

2.3.2 | Body size

We measured wing, tarsus and tail length for an average of 4.8 (range: 1–22) females and 5.7 (range: 1–23) males of each species. Individuals could only be measured during a yearly veterinarian health check and due to time constraints some of the measurements could not be taken. In these cases, measurements were taken from the book *Parrots of the World* (Forshaw, 1978) (see online data repository). Measurements for the Kākāpō were also taken from this source, as this species is not present in the LPF collection. We estimated body size for males and females, using the first principal component (PC1) from a principal component analysis (PCA) that included the three measurements for both sexes. PC1 explained 65% of the variation in the data.

2.3.3 | Clutch size

We compiled data on clutch size from the records of the LPF from the 2012 to 2015 breeding seasons. Based on these data (1–105 clutches per species, mean: 16.4), we calculated average clutch size per species. Clutch size records were missing for 13 species (see online data repository). In those cases, we used data on clutch size from the *Handbook of the Birds of the World Alive* (HBW Alive, del Hoyo et al., 2017).

2.3.4 | Sexual size dimorphism

Sexual size dimorphism (SSD) is an indicator of the intensity of sexual selection in birds (Owens & Hartley, 1998; Szekely, Lislevand, & Figuerola, 2007). We calculated SSD as $PC1_{\text{male body size}} - PC1_{\text{female body size}}$. Hence, positive values reflected species where males are larger than females.

2.3.5 | Sexual dichromatism

Sexual selection is considered to be one of the most important factors causing sexual dichromatism in birds (Dale et al., 2015). A comparative study exploring the mechanisms behind sexual dimorphism in body size and plumage colouration among passerines has shown that sexual dichromatism is associated with the frequency of EPP (Owens & Hartley, 1998). Thus, we consider sexual dichromatism as an indicator of the intensity of sexual selection in birds (Badyaev & Hill, 2003; Dale et al., 2015). We scored dichromatism as present ("yes") or absent ("no") according to (a) visual inspection of the species' colour plates and (b) information from the section "descriptive notes" in the HBW Alive (del Hoyo et al., 2017). We defined a species as dichromatic if plumage colour of any body part differed between the sexes (e.g., male and female show different colours, or the same colour but different tones). We did not classify a species as dichromatic if the colour of a patch was the same, but the patch differed in size.

2.3.6 | Gregariousness

Opportunities for extrapair mating may be higher when species nest closer together. Hence, we scored gregariousness as "yes" or "no" based on information from the "breeding" section of the HBW Alive (del Hoyo et al., 2017). We scored a "yes" for gregariousness if the description suggests that breeding pairs nest in close proximity (i.e., several pairs occupying adjacent trees, two or three nests per tree, nests with multiple breeding pairs) or if the species is described as colonial. The Kākāpō was excluded from analyses that consider gregariousness as a predictor, because this is the only lekking species among parrots.

We reported all our measurements, the conditions in which they were collected, the sample size for each variable and the reason we excluded data from our analyses.

2.4 | Phylogeny

We used a recent phylogeny of 307 parrot species produced from a 30-gene supermatrix (Provost, Joseph, & Smith, 2017). Only one of the species we studied here, the Superb parrot (*Polytelis swainsonii*), was not included in this phylogeny. We added the Superb parrot to the phylogeny using the function pinTips in the package "TREEMAN" (Bennett, Sutton, & Turvey, 2017). This function finds the branch of the phylogenetic tree common for all *Polytelis* species and adds the missing taxon at a random position within this branch.

2.5 | Statistical analysis

All the statistical analyses were performed in R 3.5.2 (R Development Core Team, 2018). All data and code are available in the online repository https://osf.io/v23bw/.

We first tested whether mean sperm length was correlated with CV_{wm} using a linear model. As these variables were negatively correlated (Figure 1), we investigated simultaneous effects of the explanatory variables on both mean sperm length and CV_{wm}. To test our hypotheses, we ran a multivariate analysis of variance (MANOVA) using these two variables as response variables and including phylogeny, testes mass, male body size (PC1), clutch size, SSD, sexual dichromatism and gregariousness as predictors. To control for phylogeny, we used phylogenetic eigenvectors (Diniz-Filho, de Sant'Ana, & Bini, 1998) calculated using the package "ADEPHYLO" v. 1.1-11 (Jombart, Dray, & Bilgrau, 2016). These phylogenetic eigenvectors are equivalent to the PC axes obtained in a PCA (Swenson, 2014). Hence, the eigenvectors that describe the phylogenetic relationship between the species considered in this study were kept in subsequent analyses. We selected these eigenvectors based on the MANOVA analysis (Desdevises, Legendre, Azouzi, & Morand, 2003).

To identify the direction and magnitude of the relationships, we ran univariate phylogenetically informed linear models separately for mean sperm length and CV_{wm} and included each of the significant predictors identified by the MANOVA analysis.

To assess the combined effect of the significant predictors identified, we ran phylogenetically informed linear models, with multiple predictors, separately for mean sperm length and CV_{wm}. We included all significant predictors as explanatory variables, except testes mass, because the sample size was too small (N = 10 species).

Finally, to explore the difference in mean sperm length and CV_{wm} among different taxonomic groups (Psittaciformes, Passeriformes and Charadriiformes), we conducted post hoc comparisons using the package "MULTCOMP" (Hothorn, Bretz, & Westfall, 2008).

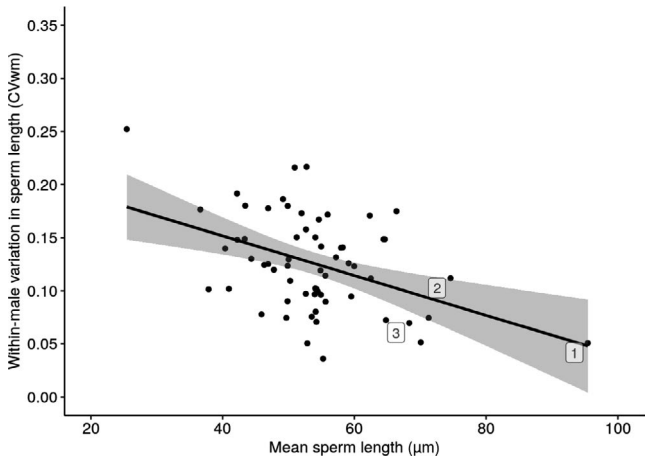

FIGURE 1 Relationship between mean sperm length and the within-male coefficient of variation in sperm length (CV_{wm}) for 62 parrot species (Pearson's r = −0.43, p < 0.001; no control for phylogeny). Plotted points represent the mean values per species. The line and the 95% CI (grey) are based on a linear model. The only two parrot species described as polygynandrous (1: Vasa parrot; 2: Eclectus parrot) and the one species described as lekking (3: Kākāpō) are highlighted

3 | RESULTS

Total sperm length varied from 25.45 µm in the Southern Festive amazon (*Amazona festiva*) to 95.43 µm in the Vasa parrot (*Coracopsis vasa*). Flagellum length ranged from 15.41 to 65.64 µm and head length from 7.9 to 29.79 µm (Figure S1). To explore the variation in the two components of sperm length, we calculated CV_{wm} for head and CV_{wm} for flagellum length separately. The CV_{wm} for head length (mean = 0.212 µm; range = 0.078–0.473 µm) was significantly larger than the CV_{wm} for flagellum length (mean = 0.152 µm; range = 0.053–0.522 µm; paired sample t test: estimate = 0.059 ± 0.012, t_{61} = 5.01, p < 0.001).

The MANOVA analysis showed a strong phylogenetic signal and a significant effect of sexual dichromatism, gregariousness and relative testes mass on both mean sperm length and CV_{wm} (Table 1). Given that only the first eigenvector explained the phylogenetic relationship between the species studied here, we included only this first eigenvector in all further models to control for phylogeny. We performed another MANOVA analysis based on those species for which we measured a minimum of 10 sperm (N = 50), as it has been shown that this sample size provides a representative estimate of the mean and variance of the sperm length (Kleven et al., 2008; Laskemoen, Kleven, Fossøy, & Lifjeld, 2007). The results of this analysis are qualitatively similar (Table S1). Therefore, we kept the complete data for the next analyses to include a larger sample size (N = 62).

Univariate, phylogenetically informed models (Table 2) showed that sperm were longer in sexually dichromatic and in gregarious species (Figure 2) and that sperm length increased with increasing relative testes mass (Figure 3a). There was a significant negative correlation between CV_{wm} and relative testes mass (Figure 3b).

TABLE 1 Results of a MANOVA analysing the effects of various predictors on both mean sperm length and the within-male coefficient of variation in sperm length (CV_{wm})

Predictors	V[b]	Statistic	p
Phylogeny			
Eigenvector 1[a]	0.122	$F_{2,57}$ = 3.97	**0.024**
Eigenvector 2[a]	0.046	$F_{2,57}$ = 1.36	0.26
Eigenvector 3[a]	0.0006	$F_{2,57}$ = 0.017	0.98
Clutch size	0.003	$F_{2,59}$ = 0.095	0.91
Body size (PC1 male)	0.044	$F_{2,59}$ = 1.35	0.27
Sexual size dimorphism	0.017	$F_{2,59}$ = 0.495	0.61
Sexual dichromatism	0.139	$F_{2,59}$ = 4.75	**0.012**
Gregariousness	0.238	$F_{2,58}$ = 9.08	**<0.001**
Body mass (male)	0.285	$F_{2,6}$ = 1.2	0.37
log(testes mass)	0.794	$F_{2,6}$ = 11.6	**0.009**

[a]Phylogenetic eigenvectors (see Methods2 for details).
[b]Pillai's trace statistic; ranges from 0 to 1. Bold p values are statistically significant.

TABLE 2 Univariate linear models analysing the relationship between various predictors and mean sperm length and the within-male coefficient of variation in sperm length (CV_{wm}) separately

Response variable	Predictors	Estimate	*SE*	Statistic	*p*
Mean sperm length	(Intercept)	51.7	1.39		
	Phylogeny[a]	−2.7	1.19	t_{59} = −2.27	**0.027**
	Sexual dichromatism[b]	7.66	2.67	t_{59} = 2.87	**0.006**
Mean sperm length	(Intercept)	51.6	1.2		
	Phylogeny[a]	−2.7	1.1	t_{58} = −2.45	**0.017**
	Gregariousness[b]	13.0	3.13	t_{58} = 4.17	**<0.001**
Mean sperm length	(Intercept)	72.7	5.88		
	Phylogeny[a]	−3.86	19.4	t_{6} = −0.199	0.85
	Body mass	−0.013	0.013	t_{6} = −1.04	0.34
	log(Testes mass)	11.1	3.63	t_{6} = 3.06	**0.022**
CV_{wm}	(Intercept)	0.129	0.007		
	Phylogeny[a]	0.011	0.006	t_{59} = 1.95	0.056
	Sexual dichromatism[b]	−0.012	0.013	t_{59} = −0.96	0.34
CV_{wm}	(Intercept)	0.129	0.006		
	Phylogeny[a]	0.011	0.006	t_{58} = 1.99	0.051
	Gregariousness[b]	−0.015	0.016	t_{58} = −0.934	0.35
CV_{wm}	(Intercept)	0.12	0.022		
	Phylogeny[a]	0.148	0.074	t_{6} = 2.0	0.092
	Body mass	$2.84x10^{-5}$	$4.78x10^{-5}$	t_{6} = 0.594	0.57
	log(Testes mass)	−0.035	0.014	t_{6} = −2.54	**0.044**

[a]Eigenvector 1 (see Methods2 for details).
[b]1, "no"; 2, "yes. Bold p values are statistically significant.

A phylogenetically informed model that included all significant predictors showed that only gregariousness remained as a significant predictor of mean sperm length (estimate = 10.6 ± 3.48, t_{57} = 3.06, p = 0.003, see Table S2). Sexual dichromatism was no longer significant, probably because the two variables are related (Fisher's exact test: p = 0.001; seven out of nine gregarious species in our data set are also sexually dichromatic). Another model that included all the predictors measured in this study—not only the predictors identified in the MANOVA analysis—also showed gregariousness to be the only significant predictor of sperm length (Table S3).

A combined univariate analysis also showed a significant interaction between sexual dichromatism and gregariousness on mean sperm length (estimate = 16.0 ± 7.26, t_{56} = 2.21, p = 0.031; Table S4), with the longest sperm in those species that are both gregarious and sexually dichromatic. The sperm length of the species that are both sexual dichromatic and gregarious is ~33% longer than of the other species, and this difference was significant (estimate = 16.6 ± 3.35, t_{59} = 4.97, p < 0.001).

Parrots showed significantly smaller sperm compared to passerines (post hoc comparison; estimate = −90.46 ± 8.90, t_{58} = −10.17, p < 0.001), but not compared to shorebirds (post hoc comparison; estimate = −21.36 ± 13.47, t_{58} = −1.59, p = 0.21; Figure 4a). However, as the shorebirds study only included 16 species, the power of that test is limited. Parrots also exhibited significantly larger variation in sperm length (within-male, CV_{wm}) compared to temperate zone passerines (Figure 4b, estimate = 0.108 ± 0.006, t_{59} = 17.57, p < 0.0001).

4 | DISCUSSION

We studied sperm morphology in 62 parrot species and found that mean sperm length and the within-male coefficient of variation in sperm length (CV_{wm}) were negatively correlated, as expected under the hypothesis that higher levels of sperm competition lead to both longer sperm and sperm that are less variable. Both measures were related to relative testes mass, another proxy of the intensity of sperm competition, though the sample size for this analysis was smaller (N = 10 species). We also found that on average, sperm were longer in sexually dichromatic and in gregarious species.

The significant relationship between relative testes mass and both mean sperm length and CV_{wm} corresponds with previous findings in passerines (Immler et al., 2008). Within passerine species, it has been shown that testes mass is associated with the level of sperm competition, as species with higher levels of extrapair paternity have larger testes (Lüpold, Linz, Rivers, et al., 2009; Møller & Briskie, 1995). It has also been reported that CV_{wm} is negatively related to the frequency of EPP (Kleven et al., 2008; Lifjeld et al.,

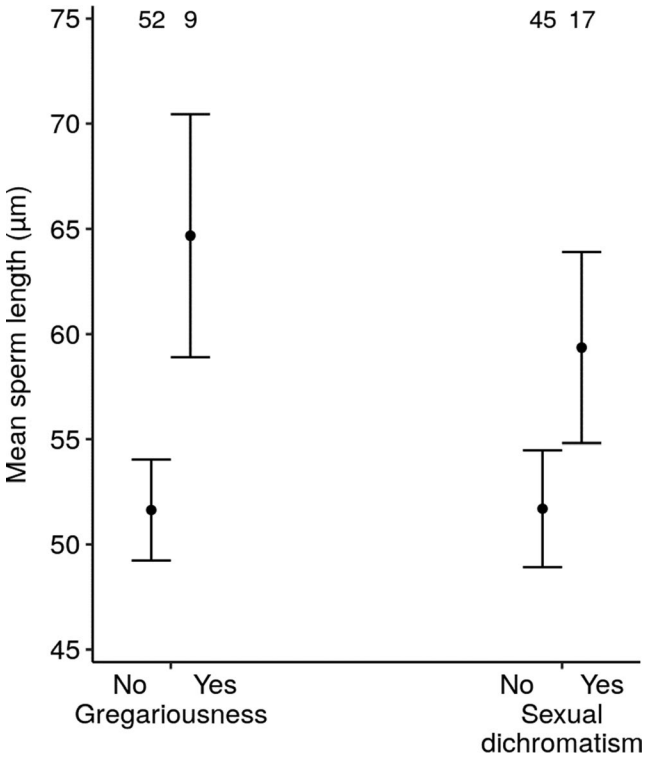

FIGURE 2 Mean total sperm length in relation to gregariousness and sexual dichromatism for 61 and 62 parrot species, respectively. Shown are estimates (dots) and their 95% CI (error bars) from the univariate models shown in Table 2. Numbers above the *X*-axis show sample sizes (number of species in each group)

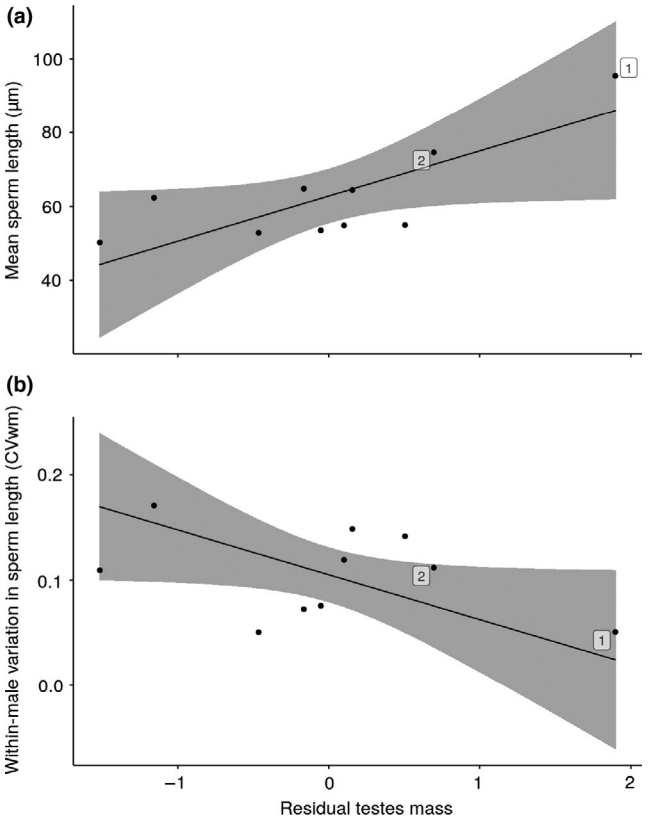

FIGURE 3 Relationship between residual testes mass (log_{10}-transformed) and (a) mean total sperm length and (b) the within-male coefficient of variation in sperm length (CV_{wm}) for the 10 parrot species for which testes mass data were available in the literature. The line and 95% CI (grey) are based on the model shown in Table 2. The only two parrot species described as polygynandrous (1: Vasa parrot; 2: Eclectus parrot) are highlighted

2010), which is clear evidence for the role of sperm competition in determining sperm morphology.

Our results also suggest that sexual dichromatism in parrots is associated with increased sperm competition, because dichromatic species had significantly longer sperm. Previous studies have suggested that sexual selection is the main driver of sexual dichromatism (Badyaev & Hill, 2003; Dale et al., 2015), and comparative analyses have shown that sexual dichromatism in birds is related to the level of extrapair paternity (Møller & Birkhead, 1994; Owens & Hartley, 1998). One possible scenario to explain this pattern is that sexual selection via sperm competition is the evolutionary force that has driven sexual dichromatism in parrots as well. This is supported by semen parameters, showing that the highly sexually dichromatic Eclectus parrot also has the highest semen density and total amount of sperm per ejaculate compared with other parrot species (Bublat et al., 2017).

Gregarious parrot species also had longer sperm, suggesting that species that breed in groups also experience higher levels of sperm competition. Breeding under higher local densities may increase opportunities to engage in mating outside the pair bond and reduce the costs of seeking extrapair copulations. Indeed, extrapair copulations seem to be more common among colonial breeders (Møller & Birkhead, 1993). Our finding also supports previous work showing that species that breed at high densities have larger testes (Pitcher et al., 2005). The significant correlation between sexual dichromatism and gregariousness, at least in the parrot species under study, further suggests that sexual ornamentation in parrots might have evolved as a consequence of sexual selection which is stronger in gregarious species. However, this does not imply a direct causal link between sexual dichromatism and sperm length. We hypothesize an evolutionary scenario where gregariousness might have driven both sexual dichromatism and increased levels of EPP, and the latter might then have driven the evolution of ejaculate traits, such as longer sperm. Our findings simply suggest that sperm competition is higher among sexually dichromatic and gregarious species, and also that sexually dichromatic species are gregarious. Further work is needed to investigate potential causal links.

The two species from our data set with the longest sperm were the Vasa parrot and the Eclectus parrot (see online data repository). These species are polygynandrous (Ekstrom, Burke, Randrianaina, & Birkhead, 2007; Heinsohn, Legge, & Endler, 2005), a mating system that is typically associated with a high level of sperm competition (Pitcher et al., 2005). The sperm measurements reported here support this view. Both of these species are also sexually dichromatic—with the Vasa parrot showing sexual differences only during the breeding season—and the Vasa parrot exhibits a unique penis-like

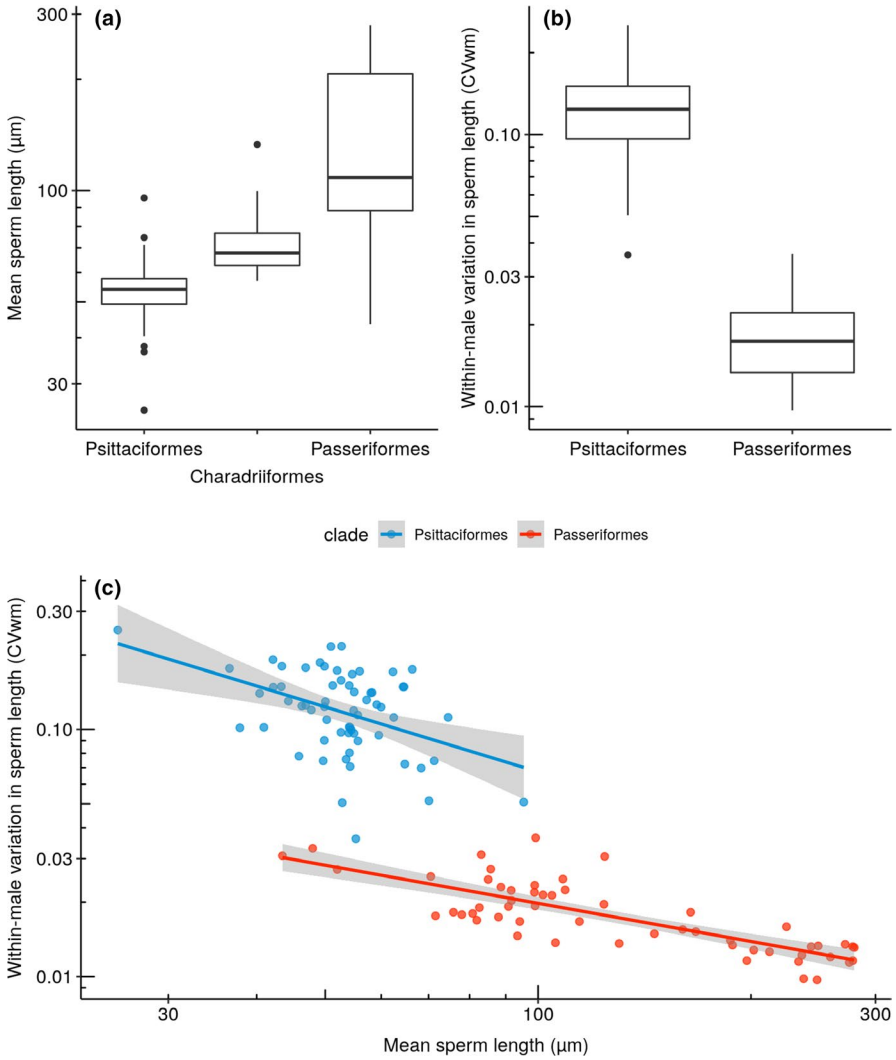

FIGURE 4 (a) Mean sperm length for 62 parrot species (Psittaciformes), 16 shorebird species (Charadriiformes) and 55 passerine species (Passeriformes); scale on *Y*-axis is $\log_{10}$ transformed. (b) Within-male coefficient of variation in total sperm length (CV_{wm}) only for the parrot and passerine species; scale on *Y*-axis is $\log_{10}$ transformed. (c) Relationship between mean sperm length and the within-male coefficient of variation for the parrot and passerine species; the lines and 95% CI (grey) are based on a linear model without controlling for phylogeny; scale on *Y*-axis and *X*-axis is $\log_{10}$ transformed. The data for passerines are from Lifjeld et al., 2010 and those for shorebirds from Johnson & Briskie, 1999

cloacal protrusion, which allows males to interlock their cloaca with the female's to prolong copulations (Wilkinson & Birkhead, 1995) and reflects the high level of sperm competition occurring in this species.

Besides the samples of the only two polygynandrous parrot species, we also obtained sperm measurements for the Kākāpō, the only lekking (and flightless) parrot species. The species is neither sexually dichromatic nor gregarious; hence, our general findings cannot explain their relatively long sperm (68.33 µm, the fifth longest, see online repository). However, it has been reported that Kākāpō females mate up to three times with the same or different males (Eason et al., 2006). Hence, sperm competition might still be high in this species and could thus be the evolutionary force that led to their relatively long sperm.

The effect of sperm competition on sperm morphology has been mainly explored for passerine species (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010). We now provide evidence suggesting that sperm competition has also shaped the morphology of parrot sperm. It is thus important to compare the variation in sperm morphology between these two taxonomic groups. Mean sperm length in our data set of 62 parrot species ranged from 25.45 to 95.43 µm (a 3.8-fold difference between the shortest and longest). A study on variation in sperm size for 196 passerine species (Immler et al., 2011) reported that mean sperm length ranged from 41.8 to 284.8 µm (6.8-fold difference). Another study focusing on 12 Afrotropical sunbird species (Omotoriogun, Laskemoen, et al., 2016) reported mean sperm length ranging from 74.1 to 115.6 µm (1.6-fold difference), whereas a study on shorebird species (Johnson & Briskie, 1999) reported mean sperm length ranging from 57 to 133.2 µm (2.3-fold difference). Parrot mean sperm length thus overlaps with that of species from other taxonomic groups. Although our results show that parrots have significantly smaller sperm compared with passerines, the extent of the variation within parrots is similar to what has been found in other groups.

In agreement with the shorter sperm length, parrots also exhibited significantly larger variation in sperm length (within-male, CV_{wm}) compared with temperate zone passerines. Even though the relationship between sperm length and CV_{wm} is negative for passerines and for parrots (Figure 4c, Table S5), the magnitude of the effect is not the same for both groups (Table S6). Where sperm length

overlaps between passerines and parrots (~40–100 μm), the CV_{wm} is much lower for passerines (Figure 4c). The lower CV_{wm} for a given sperm length, together with the generally longer sperm in passerines, suggests that the level of sperm competition is lower in parrots compared with passerines. Nevertheless, the significant negative relationship between CV_{wm} and mean sperm length, together with the correlation of these two variables with testes mass, indicates that post-copulatory sexual selection is driving variation in parrot sperm morphology as well.

We note that the negative relationship between mean sperm length and CV_{wm} could be a simple consequence of sexual selection acting on sperm length only. Indeed, if the variance does not change along with the mean, then the coefficient of variation will decrease solely due to an increase in sperm length. However, if this negative relationship was simply a statistical artefact, we would expect a similar relationship (similar slope and intercept) in passerines and in parrots, but this was clearly not the case (Table S6). Thus, the most parsimonious explanation is that post-copulatory sexual selection has both a directional and a stabilizing effect on parrot sperm length, given that the relationship between sperm length and CV_{wm} is negative for passerines and parrots (Figure 4c, Table S5 and S6; Calhim et al., 2007; Kleven et al., 2008; Lifjeld et al., 2010; Lüpold, Linz, & Birkhead, 2009).

Our results also suggest that there is stronger post-copulatory sexual selection on sperm flagellum length than on sperm head length, as the CV_{wm} of flagellum length is lower than that of head length. However, it is important to consider that when visible, we included the mid-piece in the flagellum measurements as we assumed that when the mid-piece was not visible, it would most likely be blended into the tail. If this is not the case, and the mid-piece was actually included in the head measurements, then this result would be incorrect. However, as this finding goes in agreement with what has been found in passerine species (Briskie & Montgomerie, 1992; Lüpold, Linz, & Birkhead, 2009; Omotoriogun, Laskemoen, et al., 2016; Rowe et al., 2015), we consider that sexual selection might be acting mostly on flagellum length and less on head length within parrots. The sperm head contains the acrosome and nucleus (Jamieson, 2006), two components important for the sperm–egg interaction (Rowe et al., 2015). Any alteration in the sperm head could affect the sperm function during fertilization. Hence, head length may be under stabilizing selection, whereas the flagellum seems to be the target of directional, sexual selection. An increase in flagellum length would increase sperm swimming speed (Briskie & Montgomerie, 1992; Lüpold, Calhim, et al., 2009), making it a better competitor in the race to the ova.

In summary, our results support the view that tropical species experience varying levels of sperm competition (Albrecht et al., 2012). The greatest levels of sperm competition are probably found in the two species (the Vasa and Eclectus parrots) with the rarest mating system among parrots, in which females mate promiscuously and males provide food to and copulate with multiple females (Ekstrom et al., 2007; Heinsohn et al., 2005). Additionally, within parrots, the level of sperm competition seems generally higher for species that breed at higher densities, probably because of increased opportunities to mate outside the pair bond. Our results also indicate that sexual ornamentation in parrots is related to sperm competition, though the precise evolutionary mechanism has yet to be explored.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

L.C., M.V. and B.K. conceived the study. L.C., A.Ba., M.L. and A.Bu. collected the data. K.T. measured the sperm. L.C. and M.V. analysed the data with input from B.K. L.C. wrote the paper with help of B.K. and input from M.V. L.C. is a member of the International Max Planck Research School (IMPRS) for Organismal Biology. This work was funded by the Max Planck Society (to B.K.).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary material

Table S1. Results of a MANOVA analysing the effects of various predictors on both mean sperm length and the within-male variation in sperm length (CV_{wm}). This analysis is based on the species for which we measured a minimum of 10 sperm.

Predictor	***V*†**	***Statistic***	***P***
Phylogeny			
Eigenvector 1*	0.091	$F_{2,44} = 2.21$	0.12
Eigenvector 2*	0.043	$F_{2,44} = 0.99$	0.38
Eigenvector 3*	0.0007	$F_{2,44} = 0.015$	0.99
Clutch size	0.005	$F_{2,46} = 0.122$	0.89
Body size (PC1 male)	0.058	$F_{2,46} = 1.42$	0.25
Sexual size dimorphism	0.057	$F_{2,46} = 1.39$	0.26
Sexual dichromatism	0.166	$F_{2,46} = 4.57$	**0.016**
Gregariousness	0.275	$F_{2,46} = 8.74$	**<0.001**
Body mass (male)	0.431	$F_{2,4} = 1.52$	0.32
Log(testes mass)	0.956	$F_{2,4} = 43.6$	**0.002**

*Phylogenetic eigenvectors (see Methods for details)

†Pillai's Trace statistic; ranges from 0 to 1

Table S2. Linear models analysing the combined effect of different predictor variables on mean sperm length and on the within-male variation in sperm length (CV_{wm}) separately. Only the predictors that were significant in the MANOVA analysis.

Response variable	**Predictor**	**Estimate**	**s.e.**	***Statistic***	***P***
Mean sperm length	(Intercept)	50.7	1.31		
	Phylogeny*	-2.52	1.1	$t_{57} = -2.28$	**0.026**
	Sexual dichromatism†	4.15	2.68	$t_{57} = 1.55$	0.13
	Gregariousness†	10.8	3.43	$t_{57} = 3.14$	**0.003**
CV_{wm}	(Intercept)	0.132	0.007		
	Phylogeny*	0.011	0.006	$t_{57} = 1.86$	0.069
	Sexual dichromatism†	-0.014	0.014	$t_{57} = -1.04$	0.30
	Gregariousness†	-0.007	0.018	$t_{57} = -0.395$	0.69

*Eigenvector 1 (see Methods for details)

†1, "no"; 2, "yes"

Table S3. Linear models analysing the combined effect of different predictor variables on mean sperm length and on the within-male variation in sperm length (CV_{wm}) separately.

Response variable	Predictor	Estimate	s.e.	*Statistic*	*P*
Mean sperm length	(Intercept)	55.3	3.34		
	Phylogeny*	-2.76	1.14	$t_{54} = -2.42$	**0.019**
	Body size (PC1 male)	-0.284	0.968	$t_{54} = -0.293$	0.77
	Sexual size dimorphism	-10.8	9.7	$t_{54} = -1.11$	0.27
	Clutch size	-1.1	0.958	$t_{54} = -1.15$	0.25
	Sexual dichromatism†	3.88	2.72	$t_{54} = 1.43$	0.16
	Gregariousness†	10.9	3.45	$t_{54} = 3.16$	**0.003**
CV_{wm}	(Intercept)	0.14	0.017		
	Phylogeny*	0.010	0.006	$t_{54} = 1.74$	0.088
	Body size (PC1 male)	-0.008	0.005	$t_{54} = -1.64$	0.11
	Sexual size dimorphism	0.025	0.05	$t_{54} = 0.503$	0.62
	Clutch size	-0.003	0.005	$t_{54} = -0.627$	0.53
	Sexual dichromatism†	-0.017	0.014	$t_{54} = -1.2$	0.24
	Gregariousness†	-0.005	0.018	$t_{54} = -0.261$	0.80

*Eigenvector 1 (see Methods for details)

†1, "no"; 2, "yes"

Table S4. Linear models analysing the combined effect of different predictor variables and their interaction on mean sperm length and on the within-male variation in sperm length (CV_{wm}).

Term	Estimate	s.e.	*Statistic*	*P*
(Intercept)	51.3	1.29		
Phylogeny*	-2.75	1.07	$t_{56} = -2.56$	**0.013**
Sexual dichromatism†	1.67	2.83	$t_{56} = 0.59$	0.56
Gregariousness†	-0.274	6.0	$t_{56} = -0.046$	0.96
Sexual dichromatism† x Gregariousness†	15.9	7.22	$t_{56} = 2.2$	**0.032**

*Eigenvector 1 (see Methods for details)

†1, "no"; 2, "yes"

Table S5. Linear models analysing the relation between mean sperm length and within-male variation in sperm length (CV_{wm}) for Psittaciformes and Passeriformes, separetely. This analysis did not control for phylogeny.

Clade	Term	Estimate	s.e.	*Statistic*	*P*
Psittaciformes	(Intercept)	0.223	0.028		
	Mean sperm length	-0.002	0.0005	$t_{59} = -3.45$	**0.001**
Passeriformes	(Intercept)	0.027	0.001		
	Mean sperm length	-6.23×10^{-5}	8.3×10^{-6}	$t_{53} = -7.51$	**<0.001**

Table S6. Linear models analysing the relationship between mean sperm length and within-male variation in sperm length (CV_{wm}) for two separate clades. This analysis is not controlled for phylogeny.

Term	Estimate	s.e.	*Statistic*	*P*
(Intercept)	0.223	0.021		
Mean sperm length	-0.002	0.0004	$T_{112} = -4.73$	**<0.001**
Clade*	-0.196	0.023	$T_{112} = -8.66$	**<0.001**
Mean sperm length x Clade*	0.002	0.0004	$T_{112} = 4.52$	**<0.001**

*1, "Psittaciformes"; 2, "Passeriformes"

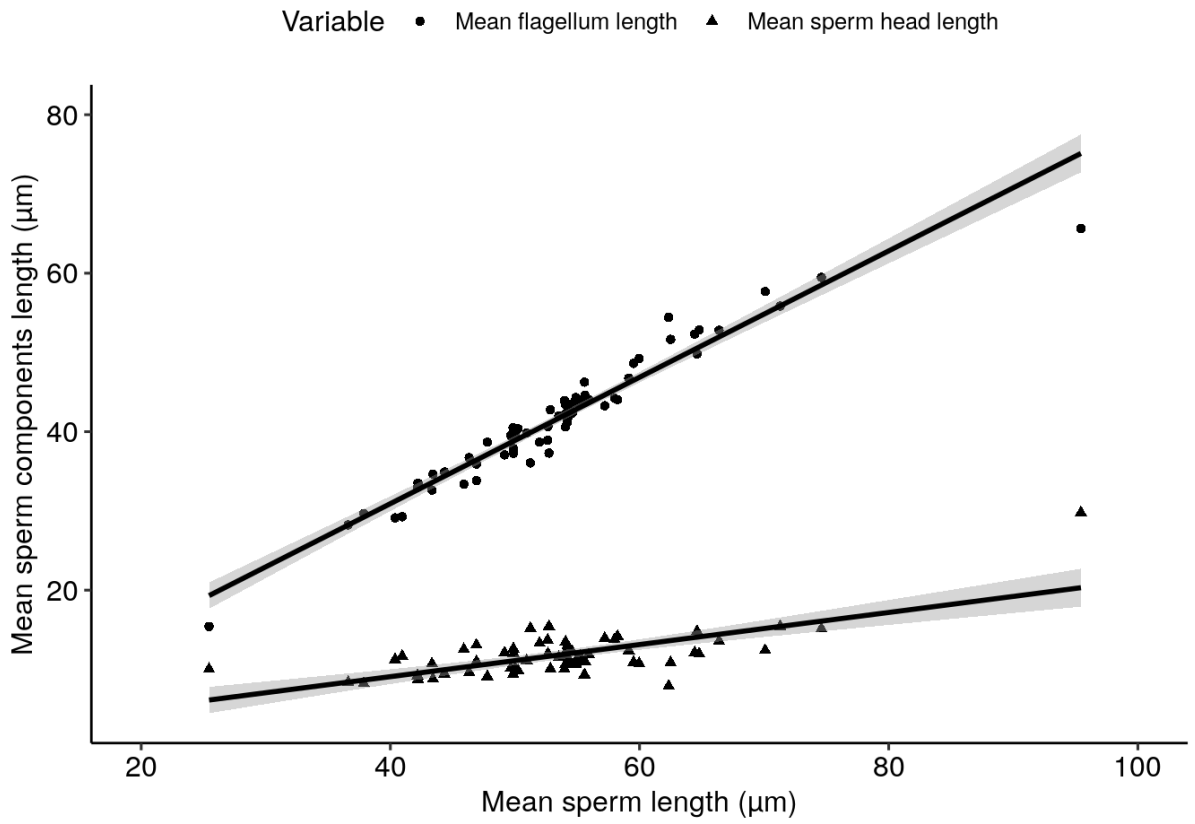

Figure S1. Relationship between mean total sperm length and mean sperm head and flagellum length for 61 parrot species. Results are based on a linear model with the total mean sperm length as the dependent variable and without controlling for phylogeny. Lines are estimates from the model with 95% CI in grey. Note that the sperm components of the largest sperm (Vasa parrot) do not fit on the model lines; this suggests that the mid-piece measures might have been included in the head measurements by mistake.

Chapter 2

Body size and climate as predictors of plumage colouration and sexual dichromatism in parrots

Luisana Carballo, Kaspar Delhey, Mihai Valcu, Bart Kempenaers

Psittaciformes (parrots, cockatoos and lorikeets) comprise one of the most colourful clades of birds. Their unique pigments and safe cavity nesting habits are two potential explanations for their colourful character. However, plumage colour varies substantially between parrot species and sometimes also between males and females of the same species. Here, we use comparative analyses to evaluate what factors correlate with colour elaboration, colour diversity and sexual dichromatism. Specifically, we test the association between different aspects of parrot colouration and (a) the intensity of sexual selection and social interactions, (b) variation along the slow-fast life-history continuum and (c) climatic variation. We show that larger species and species that live in warm environments display more elaborated colours, yet smaller species have higher levels of sexual dichromatism. Larger parrots tend to have darker and more blue and red colours. Parrots that live in wetter environments are darker and redder, whereas species inhabiting warm regions have more blue plumage colours. In general, each of the variables we considered explain small to moderate amounts of variation in parrot colouration (up to 15%). Our data suggest that sexual selection may be acting more strongly on males in small, short-lived parrots leading to sexual dichromatism. More elaborate colouration in both males and females of the larger, long-lived species with

slow tropical life histories suggests that mutual mate choice, social selection and reduced selection for crypsis may be important in these species, as has been shown for passerines.

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RESEARCH PAPER

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Body size and climate as predictors of plumage colouration and sexual dichromatism in parrots

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Abstract

Psittaciformes (parrots, cockatoos and lorikeets) comprise one of the most colourful clades of birds. Their unique pigments and safe cavity nesting habits are two potential explanations for their colourful character. However, plumage colour varies substantially between parrot species and sometimes also between males and females of the same species. Here, we use comparative analyses to evaluate what factors correlate with colour elaboration, colour diversity and sexual dichromatism. Specifically, we test the association between different aspects of parrot colouration and (a) the intensity of sexual selection and social interactions, (b) variation along the slow-fast life-history continuum and (c) climatic variation. We show that larger species and species that live in warm environments display more elaborated colours, yet smaller species have higher levels of sexual dichromatism. Larger parrots tend to have darker and more blue and red colours. Parrots that live in wetter environments are darker and redder, whereas species inhabiting warm regions have more blue plumage colours. In general, each of the variables we considered explain small to moderate amounts of variation in parrot colouration (up to 15%). Our data suggest that sexual selection may be acting more strongly on males in small, short-lived parrots leading to sexual dichromatism. More elaborate colouration in both males and females of the larger, long-lived species with slow tropical life histories suggests that mutual mate choice, social selection and reduced selection for crypsis may be important in these species, as has been shown for passerines.

KEYWORDS

body size, climate, comparative analyses, plumage colour elaboration, Psittaciformes, sexual dichromatism

1 | INTRODUCTION

Birds show great diversity in plumage colour and many studies have aimed to explain the proximate and ultimate mechanisms behind this diversity (Baker & Parker, 1979; Dale, Dey, Delhey, Kempenaers, & Valcu, 2015; Delhey, 2017, 2018; Hill & McGraw, 2006; Miller, Leighton, Freeman, Lees, & Ligon, 2019; Taysom, Stuart-Fox, & Cardoso, 2011). Among birds, Psittaciformes—parrots, cockatoos and lorikeets (from now on collectively called parrots)—show some of the most striking plumage colouration (Berg & Bennett, 2010;

Delhey, 2015). However, the evolutionary forces underlying their colourful character remain poorly understood (Berg & Bennett, 2010). It has been argued that parrots are colourful because they can synthesize and deposit red and yellow psittacofulvin pigments in their feathers, which are unique to parrots (McGraw & Nogare, 2004; Stradi, Pini, & Celentano, 2001). Because these pigments are synthesized endogenously, parrots might be able to deposit higher concentrations and display more intense colours compared with other bird species that can only obtain carotenoids (to produce yellow to red colours) through their diet (Delhey, 2015). Psittacofulvins, in combination with melanin pigments and feather microstructural components (which produce structural colours such as blue), enable parrots to display colours that encompass a large proportion of the entire avian colour gamut (Berg & Bennett, 2010; Delhey, 2015). In addition, most parrots breed in cavities, which are safe nesting sites that provide protection to parents and offspring from predators (Martin & Li, 1992), and potentially removing the need to be cryptic at the nest. Parrots, both males and females, are indeed more colourful than expected for their species richness (Delhey, 2015) and many species are mutually ornamented (Berg & Bennett, 2010).

Parrots are generally colourful, but also show great colour variation among species. For example, some cockatoo species are monochromatic and entirely white, whereas the Eclectus parrot (*Eclectus roratus*) is highly sexually dichromatic, with males being mainly green and females bright red and blue (del Hoyo, Elliott, & Christie, 2017). The selective forces behind this substantial variation in colour elaboration and sexual dichromatism within parrots (Delhey, 2015; Delhey & Peters, 2017; Taysom et al., 2011) are not yet well understood (Berg & Bennett, 2010).

Ornamental traits might be used in competitive interactions or in sexual displays. For this reason, many studies have explored how sexual and social interactions may have driven plumage colour evolution (Dale et al., 2015; Dunn, Whittingham, & Pitcher, 2001; Miller et al., 2019; Møller & Birkhead, 1994; Owens & Hartley, 1998; Rubenstein & Lovette, 2009). Colour traits can be favoured by sexual selection if the expression of the trait increases the reproductive success of individuals by gaining more access to mates, or by social selection if their expression is critical in the competition for social status or access to resources such as food or territories (West-Eberhard, 1983).

Polygynous bird species, which are subject to more intense sexual selection compared to monogamous species, exhibit multiple sexual ornaments (Møller & Pomiankowski, 1993) and higher levels of sexual dichromatism (Dale et al., 2015; Dunn et al., 2001). In lizards, two proxies for sexual selection intensity (sexual dimorphism in size and colour) correlate positively with colour diversity, that is the different colours and patterns that an individual displays (Chen, Stuart-Fox, Hugall, & Symonds, 2012). Additionally, bird species with high levels of extra-pair paternity presumably experience stronger sexual selection and also show higher levels of sexual dichromatism (Møller & Birkhead, 1994; Owens & Hartley, 1998). A large-scale comparative analysis in passerines showed that sexual selection is the strongest predictor of sexual dichromatism (Dale et al., 2015).

Colour ornamentation may have also evolved in response to the selective pressures of complex social interactions (Heinsohn, Legge, & Endler, 2005; Santana, Alfaro, Noonan, & Alfaro, 2013). For group living species, such as parrots, it might be advantageous to effectively signal status, age or identity (Bridge, Hylton, Eaton, Gamble, & Schoech, 2008; Dale et al., 2015), which may be easier to achieve with multiple signals (e.g. with higher colour diversity). Support for this idea comes from primates, where the complexity of facial markings is correlated with gregariousness (Santana et al., 2013). Further support comes from a study on the Eclectus parrot, showing that the extreme scarcity of suitable nest cavities (~1 per square kilometre) has intensified intrasexual competition (Heinsohn et al., 2005). Females spent most of their time protecting their nest (for around 11 months a year) and they may kill each other in disputes over tree hollows (Heinsohn et al., 2005). Thus, Heinsohn et al. (2005) suggested that the expression of conspicuous colours in females is a consequence of the need to display cavity ownership.

With a few exceptions, the mating system of parrots is social monogamy (Toft & Wright, 2015), which implies lower levels of sexual selection. The few studies exploring extra-pair paternity in parrots have found that some species are indeed genetically and socially monogamous (Caparroz, Miyaki, & Baker, 2011; Eastwood et al., 2018; Masello, Sramkova, Quillfeldt, Epplen, & Lubjuhn, 2002), whereas others show varying levels of extra-pair paternity (Beissinger, 2008; Heinsohn, Olah, Webb, Peakall, & Stojanovic, 2019; Martínez, de Aranzamendi, Masello, & Bucher, 2013). Furthermore, a recent study showed considerable variation in sperm length in parrots, with sexually dichromatic and gregarious species having longer sperm (Carballo et al., 2019). This study also showed that sperm length was negatively correlated with the within-male coefficient of variation in sperm length. Both longer sperm and low variation in sperm length (within and between males) are considered indicators of higher levels of sperm competition (Calhim, Immler, & Birkhead, 2007; Immler, Calhim, & Birkhead, 2008; Kleven et al., 2009; Kleven, Laskemoen, Fossøy, Robertson, & Lifjeld, 2008; Lifjeld, Laskemoen, Kleven, Albrecht, & Robertson, 2010; Lüpold, Calhim, Immler, & Birkhead, 2009). This suggests that some parrots might experience higher levels of sperm competition, for example due to increased opportunities for extra-pair mating when pairs nest in close proximity (Møller & Birkhead, 1993). We can thus ask whether variation in sexual dichromatism, colour elaboration and colour diversity are linked to indicators of the intensity of sexual selection in parrots.

The intensity of sexual selection may also depend on the species' life-history strategy (Winemiller, 1992). Given that the lifespan of parrots ranges from 8.5 to 100 years (Wasser & Sherman, 2010), one can explore whether the slow-fast life-history continuum is linked to parrot plumage colouration. In general, parrots form long-lasting pair bonds and the formation of such bonds may take time (Toft & Wright, 2015). Smaller parrot species experience a higher turnover of mates (Toft & Wright, 2015), which might be related to the higher mortality rate associated with smaller body size (de Magalhaes, Costa, & Church, 2007; Wasser & Sherman, 2010). Consequently, the expression of sexually

selected traits that help speed up the selection of mates could be more beneficial for females in species with lower adult survival if it reduces the time needed to identify a suitable male and form a pair bond. On the other hand, long-lived species with long-lasting pair bonds might experience mutual mate choice, linked to higher parental investment in both sexes (Kokko & Johnstone, 2002). In such cases, both males and females are expected to be more elaborately coloured. Larger species also experience reduced predation risk, a factor that may explain why males and females of larger passerine species have more elaborated colours (Dale et al., 2015). Furthermore, in birds, the slow-fast life-history continuum is related to extra-pair paternity: species with higher adult mortality rates and larger clutch sizes have higher levels of extra-pair paternity (Arnold & Owens, 2002). For example, a population of swift parrots (*Lathamus discolour*) where females experience high mortality due to an introduced predator shows high levels of extra-pair paternity (50.5% of nests) (Heinsohn et al., 2019).

Different studies have evaluated how abiotic factors affect bird plumage colour evolution and a variety of hypotheses have been proposed to explain colour variation both within and across avian taxa (Dale et al., 2015; Merwin, Seeholzer, & Smith, 2020; Miller et al., 2019; Ribot, Berg, Schubert, Endler, & Bennett, 2019). Previous studies showed that achromatic (light-to-dark) variation in birds is related to climate variables such as temperature and precipitation (Delhey, 2017, 2018, 2019; Heidrich et al., 2018; Merwin et al., 2020; Miller et al., 2019; Pinkert, Brandl, & Zeuss, 2017; Ribot et al., 2019). Specifically, a negative relationship between melanin pigmentation and temperature has been reported in several taxa (Delhey, 2018; Heidrich et al., 2018; Pinkert et al., 2017), in support of the thermal melanism hypothesis (Clusella Trullas, van Wyk, & Spotila, 2007). This ecogeographical rule proposes that darker animals are more common in colder environments, presumably for thermoregulation reasons (Clusella Trullas et al., 2007; Delhey, 2018). Similarly, Gloger's rule suggests a positive association between melanin pigmentation and precipitation (Delhey, 2017, 2019; Gloger, 1833), but the adaptive function of the link between darker colours and precipitation is not yet clear (Burtt & Ichida, 2004; Delhey, 2017; Zink & Remsen, 1986).

In summary, different factors may affect plumage colouration and sexual dichromatism. Therefore, to better understand what factors might explain interspecific variation in colour elaboration, colour diversity and sexual dichromatism, it is important to consider multiple variables simultaneously. So far, few studies on plumage colouration have considered multiple variables. Dale et al. (2015) used comparative analyses to explore the effects of multiple traits on plumage colour in passerines. Specifically, this study suggests that the evolution of plumage colour and sexual dichromatism are mainly driven by sexual selection and life-history traits, with stronger effects on female than on male colour. Both males and females are more colourful in larger species and in species with tropical life histories (i.e. small clutch size, low seasonality habitats), whereas sexual dichromatism was higher in smaller species and in species with male-biased sexual selection.

Here, we ask what factors affect plumage colouration in parrots. We quantified achromatic and chromatic colour variation among all 398 species of the order Psittaciformes based on colour plates and computed estimates of colour elaboration, colour diversity and sexual dichromatism. Our study had three main aims. (1) To test whether indicators of the intensity of sexual selection and social interactions relate to variation in plumage colouration in parrots. We predict higher sexual dichromatism and higher colour elaboration and colour diversity in males in species that (a) show stronger male-biased sexual size dimorphism and (b) breed at higher densities (i.e. are gregarious). (2) To test whether the slow-fast life-history continuum is associated with plumage colour variation in parrots. We predict higher sexual dichromatism and higher colour elaboration and colour diversity in males in species that (a) have smaller body size (because body size correlates positively with longevity; Wasser & Sherman, 2010) and (b) lay larger clutches. We predict lower sexual dichromatism but higher colour elaboration and colour diversity in both males and females (mutual ornamentation) in species that (c) have large body size and (d) lay smaller clutches. (3) To test whether parrots follow Gloger's rule and the thermal melanism hypothesis. If so, we predict that (a) darker species inhabit wetter and colder environments and (b) darker species inhabit densely forested rather than open habitat types (because the former are typically more humid and wet).

2 | MATERIAL AND METHODS

2.1 | Plumage colour scores

We compiled digital images of colour plates of both sexes for each of the 398 extant parrot species illustrated in the *Handbook of the Birds of the World Alive* (HBW Alive, del Hoyo et al., 2017). We imported the images into *Adobe Photoshop* (Adobe Inc. San Jose, CA), cropped them to remove the background colour and all bare parts of the birds, thus keeping only the body regions covered by plumage, and saved them as PNG files. Subsequently, we delineated 12 body patches (nape, crown, forehead, throat, upper breast, lower breast, shoulder, secondary coverts, primary coverts, secondaries, primaries and tail) for each sex and extracted RGB (red, green, blue) colour values from 400 randomly chosen pixels in each patch using the R package 'colorZapper' v.1.4.4 (Valcu & Dale, 2014). Even though the different body patches differed in size, we randomly selected 400 pixels from each patch, because body regions may vary in signalling importance. For the monochromatic species (i.e. when one plate is shown to represent both male and female, $N_{species} = 268$), the colour values were randomly extracted twice (once for the male and once for the female). In some cases ($N_{species} = 77$), the plates of one of the sexes did not show the entire body, hence the colour values of the missing body patches were extracted from the plate of the other sex. When multiple subspecies were illustrated, the nominate species was scored. Finally, we calculated mean R, G and B values for each patch, sex and species. We transformed these mean values to CIELAB coordinates (Tkalčič & Tasič, 2003) using the R package 'colorspace' v.1.4-1

(Zeileis et al., 2019). There are three CIELAB coordinates: (1) *L*, colour lightness, represents the achromatic channel (black = 0, white = 100, Figure 1a)*a*, the chromatic channel between green (low values) and red (high values) (Figure 1b) and (3) *b*, the chromatic channel between blue (low values) and yellow (high values) (Figure 1c). We used the CIELAB coordinates to compute the following colour variables:

1. *Colour elaboration score*, obtained by computing the Euclidean distance between each plumage patch and the centroid of the entire sample (joint average for *L*, *a* and *b* for all species together). These values were averaged in each species, separately for males and females. Highly elaborate colours (in this case, red, blue and yellow) are those that differ more from the average colour across parrots (here greenish brown) (Figure 1d). This index of colour elaboration yields a similar classification of elaborate colours as the one used in Dale et al. (2015) (compare Figure 1d with Figure S2 in Dale et al., 2015).
2. Sexual differences in colouration, computed in two ways: (a) *Sexual dichromatism*, as the Euclidean distance in CIELAB space between homologous patches in males and females averaged across all patches for each species (Figure 2a), and (b) *sexual difference in colour elaboration*, as the average difference in colour elaboration between males and females (Figure 2b). The first index (a) estimates the absolute difference in colouration between males and females (|male-female|) irrespective of whether males or females are more ornamented. The second one (b) indicates whether it is males or females that have more elaborated colours (male - female). Thus, positive values reflect species where males have more elaborated colours than females. Note that if males and females have different colours but with the same level of elaboration (e.g. red and blue) this index will score low.
3. Three overall plumage colour scores for each sex and species by calculating average values for *L*, *a* and *b* of all 12 body patches (Figure 1a–c, and see Figure S1 for more details of the raw colour distribution of each body patch). This allows us to assess whether explanatory variables favour the evolution of certain types of colours over others (e.g. red over green, light over dark). The downside of this approach is that species that harbour a wide range of colours may end up with intermediate average values of *L*, *a* or *b*.
4. Finally, we estimated *colour diversity*, computed as the mean of all Euclidean distances between each plumage patch and the species-specific (rather than that of the entire sample as in (a)) centroid (joint average for *L*, *a* and *b* of all plumage patches of each species). Smaller values of diversity indicate that all colours in a species are tightly clustered around the species-specific centroid (i.e. rather uniformly coloured species), whereas high values are indicative that colours are more dispersed around the centroid (i.e. species with many different colours).

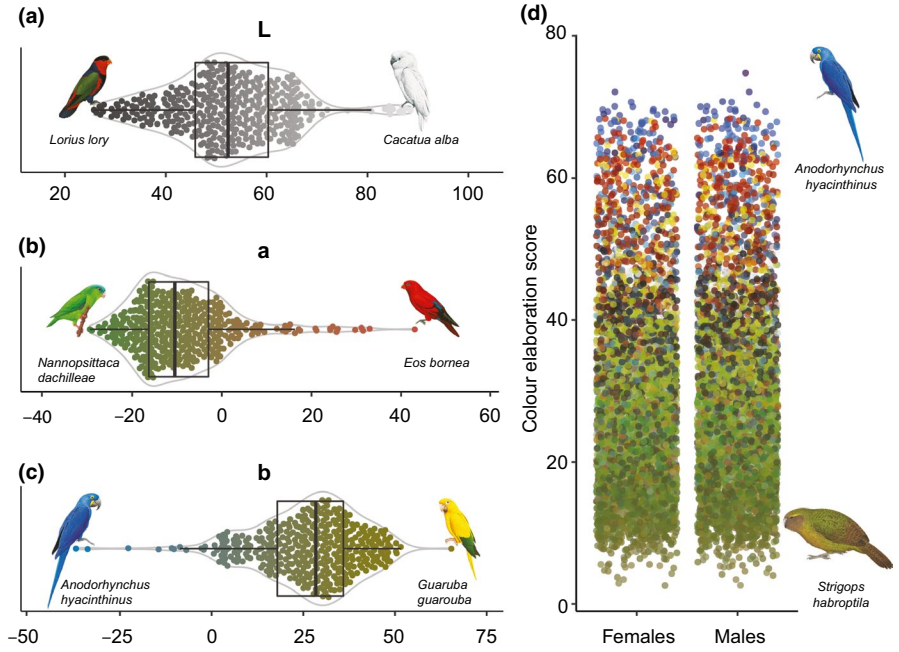

FIGURE 1 Illustration of the plumage colour scores for 398 parrot species. (a) *L*-score distribution showing dark to light colours, (b) *a*-score distribution showing green to red colours, (c) *b*-score distribution showing blue to yellow colours, and (d) colour elaboration score of females and males showing the distribution from the average colour (greenish brown) to highly elaborate colours such as red, blue and yellow. Illustrations in each panel represent the species that have the minimum and maximum scores for each variable. (a–c) Shown are box plots with median (vertical line) and interquartile range (box), and violin plots (grey lines) showing the probability density of the data. The dots in (a–c) represent the colour of each species for each colour coordinate (averaged across 12 body patches). To show the colour score of each species on the *L*, *a* and *b* coordinates separately, variation in the focal colour coordinate is shown, whereas the other two colour coordinates were fixed (a, $a = 0$, $b = 0$; b, $L = 50$, b = 26.4 (mean score for all species); c, $L = 50$, $a = -8.8$ (mean score for all species)). Illustrations © Lynx Edicions

2.2 | Measures of sexual selection and gregariousness

As a measure of the intensity of sexual selection, we calculated sexual size dimorphism (SSD) as $PC1_{\text{male body size}}$–$PC1_{\text{female body size}}$ (see below). We scored gregariousness as a categorical variable ('yes' or 'no') according to information from the 'breeding' section of the HBW Alive (del Hoyo et al., 2017). A species was classified as gregarious if the description suggested that the breeding pairs nest close together or if the species is described as colonial.

2.3 | Life-history traits

Our database contained data on body mass (N_{species} = 268), wing length (N_{species} = 359), tarsus length (N_{species} = 358) and tail length (N_{species} = 357). We measured wing, tarsus and tail length for an average of 3.3 (*range:* 1–22) females and 3.6 (*range*: 1–23) males per species (N_{species} = 214) from individuals held at the Loro Parque Fundación (LPF), Tenerife, Spain. For the species that were not present in the LPF collection, we compiled body measurements from the book *Parrots of the World* (Forshaw, 1978).

For each species, we estimated body size of males and females as the first principal component (PC1) from a principal component analysis (PCA) that included three body measurements: wing, tarsus and tail length. Species body size was estimated by calculating the average of male and female body size. We excluded body mass from our analyses, because this trait may be more condition-dependent and because the sample size for this trait was smaller, thereby decreasing the statistical power of our analyses. Note, however, that body mass correlated strongly with the other three body measurements ($r_{\text{bm-wing length}}$ = 0.87, $r_{\text{bm-tarsus length}}$ = 0.89, $r_{\text{bm-tail length}}$ = 0.66). PC1 explained 65% of the variation in the data. Wing and tarsus length had larger loadings on PC1, whilst tail length had larger loadings on PC2 (Figure S2). We kept PC1 as the species body size estimate, because tail length is more prone to wear. However, tail length was highly positively correlated with wing (r = 0.77) and tarsus length (r = 0.67).

We obtained clutch size for 250 species from the HBW Alive (del Hoyo et al., 2017). As clutch size data were not available in the HBW Alive for some species (N_{species} = 40), we completed the database using LPF records from the 2012–2015 breeding seasons (N_{species} = 21), by calculating the mean clutch size from 1–105 clutches per species (*mean* = 10.5). We also included data from the book *Parrots of the World* (N_{species} = 9) (Forshaw, 1978) and from the websites World Parrot Trust (www.parrots.org) (N_{species} = 9) and Avian Web (www.beautyofbirds.com) (N_{species} = 1). All data except those from LPF are assumed to be taken from the wild. Because captive conditions might affect clutch size, we evaluated whether LPF clutch size data differed from clutch size data from the other sources in two ways. First, we found no significant difference between the LPF data used in this study and the data from the other sources (Welch two sample t-test, mean LPF = 2.64, mean other sources = 3.29, $t_{22.4}$ = −1.99, p = 0.06). Second, we compared the clutch size for a set of 133 species for which we had data from both LPF and the HBW. A linear mixed model with family as a random factor (clutch_size_HBW-clutch_size_LPF ~ 1 + (1|family)) showed no difference (estimate = 0.23 ± 0.13, t_{163} = 1.86, p = 0.23, *df* based on Satterthwaite's method). Thus, we used the data from all the difference sources to increase sample size for the variable clutch size. The source of the body measurements and clutch size data for each species is given in the online repository.

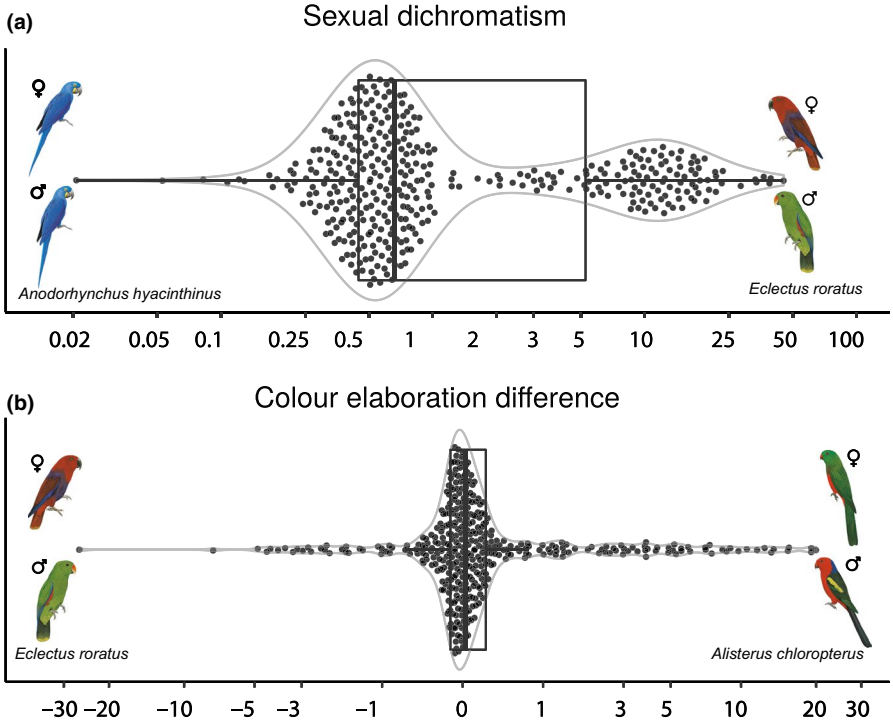

FIGURE 2 Illustration of sexual differences in colouration for 398 parrot species. (a) Distribution of the sexual dichromatism score and (b) distribution of sexual differences in colour elaboration. *X*-axes scales are $\log_{10}$ transformed and $\log_{10}$-modulus transformed (sign(*x*)*log10(abs(*x*)+1), John & Draper, 1980) for negative values. Illustrations in each panel represent the species that have the minimum and maximum scores for each variable. Shown are box plots with median (vertical line) and interquartile range (box), and violin plots (grey lines) showing the probability density of the data. Illustrations © Lynx Edicions

2.4 | Environmental variables

We considered three environmental variables: habitat type, mean annual temperature (°C) and mean annual precipitation (mm). We scored habitat type as a categorical variable (1 = 'open', 2 = 'mixed', 3 = 'forested') using the description in the 'habitat' section of the HBW Alive (del Hoyo et al., 2017). Following McNaught and Owens (2002), we classified habitat type as 'open' for species that occur in habitats such as savannah, grassland, shrubland, forest edges, arid and eucalypt woodland or cliffs, as 'forested' for species that occur in habitats such as forest, riverine forest, riparian forest, pine woodland, mangrove, evergreen lowland or wooded country and as 'mixed' for species that inhabit both 'open' and 'forested' habitat.

To estimate species-specific mean annual temperature and mean annual precipitation, we first obtained the extant breeding ranges for each parrot species using the database from BirdLife International's species distribution maps (BirdLife International, 2018). We only considered the natural distribution of each species and hence removed any breeding ranges where they were introduced. We extracted the mean annual temperature and mean annual precipitation corresponding to the breeding ranges of each species using the high-spatial resolution CHELSA climate data (Karger et al., 2017a, 2017b). Breeding ranges and environmental rasters were re-projected to an equal-area (Mollweide) projection. Spatial analyses were performed with the R package 'rangeMapper' v.0.3-7 (Valcu, Dale, & Kempenaers, 2012).

2.5 | Phylogeny

We extracted a sample of 1,000 phylogenetic trees (the 'Hackett' backbone, Hackett et al., 2008) for 351 parrot species from phylogenetic tree distributions available on *birdtree.org* (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012; Jetz et al., 2014). We added the 47 Psittaciformes species missing in these phylogenies using the function *add.species.to.genus* in the R package 'phytools' v.0.6-99 (Revell, 2012). This function finds the branch of the phylogenetic tree common to the corresponding genus and adds the missing taxon at a random position within this branch.

2.6 | Statistical analysis

All statistical and spatial analyses were performed in R 3.6.2 (R Development Core Team, 2019). The variables sexual dichromatism and sexual difference in colour elaboration were $\log_{10}$ transformed and $\log_{10}$-modulus transformed ($\text{sign}(x)*\log_{10}(\text{abs}(x)+1)$, John & Draper, 1980), respectively, to improve the data distribution for analyses. Model residuals showed no major violation of the assumptions of normality and heterogeneity of variance. All variables were standardised by centring and dividing by one standard deviation.

To explore the effect of abiotic and biotic factors on plumage colour elaboration, sexual dichromatism and colour diversity across parrots, we used species-level phylogenetic linear models. These models were fitted with the R package 'phylolm' v.2.6 (Ho & Ané, 2014) using the Pagel's λ model (Pagel, 1999), which measures the strength of the phylogenetic signal. We ran separate models for our seven response variables, that is colour elaboration, sexual dichromatism, sexual difference in colour elaboration, colour diversity and the three plumage colour scores (*L*, *a* and *b*), and we considered body size ($N = 357$), clutch size ($N = 290$), habitat type ($N = 398$), mean annual temperature ($N = 398$), mean annual precipitation ($N = 398$), sexual size dimorphism ($N = 357$) and gregariousness ($N = 350$) as predictors in our analyses. First, we ran univariate models to explore the effect of each predictor separately and allowing the use of the full dataset. For the 273 species for which all the predictors were available, we then ran a multiple predictor model to explore the effect of each predictor, whilst controlling for the others.

We estimated the proportion of variance explained by the phylogenetic linear models following Ives (2019) by using the function *R2.resid* in the R package 'rr2' v.1.0.2 (A. Ives & Li, 2018). We calculated two R^2 coefficients: (1) R^2_{full}: the total variance explained by the full model (both by phylogeny and fixed effects) and (2) R^2_{fixef}: the variance explained by the fixed effects only.

We ran species-level phylogenetic linear models for each of the 1,000 phylogenies and we averaged the model coefficients. Additionally, we computed an inference interval as the 2.5th–97.5th percentiles for p-values, Pagel's λ and the two R^2 coefficients. Therefore, the Pagel's λ and the R^2 coefficients inference intervals contain both the error of the distribution underlining the phylogenetic trees and the uncertainty of the taxonomy-based data imputation.

3 | RESULTS

3.1 | Comparing book colour plates with reflectance measurements

The colour plates in the HBW have been painted to resemble real plumage colours as perceived by humans. To determine whether our estimates approximated those obtained using direct measurements of plumage, we used reflectance measurements obtained from 51 species of Australian parrots and cockatoos (Delhey, 2015, see Supplementary Information).

In general, all variables obtained from bookplates were positively correlated with estimates from reflectance spectra (all $p < 0.001$). Colour elaboration scores showed the weakest correlations (males: $r = 0.53$, females: $r = 0.67$), followed by difference in colour elaboration between males and females ($r = 0.60$), colour diversity (males: $r = 0.83$, females: $r = 0.74$) and sexual dichromatism ($r = 0.86$). *L* scores (which depict light-to-dark variation) were also positively correlated (males: $r = 0.88$, females: $r = 0.89$). It is harder to determine whether both chromatic coordinates in the CIELAB space (*a* and *b*) correlate with the chromatic coordinates obtained from visual models (xyz, see Supplementary Information), because the latter do not

necessarily align with the former. However, if both types of chromatic coordinates represent similar colours then we would expect that a linear combination of visual model chromatic coordinates (xyz) should predict chromatic coordinates (*a*, *b*) from bookplates. This was the case: xyz predicted substantial variation in *a* (males: $R^2 = 0.78$, effects ± *SE*: $x = -0.28 \pm 0.52$, $y = -2.41 \pm 0.25$, $z = 2.48 \pm 0.35$; females: $R^2 = 0.85$, $x = -0.63 \pm 0.51$, $y = -3.12 \pm 0.26$, $z = 3.04 \pm 0.33$) and *b* (males: $R^2 = 0.68$, effects ± *SE*: $x = 2.93 \pm 0.83$, $y = 3.53 \pm 0.39$, $z = 1.11 \pm 0.56$; females: $R^2 = 0.74$, $x = 3.26 \pm 0.90$, $y = 4.65 \pm 0.46$, $z = 0.03 \pm 0.59$).

Furthermore, we tested whether missing information on ultraviolet reflectance in bookplates (which birds can perceive but humans cannot) affected the correlations between colour variables based on bookplates versus colour variables derived from reflectance measurements. First, we quantified the amount of UV reflectance for each of the 51 species with reflectance data as the stimulation of the UV-sensitive cone relative to the sum of all cones (see Supplementary Information) averaged across all measured plumage patches for males and females separately. Then, for each of the associations tested above, we extracted the residuals of the linear regression between colour variables obtained using reflectance measurements (predictor) and colour variables from book plates (response). If high ultraviolet reflectance is leading to increased error in these associations, we would expect that on average, absolute residuals should be higher for UV-rich species. This was not the case: the correlation coefficients varied between $r = -0.16$ and $r = 0.17$ (*mean* = −0.016) and in all cases $p > 0.23$ (Table 1). It could be argued that it is better to use 'raw residuals' rather than absolute residuals as we may expect that in UV-rich species our estimates of colouration (e.g. colour elaboration, colour diversity and sexual dichromatism) would be downward biased (i.e. we expect negative residuals) because UV-rich colours are often highly elaborate and conspicuous. Therefore, we also computed the correlations between raw residuals and relative UV reflectance. The correlation coefficients varied between $r = -0.31$ and $r = 0.21$ (*mean* = −0.014, Table 1). Most of these coefficients are clearly not statistically significant, except for sexual dichromatism ($r = -0.31$, $p = 0.028$) and colour diversity in males ($r = -0.27$, $p = 0.056$), indicating that we may have underestimated the true values of these variables for UV-rich species.

3.2 | Effects on plumage colouration

Both males and females of larger species and of species with smaller clutch size had more elaborated plumage colours. For body size, these effects were statistically significant in the single predictor models (Figure 3a; ♂: estimate = 0.51 ± 0.08, $t_{352} = 6.76$, $p = 1.88 \times 10^{-10}$, $\lambda = 0.82$; ♀: estimate = 0.56 ± 0.07, $t_{352} = 7.62$, $p = 6.79 \times 10^{-13}$, $\lambda = 0.81$; see Tables S1 and S2) and in the multiple predictor models (Figure 3b; ♂: estimate = 0.52 ± 0.08, $t_{262} = 6.35$, $p = 1.45 \times 10^{-9}$; ♀: estimate = 0.55 ± 0.08, $t_{262} = 6.92$, $p = 4.85 \times 10^{-11}$; see Tables S3 and S4). In the single predictor model, body size had an R^2_{fixef} ♂ = 0.12 and R^2_{fixef} ♀ = 0.14, indicating that this trait explained 12%–14% of the variation in colour elaboration after controlling for phylogenetic relatedness. The clutch size effect was statistically significant only in the single predictor models (Figure 3a; ♂: estimate = −0.19 ± 0.07, $t_{285} = -2.91$, $p = 0.004$, $\lambda = 0.84$; ♀: estimate = −0.23 ± 0.07, $t_{285} = -3.54$, $p = 0.0005$, $\lambda = 0.83$; Tables S1 and S2), and it explained 3%–4% of the variation in colour elaboration after controlling for phylogeny (R^2_{fixef}♂ = 0.03, R^2_{fixef} ♀ = 0.04). The lower effects and loss of significance of clutch size in the multiple predictor model (Figure 3b) might be due the intercorrelation between clutch size and body size ($r = -0.32$, Figure S3, Table S18).

We also found that annual mean temperature had a positive effect on colour elaboration in both males and females; this effect was significant in the single predictor models (Figure 3a; ♂: estimate = 0.14 ± 0.05, $t_{393} = 2.85$, $p = 0.006$, $\lambda = 0.86$; ♀: estimate = 0.17 ± 0.05, $t_{393} = 3.51$, $p = 0.0007$, $\lambda = 0.85$; Tables S1 and S2) and in the multiple predictor models (Figure 3b; ♂: estimate = 0.18 ± 0.06, $t_{262} = 2.98$, $p = 0.004$; ♀: estimate = 0.20 ± 0.06,

TABLE 1 Correlations between residuals (raw and absolute) and relative UV reflectance. The residuals were obtained from the linear regression between colour variables obtained using reflectance measurements (predictor) and colour variables from bookplates (response)

Variable	r (absolute residuals)	p (absolute residuals)	r (raw residuals)	p (raw residuals)
Sexual dichromatism	−0.16	0.27	−0.31	0.028
Sexual difference in colour elaboration	−0.15	0.3	−0.22	0.128
Colour elaboration ♂	−0.15	0.26	0.13	0.36
Colour elaboration ♀	0.06	0.69	0.07	0.64
Colour diversity ♂	0.08	0.56	−0.27	0.056
Colour diversity ♀	0.17	0.23	−0.22	0.118
L ♂	0.07	0.6	0.15	0.29
L ♀	0.03	0.83	0.21	0.13
a ♂	−0.15	0.27	0.05	0.73
a ♀	0.17	0.25	0.03	0.82
b ♂	−0.08	0.58	0.08	0.57
b ♀	−0.08	0.59	0.13	0.35

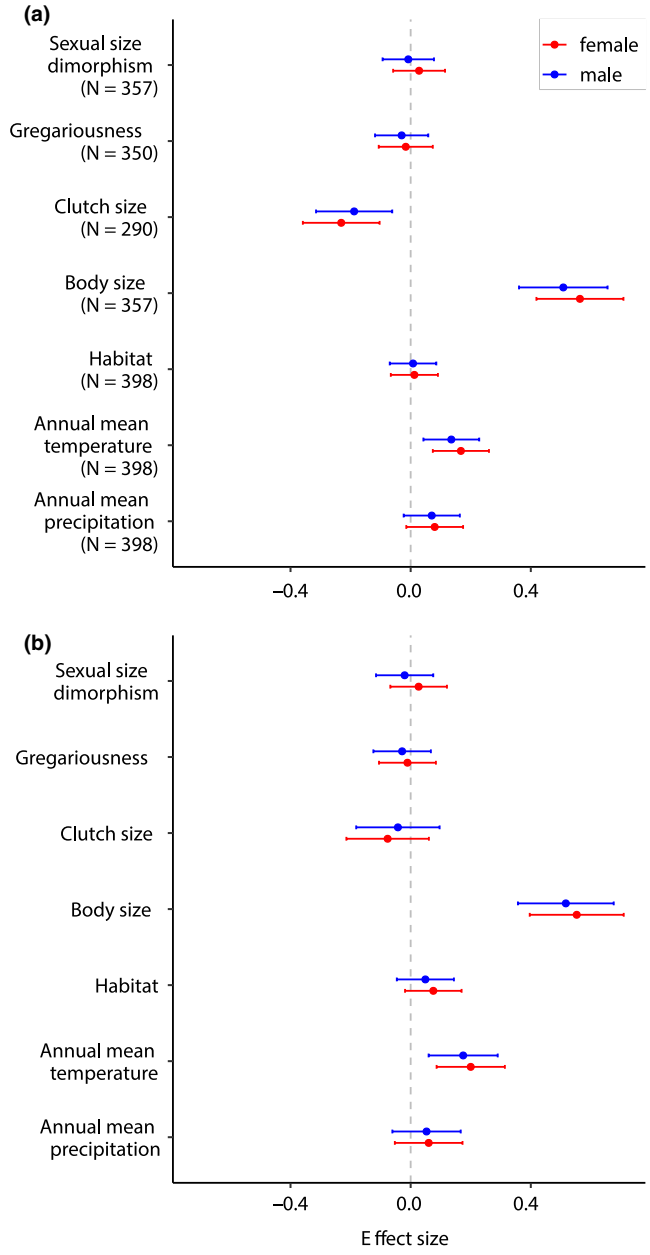

FIGURE 3 Effect sizes of predictors of colour elaboration based on (a) single predictor models and (b) a multiple predictor model ($N = 273$ species). Red denotes females and blue refers to males. Shown are the means of the model coefficients for the 1,000 phylogenetic linear models and the corresponding 95% confidence intervals. N indicates the number of species included in the analyses (determined by data availability)

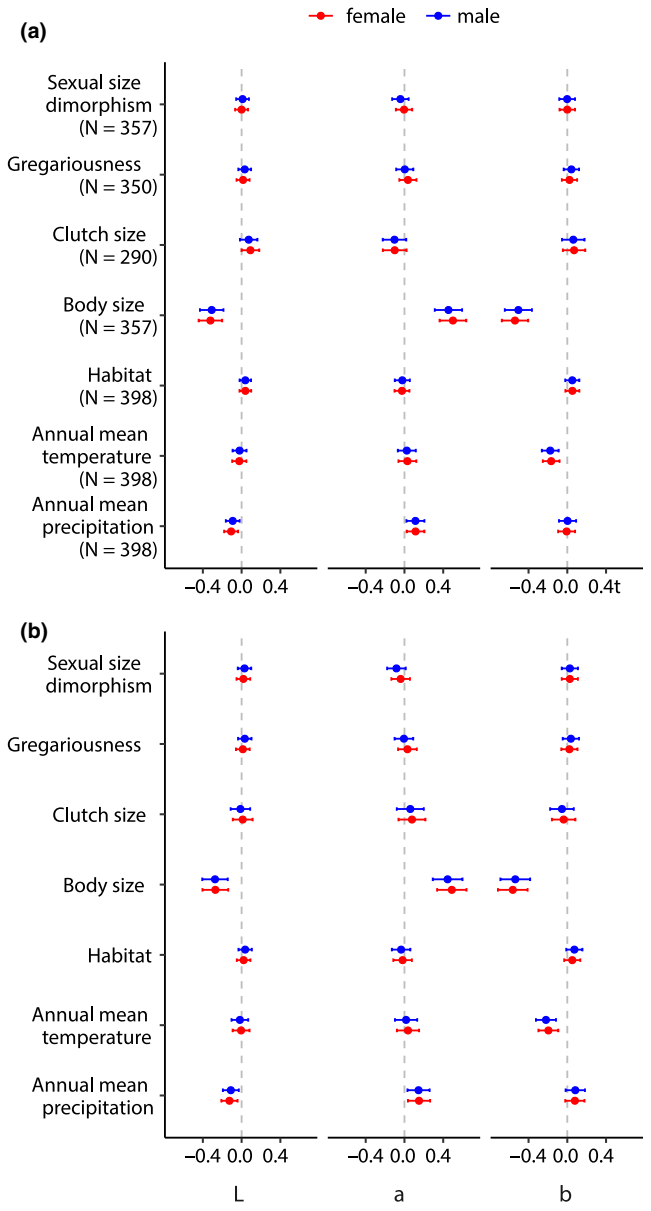

FIGURE 4 Effect sizes for each of the predictor variables on the three CIELAB colour coordinates (L = dark-to-light variation, a = green-to-red variation, b = blue-to-yellow variation), based on (a) single predictor models and (b) multiple predictor models ($N = 273$ species). Red denotes females and blue refers to males. Shown are the means of the model coefficients for the 1,000 phylogenetic linear models and the corresponding 95% confidence intervals. N indicates the number of species included in the analyses (determined by data availability)

$t_{262} = 3.45$, $p = 0.0007$; Tables S3 and S4). Annual mean temperature explained 2%–3% of the variation in colour elaboration after controlling for phylogeny ($R^2_{\text{fixef}}\text{♂} = 0.02$, $R^2_{\text{fixef}}\text{♀} = 0.03$).

In both sexes, body size was significantly negatively associated with L and b scores and positively associated with a scores, both in the single predictor models (Figure 4a; L ♂: estimate = −0.31 ± 0.06, $t_{352} = -4.99$, $p = 4.94 \times 10^{-6}$, $\lambda = 0.87$; b ♂: estimate = −0.51 ± 0.07, $t_{352} = -7.06$, $p = 8.87 \times 10^{-11}$, $\lambda = 0.81$; a ♂: estimate = 0.45 ± 0.07, $t_{352} = 6.30$, $p = 2.33 \times 10^{-9}$, $\lambda = 0.77$; L ♀: estimate = −0.32 ± 0.06, $t_{352} = -5.18$, $p = 2.19 \times 10^{-6}$, $\lambda = 0.86$; b ♀: estimate = −0.54 ± 0.07, $t_{352} = -7.75$, $p = 3.39 \times 10^{-12}$, $\lambda = 0.81$; a ♀: estimate = 0.50 ± 0.07, $t_{352} = 7.10$, $p = 1.92 \times 10^{-11}$, $\lambda = 0.76$; Tables S5 and S6) and in the multiple predictor models (Figure 4b; L ♂: estimate = −0.28 ± 0.07, $t_{262} = -4.08$, $p = .0002$; b ♂: estimate = −0.54 ± 0.08, $t_{262} = -6.84$,

$p = 5.67 \times 10^{-10}$; *a* ♂: estimate = 0.45 ± 0.08, $t_{262} = 5.68$, $p = 4.74 \times 10^{-8}$; *L* ♀: estimate = −0.27 ± 0.07, $t_{262} = -3.99$, $p = .0002$; *b* ♀: estimate = −0.56 ± 0.08, $t_{262} = -7.20$, $p = 1.56 \times 10^{-10}$; *a* ♀: estimate = 0.49 ± 0.08, $t_{262} = 6.31$, $p = 1.53 \times 10^{-9}$; Tables S7 and S8). Body size explained 7% of variation on *L* score (R^2_{fixef} ♂ = 0.07, R^2_{fixef} ♀ = 0.07), 13%–15% on *b* scores (R^2_{fixef} ♂ = 0.13, R^2_{fixef} ♀ = 0.15) and 10%–12% on *a* scores (R^2_{fixef} ♂ = 0.10, R^2_{fixef} ♀ = 0.12) after controlling for phylogeny. These results suggested that males and females of larger species are darker, redder and more blue-coloured.

In both sexes, precipitation had a negative effect on *L* scores, in both the single predictor models (Figure 4a; ♂: estimate = −0.09 ± 0.04, $t_{393} = -2.52$, $p = 0.017$, $\lambda = 0.88$; ♀: estimate = −0.11 ± 0.04, $t_{393} = -2.93$, $p = 0.005$, $\lambda = 0.87$; Tables S5 and S6) and in the multiple predictor models (Figure 4b; ♂: estimate = −0.11 ± 0.04, $t_{262} = -2.64$, $p = 0.012$; ♀: estimate = −0.13 ± 0.04, $t_{262} = -2.93$, $p = 0.005$; Tables S7 and S8), and a positive effect on *a* scores, in the single predictor models (Figure 4a; ♂: estimate = 0.11 ± 0.05, $t_{393} = 2.39$, $p = 0.02$, $\lambda = 0.83$; ♀: estimate = 0.11 ± 0.05, $t_{393} = 2.43$, $p = 0.019$, $\lambda = 0.83$; Tables S5 and S6) and in the multiple predictor models (Figure 4b; ♂: estimate = 0.15 ± 0.06, $t_{262} = 2.48$, $p = 0.014$, $\lambda = 0.66$; ♀: estimate = 0.15 ± 0.06, $t_{262} = 2.56$, $p = 0.01$, $\lambda = 0.63$; Tables S7 and S8). Temperature had a negative effect on *b* scores in the single predictor models (Figure 4a; ♂: estimate = −0.18 ± 0.04, $t_{393} = -3.96$, $p = .0.0002$, $\lambda = 0.89$; ♀: estimate = −0.17 ± 0.04, $t_{393} = -3.77$, $p = 0.0003$, $\lambda = 0.89$; Tables S5 and S6) and in the multiple predictor models (Figure 4b; ♂: estimate = −0.22 ± 0.05, $t_{262} = -4.17$, $p = 5.55 \times 10^{-5}$; ♀: estimate = −0.20 ± 0.05, $t_{262} = -3.71$, $p = 0.0003$; Tables S7 and S8). Mean annual precipitation explained 2% of the variation on *L* scores (R^2_{fixef} ♂ = 0.02, R^2_{fixef} ♀ = 0.02) and 1%–2% on *a* scores (R^2_{fixef} ♂ = 0.01, R^2_{fixef} ♀ = 0.02) after controlling for phylogeny, whereas mean annual temperature explained 3%–4% of the variation on *b* scores after controlling for phylogeny (R^2_{fixef} ♂ = 0.04, R^2_{fixef} ♀ = 0.03). These results indicate that species that are darker and redder inhabit areas of higher mean annual precipitation and that more blue-coloured species inhabit areas of higher mean annual temperature.

Habitat type did not have an effect on plumage colour in parrots (Figures 3 and 4, Tables S1–S8), at least based on the data and classification used in this study.

3.3 | Effects on colour diversity

None of the predictors used in this study had a statistically significant effect on colour diversity in parrots, either in the single or in the multiple predictor models (Figure 5, Tables S9–S12).

3.4 | Effects on sexual dichromatism

The single predictor models showed that body size was negatively related to sexual dichromatism (Figure 6a; estimate = −0.29 ± 0.07, $t_{352} = -4.09$, $p = 7.62 \times 10^{-5}$, $\lambda = 0.76$; Table S13). Additionally, sexual dichromatism was more pronounced in more closed or forested habitats (Figure 6a; estimate = 0.08 ± 0.04, $t_{393} = 2.14$, $p = 0.038$, $\lambda = 0.78$; Table S13). Body size explained 4% ($R^2_{fixef} = 0.04$) of the variation in sexual dichromatism, and habitat explained 1% ($R^2_{fixef} = 0.01$). In the multiple predictor models, the only effect that remained significant was that of body size on sexual dichromatism

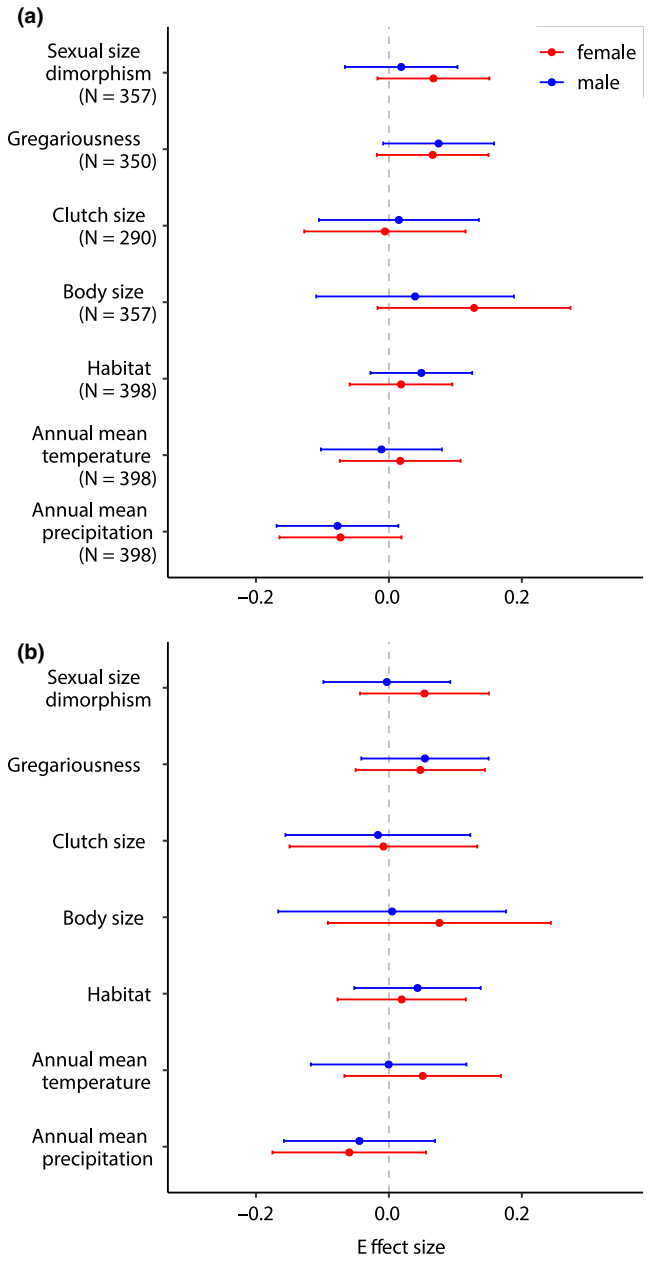

FIGURE 5 Effect sizes of predictors of colour diversity based on (a) single predictor models and (b) a multiple predictor model ($N = 273$ species). Red denotes females and blue refers to males. Shown are the means of the model coefficients for the 1,000 phylogenetic linear models and the corresponding 95% confidence intervals. N indicates the number of species included in the analyses (determined by data availability)

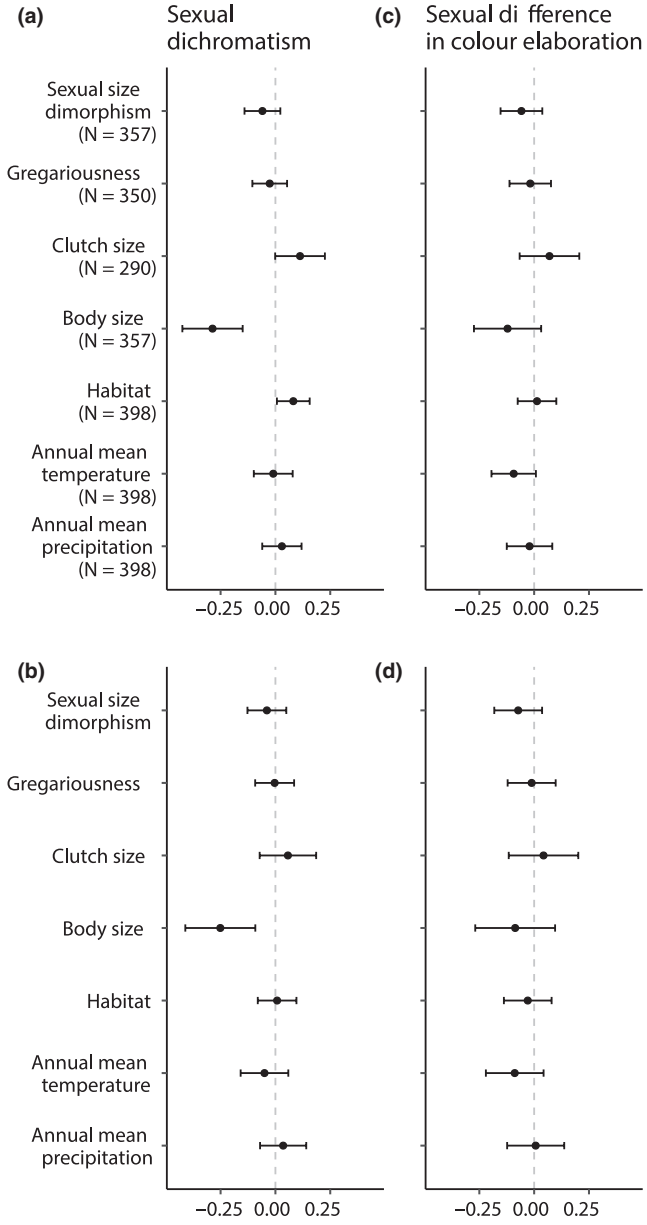

FIGURE 6 Effect sizes of predictors of difference in plumage colour between the sexes. Effect size of (a) sexual dichromatism and (b) sexual difference in colour elaboration based on single predictor models. Effect size of (c) sexual dichromatism and (d) sexual difference in colour elaboration based on multiple predictor models (N = 273 species). Shown are the means of the model coefficients for the 1,000 phylogenetic linear models and the corresponding 95% confidence intervals. N indicates the number of species included in the analyses (determined by data availability)

(Figure 6c; estimate = −0.25 ± 0.08, t_{262} = −3.09, p = 0.003; Table S15). The effect of habitat type on sexual dichromatism (Figure 6c, Table S15) was somewhat smaller and no longer significant, possibly due to reduced statistical power related to lower sample size (from $N_{species}$ = 357 to $N_{species}$ = 273). We found no effect of any of the predictors on the sexual difference in colour elaboration (Figure 6b,d, Tables S14 and S16).

3.5 | Variance explained by phylogeny and fixed effects

In all the models where we found significant effects of the predictors on the plumage colour elaboration score, the colour scores (L, a and b) and sexual dichromatism, the variance explained by both phylogeny and fixed effects together was higher (R^2_{full}: *range* = 0.39 – 0.67) than that explained by the fixed effects alone (R^2_{fixef}: *range* = 0.01 – 0.15). Thus, after controlling for phylogeny, the fixed effects separately explained up to 15% of the variation in the different plumage colour variables (Tables S1, S2, S5, S6 and S13). In the multiple predictor models, the fixed effects together explained up to 23% of the variance in the data after controlling for phylogeny (Tables S3, S4, S7, S8 and S15).

4 | DISCUSSION

Our study shows that variation in plumage colouration across all species of parrots, whilst strongly phylogenetically conserved, can be partly explained by key life-history traits and environmental variables. Among the former, body size seems the most important: larger species display more elaborate colours, such as red or blue, whereas smaller species had less elaborate plumage yet higher levels of sexual dichromatism (Figure 7 and Figure S4). Environmental effects were largely restricted to climatic variables and were partially in agreement with ecogeographical rules of colour variation. Two climatic variables correlate with plumage colour variation in parrots: temperature and precipitation.

Darker parrots are more frequent in wetter environments, as predicted by Gloger's rule (Rensch, 1936). Support for Gloger's rule has already been found at the intraspecific level in parrots (in the crimson rosella *Platycercus elegans*; Ribot et al., 2019) and also at the interspecific level among lorikeets (Merwin et al., 2020). We now show that it is a general pattern that applies at the interspecific level based on all 398 extant parrot species. There are two plausible adaptive explanations for the correlation between higher precipitation and darker colours (Delhey, 2017). First, darker colours would be favoured for camouflage in wetter environments as these harbour more vegetation and low light conditions. Second, as the presence of feather-degrading bacteria is higher in wetter environments, darker animals (with higher melanin concentration in their feathers) would be more resistant to feather degradation (parasite-resistance hypothesis). Melanin deposition thickens the cortex of the barb and this makes feathers more resistant to feather-degrading bacteria (Bonser, 1995), which is more important in wetter and warmer environments (Burtt & Ichida, 1999, 2004). Because we did not find an effect of habitat type on colour darkness, we consider the parasite-resistance hypothesis the more plausible scenario behind Gloger's rule for parrots. Furthermore, we found that parrots are redder in wetter environments. Psittacofulvin concentration, which is higher in redder colours, might also provide more

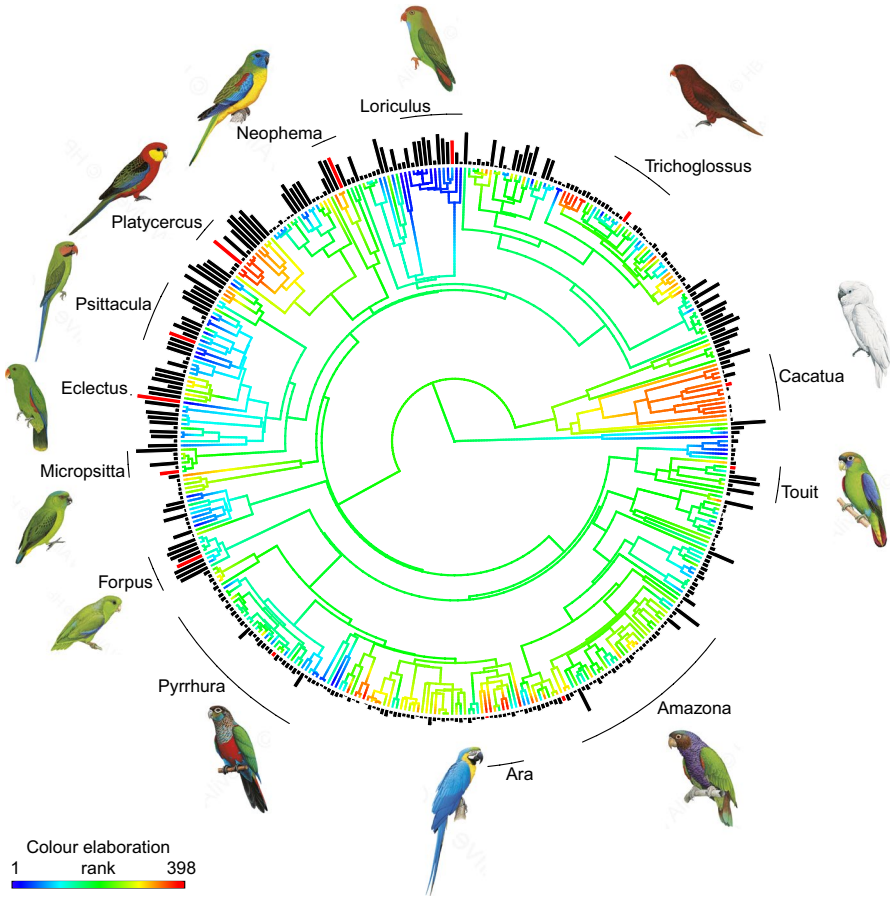

FIGURE 7 Parrots and cockatoos with more elaborate colours have lower levels of sexual dichromatism. Phylogeny of Psittaciformes depicting a reconstruction of evolutionary changes in male colour elaboration (branch colours, red = high, blue = low) using function *contMap* in R package 'phytools' v.0.6-99 (Revell, 2012) and levels of sexual dichromatism (bar lengths at the tips). Note how species with low levels of colour elaboration have higher levels of sexual dichromatism. The plot is based on one phylogeny in the sample, but comparative analyses were carried out on 1,000 phylogenetic reconstructions to account for phylogenetic uncertainty. Selected genera have been highlighted and species in illustrations are represented with red bars. Illustrations © Lynx Edicions

protection against feather-degrading bacteria (Burtt, Schroeder, Smith, Sroka, & McGraw, 2010). These findings thus provide further support for selection on plumage colours that strengthen feathers in parrot species living in wetter environments.

Our results also show that males and females have more elaborated colours in warmer environments. As variation in temperature closely follows variation in latitude, this means that tropical parrots tend to be more colourful. Whether tropical birds are more colourful than their temperate counterparts has been a contested issue for nearly 200 years. Gloger, for example, suggested that tropical birds should be more pigmented and colourful because the environment was more benign allowing the production of such colours (Gloger, 1833). Proper tests of latitudinal patterns of colouration in birds have yielded conflicting results, some studies reporting no such correlation or even the opposite pattern (Bailey, 1978; Dalrymple et al., 2015), and others confirming the more elaborate colours of tropical species (Dale et al., 2015; Willson & von Neumann, 1972). Our findings agree with the latter and are consistent with two non-mutually exclusive hypotheses (Dale et al., 2015): first, that tropical species are more colourful because mutual mate choice is stronger in those species; and second, because resource competition is stronger in the tropics, colour ornamentation might signal status in aggressive contexts (social selection) (Tobias, Montgomerie, & Lyon, 2012). These effects are thought to be mediated by selection pressures associated with slow life histories typical of large species living in tropical environments.

We found that larger species display on average more elaborate colours and also show darker, redder and more blue colours in their plumage. A similar finding has been reported in a large-scale comparative analysis of passerine plumage colour (Dale et al., 2015). Together, our results and those in Dale et al. (2015) disagree with the hypothesis that body size represents an evolutionary constraint on plumage colouration, as suggested by Galván, Negro, Rodríguez, and Carrascal (2013). Firstly, Galván et al. (2013) suggested that larger species might be less colourful compared to smaller species because, proportionally to their size, the latter consume higher quantities of food (Tella et al., 2004). Hence, smaller species would have higher concentrations of limiting carotenoids pigments in their blood to colour their feathers. This explanation does not apply to parrots, since they do not deposit carotenoids in their plumage (Berg & Bennett, 2010). Secondly, they suggested that larger species might be able to detect other individuals at longer distances, whereas smaller species might have been forced to develop more conspicuous signals to communicate with conspecifics. Our results, on the contrary, are more consistent with the hypothesis that larger species experience lower predation pressure (Ricklefs, 2010), hence reducing selection for crypsis.

Our analyses further indicate that smaller parrot species—whilst displaying on average less elaborate colours—are more sexually dichromatic, in most cases (but not all) due to males having more elaborate colours than females (Figure S4). This suggests that smaller parrots are not only constrained from having highly elaborate colours,

but also that the cost-benefit ratio of ornamental plumage colours varies between the sexes. Smaller species tend to have shorter lifespans (Bennett & Owens, 2002; de Magalhaes et al., 2007; Wasser & Sherman, 2010), which reduces the probability that a pair breeds together in subsequent seasons (Mauck, Marschall, & Parker, 1999). Under this scenario, higher levels of extra-pair paternity may be tolerated, that is it might not lead to reduced male investment, because males might invest more in current rather than in uncertain future reproduction (Arnold & Owens, 2002; Mauck et al., 1999). Studies on extra-pair paternity in parrots are few and the findings are diverse. Some parrot species appear to be genetically monogamous, such as the burrowing parrot (*Cyanoliseus patagonus*, Masello et al., 2002), the blue and yellow macaw (*Ara ararauna*, Caparroz et al., 2011) and the crimson rosella (*Platycercus elegans*, Eastwood et al., 2018), whereas others show varying levels of extra-pair paternity (EPP), such as the green-rumped parrotlet (*Forpus passerines*, 14% of nests with EPP; Beissinger, 2008), the monk parakeet (*Myiopsitta monachus*, 40% of nests with EPP; Martínez et al., 2013) and the swift parrot (50.5% of nests with EPP; Heinsohn et al., 2019). Additionally, a study looking into sperm morphology of 62 parrot species showed that sperm length (a proxy of sperm competition) was related to sexual dichromatism, indicating that these species potentially have higher levels of extra-pair paternity (Carballo et al., 2019). Furthermore, previous studies showed that the frequency of extra-pair paternity in birds is related to sexual dichromatism in birds (Møller & Birkhead, 1994; Owens & Hartley, 1998). Thus, our finding that smaller parrot species are more dichromatic (with a tendency of males having more elaborated colours than females, Figure S4) may be a consequence of sexual selection via female choice for (extra-pair) mates. However, more research is needed to explore whether the levels of extra-pair paternity in smaller parrot species are indeed higher, as suggested by our results. If sexual selection has an effect on parrot plumage colouration, then this could also explain the observed relationship between habitat type and sexual dichromatism. Species inhabiting more forested habitats are more dichromatic possibly because bright colours would be favoured to help maximizing conspicuousness of the sex under stronger sexual selection (Marchetti, 1993).

Many parrots form long-lasting pair bonds (Toft & Wright, 2015). Thus, our finding that larger species with longer lifespans (de Magalhaes et al., 2007; Wasser & Sherman, 2010) are less dichromatic, but display more elaborate colours, might be a consequence of mutual mate choice. As parrots are generally long-lived, especially compared with other bird species (Wasser & Sherman, 2010), we expect that both sexes are typically equally ornamented due to mutual mate choice, as observed in other tropical species (Bailey, 1978; Dale et al., 2015). Larger species of parrots may also experience stronger competition for suitable nesting sites, because they need larger nest chambers, which are rarer than smaller ones, and they are thus more limited by suitable nesting cavities than smaller parrots (Renton, Salinas-Melgoza, De Labra-Hernández, & de la Parra-Martínez, 2015). Moreover, the fact that suitable cavities are often a scarce resource may lead to strong competition between females

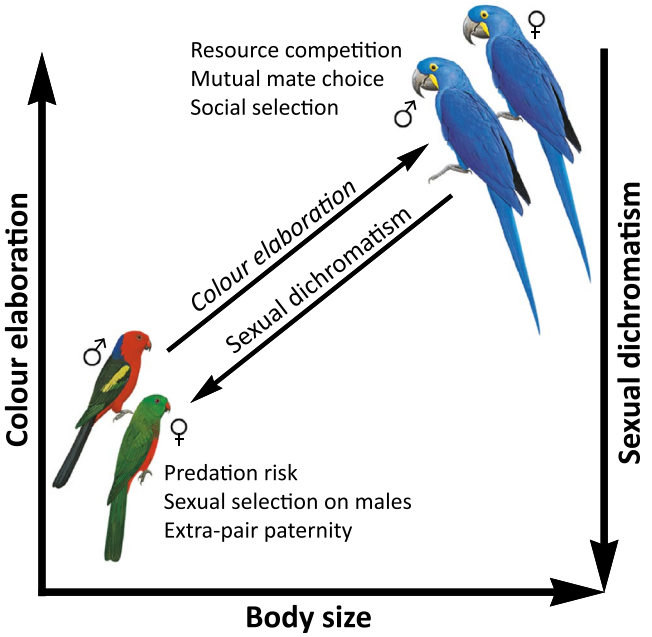

FIGURE 8 Variation in colour elaboration and sexual dichromatism in parrots is correlated with body size. Lager parrots have more elaborated colours and lower levels of sexual dichromatism, whilst smaller parrots are less colourful but show higher levels of sexual dichromatism. Resource competition, mutual mate choice, social selection, predation risk, sexual selection on males and extra-pair paternity are the possible processes that led to the varying patterns of colour elaboration and sexual dichromatism in large and small parrot species. Illustrations © Lynx Edicions

(Heinsohn et al., 2005) for access to these resources and elaborate colouration may be selected as a signal of competitive ability or to advertise territory ownership.

In conclusion, our results are consistent with the idea that life-history traits reflecting predation pressure, the abiotic environment and possibly social and sexual selection have all shaped the evolution of plumage colouration in parrots. Body size had a pervasive effect, suggesting that this life-history trait plays a key role mediating variation in colour elaboration and sexual dichromatism in parrots (Figure 8). Phylogenetic analyses indicated that an important component of the variation in parrot colouration and sexual dichromatism can be explained simply by shared evolutionary history. However, even though phylogeny explained most of the variation, we still found significant effects of life-history and environment on plumage colouration and sexual differences in parrots. Additionally, even though using bookplates to estimate parrot plumage colouration may not provide colour measures as accurate as those obtained by reflectance measurements taken from museum specimens, and this may be more marked in UV-rich species, our results should generally provide a reasonable approximation of the true colour variation, as shown in other studies (Bergeron & Fuller, 2018; Dale et al., 2015). Our comparative study leads to several testable hypotheses that could guide future field work. Specifically, we make

five predictions. (a) In larger, colourful species, both males and females defend scarce cavities and colours should play an important role in mediating these aggressive interactions. Conversely, in smaller species, competition for cavities should be weaker and not necessarily associated with plumage colours, especially female colours. (b) Mutual mate choice based on coloration should be more common in large parrots. (c) Large parrots should experience lower predation risk. (d) Sex differences in the variance in reproductive success should be size-dependent. In smaller species male variance should be larger than female variance, whereas there should be little difference in larger species. (e) Extra-pair paternity may be the mechanism allowing higher male variance in spite of social monogamy, and hence, we expect higher levels of extra-pair paternity in smaller parrots.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

L.C., M.V. and B.K conceived the study. L.C collected the data. L.C., M.V. and K.D. analysed the data with input from B.K. L.C. wrote the paper with help of B.K and K.D. and input from M.V. L.C. is a member of the International Max Planck Research School (IMPRS) for Organismal Biology. This work was funded by the Max Planck Society (to B.K.).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jeb.13690.

DATA AVAILABILITY STATEMENT

All data, scripts and supplementary information accompanies this paper at https://osf.io/2xr4v/.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary material

Table S1. Univariate models analysing the effect of each predictor on male colour elaboration.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,357	0,662	-0,541	0,590	0,549	0,710	-1,653	0,940	357
Sexual size dimorphism	-0,008	0,044	-0,183	0,832	0,756	0,991	-0,094	0,078	357
Pagel's λ	0,850						0,825	0,915	357
R^2full	0,414						0,406	0,438	357
R^2fixef	0,000						0,000	0,000	357
Intercept	-0,174	0,666	-0,261	0,795	0,755	0,922	-1,479	1,131	398
Annual mean temperature	0,135	0,047	2,851	0,006	0,003	0,019	0,042	0,228	398
Pagel's λ	0,855						0,832	0,915	398
R^2full	0,437						0,429	0,460	398
R^2fixef	0,022						0,020	0,028	398
Intercept	-0,338	0,677	-0,499	0,619	0,575	0,753	-1,665	0,989	398
Annual mean precipitation	0,070	0,048	1,469	0,157	0,097	0,367	-0,023	0,164	398
Pagel's λ	0,860						0,838	0,919	398
R^2full	0,428						0,421	0,452	398
R^2fixef	0,006						0,005	0,010	398
Intercept	-0,887	0,593	-1,498	0,138	0,114	0,211	-2,048	0,275	357
Body size	0,508	0,075	6,755	0,000	0,000	0,000	0,361	0,656	357
Pagel's λ	0,823						0,799	0,889	357
R^2full	0,482						0,475	0,503	357
R^2fixef	0,116						0,111	0,130	357
Intercept	-0,466	0,647	-0,725	0,470	0,436	0,573	-1,734	0,802	290
Clutch size	-0,188	0,065	-2,906	0,004	0,003	0,010	-0,315	-0,061	290
Pagel's λ	0,839						0,813	0,917	290
R^2full	0,394						0,385	0,420	290
R^2fixef	0,030						0,028	0,036	290
Intercept	-0,071	0,653	-0,109	0,910	0,878	0,992	-1,351	1,209	350
Gregariousness	-0,030	0,045	-0,661	0,518	0,419	0,846	-0,119	0,059	350
Pagel's λ	0,800						0,768	0,884	350
R^2full	0,388						0,381	0,410	350
R^2fixef	0,001						0,001	0,003	350
Intercept	-0,341	0,684	-0,499	0,619	0,578	0,744	-1,682	1,001	398
Habitat	0,008	0,040	0,201	0,806	0,720	0,991	-0,070	0,085	398
Pagel's λ	0,864						0,841	0,923	398
R^2full	0,425						0,417	0,449	398
R^2fixef	0,000						0,000	0,001	398

Table S2. Univariate models analysing the effect of each predictor on female colour elaboration.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,373	0,656	-0,573	0,568	0,534	0,666	-1,658	0,912	357
Sexual size dimorphism	0,028	0,044	0,633	0,537	0,426	0,891	-0,058	0,115	357
Pagel's λ	0,842						0,817	0,912	357
R^2full	0,406						0,398	0,427	357
R^2fixef	0,002						0,001	0,004	357
Intercept	-0,165	0,667	-0,247	0,805	0,774	0,905	-1,471	1,142	398
Annual mean temperature	0,167	0,048	3,505	0,001	0,000	0,003	0,074	0,261	398
Pagel's λ	0,853						0,829	0,918	398
R^2full	0,431						0,424	0,454	398
R^2fixef	0,032						0,030	0,039	398
Intercept	-0,366	0,678	-0,542	0,589	0,554	0,696	-1,695	0,963	398
Annual mean precipitation	0,080	0,048	1,657	0,111	0,066	0,265	-0,015	0,175	398
Pagel's λ	0,857						0,834	0,921	398
R^2full	0,417						0,410	0,439	398
R^2fixef	0,008						0,007	0,012	398
Intercept	-0,960	0,573	-1,681	0,096	0,078	0,155	-2,082	0,163	357
Body size	0,564	0,074	7,623	0,000	0,000	0,000	0,419	0,709	357
Pagel's λ	0,809						0,786	0,877	357
R^2full	0,489						0,483	0,508	357
R^2fixef	0,142						0,137	0,157	357
Intercept	-0,495	0,639	-0,782	0,436	0,406	0,526	-1,748	0,757	290
Clutch size	-0,231	0,065	-3,543	0,001	0,000	0,001	-0,359	-0,103	290
Pagel's λ	0,830						0,800	0,912	290
R^2full	0,386						0,378	0,411	290
R^2fixef	0,043						0,041	0,050	290
Intercept	-0,112	0,652	-0,174	0,862	0,834	0,950	-1,389	1,166	350
Gregariousness	-0,016	0,046	-0,354	0,720	0,625	0,974	-0,106	0,074	350
Pagel's λ	0,791						0,757	0,886	350
R^2full	0,371						0,363	0,391	350
R^2fixef	0,000						0,000	0,002	350
Intercept	-0,369	0,687	-0,541	0,590	0,556	0,690	-1,716	0,977	398
Habitat	0,012	0,040	0,309	0,749	0,652	0,983	-0,066	0,091	398
Pagel's λ	0,861						0,838	0,925	398
R^2full	0,413						0,405	0,436	398
R^2fixef	0,000						0,000	0,001	398

Table S3. Multivariate models analysing the effect of each predictor whilst controlling for the others on male colour elaboration.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,312	0,532	-0,585	0,560	0,538	0,629	-1,356	0,731	273
Annual mean precipitation	0,053	0,058	0,910	0,369	0,313	0,569	-0,061	0,167	273
Annual mean temperature	0,175	0,059	2,977	0,004	0,002	0,008	0,060	0,290	273
Clutch size	-0,042	0,071	-0,600	0,555	0,474	0,780	-0,181	0,096	273
Body size	0,517	0,081	6,348	0,000	0,000	0,000	0,357	0,676	273
Sexual size dimorphism	-0,020	0,049	-0,412	0,682	0,627	0,851	-0,115	0,075	273
Gregariousness	-0,028	0,049	-0,582	0,566	0,491	0,800	-0,124	0,067	273
Habitat	0,049	0,049	1,009	0,318	0,274	0,445	-0,046	0,144	273
Pagel's λ	0,731						0,700	0,817	273
R^2full	0,480						0,474	0,498	273
R^2fixef	0,186						0,180	0,203	273

Table S4. Multivariate models analysing the effect of each predictor whilst controlling for the others on female colour elaboration.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,333	0,515	-0,648	0,518	0,494	0,594	-1,343	0,676	273
Annual mean precipitation	0,060	0,057	1,043	0,303	0,254	0,467	-0,053	0,173	273
Annual mean temperature	0,200	0,058	3,448	0,001	0,000	0,002	0,086	0,314	273
Clutch size	-0,077	0,070	-1,094	0,281	0,231	0,423	-0,214	0,061	273
Body size	0,553	0,080	6,923	0,000	0,000	0,000	0,396	0,709	273
Sexual size dimorphism	0,027	0,048	0,552	0,585	0,522	0,796	-0,068	0,121	273
Gregariousness	-0,011	0,048	-0,221	0,819	0,746	0,993	-0,105	0,084	273
Habitat	0,075	0,048	1,570	0,120	0,099	0,183	-0,019	0,170	273
Pagel's λ	0,719						0,688	0,807	273
R^2full	0,492						0,486	0,508	273
R^2fixef	0,229						0,224	0,245	273

Table S5. Univariate models analysing the effect of each predictor on the three colour scores (L, a and b) in males.

Model_term	depvar	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	L_m	-0,307	0,544	-0,572	0,570	0,523	0,723	-1,372	0,759	357
Sexual size dimorphism	L_m	0,010	0,034	0,283	0,733	0,617	0,992	-0,056	0,076	357
Pagel's λ	L_m	0,877						0,860	0,923	357
R^2full	L_m	0,645						0,640	0,662	357
R^2fixef	L_m	0,001						0,000	0,005	357
Intercept	L_m	-0,338	0,551	-0,622	0,537	0,489	0,687	-1,418	0,741	398
Annual mean temperature	L_m	-0,021	0,037	-0,576	0,578	0,450	0,934	-0,093	0,051	398
Pagel's λ	L_m	0,882						0,866	0,925	398
R^2full	L_m	0,657						0,651	0,674	398
R^2fixef	L_m	0,001						0,000	0,002	398
Intercept	L_m	-0,314	0,546	-0,584	0,562	0,513	0,713	-1,385	0,757	398
Annual mean precipitation	L_m	-0,092	0,036	-2,522	0,017	0,006	0,058	-0,163	-0,020	398
Pagel's λ	L_m	0,883						0,868	0,925	398
R^2full	L_m	0,662						0,657	0,678	398
R^2fixef	L_m	0,015						0,013	0,022	398
Intercept	L_m	0,029	0,515	0,050	0,911	0,870	0,996	-0,980	1,038	357
Body size	L_m	-0,309	0,062	-4,986	0,000	0,000	0,000	-0,430	-0,187	357
Pagel's λ	L_m	0,865						0,848	0,913	357
R^2full	L_m	0,670						0,664	0,685	357
R^2fixef	L_m	0,069						0,065	0,082	357
Intercept	L_m	-0,275	0,513	-0,546	0,588	0,536	0,747	-1,280	0,729	290
Clutch size	L_m	0,073	0,046	1,586	0,123	0,085	0,256	-0,017	0,164	290
Pagel's λ	L_m	0,886						0,871	0,925	290
R^2full	L_m	0,682						0,677	0,697	290
R^2fixef	L_m	0,011						0,009	0,016	290
Intercept	L_m	-0,327	0,581	-0,573	0,570	0,519	0,717	-1,467	0,812	350
Gregariousness	L_m	0,032	0,034	0,951	0,361	0,252	0,712	-0,034	0,098	350
Pagel's λ	L_m	0,875						0,858	0,922	350
R^2full	L_m	0,643						0,638	0,659	350
R^2fixef	L_m	0,003						0,002	0,007	350
Intercept	L_m	-0,313	0,546	-0,582	0,564	0,515	0,719	-1,382	0,756	398
Habitat	L_m	0,038	0,030	1,264	0,224	0,147	0,485	-0,021	0,098	398
Pagel's λ	L_m	0,880						0,864	0,923	398
R^2full	L_m	0,658						0,653	0,675	398
R^2fixef	L_m	0,005						0,004	0,008	398
Intercept	a_m	0,377	0,608	0,625	0,534	0,497	0,649	-0,814	1,568	357
Sexual size dimorphism	a_m	-0,042	0,044	-0,960	0,347	0,281	0,544	-0,127	0,043	357
Pagel's λ	a_m	0,813						0,784	0,899	357

R^2full	a_m	0,429						0,418	0,462	357
R^2fixef	a_m	0,003						0,002	0,006	357
Intercept	a_m	0,440	0,635	0,699	0,487	0,449	0,595	-0,805	1,686	398
Annual mean temperature	a_m	0,024	0,047	0,512	0,618	0,500	0,928	-0,069	0,117	398
Pagel's λ	a_m	0,832						0,806	0,910	398
R^2full	a_m	0,438						0,428	0,470	398
R^2fixef	a_m	0,001						0,000	0,002	398
Intercept	a_m	0,414	0,628	0,667	0,507	0,468	0,619	-0,817	1,646	398
Annual mean precipitation	a_m	0,113	0,047	2,392	0,021	0,010	0,062	0,020	0,206	398
Pagel's λ	a_m	0,832						0,806	0,909	398
R^2full	a_m	0,446						0,436	0,476	398
R^2fixef	a_m	0,015						0,013	0,021	398
Intercept	a_m	-0,096	0,535	-0,178	0,859	0,828	0,948	-1,145	0,952	357
Body size	a_m	0,454	0,072	6,303	0,000	0,000	0,000	0,313	0,595	357
Pagel's λ	a_m	0,765						0,726	0,869	357
R^2full	a_m	0,483						0,472	0,515	357
R^2fixef	a_m	0,098						0,093	0,111	357
Intercept	a_m	0,286	0,589	0,490	0,625	0,593	0,722	-0,868	1,440	290
Clutch size	a_m	-0,103	0,062	-1,656	0,106	0,074	0,215	-0,225	0,019	290
Pagel's λ	a_m	0,812						0,778	0,902	290
R^2full	a_m	0,445						0,435	0,475	290
R^2fixef	a_m	0,010						0,009	0,014	290
Intercept	a_m	0,401	0,586	0,692	0,491	0,448	0,614	-0,748	1,550	350
Gregariousness	a_m	0,003	0,045	0,074	0,857	0,794	0,994	-0,085	0,092	350
Pagel's λ	a_m	0,744						0,704	0,857	350
R^2full	a_m	0,395						0,385	0,422	350
R^2fixef	a_m	0,000						0,000	0,001	350
Intercept	a_m	0,411	0,628	0,661	0,510	0,473	0,622	-0,819	1,642	398
Habitat	a_m	-0,022	0,040	-0,558	0,586	0,467	0,930	-0,100	0,056	398
Pagel's λ	a_m	0,828						0,801	0,909	398
R^2full	a_m	0,439						0,428	0,470	398
R^2fixef	a_m	0,001						0,001	0,003	398
Intercept	b_m	-0,621	0,675	-0,937	0,354	0,307	0,498	-1,944	0,702	357
Sexual size dimorphism	b_m	-0,002	0,042	-0,038	0,880	0,831	0,995	-0,083	0,080	357
Pagel's λ	b_m	0,878						0,860	0,928	357
R^2full	b_m	0,457						0,449	0,481	357
R^2fixef	b_m	0,000						0,000	0,001	357
Intercept	b_m	-0,852	0,680	-1,273	0,209	0,171	0,321	-2,184	0,480	398
Annual mean temperature	b_m	-0,176	0,044	-3,959	0,000	0,000	0,001	-0,263	-0,089	398
Pagel's λ	b_m	0,889						0,872	0,933	398
R^2full	b_m	0,496						0,489	0,519	398
R^2fixef	b_m	0,035						0,032	0,044	398
Intercept	b_m	-0,635	0,680	-0,952	0,347	0,299	0,487	-1,968	0,697	398
Annual mean precipitation	b_m	0,003	0,045	0,057	0,822	0,740	0,992	-0,086	0,091	398

Pagel's λ	b_m	0,883					0,866	0,929	398
R²full	b_m	0,478					0,470	0,502	398
R²fixef	b_m	0,000					0,000	0,001	398
Intercept	b_m	-0,083	0,557	-0,166	0,860	0,798	0,993	-1,174	1,008	357
Body size	b_m	-0,505	0,072	-7,064	0,000	0,000	0,000	-0,645	-0,365	357
Pagel's λ	b_m	0,813					0,786	0,882	357
R²full	b_m	0,525					0,519	0,545	357
R²fixef	b_m	0,125					0,118	0,147	357
Intercept	b_m	-0,501	0,668	-0,765	0,448	0,400	0,589	-1,809	0,807	290
Clutch size	b_m	0,062	0,059	1,040	0,312	0,237	0,554	-0,055	0,178	290
Pagel's λ	b_m	0,888					0,870	0,942	290
R²full	b_m	0,470					0,461	0,494	290
R²fixef	b_m	0,005					0,004	0,008	290
Intercept	b_m	-0,707	0,724	-0,995	0,325	0,283	0,452	-2,126	0,711	350
Gregariousness	b_m	0,042	0,041	1,032	0,323	0,221	0,648	-0,038	0,122	350
Pagel's λ	b_m	0,885					0,867	0,935	350
R²full	b_m	0,473					0,465	0,498	350
R²fixef	b_m	0,003					0,002	0,006	350
Intercept	b_m	-0,638	0,679	-0,957	0,344	0,296	0,483	-1,968	0,693	398
Habitat	b_m	0,051	0,037	1,383	0,183	0,120	0,408	-0,021	0,124	398
Pagel's λ	b_m	0,883					0,866	0,928	398
R²full	b_m	0,480					0,473	0,504	398
R²fixef	b_m	0,005					0,004	0,008	398

Table S6. Univariate models analysing the effect of each predictor on the three colour scores (L, a and b) in females.

Model_term	depvar	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	L_f	-0,320	0,541	-0,600	0,552	0,507	0,699	-1,381	0,741	357
Sexual size dimorphism	L_f	0,000	0,034	-0,018	0,739	0,633	0,986	-0,067	0,067	357
Pagel's λ	L_f	0,869						0,852	0,917	357
R²full	L_f	0,636						0,629	0,653	357
R²fixef	L_f	0,000						0,000	0,003	357
Intercept	L_f	-0,350	0,546	-0,648	0,520	0,474	0,669	-1,421	0,722	398
Annual mean temperature	L_f	-0,024	0,037	-0,645	0,534	0,417	0,875	-0,098	0,049	398
Pagel's λ	L_f	0,872						0,856	0,919	398
R²full	L_f	0,647						0,641	0,664	398
R²fixef	L_f	0,001						0,000	0,003	398
Intercept	L_f	-0,323	0,542	-0,606	0,548	0,500	0,697	-1,385	0,739	398
Annual mean precipitation	L_f	-0,108	0,037	-2,928	0,005	0,002	0,019	-0,181	-0,036	398
Pagel's λ	L_f	0,874						0,858	0,920	398

R^2full	L_f	0,654						0,648	0,670	398
R^2fixef	L_f	0,020						0,018	0,028	398
Intercept	L_f	0,030	0,510	0,053	0,911	0,874	0,996	-0,970	1,031	357
Body size	L_f	-0,322	0,062	-5,177	0,000	0,000	0,000	-0,444	-0,200	357
Pagel's λ	L_f	0,855						0,837	0,906	357
R^2full	L_f	0,662						0,656	0,678	357
R^2fixef	L_f	0,073						0,069	0,086	357
Intercept	L_f	-0,279	0,512	-0,554	0,582	0,532	0,739	-1,283	0,724	290
Clutch size	L_f	0,091	0,047	1,938	0,059	0,038	0,132	-0,001	0,183	290
Pagel's λ	L_f	0,880						0,864	0,923	290
R^2full	L_f	0,675						0,670	0,690	290
R^2fixef	L_f	0,014						0,013	0,020	290
Intercept	L_f	-0,335	0,579	-0,588	0,560	0,509	0,703	-1,470	0,800	350
Gregariousness	L_f	0,015	0,034	0,437	0,659	0,531	0,970	-0,052	0,082	350
Pagel's λ	L_f	0,866						0,849	0,915	350
R^2full	L_f	0,632						0,626	0,647	350
R^2fixef	L_f	0,001						0,000	0,003	350
Intercept	L_f	-0,321	0,542	-0,600	0,551	0,505	0,704	-1,384	0,741	398
Habitat	L_f	0,039	0,031	1,253	0,227	0,152	0,469	-0,022	0,099	398
Pagel's λ	L_f	0,871						0,854	0,918	398
R^2full	L_f	0,648						0,643	0,665	398
R^2fixef	L_f	0,004						0,003	0,007	398
Intercept	a_f	0,387	0,601	0,649	0,518	0,481	0,631	-0,791	1,565	357
Sexual size dimorphism	a_f	-0,004	0,043	-0,092	0,852	0,791	0,994	-0,089	0,081	357
Pagel's λ	a_f	0,809						0,776	0,904	357
R^2full	a_f	0,435						0,424	0,467	357
R^2fixef	a_f	0,000						0,000	0,001	357
Intercept	a_f	0,456	0,629	0,731	0,467	0,428	0,579	-0,776	1,688	398
Annual mean temperature	a_f	0,030	0,047	0,642	0,533	0,427	0,836	-0,062	0,123	398
Pagel's λ	a_f	0,829						0,800	0,914	398
R^2full	a_f	0,446						0,435	0,477	398
R^2fixef	a_f	0,001						0,001	0,003	398
Intercept	a_f	0,423	0,625	0,684	0,496	0,457	0,613	-0,803	1,649	398
Annual mean precipitation	a_f	0,114	0,047	2,434	0,019	0,009	0,054	0,022	0,207	398
Pagel's λ	a_f	0,833						0,805	0,913	398
R^2full	a_f	0,454						0,443	0,484	398
R^2fixef	a_f	0,015						0,013	0,021	398
Intercept	a_f	-0,138	0,522	-0,263	0,793	0,764	0,874	-1,161	0,886	357
Body size	a_f	0,500	0,070	7,098	0,000	0,000	0,000	0,362	0,638	357
Pagel's λ	a_f	0,762						0,723	0,867	357
R^2full	a_f	0,503						0,493	0,532	357
R^2fixef	a_f	0,121						0,116	0,135	357
Intercept	a_f	0,286	0,582	0,496	0,621	0,589	0,720	-0,855	1,427	290
Clutch size	a_f	-0,101	0,063	-1,606	0,116	0,081	0,238	-0,224	0,022	290
Pagel's λ	a_f	0,800						0,762	0,901	290

R^2full	a_f	0,435					0,425	0,465	290
R^2fixef	a_f	0,009					0,008	0,013	290
Intercept	a_f	0,401	0,575	0,704	0,485	0,438	0,607	-0,727	1,529	350
Gregariousness	a_f	0,036	0,045	0,787	0,441	0,363	0,696	-0,053	0,124	350
Pagel's λ	a_f	0,733					0,692	0,860	350
R^2full	a_f	0,397					0,386	0,423	350
R^2fixef	a_f	0,002					0,001	0,004	350
Intercept	a_f	0,420	0,622	0,681	0,498	0,461	0,611	-0,799	1,639	398
Habitat	a_f	-0,026	0,039	-0,648	0,527	0,418	0,879	-0,103	0,052	398
Pagel's λ	a_f	0,826					0,796	0,912	398
R^2full	a_f	0,446					0,436	0,477	398
R^2fixef	a_f	0,001					0,001	0,003	398
Intercept	b_f	-0,651	0,675	-0,981	0,332	0,284	0,473	-1,973	0,672	357
Sexual size dimorphism	b_f	0,000	0,041	-0,006	0,873	0,819	0,995	-0,081	0,080	357
Pagel's λ	b_f	0,883					0,865	0,933	357
R^2full	b_f	0,470					0,462	0,493	357
R^2fixef	b_f	0,000					0,000	0,001	357
Intercept	b_f	-0,867	0,680	-1,294	0,202	0,163	0,316	-2,199	0,465	398
Annual mean temperature	b_f	-0,166	0,044	-3,773	0,000	0,000	0,001	-0,253	-0,080	398
Pagel's λ	b_f	0,892					0,876	0,935	398
R^2full	b_f	0,504					0,496	0,526	398
R^2fixef	b_f	0,031					0,028	0,039	398
Intercept	b_f	-0,662	0,675	-0,998	0,324	0,275	0,467	-1,985	0,662	398
Annual mean precipitation	b_f	-0,007	0,045	-0,161	0,796	0,712	0,993	-0,095	0,080	398
Pagel's λ	b_f	0,884					0,867	0,931	398
R^2full	b_f	0,488					0,480	0,510	398
R^2fixef	b_f	0,000					0,000	0,001	398
Intercept	b_f	-0,073	0,542	-0,152	0,866	0,806	0,997	-1,135	0,988	357
Body size	b_f	-0,540	0,070	-7,747	0,000	0,000	0,000	-0,677	-0,403	357
Pagel's λ	b_f	0,811					0,784	0,882	357
R^2full	b_f	0,547					0,541	0,565	357
R^2fixef	b_f	0,146					0,138	0,169	357
Intercept	b_f	-0,520	0,669	-0,793	0,432	0,383	0,574	-1,831	0,790	290
Clutch size	b_f	0,070	0,059	1,180	0,251	0,185	0,452	-0,046	0,186	290
Pagel's λ	b_f	0,890					0,871	0,944	290
R^2full	b_f	0,473					0,464	0,496	290
R^2fixef	b_f	0,006					0,005	0,010	290
Intercept	b_f	-0,731	0,721	-1,031	0,308	0,263	0,440	-2,144	0,681	350
Gregariousness	b_f	0,023	0,040	0,562	0,586	0,452	0,967	-0,056	0,101	350
Pagel's λ	b_f	0,887					0,870	0,937	350
R^2full	b_f	0,482					0,475	0,504	350
R^2fixef	b_f	0,001					0,000	0,003	350
Intercept	b_f	-0,664	0,674	-1,002	0,322	0,274	0,463	-1,985	0,657	398
Habitat	b_f	0,052	0,037	1,426	0,171	0,110	0,399	-0,020	0,125	398

Pagel's λ	b_f	0,884			0,868	0,932	398
R^2full	b_f	0,490			0,482	0,512	398
R^2fixef	b_f	0,005			0,004	0,008	398

Table S7. Multivariate models analysing the effect of each predictor whilst controlling for the others on the three colour scores (L, a and b) in males.

Model_term	depvar	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	L_m	-0,155	0,528	-0,306	0,762	0,694	0,963	-1,190	0,880	273
Annual mean precipitation	L_m	-0,111	0,042	-2,643	0,012	0,005	0,039	-0,194	-0,029	273
Annual mean temperature	L_m	-0,018	0,044	-0,408	0,688	0,577	0,976	-0,104	0,068	273
Clutch size	L_m	-0,012	0,052	-0,240	0,794	0,713	0,988	-0,113	0,089	273
Body size	L_m	-0,275	0,067	-4,082	0,000	0,000	0,001	-0,407	-0,143	273
Sexual size dimorphism	L_m	0,030	0,036	0,822	0,478	0,277	0,921	-0,040	0,099	273
Gregariousness	L_m	0,033	0,036	0,920	0,382	0,252	0,798	-0,037	0,102	273
Habitat	L_m	0,036	0,035	1,034	0,314	0,242	0,537	-0,033	0,106	273
Pagel's λ	L_m	0,877						0,862	0,918	273
R^2full	L_m	0,701						0,695	0,715	273
R^2fixef	L_m	0,111						0,103	0,132	273
Intercept	a_m	0,073	0,477	0,162	0,871	0,836	0,979	-0,861	1,007	273
Annual mean precipitation	a_m	0,145	0,059	2,483	0,015	0,010	0,030	0,031	0,260	273
Annual mean temperature	a_m	0,017	0,059	0,288	0,774	0,701	0,972	-0,098	0,132	273
Clutch size	a_m	0,060	0,071	0,847	0,401	0,351	0,545	-0,079	0,200	273
Body size	a_m	0,446	0,078	5,684	0,000	0,000	0,000	0,292	0,599	273
Sexual size dimorphism	a_m	-0,083	0,049	-1,687	0,098	0,074	0,164	-0,179	0,013	273
Gregariousness	a_m	-0,006	0,049	-0,114	0,856	0,795	0,994	-0,102	0,091	273
Habitat	a_m	-0,035	0,049	-0,706	0,484	0,438	0,614	-0,130	0,061	273
Pagel's λ	a_m	0,659						0,615	0,780	273
R^2full	a_m	0,475						0,467	0,497	273
R^2fixef	a_m	0,135						0,128	0,154	273
Intercept	b_m	-0,411	0,588	-0,717	0,477	0,426	0,618	-1,563	0,741	273
Annual mean precipitation	b_m	0,082	0,051	1,617	0,114	0,081	0,226	-0,017	0,182	273
Annual mean temperature	b_m	-0,220	0,053	-4,170	0,000	0,000	0,000	-0,324	-0,117	273
Clutch size	b_m	-0,055	0,062	-0,884	0,387	0,310	0,617	-0,177	0,067	273
Body size	b_m	-0,537	0,078	-6,844	0,000	0,000	0,000	-0,691	-0,383	273
Sexual size dimorphism	b_m	0,026	0,043	0,610	0,548	0,471	0,787	-0,058	0,110	273
Gregariousness	b_m	0,037	0,043	0,855	0,409	0,299	0,742	-0,048	0,121	273
Habitat	b_m	0,073	0,043	1,707	0,095	0,067	0,191	-0,011	0,157	273

Pagel's λ	b_m	0,843			0,818	0,908	273
R^2full	b_m	0,577			0,571	0,594	273
R^2fixef	b_m	0,214			0,205	0,241	273

Table S8. Multivariate models analysing the effect of each predictor whilst controlling for the others on the three colour scores (L, a and b) in females.

Model_term	depvar	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	L_f	-0,159	0,530	-0,314	0,756	0,688	0,953	-1,198	0,880	273
Annual mean precipitation	L_f	-0,126	0,043	-2,932	0,005	0,002	0,017	-0,209	-0,042	273
Annual mean temperature	L_f	-0,005	0,045	-0,123	0,840	0,770	0,995	-0,093	0,082	273
Clutch size	L_f	0,011	0,052	0,215	0,810	0,727	0,987	-0,091	0,114	273
Body size	L_f	-0,271	0,068	-3,986	0,000	0,000	0,001	-0,405	-0,138	273
Sexual size dimorphism	L_f	0,018	0,036	0,486	0,641	0,463	0,987	-0,053	0,089	273
Gregariousness	L_f	0,013	0,036	0,351	0,695	0,562	0,984	-0,058	0,084	273
Habitat	L_f	0,021	0,036	0,579	0,571	0,473	0,858	-0,050	0,091	273
Pagel's λ	L_f	0,872						0,855	0,915	273
R^2full	L_f	0,692						0,687	0,707	273
R^2fixef	L_f	0,109						0,103	0,130	273
Intercept	a_f	0,067	0,459	0,154	0,878	0,842	0,982	-0,833	0,968	273
Annual mean precipitation	a_f	0,150	0,059	2,563	0,012	0,009	0,022	0,035	0,265	273
Annual mean temperature	a_f	0,037	0,058	0,625	0,536	0,480	0,696	-0,078	0,151	273
Clutch size	a_f	0,078	0,071	1,090	0,280	0,243	0,391	-0,062	0,217	273
Body size	a_f	0,489	0,077	6,311	0,000	0,000	0,000	0,337	0,641	273
Sexual size dimorphism	a_f	-0,039	0,049	-0,800	0,430	0,373	0,601	-0,136	0,057	273
Gregariousness	a_f	0,031	0,049	0,623	0,540	0,455	0,785	-0,066	0,127	273
Habitat	a_f	-0,019	0,049	-0,392	0,696	0,650	0,831	-0,115	0,077	273
Pagel's λ	a_f	0,635						0,591	0,761	273
R^2full	a_f	0,473						0,465	0,495	273
R^2fixef	a_f	0,152						0,146	0,169	273
Intercept	b_f	-0,394	0,587	-0,689	0,495	0,442	0,638	-1,544	0,757	273
Annual mean precipitation	b_f	0,078	0,051	1,543	0,132	0,093	0,257	-0,021	0,178	273
Annual mean temperature	b_f	-0,195	0,053	-3,714	0,000	0,000	0,001	-0,298	-0,092	273
Clutch size	b_f	-0,038	0,062	-0,606	0,553	0,456	0,832	-0,159	0,084	273
Body size	b_f	-0,563	0,078	-7,197	0,000	0,000	0,000	-0,716	-0,409	273
Sexual size dimorphism	b_f	0,026	0,043	0,609	0,549	0,471	0,794	-0,058	0,110	273
Gregariousness	b_f	0,022	0,043	0,512	0,614	0,487	0,953	-0,062	0,106	273
Habitat	b_f	0,051	0,043	1,203	0,240	0,181	0,430	-0,032	0,134	273
Pagel's λ	b_f	0,844						0,819	0,915	273

R^2full	b_f	0,580					0,575	0,597	273
R^2fixef	b_f	0,215					0,205	0,242	273

Table S9. Univariate models analysing the effect of each predictor on male colour diversity.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,723	0,566	-1,295	0,201	0,166	0,300	-1,833	0,387	357
Sexual size dimorphism	0,019	0,043	0,433	0,649	0,527	0,971	-0,066	0,103	357
Pagel's λ	0,785						0,760	0,862	357
R^2full	0,445						0,437	0,468	357
R^2fixef	0,001						0,000	0,003	357
Intercept	-0,767	0,586	-1,329	0,189	0,154	0,287	-1,914	0,381	398
Annual mean temperature	-0,011	0,046	-0,243	0,787	0,686	0,992	-0,102	0,080	398
Pagel's λ	0,804						0,780	0,874	398
R^2full	0,465						0,458	0,488	398
R^2fixef	0,000						0,000	0,001	398
Intercept	-0,756	0,581	-1,322	0,192	0,155	0,297	-1,894	0,383	398
Annual mean precipitation	-0,077	0,047	-1,657	0,107	0,071	0,223	-0,169	0,014	398
Pagel's λ	0,804						0,780	0,874	398
R^2full	0,469						0,462	0,492	398
R^2fixef	0,007						0,006	0,011	398
Intercept	-0,768	0,575	-1,352	0,182	0,149	0,278	-1,895	0,359	357
Body size	0,039	0,076	0,518	0,614	0,488	0,950	-0,109	0,188	357
Pagel's λ	0,788						0,763	0,864	357
R^2full	0,445						0,438	0,468	357
R^2fixef	0,001						0,000	0,003	357
Intercept	-0,659	0,581	-1,149	0,255	0,219	0,359	-1,798	0,481	290
Clutch size	0,015	0,061	0,244	0,796	0,713	0,989	-0,105	0,135	290
Pagel's λ	0,815						0,791	0,884	290
R^2full	0,462						0,453	0,486	290
R^2fixef	0,000						0,000	0,002	290
Intercept	-0,625	0,606	-1,052	0,298	0,253	0,424	-1,812	0,562	350
Gregariousness	0,075	0,043	1,757	0,094	0,051	0,266	-0,009	0,158	350
Pagel's λ	0,795						0,770	0,870	350
R^2full	0,461						0,453	0,485	350
R^2fixef	0,009						0,007	0,016	350
Intercept	-0,755	0,582	-1,316	0,194	0,159	0,297	-1,895	0,386	398
Habitat	0,049	0,039	1,249	0,222	0,164	0,401	-0,028	0,125	398
Pagel's λ	0,805						0,780	0,875	398
R^2full	0,467						0,460	0,490	398
R^2fixef	0,004						0,003	0,007	398

Table S10. Univariate models analysing the effect of each predictor on female colour diversity.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,740	0,533	-1,414	0,165	0,125	0,280	-1,784	0,304	357
Sexual size dimorphism	0,067	0,043	1,560	0,132	0,087	0,341	-0,017	0,151	357
Pagel's λ	0,755						0,727	0,837	357
R^2full	0,453						0,445	0,475	357
R^2fixef	0,007						0,005	0,011	357
Intercept	-0,760	0,551	-1,404	0,168	0,128	0,286	-1,840	0,320	398
Annual mean temperature	0,017	0,046	0,368	0,714	0,612	0,973	-0,074	0,108	398
Pagel's λ	0,772						0,746	0,848	398
R^2full	0,464						0,457	0,486	398
R^2fixef	0,000						0,000	0,002	398
Intercept	-0,782	0,546	-1,458	0,153	0,113	0,272	-1,853	0,289	398
Annual mean precipitation	-0,073	0,047	-1,555	0,128	0,091	0,240	-0,165	0,019	398
Pagel's λ	0,772						0,745	0,847	398
R^2full	0,467						0,460	0,489	398
R^2fixef	0,006						0,005	0,010	398
Intercept	-0,883	0,547	-1,635	0,109	0,079	0,197	-1,956	0,190	357
Body size	0,128	0,074	1,730	0,094	0,060	0,207	-0,017	0,273	357
Pagel's λ	0,766						0,740	0,844	357
R^2full	0,454						0,448	0,476	357
R^2fixef	0,009						0,007	0,015	357
Intercept	-0,702	0,547	-1,304	0,199	0,159	0,317	-1,775	0,371	290
Clutch size	-0,006	0,062	-0,096	0,871	0,816	0,992	-0,127	0,115	290
Pagel's λ	0,778						0,752	0,856	290
R^2full	0,454						0,446	0,477	290
R^2fixef	0,000						0,000	0,001	290
Intercept	-0,673	0,573	-1,201	0,239	0,186	0,386	-1,796	0,450	350
Gregariousness	0,066	0,043	1,540	0,139	0,085	0,332	-0,018	0,150	350
Pagel's λ	0,764						0,738	0,841	350
R^2full	0,459						0,451	0,482	350
R^2fixef	0,007						0,005	0,013	350
Intercept	-0,780	0,549	-1,448	0,155	0,117	0,269	-1,856	0,295	398
Habitat	0,018	0,039	0,464	0,648	0,555	0,916	-0,059	0,096	398
Pagel's λ	0,772						0,746	0,847	398
R^2full	0,464						0,457	0,486	398
R^2fixef	0,001						0,000	0,002	398

Table S11. Multivariate models analysing the effect of each predictor whilst controlling for the others on male colour diversity.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,558	0,638	-0,890	0,378	0,333	0,499	-1,809	0,693	273
Annual mean precipitation	-0,044	0,058	-0,766	0,453	0,366	0,678	-0,158	0,069	273
Annual mean temperature	0,000	0,060	-0,007	0,884	0,828	0,996	-0,117	0,117	273
Clutch size	-0,017	0,071	-0,234	0,796	0,708	0,988	-0,156	0,123	273
Body size	0,005	0,088	0,056	0,826	0,754	0,991	-0,167	0,176	273
Sexual size dimorphism	-0,003	0,049	-0,059	0,820	0,737	0,993	-0,099	0,092	273
Gregariousness	0,054	0,049	1,111	0,287	0,194	0,575	-0,042	0,150	273
Habitat	0,043	0,049	0,888	0,384	0,312	0,602	-0,052	0,138	273
Pagel's λ	0,823						0,797	0,890	273
R^2full	0,462						0,453	0,487	273
R^2fixef	0,012						0,010	0,018	273

Table S12. Multivariate models analysing the effect of each predictor whilst controlling for the others on female colour diversity.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,614	0,590	-1,061	0,297	0,241	0,451	-1,771	0,543	273
Annual mean precipitation	-0,060	0,059	-1,013	0,319	0,261	0,476	-0,175	0,056	273
Annual mean temperature	0,051	0,060	0,847	0,404	0,344	0,598	-0,067	0,169	273
Clutch size	-0,008	0,072	-0,114	0,865	0,809	0,994	-0,150	0,133	273
Body size	0,076	0,086	0,888	0,388	0,308	0,632	-0,092	0,244	273
Sexual size dimorphism	0,053	0,049	1,085	0,294	0,211	0,624	-0,043	0,150	273
Gregariousness	0,047	0,050	0,953	0,356	0,263	0,639	-0,050	0,145	273
Habitat	0,019	0,049	0,391	0,699	0,624	0,927	-0,077	0,116	273
Pagel's λ	0,778						0,752	0,858	273
R^2full	0,457						0,449	0,480	273
R^2fixef	0,018						0,016	0,025	273

Table S13. Univariate models analysing the effect of each predictor on sexual dichromatism.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	0,140	0,529	0,281	0,780	0,722	0,940	-0,897	1,178	357
Sexual size dimorphism	-0,059	0,042	-1,418	0,175	0,109	0,431	-0,141	0,022	357
Pagel's λ	0,767						0,732	0,861	357
R^2full	0,484						0,474	0,510	357
R^2fixef	0,005						0,004	0,011	357
Intercept	0,125	0,547	0,243	0,809	0,750	0,966	-0,948	1,197	398
Annual mean temperature	-0,010	0,045	-0,219	0,802	0,719	0,990	-0,099	0,079	398
Pagel's λ	0,781						0,749	0,867	398
R^2full	0,490						0,481	0,517	398
R^2fixef	0,000						0,000	0,001	398
Intercept	0,137	0,544	0,268	0,790	0,732	0,948	-0,929	1,204	398
Annual mean precipitation	0,030	0,046	0,646	0,529	0,421	0,836	-0,060	0,119	398
Pagel's λ	0,781						0,750	0,867	398
R^2full	0,491						0,481	0,517	398
R^2fixef	0,001						0,001	0,003	398
Intercept	0,441	0,515	0,875	0,387	0,332	0,533	-0,568	1,450	357
Body size	-0,287	0,070	-4,089	0,000	0,000	0,000	-0,424	-0,149	357
Pagel's λ	0,757						0,723	0,853	357
R^2full	0,504						0,495	0,530	357
R^2fixef	0,043						0,041	0,053	357
Intercept	0,228	0,567	0,420	0,677	0,617	0,851	-0,884	1,339	290
Clutch size	0,112	0,058	1,940	0,059	0,038	0,131	-0,001	0,226	290
Pagel's λ	0,830						0,800	0,913	290
R^2full	0,518						0,510	0,541	290
R^2fixef	0,013						0,011	0,019	290
Intercept	0,297	0,580	0,530	0,599	0,548	0,742	-0,840	1,435	350
Gregariousness	-0,026	0,040	-0,642	0,533	0,421	0,853	-0,105	0,053	350
Pagel's λ	0,798						0,769	0,877	350
R^2full	0,513						0,504	0,538	350
R^2fixef	0,001						0,001	0,004	350
Intercept	0,134	0,539	0,265	0,793	0,734	0,953	-0,923	1,191	398
Habitat	0,082	0,038	2,136	0,038	0,022	0,095	0,007	0,156	398
Pagel's λ	0,779						0,748	0,863	398
R^2full	0,496						0,487	0,522	398
R^2fixef	0,011						0,010	0,017	398

Table S14. Univariate models analysing the effect of each predictor on the sexual differences in colour elaboration.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	0,094	0,517	0,194	0,846	0,792	0,988	-0,918	1,107	357
Sexual size dimorphism	-0,058	0,049	-1,194	0,239	0,196	0,387	-0,153	0,037	357
Pagel's λ	0,660						0,603	0,826	357
R^2full	0,301						0,287	0,341	357
R^2fixef	0,004						0,002	0,007	357
Intercept	0,009	0,547	0,026	0,930	0,898	0,997	-1,064	1,081	398
Annual mean temperature	-0,093	0,052	-1,806	0,075	0,059	0,133	-0,195	0,008	398
Pagel's λ	0,695						0,645	0,834	398
R^2full	0,317						0,303	0,354	398
R^2fixef	0,010						0,008	0,014	398
Intercept	0,111	0,537	0,218	0,828	0,774	0,973	-0,942	1,164	398
Annual mean precipitation	-0,021	0,053	-0,398	0,693	0,613	0,940	-0,125	0,083	398
Pagel's λ	0,684						0,633	0,829	398
R^2full	0,311						0,296	0,349	398
R^2fixef	0,000						0,000	0,001	398
Intercept	0,220	0,531	0,425	0,673	0,617	0,822	-0,821	1,260	357
Body size	-0,121	0,078	-1,550	0,126	0,101	0,210	-0,275	0,032	357
Pagel's λ	0,672						0,617	0,826	357
R^2full	0,305						0,291	0,343	357
R^2fixef	0,008						0,007	0,013	357
Intercept	0,126	0,551	0,241	0,811	0,758	0,960	-0,953	1,206	290
Clutch size	0,070	0,069	1,006	0,322	0,267	0,502	-0,066	0,206	290
Pagel's λ	0,700						0,632	0,878	290
R^2full	0,297						0,281	0,338	290
R^2fixef	0,003						0,002	0,006	290
Intercept	0,145	0,567	0,269	0,790	0,736	0,933	-0,966	1,256	350
Gregariousness	-0,018	0,048	-0,369	0,715	0,626	0,970	-0,113	0,077	350
Pagel's λ	0,685						0,629	0,836	350
R^2full	0,312						0,298	0,350	350
R^2fixef	0,000						0,000	0,002	350
Intercept	0,112	0,539	0,220	0,827	0,773	0,975	-0,944	1,169	398
Habitat	0,013	0,045	0,289	0,770	0,696	0,976	-0,075	0,101	398
Pagel's λ	0,686						0,637	0,831	398
R^2full	0,311						0,296	0,348	398
R^2fixef	0,000						0,000	0,002	398

Table S15. Multivariate models analysing the effect of each predictor whilst controlling for the others on the sexual dichromatism score.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	0,483	0,597	0,828	0,413	0,360	0,556	-0,687	1,653	273
Annual mean precipitation	0,035	0,054	0,659	0,518	0,440	0,777	-0,070	0,141	273
Annual mean temperature	-0,050	0,055	-0,900	0,377	0,310	0,577	-0,159	0,059	273
Clutch size	0,057	0,066	0,869	0,396	0,320	0,635	-0,072	0,186	273
Body size	-0,251	0,081	-3,086	0,003	0,001	0,008	-0,411	-0,092	273
Sexual size dimorphism	-0,039	0,045	-0,858	0,414	0,276	0,837	-0,128	0,050	273
Gregariousness	-0,003	0,045	-0,077	0,847	0,780	0,994	-0,092	0,085	273
Habitat	0,008	0,045	0,170	0,831	0,751	0,994	-0,080	0,096	273
Pagel's λ	0,826						0,797	0,908	273
R^2full	0,536						0,528	0,558	273
R^2fixef	0,054						0,050	0,065	273

Table S16. Multivariate models analysing the effect of each predictor whilst controlling for the others on the sexual differences in colour elaboration.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	0,112	0,607	0,194	0,846	0,794	0,989	-1,078	1,302	273
Annual mean precipitation	0,007	0,066	0,108	0,872	0,824	0,995	-0,123	0,137	273
Annual mean temperature	-0,089	0,067	-1,321	0,193	0,156	0,303	-0,220	0,043	273
Clutch size	0,043	0,081	0,528	0,603	0,546	0,802	-0,116	0,201	273
Body size	-0,087	0,093	-0,931	0,357	0,311	0,491	-0,269	0,096	273
Sexual size dimorphism	-0,073	0,056	-1,307	0,198	0,159	0,351	-0,182	0,036	273
Gregariousness	-0,011	0,056	-0,204	0,823	0,749	0,992	-0,121	0,098	273
Habitat	-0,029	0,056	-0,526	0,603	0,542	0,806	-0,138	0,080	273
Pagel's λ	0,722						0,667	0,878	273
R^2full	0,316						0,302	0,352	273
R^2fixef	0,023						0,021	0,030	273

Table S17. Multivariate models analysing the effect of each predictor whilst controlling for the others on sexual size dimorphism.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,073	0,183	-0,135	0,899	0,980	1,000	-0,432	0,285	325
Annual mean precipitation	-0,014	0,059	-0,239	0,778	0,752	0,977	-0,130	0,102	325
Annual mean temperature	-0,041	0,058	-0,683	0,517	0,597	0,597	-0,155	0,074	325
Habitat	-0,067	0,055	-1,234	0,220	0,211	0,331	-0,174	0,040	325
Body size	0,230	0,062	3,863	0,004	0,000	0,042	0,109	0,352	325
Gregariousness	0,017	0,055	0,302	0,749	0,720	0,968	-0,092	0,125	325
Pagel's λ	0,149						0,000	0,868	325
R^2full	0,102						0,067	0,269	325
R^2fixef	-0,020						-0,043	0,067	325

Table S18. Multivariate models analysing the effect of each predictor whilst controlling for the others on clutch size.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	-0,030	0,388	-0,083	0,910	0,868	0,997	-0,790	0,730	273
Annual mean precipitation	-0,221	0,049	-4,546	0,000	0,000	0,000	-0,316	-0,126	273
Annual mean temperature	-0,157	0,049	-3,191	0,002	0,001	0,004	-0,253	-0,060	273
Habitat	0,061	0,042	1,452	0,150	0,130	0,214	-0,021	0,143	273
Body size	-0,316	0,063	-5,039	0,000	0,000	0,000	-0,440	-0,193	273
Gregariousness	0,051	0,042	1,211	0,233	0,186	0,368	-0,032	0,134	273
Pagel's λ	0,625						0,594	0,718	273
R^2full	0,607						0,604	0,617	273
R^2fixef	0,196						0,191	0,213	273

Table S19. Multivariate models analysing the effect of each predictor whilst controlling for the others on body size.

Model_term	Estimate	SE	t.value	p.value	p.val_2.5	p.val_97.5	CI_2.5	CI_97.5	N
Intercept	0,801	0,628	1,283	0,204	0,173	0,298	-0,429	2,031	325
Annual mean precipitation	0,014	0,027	0,495	0,575	0,372	0,978	-0,040	0,067	325
Annual mean temperature	-0,029	0,029	-1,014	0,379	0,143	0,959	-0,085	0,028	325
Habitat	-0,014	0,022	-0,632	0,525	0,313	0,970	-0,058	0,029	325
Gregariousness	0,006	0,023	0,280	0,544	0,317	0,971	-0,039	0,051	325
Pagel's λ	0,981						0,977	0,994	325
R^2full	0,759						0,753	0,778	325
R^2fixef	0,004						0,002	0,012	325

Figure S1. Raw colour distribution of each body patch.

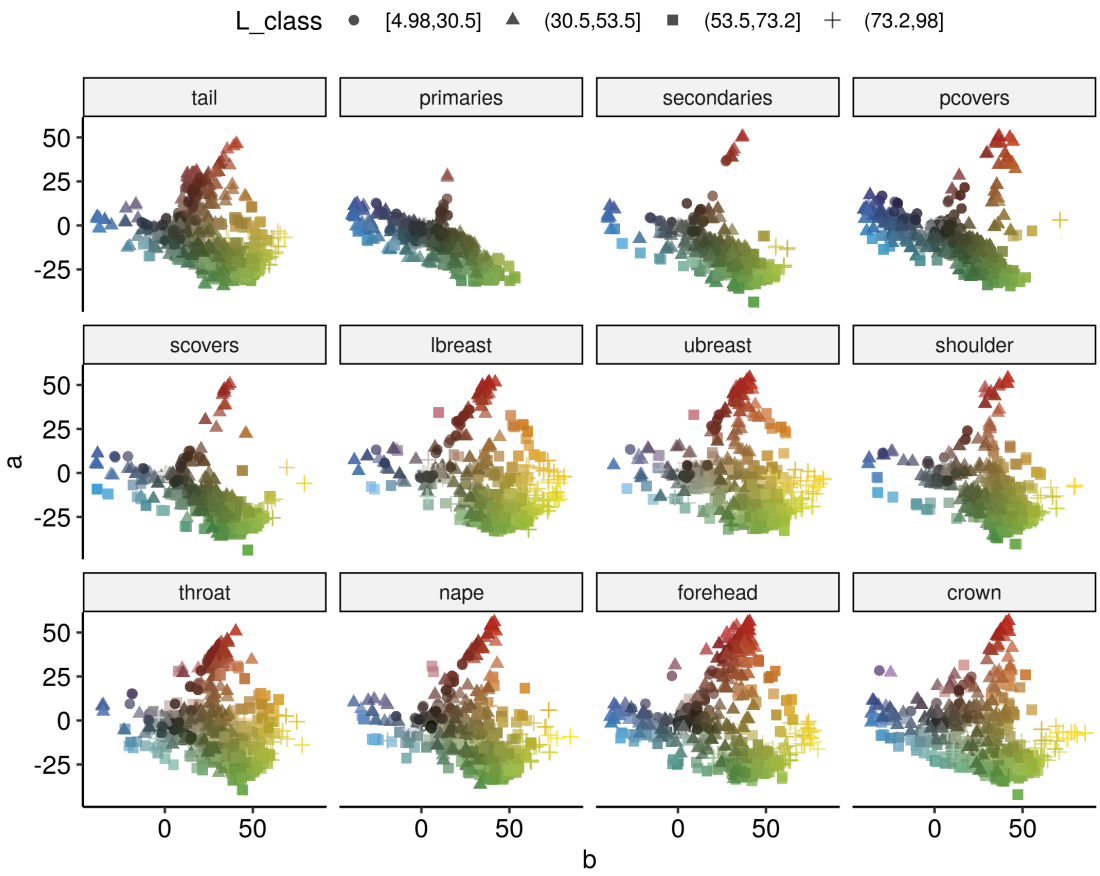


Figure S2. Principal component analysis to estimate body size given tail, tarsus and wing length.

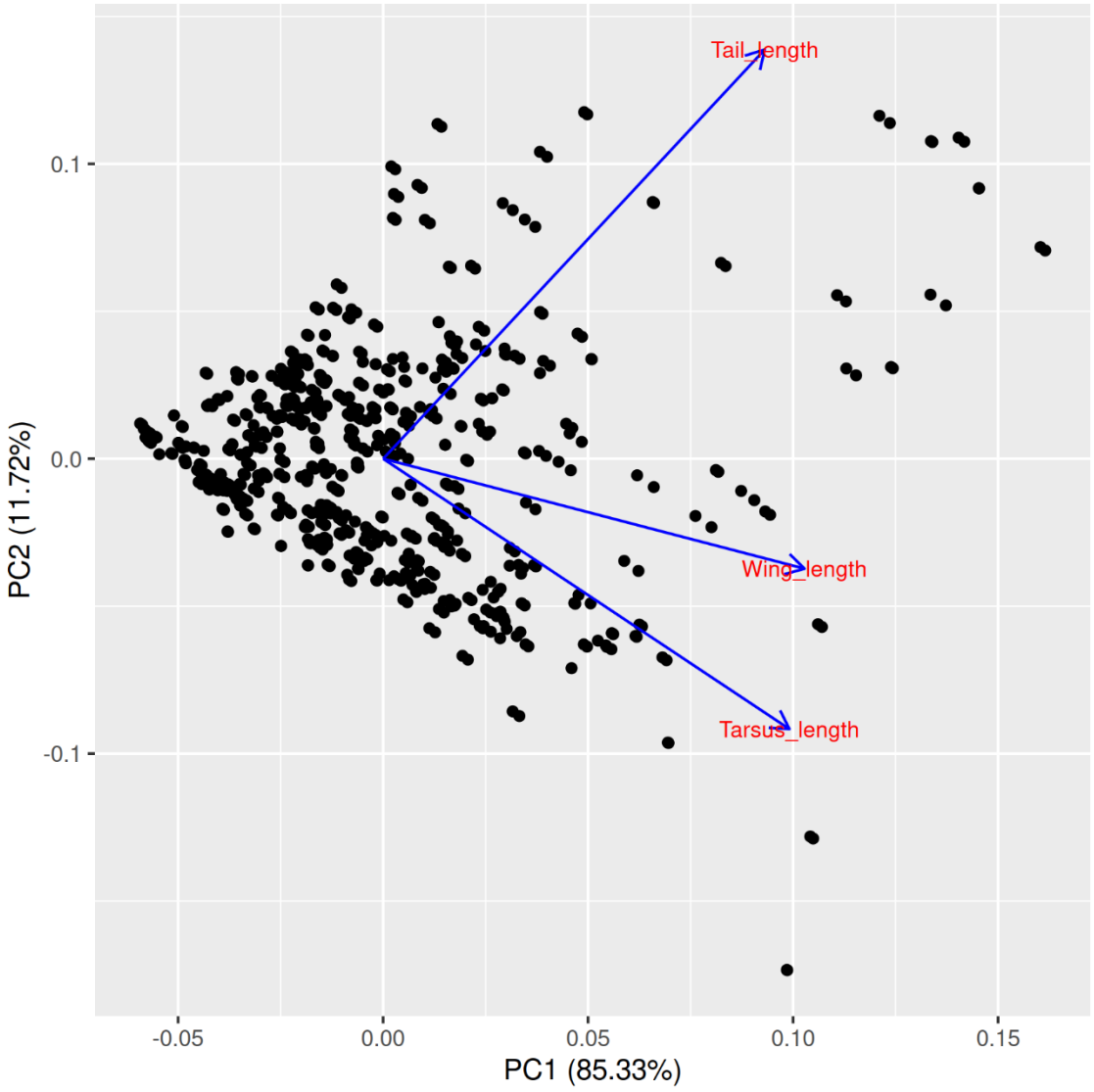


Figure S3. Effect sizes of the predictor variables on body size (PC1), clutch size and sexual size dimorphism (SSD).

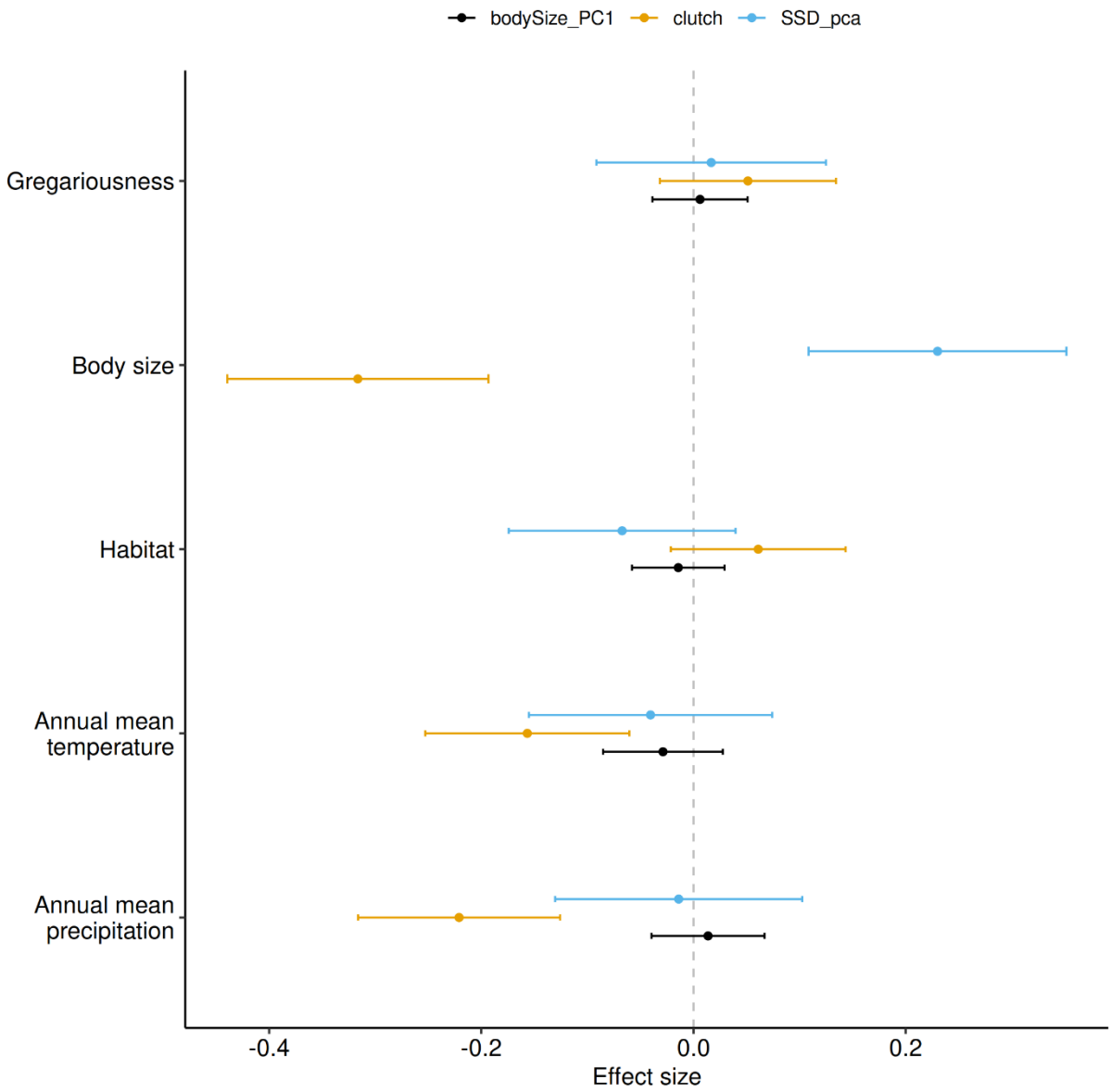
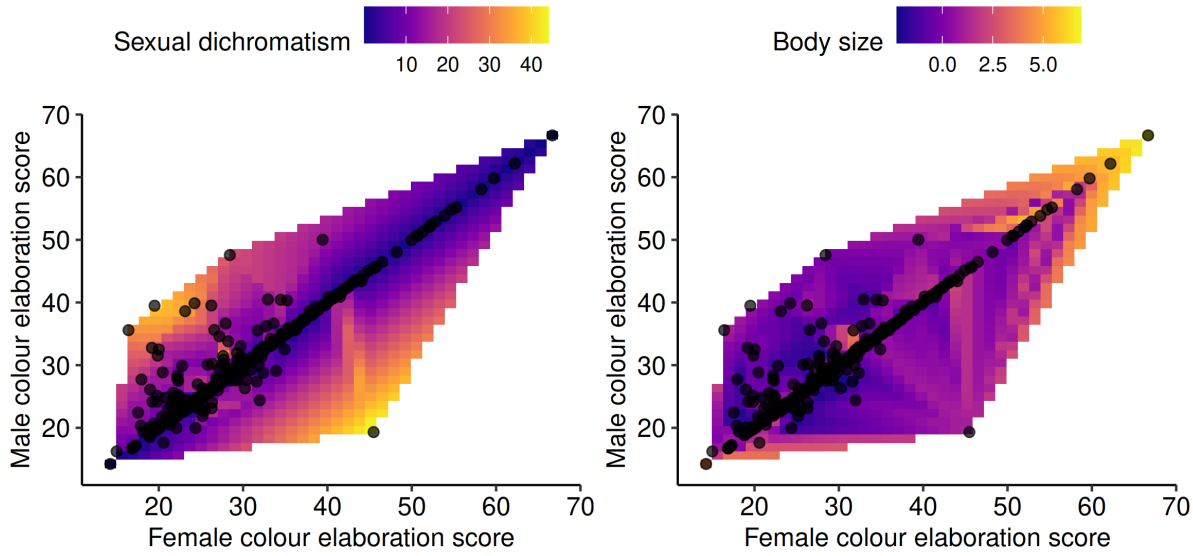


Figure S4. Heatmap of relationship between female colour elaboration score and male colour elaboration score by sexual dichromatism score (left panel) and body size (right panel).



For details of supplementary table and figures, please see online supporting information for "Body size and climate as predictors of plumage colouration and sexual dichromatism in parrots": https://osf.io/2xr4v/

Chapter 3

Between-species variation in within-pair copulation behaviour in parrots

Luisana Carballo, Mihai Valcu, Bart Kempenaers

In birds, a single insemination would – in principle – be sufficient to fertilise all the eggs in a clutch. However, the frequency and duration of within-pair copulations vary greatly among bird species. Different hypotheses have been proposed to explain this variation, of which two are highly relevant for explaining variation during the fertile period. The sperm competition hypothesis suggests that within- and between-species variation in copulation behaviour patterns is linked to variation in the intensity of sperm competition. Males of socially monogamous species vary in the risk of losing paternity due to extra-pair copulations. To protect their paternity, individuals can closely guard their fertile female or they can copulate frequently or for longer with her. The predation risk hypothesis suggests that copulations should be shorter and less frequent when predation risk is higher. Here, we explored variation in copulation behaviour among 103 parrot species held in pairs in captivity. We assessed whether the variation in copulation frequency and duration of Psittaciformes has been driven by sperm competition or by other aspects of the species' social environment or life-history. Even though we found trends in line with expectations based on prior research, such as that species in which males are larger and more colourful than females have longer copulation duration, none of the factors considered in this study had a significant effect. Our findings might reflect that most parrots are "truly" monogamous (socially as well as genetically), but we cannot exclude that our results are affected by the captive conditions. Thus, in natural

environments, males might adapt their copulation behaviour strategy in response to a higher risk of sperm competition or to a higher predation risk.

Unpublished manuscript

Introduction

The frequency and duration of within-pair copulations varies greatly among birds (Birkhead et al. 1987). Because the amount of sperm transferred in a single ejaculate would be sufficient to fertilise all the eggs in a clutch (Birkhead et al. 1989), the observed variation in copulation behaviour among birds needs an explanation (Birkhead and Moller 1992).

Several non-mutually exclusive hypotheses have been proposed to explain variation in within-pair copulation frequency (Birkhead and Moller 1992). The fertilisation hypothesis states that copulations occur during the fertile period and frequently enough to ensure that sufficient sperm is available to fertilise the eggs in a clutch. The social-bond hypothesis posits that copulations occur to strengthen the social-bond between the breeding partners. The predation hypothesis suggests that copulation behaviour is influenced by predation risk, such that copulations are less frequent (and shorter) when predation risk is higher. Finally, the sperm competition hypothesis states that the high frequency (and long duration) of within-pair copulations is a mechanism to reduce the risk of paternity loss. For birds, it has been suggested that the sperm competition hypothesis is the most plausible explanation for the variation of within-pair copulation frequency observed during the fertile period, whereas the social-bond hypothesis is the most plausible explanation for copulations occurring before the fertile period (Birkhead and Moller 1992). Males of socially monogamous species risk losing paternity because their female can engage in extra-pair copulations. This has led to the evolution of paternity protection mechanisms, including two behavioural tactics. First, if the risk of losing paternity is higher, males should invest more in mate guarding, i.e. closely following their fertile female (Birkhead and Moller 1992; Harts et al. 2016). Second, under higher risk of sperm competition males can avoid paternity loss and hence increase their reproductive success by copulating more frequently with their mate (Mougeot 2004; Wysocki

and Halupka 2004), or by copulating longer to transfer more sperm (Mougeot 2004). For example, a field study on European blackbirds (*Turdus merula*) showed that males increased their within-pair copulation frequency during the fertile period and when there was a higher risk of paternity loss (Wysocki and Halupka 2004). In this blackbird population forced extra-pair copulation attempts posed an important cuckoldry threat, which could explain why territorial males had higher within-pair copulation rates than expected if only the fertilisation hypothesis holds true, and solicited copulations more frequently than females did. Additionally, a comparative study on raptors showed that copulations lasted longer and were more frequent in species that breed at higher densities (Mougeot 2004). In such species, a higher percentage of females engaged in extra-pair copulations, suggesting that males faced an increased risk of losing paternity, which may then lead to selection for a higher copulation frequency and duration (Mougeot 2004).

The previous studies suggest that sperm competition might be the driving force explaining variation in within-pair copulation frequency and duration in birds, especially during the fertile period. However, other selective pressures or factors might also influence copulation frequency and duration. For example, smaller species might have fewer and more brief copulations because they suffer from an increased risk of predation compared to larger species (Bennett and Owens 2002; Ricklefs 2010). Smaller species might also be able to have shorter copulations because they are more agile than larger species and can more easily make cloacal contact to transfer sperm (Birkhead and Moller 1992). However, empirical evidence supporting this hypothesis remains limited (Birkhead and Moller 1992).

Here, we use a comparative approach to study variation in within-pair copulation frequency and duration in parrots. Parrots are mainly socially monogamous, with long-term pair-bonds (Toft and Wright 2015). However, a study exploring sperm morphology across 62 parrot

species showed that sperm was longer in sexually dichromatic species and in gregarious species, suggesting that these species might have higher levels of sperm competition (Carballo et al. 2019). Furthermore, even though parentage studies in parrots have found that some species are genetically monogamous (Masello et al. 2002; Caparroz et al. 2011; Eastwood et al. 2018), others have shown varying levels of extra-pair paternity (Beissinger 2008; Taylor and Parkin 2009; Martínez et al. 2013; Heinsohn et al. 2019), with up to 50.5% of swift parrot (*Lathamus discolour)* broods containing extra-pair offspring (Heinsohn et al. 2019). Additionally, a recent comparative study of 398 parrot species reported that sexual dichromatism and male plumage colour elaboration were associated with body size (Carballo et al. 2020). Larger parrot species have more colourful plumage (more blue and red colours), but show lower levels of sexual dichromatism. These results suggest that post-copulatory sexual selection via sperm competition might be stronger in smaller parrot species, whereas the higher levels of colour elaboration in larger parrot species might be a consequence of mutual mate choice, social selection or reduced selection for crypsis related to lower predation risk (Carballo et al. 2020). The aim of the current study is to explore whether the sperm competition and predation risk hypotheses can explain variation in within-pair copulation behaviour in parrots. Thus, we ask whether between-species variation in copulation behaviour (frequency and duration) during the presumed fertile period relates to measures reflecting the intensity of sperm competition and the risk of predation. More specifically, we explored whether body size (as an estimate of lifespan), gregariousness, sperm length, sexual dimorphism in body size and plumage colouration, and male colour elaboration explain variation in within-pair copulation frequency and duration across parrots. If the sperm competition hypothesis is driving the variation in within-pair copulation behaviour in parrots then we expect that species with shorter lifespans, that breed gregariously, that have longer sperm and that show male-biased sexual dimorphism and

dichromatism will have a higher within-pair copulation frequency and longer copulations. If the predation risk hypothesis is shaping the variation in parrot copulation behaviour, we expect that smaller species, that have evolved under higher predation risk, will show fewer and shorter copulations compared to larger species.

Methods

Study system and site

We studied the copulation behaviour of 103 parrot species held in single pairs in the Loro Parque Fundacion (LPF), Tenerife, Spain. During the breeding seasons of 2016 and 2017 we used video recordings to observe copulations of a total of 133 pairs (1–4 pairs per species, *mean*: 1.3, Table S1). We recorded each pair for an average of 68.1 hours (*range*: 5.8–177.5) across an average of 9.5 days (*range*: 1–27). All recorded individuals had been raised in the facilities of LPF, and once pairs were formed they were kept in separate aviaries. From February to August of each year, LPF staff attached a nest box to each aviary and provided nesting material, which triggered breeding.

Video recordings and coding procedure

Video recording protocol

The curator and the animal care-takers at LPF monitored the breeding status of all pairs in the facility. They did so by checking the nest boxes to identify the pairs that were collecting nesting material inside the nest box. This information allowed us to identify the pairs that were ready to breed. As some females laid more than one clutch per season, either after

raising a brood or after a clutch had been removed to be hand-raised, we recorded pairs during the pre-laying period of the first or any of the subsequent clutches.

As soon as a suitable pair was identified, we placed a video camera (Sony FDR-X1000 Camcorder) connected to a fully-charged external power bank (Powerbank Voltcraft PB 13 Outdoor Li-Ion 9000 mAh) on a tripod (Mantona Scout Tripod with Ballhead) outside its aviary. Cameras were placed outside the aviaries to reduce disturbance to the pair and to avoid damage to the equipment.

Behaviour coding

We used the free software Solomon Coder beta 17.03.22 (https://solomon.andraspeter.com) to analyse videos and code behaviours. We defined a copulation as the action involving the male mounting the female followed by sideways movements of the tail, which suggested that cloacal contact was made. We measured the duration of each copulation (i.e. the duration the pair was mounted), and calculated the copulation frequency as the total number of copulations divided by the total observation time (in hours).

We recorded a total of 1589 copulations across all pairs, with an average of 15.4 copulations per species (*range*: 1–96). Of all the observed pairs, only 44 (33.1%) pairs laid eggs during the observation period, thus some of the observations might have been outside the fertile period. We therefore analysed the data including all observations, and including only those pairs that laid eggs (from now on called “successful breeders”) separately.

Explanatory variables

Body size and sexual size dimorphism

We used three body size measurements (wing, tarsus and tail length) for females ($N_{species}$ = 94) and males ($N_{species}$ = 93) of individuals held at LPF from Carballo et al. (2020). For the species for which male or female body size measures could not be obtained at LPF (Table S2), we used data from Forshaw (1978). We estimated body size for males and females using the first principal component (PC1) from a principal component analysis (PCA) that included the three measurements for both sexes. PC1 explained 65% of the variation in the data. Species body size was then estimated as the average of male and female body size (PC1, Table S3). As a measure of the intensity of sexual selection, we calculated sexual size dimorphism (SSD) as $PC1_{\text{male body size}} - PC1_{\text{female body size}}$ (Table S3).

Plumage colour scores

For each species, we used the male colour elaboration score and two indices of sexual dichromatism from Carballo et al. (2020) (Table S3). Highly elaborate colours (i.e. red, blue and yellow) were those that differed more from the average colour across all parrots (i.e. greenish brown). "Sexual dichromatism" was estimated as the absolute difference in colouration between males and females (| male − female |) irrespective of whether males or females were more ornamented. "Sexual difference in colour elaboration" is a variable that indicates which sex has more elaborated colours in their plumage (male − female). Thus, positive values reflected species where males had more elaborated colours than females. Even though the first index did not provide direction in the difference of colouration between the sexes, we considered it for our analyses because from the two sexual dichromatism

indices this was the only one showing a significant relationship with species body size (Carballo et al. 2020).

Gregariousness

When species nest close together there are more opportunities to engage in extra-pair copulations. Thus, we took gregariousness data – a categorical variable ("yes" or "no") – from Carballo et al. (2020) (Table S3). A species was classified as gregarious if the description in the "breeding" section of the *Handbook of the Birds of the World Alive* (del Hoyo et al. 2017) suggested that the pairs nest close together or if the species was described as colonial.

Sperm length

Longer sperm is considered an indicator of higher levels of sperm competition in birds (Kleven et al. 2009; Lüpold et al. 2009). Thus, we used data on mean sperm length for the 33 parrot species that were available from Carballo et al. (2019) (Table S3).

Phylogeny

We extracted a sample of 1000 phylogenetic trees (the "Hackett" backbone, Hackett et al. 2008) from phylogenetic tree distributions available on birdtree.org (Jetz et al. 2012; Jetz et al. 2014) for 96 parrot species. We added the 7 Psittaciformes species for which we obtained copulation observations but that were missing in these phylogenies, using the function

add.species.to.genus in the R package "phytools" v.0.6-99 (Revell 2012). This function finds the branch of the phylogenetic tree common to the corresponding genus and adds the missing taxon at a random position within this branch. We constructed a consensus tree with minimum clade frequency threshold of 0.5 (Rubolini et al. 2015) using the function *SumTrees* from the package "DendroPy" v.4.4.0 (Sukumaran and Holder 2010).

Statistical analysis

All statistical analyses were performed in R 4.0.3 (R Development Core Team 2020). The response variables "copulation duration" and "copulation frequency", and the predictor "sexual dichromatism" were $\log_{10}$ transformed to improve data distribution for analyses.

We ran Bayesian mixed models using the R package "brms" (Bürkner 2017) with four parallel chains of 5000 iterations and a warm-up period of at least 1000 iterations to derive the posterior distributions and associate 95% credible intervals (CIs) for the following fitted parameters: sexual size dimorphism ($N_{species}$ = 102), SSD ($N_{species}$ = 103), sexual difference in colour elaboration ($N_{species}$ = 103), gregariousness ($N_{species}$ = 99), male colour elaboration ($N_{species}$ = 103) and body size ($N_{species}$ = 102). We ran models using the entire dataset ($N_{species}$ = 98 for which data on all predictors were available) and additional models only including successful breeders ($n_{species}$ = 40 for which data on all predictors were available). Because mean sperm length data were only available for 33 species, we also ran a separate model to explore the effect of this predictor on copulation duration and copulation frequency.

We used a hierarchical modelling approach with species-level and pair identity as random effects. Convergence of all models was confirmed with Gelman-Rubin diagnostics (Gelman and Rubin 1992), which means that R-hat for all models was <1.05.

Results

The mean within-pair copulation duration per species varied between 3 and 1781 seconds, and copulation frequency from 0.1 to 1.1 copulations/hour across all parrot species observed (Figure 1). The shortest copulation was observed in the Cordilleran parakeet (*Psittacara frontatus*) and the longest copulation in the lesser vasa parrot (*Coracopsis nigra*). The lowest copulation frequency was observed in the lesser vasa parrot and the highest frequency in the blue-throated macaw (*Ara glaucogularis*).

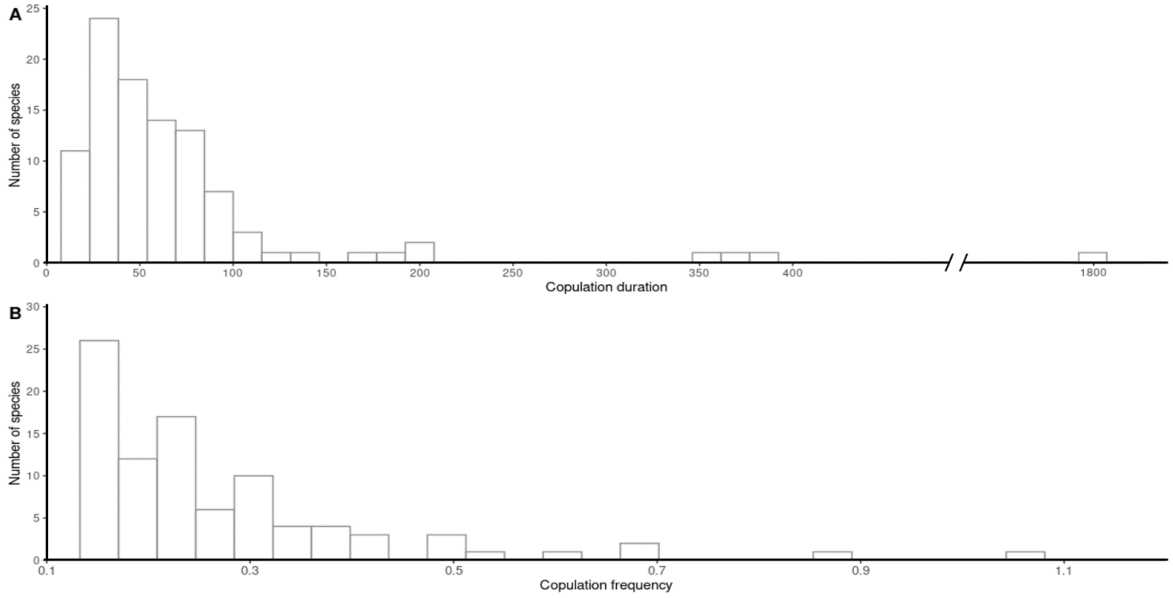


Figure 1. Frequency distribution of A mean copulation duration (in seconds), and B mean copulation frequency (number per hour) per species (N = 103).

Copulation duration and copulation frequency varied substantially between pairs and between species. Pair ID explained 11–21% of the variation in copulation duration, whilst species explained 12–28% of the variation (Table 1). Pair ID also explained 22–34% of the variation in copulation frequency, whilst species explained 21–33 % (Table 2). However, the Pair ID effect was only significant for copulation duration considering all data and all predictors

(including sperm length), and the species effect was only significant for copulation duration when considering all data and all predictors, but excluding sperm length (Table 1).

Table 1. Proportion of the variance in within-pair copulation duration in parrots explained by the random effects pair identity and species.

Random effects	Dataset	Predictors	Estimate	SE	l.95 CI	u.95 CI	*p*
Pair ID	All data	All, except sperm length	0.14	0.04	0.07	0.24	0
		Mean sperm length	0.21	0.07	0.09	0.37	0
	Data from pairs that laid an egg	All, except sperm length	0.11	0.06	0.01	0.24	0.36
		Mean sperm length	0.11	0.12	0	0.43	0.71
Species	All data	All, except sperm length	0.28	0.10	0.08	0.48	0.03
		Mean sperm length	0.16	0.11	0.01	0.42	0.33
	Data from pairs that laid an egg	All, except sperm length	0.12	0.12	0	0.42	0.72
		Mean sperm length	0.17	0.16	0	0.58	0.62

Predictors: body size, sexual size dimorphism, male colour elaboration, sexual dichromatism, sexual difference in colour elaboration, gregariousness

Table 2. Proportion of the variance in copulation frequency in parrots explained by the random effects pair identity and species.

Random effects	Dataset	Predictors	Estimate	SE	l.95 CI	u.95 CI	p
Pair ID	All data	All, except sperm length	0.22	0.17	0	0.57	0.52
		Mean sperm length	0.34	0.28	0	0.9	0.45
	Data from pairs that laid an egg	All, except sperm length	0.24	0.23	0	0.78	0.54
		Mean sperm length	0.32	0.29	0	0.94	0.48
Species	All data	All, except sperm length	0.29	0.15	0.04	0.59	0.12
		Mean sperm length	0.21	0.17	0	0.6	0.48
	Data from pairs that laid an egg	All, except sperm length	0.27	0.23	0	0.77	0.48
		Mean sperm length	0.33	0.28	0	0.92	0.45

Predictors: body size, sexual size dimorphism, male colour elaboration, sexual dichromatism, sexual difference in colour elaboration, gregariousness

When considering all pairs, Bayesian mixed models showed that copulation duration was longer with higher SSD, sexual difference in colour elaboration and body size, and decreased with sexual dichromatism, gregariousness and male colour elaboration (Figure 2A). The direction of these effects was consistent when only successful breeders were considered (Figure 2A). However, none of the effects were significant. Copulation duration was negatively associated with mean sperm length when considering all pairs, but the association was positive when only successful breeders were included in the model (Figure 2B). However, again, the effect of mean sperm length was not significant in either of the models.

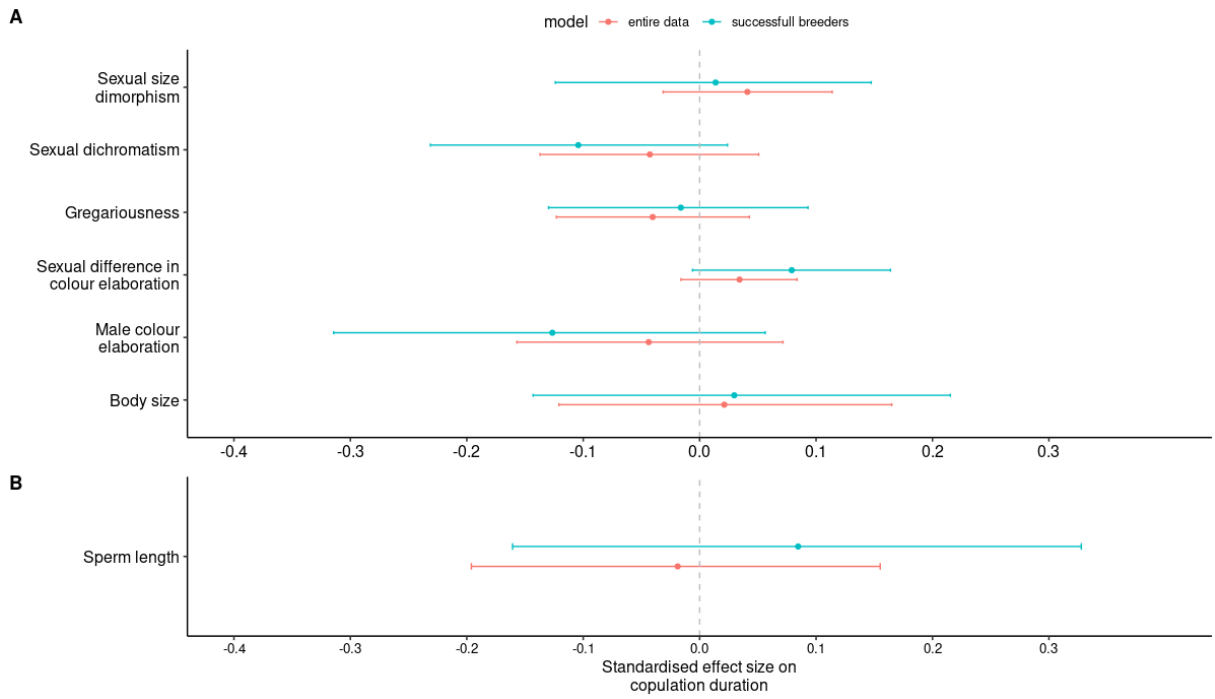


Figure 2. Standardised effect sizes of predictors of copulation duration in parrots. A, Results from Bayesian mixed models with all predictors, except sperm length. Blue: all pairs included ($N_{species}$ = 98, N_{pairs} = 127, $N_{copulations}$ = 1529), red: only pairs that laid an egg during the observation period included (successful breeders; $n_{species}$ = 40, n_{pairs} = 41, $n_{copulations}$ = 651). B, Results from Bayesian mixed models with mean sperm length as sole predictor. Blue: all pairs included ($N_{species}$ = 33, n_{pairs} = 51, $n_{copulations}$ = 622), red: only successful breeders included ($n_{species}$ = 12, n_{pairs} = 12, $n_{copulations}$ = 236).

Species with higher sexual dichromatism, a stronger sexual difference in colour elaboration and more elaborated male colour copulated more frequently, whilst those with higher SSD, gregariousness and body size showed lower copulation frequencies (Figure 3A). When considering only successful breeders, copulation frequency was positively correlated with SSD, sexual dichromatism, gregariousness and body size, and negatively with the sexual difference in colour elaboration and with male colour elaboration. Copulation frequency was

negatively associated with mean sperm length (Figure 3B). However, none of these effects were significant.

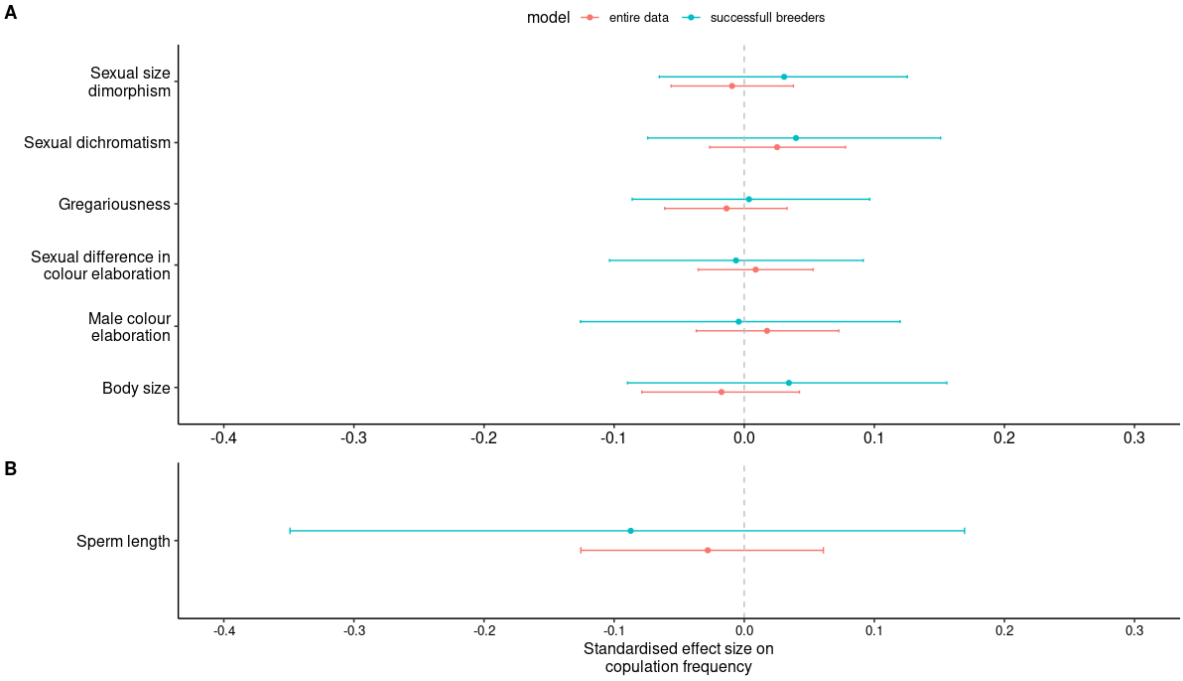


Figure 3. Standardised effect sizes of predictors of copulation frequency. A, Results from Bayesian mixed models with all predictors, except sperm length. Blue: all pairs included ($N_{species}$ = 98, N_{pairs} = 127, $N_{copulation\text{-}frequency}$ = 132), red: only pairs that laid an egg during the observation period included (successful breeders; $n_{species}$ = 40, n_{pairs} = 41, $n_{copulation\text{-}frequency}$ = 42). B, Results from Bayesian mixed models with mean sperm length as sole predictor. Blue: all pairs included ($N_{species}$ = 33, n_{pairs} = 51, $n_{copulation\text{-}frequency}$ = 51), red: only successful breeders included ($n_{species}$ = 12, n_{pairs} = 12, $n_{copulation\text{-}frequency}$ = 12).

Discussion

In this study, we describe the copulation behaviour of 103 parrot species. For the 98 species for which we had data available for all predictors considered in the analyses, we explored whether variation in copulation duration and copulation frequency could be explained by SSD, sexual dichromatism, sexual difference in colour elaboration, male colour elaboration, gregariousness, body size and sperm length. We found no significant effect of any of these predictors on the variation in within-pair copulation frequency and duration in parrots.

Parrots have been considered by some researchers as the only "true" monogamous species (Toft and Wright 2015), and studies have found that some parrots are in fact genetically monogamous (Masello et al. 2002; Caparroz et al. 2011; Eastwood et al. 2018). However, other studies have shown that extra-pair paternity is common in other parrot species (Beissinger 2008; Taylor and Parkin 2009; Martínez et al. 2013; Heinsohn et al. 2019), and that gregarious and sexually dichromatic species have longer sperm (a proxy of sperm competition) (Carballo et al. 2019). These findings suggest that there is variation among parrot species in the level of sperm competition. A previous comparative study exploring variation in parrot plumage colouration suggested that phenotypic variation is affected not only by sperm competition, but also by other factors such as mutual mate choice, social selection and predation risk (Carballo et al. 2020). We therefore investigated whether variation in copulation behaviour could also be linked to the prevalence of sperm competition and/or variation in predation risk among parrots. If sperm competition was affecting the variation in within-pair copulation behaviour in parrots, we expected that species that experience higher mortality, breed gregariously, show male-biased sexual dimorphism and male-biased sexual dichromatism, and have longer sperm would copulate more frequently and for longer. If predation risk is the main underlying factor explaining copulation

behaviour, we expected that larger species that experience lower mortality (lower predation risk) would copulate more frequently and for longer. Even though we found trends that went in the expected direction, none of these factors had a significant effect on within-pair copulation frequency and copulation duration.

One possible explanation for the lack of an effect is that most parrot species are both socially and genetically monogamous. However, a previous comparative study, which included 398 parrot species, found that smaller parrots were generally less colourful but more sexually dichromatic than larger species (Carballo et al. 2020), and suggested that as smaller species tend to have shorter lifespans, they might tolerate higher levels of extra-pair paternity. Thus, the sexual dichromatism found in smaller species may be a consequence of sexual selection via female choice for (extra-pair) mates. If few parrot species experience sperm competition, we might not have captured this effect in the current study with a smaller sample size ($N_{species}$ = 98).

An alternative explanation for the lack of significant effects is that our study was conducted with parrots that were kept as separate pairs in aviaries, i.e. under circumstances where both predation and extra-pair paternity are excluded. Under natural circumstances, in free-living populations, individuals might flexibly adjust their copulation behaviour in response to variation in the perceived risk of sperm competition (Birkhead et al. 1987; Mougeot 2004) or predation. Nevertheless, we find consistent differences between species in copulation duration and frequency (Table 1 and 2), which should be shaped by selection in response to variation in their life-history.

Observing parrots' copulation behaviour in the wild is difficult as these species breed high in the forest canopies (Taylor and Parkin 2009; Toft and Wright 2015). Thus, captive studies provide a valuable setting to study parrot copulation behaviour. For example, the peculiar

copulation behaviour of the polyandrous lesser and greater vasa parrot (*Coracopsis vasa*) was studied in captivity (Wilkinson and Birkhead 1995). Males of these two species have an enlarged cloacal protrusion that allows them to interlock with the female for longer periods of time, which was confirmed in our study (copulation duration of up to ~30 minutes). This peculiar morphology and behaviour presumably evolved due to intense sperm competition.

To be able to corroborate whether the predictors used in this study truly have no effect on within-pair copulation behaviour in parrots, it would be important to increase the sample size, either by including more species or more pairs per species. Our data show that there is considerable variation in both frequency and duration of copulations among parrots. Psittaciformes are one of the order of birds with more endangered species, with 13.9% of parrot species classified as "endangered" or "critically endangered" on the IUCN (2020), but we still lack an understanding of the mating system of many species.

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Supplementary material

Table S1. Pairs observed during 2016 and 2017 breeding seasons and their egg laying status.

Scinam	Pair ID	Year	Egg laid
Agapornis taranta	957	2016	0
Alipiopsitta xanthops	788	2016	0
Alisterus chloropterus	776	2016	1
Amazona autumnalis	838	2017	0
Amazona barbadensis	813	2016	1
Amazona finschi	804	2017	0
Amazona lilacina	794	2017	0
Amazona ochrocephala	809	2016	1
Amazona oratrix	881	2017	0
Amazona oratrix	833	2016	1
Amazona oratrix	858	2017	0
Amazona xantholora	870	2016	0
Amazona xantholora	814	2016	1
Anodorhynchus hyacinthinus	683	2016	1
Anodorhynchus hyacinthinus	653	2016	0
Ara ambiguus	585	2016	0
Ara ararauna	637	2016	1
Ara chloroptera	608	2017	0
Ara chloroptera	594	2016	0
Ara glaucogularis	601	2016	1
Ara macao	636	2016	1
Ara militaris	603	2016	0
Ara rubrogenys	597	2016	1
Ara rubrogenys	597	2017	1
Aratinga auricapillus	187	2017	1
Aratinga jandaya	165	2016	0
Aratinga solstitialis	189	2016	1
Aratinga solstitialis	176	2017	0
Aratinga weddellii	167	2016	0
Aratinga weddellii	171	2017	0
Brotogeris chrysoptera	318	2016	0
Brotogeris cyanoptera	321	2016	0
Brotogeris pyrrhoptera	315	2017	0
Brotogeris tirica	325	2016	0
Brotogeris tirica	323	2016	0
Cacatua ducorpsii	494	2016	0
Cacatua galerita	38	2016	0
Cacatua galerita	4	2017	1
Cacatua haematuropygia	20	2017	0
Cacatua leadbeateri	8	2017	1
Cacatua moluccensis	43	2016	0
Cacatua ophthalmica	36	2017	0
Cacatua pastinator	3	2017	0
Cacatua sulphurea	26	2017	0
Cacatua sulphurea	2	2016	0
Callocephalon fimbriatum	35	2016	1
Callocephalon fimbriatum	46	2017	1
Calyptorhynchus funereus	614	2016	1
Chalcopsitta atra	80	2016	0
Chalcopsitta cardinalis	85	2016	0
Chalcopsitta duivenbodei	112	2017	0
Chalcopsitta scintillata	90	2016	0

Charmosyna josefinae	952	2016	1
Charmosyna papou	967	2016	1
Coracopsis nigra	777	2016	0
Cyanoliseus patagonus	1019	2017	0
Deroptyus accipitrinus	651	2016	0
Diopsittaca nobilis	730	2016	0
Eclectus roratus	266	2016	0
Eclectus roratus	255	2016	0
Eclectus roratus	257	2016	0
Eolophus roseicapilla	16	2017	0
Eolophus roseicapilla	23	2016	0
Eos bornea	79	2016	0
Eos bornea	52	2017	0
Eos histrio	88	2017	0
Eos reticulata	102	2016	0
Eos reticulata	102	2016	1
Eos squamata	95	2016	0
Eupsittula aurea	190	2016	1
Eupsittula pertinax	168	2017	0
Forpus coelestis	993	2016	1
Forpus conspicillatus	1006	2016	1
Forpus passerinus	1005	2016	0
Guaruba guarouba	706	2016	0
Lathamus discolor	932	2016	1
Lorius chlorocercus	101	2017	1
Lorius garrulus	74	2017	0
Lorius garrulus	111	2016	1
Lorius hypoinochrous	120	2017	0
Lorius lory	93	2017	0
Lorius lory	87	2016	0
Neophema chrysostoma	352	2017	0
Neophema pulchella	345	2017	1
Nestor notabilis	595	2016	1
Pionites leucogaster	737	2016	1
Pionites xanthomerius	712	2017	1
Pionites xanthomerius	708	2016	0
Pionopsitta pileata	703	2016	1
Pionus chalcopterus	731	2016	0
Poicephalus meyeri	551	2017	0
Poicephalus robustus	638	2016	0
Poicephalus rufiventris	562	2017	0
Primolius auricollis	678	2017	0
Primolius couloni	663	2016	1
Primolius couloni	660	2016	0
Probosciger aterrimus	627	2016	0
Probosciger aterrimus	627	2017	0
Pseudeos fuscata	50	2016	0
Psittacara acuticaudatus	693	2017	0
Psittacara frontatus	199,1	2017	0
Psittacula alexandri	210	2017	1
Psittacula alexandri	216	2017	0
Psittacula alexandri	234	2016	0
Psittacula alexandri	208	2016	0
Psittacula cyanocephala	230	2017	1
Psittaculirostris edwardsii	936	2016	1
Psittacus erithacus	542	2017	0
Psitteuteles goldiei	946	2017	0
Psitteuteles iris	931	2016	1
Psittrichas fulgidus	1020	2016	1
Pyrrhura griseipectus	126	2016	0
Pyrrhura griseipectus	140	2017	0
Pyrrhura griseipectus	145	2017	0
Pyrrhura lepida	159	2017	0
Pyrrhura melanura	135	2017	1

Pyrrhura perlata	149	2017	1
Pyrrhura rhodocephala	150	2017	0
Pyrrhura roseifrons	161	2016	1
Pyrrhura rupicola	136	2017	1
Pyrrhura rupicola	146	2017	0
Rhynchopsitta pachyrhyncha	654	2017	0
Rhynchopsitta pachyrhyncha	654	2016	0
Rhynchopsitta terrisi	641	2016	0
Trichoglossus capistratus	69	2017	0
Trichoglossus capistratus	98	2016	0
Trichoglossus capistratus	98	2016	1
Trichoglossus chlorolepidotus	97	2017	0
Trichoglossus euteles	83	2017	0
Trichoglossus haematodus	53	2017	0
Trichoglossus haematodus	62	2017	0
Trichoglossus haematodus	99	2016	0
Trichoglossus johnstoniae	944	2017	0
Trichoglossus moluccanus	48	2017	0
Trichoglossus moluccanus	121	2016	0
Trichoglossus rubritorquis	74	2016	1
Trichoglossus rubritorquis	84	2017	0
Vini australis	1004	2016	1

Egg laid ("yes"=1, "no"=0)

Table S2. Body measurements considered to estimate species body size using PCA.

	Female						Male					
Species	**Wing length (mm)**	**Source**	**Tail length (mm)**	**Source**	**Tarsus length (mm)**	**Source**	**Wing length (mm)**	**Source**	**Tail length (mm)**	**Source**	**Tarsus length (mm)**	**Source**
Agapornis taranta	99 .8	LPF	47 .6	LPF	14 .27	LPF	101 .00	LPF	48 .43	LPF	14 .06	LPF
Alipiopsitta xanthops	193	LPF	75	LPF	21 .37	LPF	198 .00	LPF	85 .00	LPF	22 .05	LPF
Alisterus chloropterus	181 .5	LPF	200	LPF	20 .72	LPF	179 .50	LPF	192 .00	LPF	20 .78	LPF
Amazona autumnalis	211	LPF	118	LPF	24 .91	LPF	215 .00	LPF	118 .00	LPF	25 .39	LPF
Amazona barbadensis	201	LPF	124 .67	LPF	22 .52	LPF	212 .56	LPF	129 .63	LPF	23 .04	LPF
Amazona finschi	199 .67	LPF	121 .67	LPF	23 .12	LPF	206 .57	LPF	123 .71	LPF	23 .19	LPF
Amazona lilacina	192	LPF	110	LPF	21 .87	LPF	199 .00	LPF	103 .00	LPF	23 .31	LPF
Amazona ochrocephala	220	LPF	117 .33	LPF	26 .67	LPF	223 .83	LPF	113 .00	LPF	26 .27	LPF
Amazona oratrix	230 .5	LPF	120	LPF	25 .63	LPF	238 .29	LPF	124 .29	LPF	26 .44	LPF
Amazona xantholora	166	LPF	82	LPF	19 .89	LPF	174 .50	LPF	89 .25	LPF	19 .75	LPF
Anodorhynchus hyacinthinus	417	LPF	502 .33	LPF	43 .34	LPF	422 .86	LPF	504 .00	LPF	42 .92	LPF
Ara ambiguus	400 .4	LPF	437 .75	LPF	36 .93	LPF	419 .40	LPF	429 .67	LPF	37 .37	LPF
Ara ararauna	395 .67	LPF	497 .33	LPF	35 .11	LPF	402 .67	LPF	498 .00	LPF	35 .83	LPF
Ara chloroptera	406	LPF	450 .33	LPF	38 .11	LPF	405 .56	LPF	490 .57	LPF	37 .83	LPF
Ara glaucogularis	347 .63	LPF	464 .55	LPF	31 .84	LPF	360 .41	LPF	472 .94	LPF	33 .07	LPF
Ara macao	381 .33	LPF	491 .67	LPF	34 .39	LPF	383 .00	LPF	485 .00	LPF	34 .97	LPF
Ara militaris	365	LPF	344	LPF	32 .10	LPF	375 .83	LPF	385 .50	LPF	33 .23	LPF
Ara rubrogenys	307 .75	LPF	336 .75	LPF	26 .70	LPF	313 .33	LPF	345 .00	LPF	28 .22	LPF
Aratinga auricapillus	170	LPF	140	LPF	16 .33	LPF	173 .60	LPF	156 .40	LPF	16 .80	LPF
Aratinga jandaya	159	LPF	143	LPF	15 .33	LPF	159 .00	LPF	148 .00	LPF	15 .68	LPF
Aratinga solstitialis	157 .4	LPF	147 .67	LPF	15 .17	LPF	160 .70	LPF	149 .60	LPF	15 .27	LPF
Aratinga weddellii	142 .5	LPF	115 .67	LPF	15 .04	LPF	142 .67	LPF	117 .33	LPF	15 .35	LPF
Brotogeris chrysoptera	113	LPF	69	LPF	12 .49	LPF	119 .33	LPF	71 .00	LPF	12 .59	LPF

Brotogeris cyanoptera	120 .89	LPF	70 .63	LPF	13 .57	LPF	120 .15	LPF	72 .50	LPF	13 .31	LPF
Brotogeris pyrrhoptera	117 .5	LPF	79	LPF	12 .78	LPF	116 .50	LPF	76 .25	LPF	13 .09	LPF
Brotogeris tirica	118	LPF	118 .67	LPF	13 .59	LPF	122 .67	LPF	119 .00	LPF	13 .66	LPF
Cacatua ducorpsii	258 .5	LPF	121 .5	LPF	24 .41	LPF	258 .00	LPF	118 .00	LPF	25 .09	LPF
Cacatua galerita	302	LPF	159 .2	LPF	29 .80	LPF	308 .80	LPF	156 .40	LPF	29 .87	LPF
Cacatua haematuropygia	223	LPF	112	LPF	23 .62	LPF	235 .00	LPF	111 .00	LPF	25 .46	LPF
Cacatua leadbeateri	272 .33	LPF	156 .67	LPF	23 .83	LPF	269 .83	LPF	149 .83	LPF	25 .01	LPF
Cacatua moluccensis	308 .33	LPF	187 .67	LPF	32 .17	LPF	313 .33	LPF	183 .50	LPF	33 .79	LPF
Cacatua ophthalmica	307 .5	LPF	179 .5	LPF	31 .94	LPF	309 .00	LPF	175 .00	LPF	31 .37	LPF
Cacatua pastinator	296	LPF	155	LPF	28 .03	LPF	307 .00	LPF	155 .00	LPF	29 .61	LPF
Cacatua sulphurea	252	LPF	133	LPF	24 .36	LPF	266 .00	LPF	140 .67	LPF	24 .58	LPF
Callocephalon fimbriatum	257 .33	LPF	136 .33	LPF	20 .13	LPF	249 .00	LPF	130 .33	LPF	19 .68	LPF
Calyptorhynchus funereus	405	LPF	359	LPF	30 .05	LPF	411 .00	LPF	262 .50	LPF	30 .51	LPF
Chalcopsitta atra	170 .83	LPF	127	LPF	24 .72	LPF	182 .00	LPF	136 .00	LPF	24 .38	LPF
Chalcopsitta cardinalis	180	LPF	144	LPF	22 .00	LPF	183 .00	LPF	151 .00	LPF	23 .22	LPF
Chalcopsitta duivenbodei	175	LPF	130 .5	LPF	22 .80	LPF	171 .50	LPF	134 .00	LPF	23 .62	LPF
Chalcopsitta scintillata	174 .4	LPF	116 .4	LPF	22 .58	LPF	179 .50	LPF	115 .75	LPF	22 .42	LPF
Charmosyna josefinae	118	LPF	127	LPF	14 .41	LPF	120 .00	LPF	140 .00	LPF	15 .47	LPF
Charmosyna papou	133 .5	LPF	266 .8	LPF	16 .69	LPF	138 .33	LPF	275 .38	LPF	16 .99	LPF
Coracopsis nigra	238	LPF	162 .33	LPF	23 .88	LPF	249 .67	LPF	174 .50	LPF	25 .20	LPF
Cyanoliseus patagonus	251 .63	LPF	253 .5	LPF	25 .10	LPF	251 .00	LPF	255 .00	LPF	25 .39	LPF
Deroptyus accipitrinus	197 .33	LPF	140 .67	LPF	21 .99	LPF	202 .50	LPF	150 .00	LPF	22 .59	LPF
Diopsittaca nobilis	172 .5	LPF	136 .33	LPF	18 .51	LPF	172 .83	LPF	146 .00	LPF	18 .02	LPF
Eclectus roratus	241 .41	LPF	115 .36	LPF	23 .95	LPF	253 .65	LPF	127 .52	LPF	24 .39	LPF
Eolophus roseicapilla	261	LPF	147	LPF	21 .90	LPF	267 .00	LPF	144 .00	LPF	23 .50	LPF
Eos bornea	155 .8	LPF	107 .6	LPF	21 .38	LPF	157 .67	LPF	109 .67	LPF	21 .10	LPF
Eos histrio	165 .2	LPF	126 .2	LPF	21 .90	LPF	165 .00	LPF	134 .25	LPF	21 .75	LPF
Eos reticulata	167 .8	LPF	128 .5	LPF	21 .44	LPF	173 .00	LPF	133 .67	LPF	22 .43	LPF
Eos squamata	142 .5	LPF	101 .5	LPF	17 .62	LPF	141 .33	LPF	104 .00	LPF	18 .23	LPF
Eupsittula aurea	148	LPF	133	LPF	14 .04	LPF	148 .75	LPF	135 .50	LPF	14 .13	LPF
Eupsittula pertinax	137 .2	LPF	117 .2	LPF	14 .71	LPF	139 .67	LPF	124 .17	LPF	14 .75	LPF
Forpus coelestis	81	LPF	43	LPF	10 .84	LPF	83 .60	PW	39 .40	PW	12 .10	PW
Forpus conspicillatus	80 .2	PW	36 .6	PW	10 .80	PW	79 .30	PW	38 .10	PW	10 .40	PW
Forpus passerinus	79 .5	LPF	43 .5	LPF	10 .48	LPF	81 .00	LPF	45 .00	LPF	10 .00	LPF
Guaruba guarouba	217 .5	LPF	159 .5	LPF	22 .40	LPF	215 .13	LPF	160 .25	LPF	21 .49	LPF
Lathamus discolor	118 .5	LPF	115 .5	LPF	14 .28	LPF	120 .50	LPF	136 .00	LPF	13 .92	LPF
Lorius chlorocercus	157 .33	LPF	96	LPF	21 .46	LPF	165 .75	LPF	104 .75	LPF	21 .70	LPF
Lorius garrulus	175	LPF	112 .67	LPF	23 .29	LPF	177 .00	LPF	107 .67	LPF	23 .60	LPF
Lorius hypoinochrous	171 .25	LPF	96 .75	LPF	24 .08	LPF	171 .83	LPF	101 .17	LPF	24 .36	LPF
Lorius lory	151 .2	LPF	96	LPF	23 .00	LPF	162 .00	LPF	103 .80	LPF	24 .60	LPF
Neophema chrysostoma	110	LPF	114 .5	LPF	14 .54	LPF	109 .00	LPF	117 .00	LPF	14 .85	LPF
Neophema pulchella	108 .67	LPF	113	LPF	13 .62	LPF	108 .00	LPF	110 .00	LPF	14 .30	LPF
Nestor notabilis	313	LPF	159 .8	LPF	46 .38	LPF	312 .50	LPF	153 .50	LPF	50 .27	LPF
Pionites leucogaster	142 .2	LPF	71 .8	LPF	18 .67	LPF	145 .18	LPF	73 .82	LPF	18 .52	LPF
Pionites xanthomerius	140	PW	68	PW	18 .00	PW	140 .50	PW	67 .70	PW	18 .30	PW
Pionopsitta pileata	144 .75	LPF	73 .5	LPF	15 .54	LPF	152 .33	LPF	79 .00	LPF	15 .11	LPF
Pionus chalcopterus	197	LPF	120 .25	LPF	21 .91	LPF	185 .40	LPF	81 .80	LPF	20 .25	LPF
Poicephalus meyeri	155	LPF	78 .67	LPF	17 .42	LPF	154 .00	LPF	79 .00	LPF	16 .58	LPF
Poicephalus robustus	209 .43	LPF	96 .57	LPF	22 .43	LPF	215 .57	LPF	97 .86	LPF	23 .66	LPF
Poicephalus rufiventris	158	LPF	85	LPF	17 .62	LPF	160 .67	LPF	79 .33	LPF	18 .34	LPF
Primolius auricollis	220	LPF	205	LPF	20 .40	LPF	221 .50	LPF	215 .50	LPF	21 .30	LPF
Primolius couloni	228 .38	LPF	239 .25	LPF	21 .98	LPF	231 .38	LPF	247 .00	LPF	22 .17	LPF
Probosciger aterrimus	328	LPF	235	LPF	29 .29	LPF	347 .67	LPF	242 .67	LPF	31 .21	LPF
Pseudeos fuscata	162 .5	LPF	96 .5	LPF	20 .19	LPF	162 .00	LPF	95 .50	LPF	19 .80	LPF

Psittacara acuticaudatus	191	LPF	188 .67	LPF	17 .88	LPF	200 .00	LPF	197 .50	LPF	18 .98	LPF
Psittacara frontatus	–	–	–	–	–	–	–	–	–	–	–	
Psittacula alexandri	155 .73	LPF	151 .64	LPF	16 .48	LPF	156 .00	LPF	155 .60	LPF	16 .90	LPF
Psittacula cyanocephala	140	LPF	173 .67	LPF	14 .15	LPF	145 .00	LPF	233 .00	LPF	14 .24	LPF
Psittaculirostris edwardsii	112	LPF	67	LPF	16 .12	LPF	110 .50	LPF	62 .75	LPF	16 .83	LPF
Psittacus erithacus	245 .17	LPF	92 .33	LPF	26 .84	LPF	253 .33	LPF	97 .33	LPF	28 .93	LPF
Psitteuteles goldiei	110	LPF	84 .33	LPF	14 .16	LPF	107 .00	LPF	82 .00	LPF	14 .18	LPF
Psitteuteles iris	113	LPF	80	LPF	15 .40	LPF	118 .00	LPF	82 .00	LPF	15 .52	LPF
Psittrichas fulgidus	305	LPF	178 .75	LPF	30 .86	LPF	308 .25	LPF	181 .67	LPF	31 .55	LPF
Pyrrhura griseipectus	115 .8	LPF	116	LPF	13 .35	LPF	118 .67	LPF	122 .00	LPF	13 .37	LPF
Pyrrhura lepida	128 .25	LPF	120	LPF	13 .62	LPF	130 .33	LPF	124 .00	LPF	13 .67	LPF
Pyrrhura melanura	125 .5	LPF	108 .5	PW	13 .90	LPF	128 .00	LPF	104 .33	LPF	14 .33	LPF
Pyrrhura perlata	136 .5	LPF	106	LPF	15 .03	LPF	135 .83	LPF	112 .67	LPF	15 .20	LPF
Pyrrhura rhodocephala	127 .67	LPF	107	LPF	14 .48	LPF	126 .40	LPF	109 .00	LPF	15 .02	LPF
Pyrrhura roseifrons	122 .1	PW	99 .5	PW	14 .00	PW	124 .70	PW	102 .40	PW	13 .80	PW
Pyrrhura rupicola	123 .25	LPF	102 .5	LPF	13 .96	LPF	126 .50	LPF	109 .75	LPF	13 .76	LPF
Rhynchopsitta pachyrhyncha	263	LPF	184	LPF	22 .35	LPF	261 .00	LPF	183 .00	LPF	22 .13	LPF
Rhynchopsitta terrisi	275	LPF	194	LPF	24 .64	LPF	290 .00	LPF	196 .00	LPF	23 .84	LPF
Trichoglossus capistratus	144 .7	PW	113 .4	PW	18 .00	PW	146 .10	PW	116 .10	PW	18 .80	PW
Trichoglossus chrolepidotus	125 .2	PW	96 .9	PW	15 .20	PW	132 .40	PW	102 .60	PW	14 .90	PW
Trichoglossus euteles	129 .5	LPF	112 .5	LPF	15 .43	LPF	134 .20	LPF	113 .80	LPF	15 .82	LPF
Trichoglossus haematodus	142 .07	LPF	116 .19	LPF	17 .96	LPF	144 .74	LPF	121 .03	LPF	18 .30	LPF
Trichoglossus johnstoniae	105 .5	LPF	69 .67	LPF	13 .95	LPF	107 .60	LPF	73 .60	LPF	13 .86	LPF
Trichoglossus moluccanus	146 .3	PW	127 .9	PW	17 .00	PW	150 .80	PW	130 .90	PW	17 .00	PW
Trichoglossus rubritorquis	148 .4	PW	133 .3	PW	17 .40	PW	151 .10	PW	130 .40	PW	17 .50	PW
Vini australis	109	PW	64 .9	PW	14 .40	PW	107 .50	PW	65 .30	PW	14 .70	PW

LPF, Loro Parque Fundacion; PW, Parrots of the world

Table S3. Body size, sexual dichromatism (SSD), gregariousness, male colour elaboration (male CEB), sexual dichromatism (SexDicr), sexual difference in colour elaboration (ElabDiff) and sperm length values per species.

Species	Body size (PC1)	SSD	Gregariousness	Male CEB	SexDicr	ElabDiff	Sperm length
Agapornis taranta	-1 .540	0 .010	0	32 .408	9 .345	0 .462	–
Alipiopsitta xanthops	0 .232	0 .042	0	29 .799	0 .365	0 .094	62 .636
Alisterus chloropterus	0 .729	-0 .017	0	39 .485	39 .319	19 .981	–
Amazona autumnalis	0 .906	0 .034	0	33 .319	0 .438	-0 .026	–
Amazona barbadensis	0 .724	0 .098	0	31 .186	0 .420	-0 .133	40 .377
Amazona finschi	0 .666	0 .059	0	30 .666	0 .437	0 .152	42 .215
Amazona lilacina	0 .469	0 .059	0	32 .886	0 .558	0 .125	–
Amazona ochrocephala	1 .026	0 .033	0	34 .179	0 .328	-0 .044	–
Amazona oratrix	1 .225	0 .066	0	41 .226	0 .512	-0 .040	44 .300
Amazona xantholora	-0 .158	0 .072	0	24 .394	13 .114	-7 .578	–
Anodorhynchus hyacinthinus	6 .890	0 .050	0	66 .672	0 .021	0 .003	–
Ara ambiguus	5 .797	0 .161	0	40 .731	0 .406	-0 .057	42 .882
Ara ararauna	6 .040	0 .059	0	52 .918	0 .291	0 .090	–
Ara chloroptera	6 .223	-0 .004	0	54 .803	0 .357	0 .090	43 .340
Ara glaucogularis	5 .238	0 .108	0	52 .382	0 .318	0 .090	40 .947
Ara macao	5 .729	0 .014	0	59 .800	0 .455	0 .071	–
Ara militaris	4 .788	0 .092	1	36 .155	0 .394	-0 .106	49 .287
Ara rubrogenys	3 .553	0 .047	1	26 .570	0 .433	0 .037	–
Aratinga auricapillus	0 .055	0 .031	0	35 .518	0 .612	-0 .195	–
Aratinga jandaya	-0 .212	0 .000	0	44 .375	0 .184	0 .040	–
Aratinga solstitialis	-0 .237	0 .028	0	42 .531	0 .321	0 .108	46 .907
Aratinga weddellii	-0 .592	0 .001	0	28 .319	0 .708	-0 .179	–
Brotogeris chrysoptera	-1 .383	0 .054	0	21 .290	0 .406	-0 .007	–
Brotogeris cyanoptera	-1 .271	-0 .006	0	29 .084	0 .709	0 .184	54 .947
Brotogeris pyrrhoptera	-1 .295	-0 .008	0	29 .978	0 .536	-0 .063	–
Brotogeris tirica	-0 .920	0 .040	0	29 .038	0 .402	-0 .283	–

Cacatua ducorpsii	1 .264	-0 .004	0	43 .169	0 .335	0 .084	53 .828
Cacatua galerita	2 .355	0 .058	0	43 .685	0 .082	-0 .055	49 .974
Cacatua haematuropygia	1 .000	0 .102	0	40 .491	0 .363	0 .051	55 .467
Cacatua leadbeateri	1 .585	-0 .021	0	40 .932	2 .584	0 .767	46 .914
Cacatua moluccensis	2 .939	0 .042	0	39 .731	0 .344	-0 .120	55 .929
Cacatua ophthalmica	2 .642	0 .013	0	43 .398	2 .067	-0 .638	–
Cacatua pastinator	2 .290	0 .093	0	40 .896	0 .129	0 .004	59 .130
Cacatua sulphurea	1 .381	0 .119	0	41 .377	0 .344	-0 .007	53 .626
Callocephalon fimbriatum	0 .823	-0 .071	0	30 .209	17 .002	3 .855	–
Calyptorhynchus funereus	4 .016	0 .051	0	32 .932	1 .332	0 .512	53 .418
Chalcopsitta atra	0 .630	0 .095	0	36 .544	0 .351	-0 .144	–
Chalcopsitta cardinalis	0 .673	0 .025	0	46 .476	0 .264	-0 .009	–
Chalcopsitta duivenbodei	0 .521	-0 .030	0	29 .522	0 .683	0 .078	–
Chalcopsitta scintillata	0 .320	0 .043	0	23 .777	0 .482	0 .155	–
Charmosyna josefinae	-0 .625	0 .017	0	35 .957	5 .619	0 .605	–
Charmosyna papou	0 .588	0 .041	–	34 .504	0 .107	0 .063	–
Coracopsis nigra	1 .541	0 .099	0	35 .735	0 .462	-0 .016	–
Cyanoliseus patagonus	2 .176	-0 .005	1	27 .074	0 .394	0 .146	–
Deroptyus accipitrinus	0 .766	0 .044	0	29 .319	0 .809	0 .199	–
Diopsittaca nobilis	0 .099	0 .003	0	25 .730	0 .647	0 .255	–
Eclectus roratus	1 .176	0 .104	1	19 .315	45 .205	-26 .160	74 .469
Eolophus roseicapilla	1 .350	0 .051	1	38 .754	1 .628	0 .066	–
Eos bornea	-0 .011	0 .016	0	58 .055	0 .556	-0 .194	59 .973
Eos histrio	0 .287	-0 .002	0	50 .656	0 .801	0 .048	–
Eos reticulata	0 .388	0 .044	–	52 .033	0 .722	-0 .039	–
Eos squamata	-0 .432	-0 .010	–	52 .269	0 .749	0 .048	–
Eupsittula aurea	-0 .526	0 .006	0	37 .162	0 .521	-0 .032	–
Eupsittula pertinax	-0 .634	0 .021	1	30 .566	0 .511	-0 .101	–
Forpus coelestis	-1 .932	0 .022	0	25 .005	16 .611	2 .743	–
Forpus conspicillatus	-2 .114	-0 .008	0	27 .582	14 .454	-1 .727	–
Forpus passerinus	-2 .098	0 .013	0	27 .368	10 .899	-4 .238	–
Guaruba guarouba	0 .878	-0 .020	1	49 .955	0 .169	-0 .008	–

Lathamus discolor	-0 .787	0 .017	1	35 .333	0 .649	0 .280	54 .598
Lorius chlorocercus	0 .049	0 .071	–	38 .779	0 .768	-0 .125	–
Lorius garrulus	0 .361	0 .017	0	40 .144	0 .761	0 .037	66 .383
Lorius hypoinochrous	0 .346	0 .005	0	38 .071	0 .504	0 .159	49 .852
Lorius lory	0 .259	0 .092	0	34 .964	0 .338	0 .002	–
Neophema chrysostoma	-0 .920	-0 .008	1	30 .023	7 .464	-1 .826	–
Neophema pulchella	-1 .028	-0 .006	0	40 .433	18 .233	5 .982	–
Nestor notabilis	4 .219	-0 .004	0	21 .960	1 .249	-0 .308	–
Pionites leucogaster	-0 .600	0 .025	0	34 .409	0 .707	0 .082	–
Pionites xanthomerius	-0 .691	0 .004	0	33 .214	0 .510	0 .207	–
Pionopsitta pileata	-0 .827	0 .064	0	30 .785	11 .060	1 .496	–
Pionus chalcopterus	0 .013	-0 .098	0	41 .724	0 .631	0 .002	–
Poicephalus meyeri	-0 .646	-0 .008	0	23 .987	0 .470	-0 .210	–
Poicephalus robustus	0 .609	0 .052	0	23 .572	4 .411	-1 .733	–
Poicephalus rufiventris	-0 .445	0 .023	1	25 .212	8 .071	2 .924	–
Primolius auricollis	1 .280	0 .013	0	28 .638	0 .741	0 .341	55 .618
Primolius couloni	1 .652	0 .026	0	26 .038	0 .281	0 .192	–
Probosciger aterrimus	3 .346	0 .167	0	35 .914	0 .334	0 .117	–
Pseudeos fuscata	-0 .179	-0 .004	0	33 .893	0 .973	0 .284	–
Psittacara acuticaudatus	0 .734	0 .076	0	33 .157	0 .316	-0 .183	49 .443
Psittacara frontatus	–	–	1	39 .533	0 .214	-0 .118	–
Psittacula alexandri	-0 .077	0 .002	1	19 .954	5 .299	-0 .035	71 .322
Psittacula cyanocephala	0 .106	0 .042	1	32 .504	17 .992	12 .491	–
Psittaculirostris edwardsii	-1 .102	-0 .013	0	22 .786	8 .928	-2 .690	–
Psittacus erithacus	1 .388	0 .069	1	32 .805	0 .244	0 .087	70 .085
Psitteuteles goldiei	-1 .230	-0 .025	0	24 .189	0 .483	-0 .120	–
Psitteuteles iris	-1 .051	0 .042	0	27 .224	0 .459	-0 .076	–
Psittrichas fulgidus	2 .690	0 .028	0	39 .656	1 .853	0 .042	–
Pyrrhura griseipectus	-0 .952	0 .024	0	27 .522	0 .542	0 .011	57 .209
Pyrrhura lepida	-0 .809	0 .018	0	31 .865	0 .890	0 .242	54 .225
Pyrrhura melanura	-0 .908	0 .021	0	23 .907	0 .780	0 .179	–
Pyrrhura perlata	-0 .693	-0 .006	0	30 .760	0 .614	0 .122	58 .23

Pyrrhura rhodocephala	-0 .811	-0 .011	0	37 .339	0 .643	0 .154	–
Pyrrhura roseifrons	-0 .996	0 .022	0	30 .636	0 .745	0 .041	–
Pyrrhura rupicola	-0 .937	0 .028	0	21 .835	0 .959	0 .064	47 .765
Rhynchopsitta pachyrhyncha	1 .480	-0 .017	0	24 .393	0 .307	-0 .097	–
Rhynchopsitta terrisi	1 .896	0 .127	1	20 .357	0 .511	0 .045	–
Trichoglossus capistratus	-0 .269	0 .012	0	30 .652	0 .522	-0 .175	–
Trichoglossus chlorolepidotus	-0 .851	0 .061	0	21 .160	0 .366	-0 .103	62 .338
Trichoglossus euteles	-0 .666	0 .040	0	22 .620	0 .418	0 .130	–
Trichoglossus haematodus	-0 .296	0 .023	0	28 .324	0 .315	0 .061	52 .850
Trichoglossus johnstoniae	-1 .333	0 .018	0	24 .839	0 .839	-0 .302	–
Trichoglossus moluccanus	-0 .301	0 .038	0	30 .987	0 .618	0 .023	–
Trichoglossus rubritorquis	-0 .249	0 .023	0	29 .718	0 .527	-0 .041	–
Vini australis	-1 .300	-0 .013	0	27 .366	0 .446	-0 .127	–

Gregariousness (“yes”=1, “no”=0)

CEB, colour elaboration; ElabDiff, sexual difference in colour elaboration; PC1, first principal component; SexDicr, sexual dichromatism; SSD, sexual size dimorphism

General discussion

Looking for evidence of sexual selection in parrots

The aim of my PhD was to investigate whether sexual selection has had an effect on the evolution of morphological traits and behaviours associated with reproduction in the order Psittaciformes. As there are other factors that can affect the evolution of these characteristics, I specifically explored whether environmental factors, social interactions and life-history traits, together with sexual selection, have shaped the evolution of sperm morphology, sexual dichromatism and mating behaviour across parrots by using comparative analyses.

Parrots are well known for their fascinating plumage colouration and their practice of life-long monogamy (Toft & Wright, 2015). However, detailed studies exploring the mechanisms driving the evolution of plumage colouration and the social and genetic mating systems of parrots are relatively scarce (Berg & Bennett, 2010; Toft & Wright, 2015). According to previous studies, it appears that the majority of parrots are socially monogamous, with some species also being genetically monogamous (Caparroz et al., 2011; Eastwood et al., 2018; Masello et al., 2002) and some others showing varying levels of extrapair paternity (Waltman & Beissinger, 2016; Heinsohn et al., 2019; Martínez et al., 2013). To further understand whether parrots as a group experience post-copulatory sexual selection via sperm competition, it is necessary to further explore the levels of extrapair paternity in different species. Extrapair paternity rates are not easy to estimate because parental analysis requires detailed data that can only be obtained with established, long-term studies. An alternative proxy to understand whether species experience sperm competition is to measure their sperm morphology.

In **Chapter 1**, I found that within the 62 parrot species included in the analyses there was variation in the mean sperm length and the within-male coefficient of variation in sperm length. Both of these sperm competition proxies were also related to relative testes mass, which has long been used as an indicator of sperm competition intensity because species with high levels of extrapair paternity have relatively larger testes (Lüpold et al., 2009; Møller & Briskie, 1995). Additionally, by using the sperm competition proxies (mean sperm length and within-male coefficient of variation in sperm length), the results reported in **Chapter 1** indicate that different parrot species *do* have different levels of sperm competition. This finding provides evidence to suggest that not all parrot species are strictly monogamous as it has been previously suggested.

The next question to address is what factors are behind the varying levels of sperm competition in the different parrot species. Among the different predictors used to evaluate their effect on mean sperm length and within-male coefficient of variation in sperm length, I found that sexual dichromatism and gregariousness were significantly positively correlated with mean sperm length, indicating that sexually dichromatic species and gregarious species potentially have stronger sperm competition. A link between sexual dichromatism and extrapair paternity rates has been previously found in a comparative analysis that included 73 bird species (Owens & Hartley, 1998). It is therefore possible that among parrots, those species that are sexually dichromatic and those that breed gregariously also have higher extrapair paternity rates. When pairs breed close together there are more opportunities for individuals to engage in extrapair copulations, as the cost of looking for extra mates is low. In **Chapter 1**, I also found a significant positive relationship between gregariousness and sexual dichromatism. For this reason, I suggested that sexual ornamentation in parrots might have evolved in gregarious species as sexual selection via extrapair paternity might be stronger in

these species, however, causal links need to be further investigated to corroborate this suggestion.

Further understanding sexual dichromatism in parrots

In **Chapter 1**, I used a binary variable to describe a species as sexually dichromatic or monochromatic, thus the level of sexual dichromatism of a given species was assigned as 'present' or 'absent'. The downside of this approach is that it was not possible to estimate different levels of sexual dichromatism. For example, a species where males and females differ in colouration in a single patch on the tail and a species where males and females show completely different colours across all their body would both be equally classified as sexually dichromatic. For this reason, in **Chapter 2**, I performed a more detailed assessment of the plumage colouration of males and females, and with this more detailed measure I then estimated the *degree* of sexual dichromatism of each parrot species. By using bookplates, I was able to measure the colour of 12 different body patches of each sex per species, and then used these values to calculate a colour elaboration score and sexual dichromatism indexes for each of the 398 extant parrot species illustrated in the *Handbook of the Birds of the World* (del Hoyo et al., 2017). This much larger dataset allowed me to explore what factors are behind the fascinating variation of plumage colour elaboration and sexual dichromatism in parrots.

For this part of the analysis, in **Chapter 2**, I explored whether the intensity of sexual selection, social interactions, life-history traits and environmental variables were correlated with colour elaboration and sexual dichromatism in parrots. I found that larger parrot species are more colour elaborated but are less dichromatic than smaller parrot species. Additionally,

regarding environmental factors, I found that species that live in wetter habitats have darker and redder colours than species living in drier habitats, while species that live in warmer habitats have bluer colours compared with species living in colder habitats. Overall, these predictors explained a small to moderate variation (up to 15%) of the colour elaboration and sexual dichromatism in parrots.

The relationship found between colour elaboration and body size supports the hypothesis that larger species experience less predation risk and therefore can pay the cost of displaying colourful plumage (Ricklefs, 2010). The opposite side of this coin is that the plumage colouration in smaller parrots is less elaborated as these species likely experience higher selective pressure for crypsis compared with larger parrots. Interestingly, smaller species showed higher levels of sexual dichromatism than larger species which might be explained by the difference in lifespan. Due to their shorter lifespan (Bennett & Owens, 2002; de Magalhaes et al., 2007; Wasser & Sherman, 2010), smaller parrots have a reduced chance to mate with the same partner in subsequent breeding seasons (as they have higher mortality rates) (Mauck et al., 1999), and under this scenario, higher levels of extrapair paternity might be endured. As previously mentioned, it has been found that sexual dichromatism is related with the level of extrapair paternity (Owens & Hartley, 1998), and this can therefore explain why smaller parrots are more dichromatic; those that advertise themselves to more mating opportunities during their short lives are selected for due to more offspring. On the other hand, larger parrots are less dichromatic, but I have also found that these are generally more colour elaborated. These results are likely explained by mutual mate choice. As larger parrots live longer (Wasser & Sherman, 2010), they might take more time selecting the right partner, and colour elaboration may be the cue that both sexes use to select with whom they bond. Colour elaboration in parrots, however, is not only explained by sexual selection. I found that

environmental variables, such as precipitation and temperature, also play a role in the variation of plumage colouration in parrots. Although, the adaptive explanation is yet to be understood, I showed that parrots display darker colours in wetter environments, following Gloger's Rule (Rensch, 1936). Additionally, I found that species that are more colour elaborated inhabit warmer environments. This observation supports previous findings showing that tropical species (where temperatures are higher) are more colourful than temperate species (Dale et al., 2015; Willson & von Neumann, 1972), possibly explained by mutual mate choice which is stronger in the tropics or by social selection via resource competition, with colour elaboration evolving as a signalling status in aggressive encounters (Tobias et al., 2012). There are likely to be number of complex interactions between these variables that have led to this snapshot of current colourations in extant parrots. Without more detail in the historical colorations of parrots' ancestors, parsing apart these interactions is a difficult task.

Mating behaviour in parrots

So far, I have shown how the variation in morphological traits at the phenotypic and cellular level (plumage colouration and sperm length) have been shaped to some extent by sexual selection but also by life-history traits, environmental factors and social interactions. The next question I wanted to address was whether these factors have also driven the evolution of mating behaviour in parrots.

Different hypotheses have been proposed to understand the great variation on the frequency and duration of within-pair copulations in birds (Birkhead & Moller, 1992). However, the sperm competition and the predation risk hypotheses are the most plausible explanations to

describe the variation in the frequency and duration of within-pair copulations during the fertile period (Birkhead & Moller, 1992). Under the sperm competition scenario, it would be expected that species with higher risk of extrapair paternity would have more frequent and longer within-pair copulations as a behavioural mechanism to counteract the risk of paternity loss. Under the predation risk scenario, it would be expected that species with higher mortality risk would show less frequent and shorter within-pair copulations.

In **Chapter 3**, I explored whether these two hypotheses could explain the variation in mating behaviour across 103 parrot species breeding in captivity. Specifically, using a comparative approach, I evaluated whether the variation in frequency and duration of within-pair copulations could be explained by body size, gregariousness, sexual dimorphism, sexual dichromatism, male colour elaboration or sperm length. However, I found no statistically significant effect of any of the predictors included in this study on the frequency and duration of within-pair copulations in parrots.

With these results, it would be possible to suggest that the non-significant effect indicates that parrots do not show varying levels on the duration and frequency of within-pair copulations because they are socially and genetically monogamous. However, in **Chapter 1** and **Chapter 2** I showed evidence that sexual selection via sperm competition seems to have an effect on the evolution of sperm morphology and plumage colouration across parrots. Therefore, I consider that the captive conditions under which the individuals I observed were bred and held might explain the lack of effects on mating behaviour found in **Chapter 3**. By living in captive conditions, the breeding pairs are not exposed to the predation risk and sperm competition risk that their counterparts will do in wild conditions. In the wild, individuals might adjust the duration and frequency of the within-pair copulations in response to the potential risk of being predated (Birkhead, 1987; Mougeot, 2004) or cuckolded.

As collecting mating behaviour observations in natural conditions might be challenging due to parrots breeding high in the forest canopies, more studies (including more breeding pairs per species and more species) would need to be done in captive conditions to further understand parrot mating behaviour.

Conclusions

The findings of my PhD thesis showed that different factors have shaped the evolution of parrots, and that contrary to previous beliefs, parrots are not all strictly monogamous species. Although I found evidence of sexual selection via sperm competition affecting parrot plumage colouration and sperm morphology variation, there are many other factors, such as environmental variables, life-history traits and social interactions, driving the evolution of the species in this group. Additionally, my thesis reflects the lack of knowledge we currently have about parrots and how much more it needs to be explored to be able to provide necessary tools to contribute to the conservation of this magnificent group of birds that are currently at great risk of extinction.

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Luisana Carballo González

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Chapter 1: L.C., M.V. and B.K. conceived the study. L.C., A.Ba., M.L. and A.Bu. collected the data. K.T. measured the sperm. L.C. and M.V. analysed the data with input from B.K. L.C. wrote the paper with help of B.K. and input from M.V.

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Chapter 3: L.C., M.V. and B.K conceived the study. L.C collected the data. L.C. and M.V. analysed the data with input from B.K. L.C. wrote the paper with help of B.K. and input from M.V.

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Publications

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- **Carballo, L**., Battistotti, A., Teltscher, K., Lierz, M., Bublat, A., Valcu, M. & Kempenaers, B. (2019). Sperm morphology and evidence for sperm competition among parrots. *Journal of Evolutionary Biology*. 32, 856–867. doi: 10.1111/jeb.13487
- **Carballo, L**., Kaspar, D., Valcu, M. & Kempenaers, B. (2020). *Body size and climate as predictors of plumage colouration and sexual dichromatism in parrots*. *Journal of Evolutionary Biology*. 33, 1543–1557. doi: 10.1111/jeb.13690
- Czarkwiani, A., Dylus, D. V., **Carballo, L**. & Oliveri, P. (2021). FGF signalling plays similar roles in development and regeneration of the skeleton in the brittle star *Amphiura filiformis*. *Development.* 148(10), dev180760. doi:10.1242/dev.180760

Fieldwork experience

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Methods skill-set

- Microsoft office (Word, Excel, PowerPoint).
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- Laboratory techniques: Cloning, PCR, QPCR and In situ hybridization.

Statutory declaration and statement

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den 11/10/2022 Luisana Carballo González

(Unterschrift)

Erklärung

Hiermit erkläre ich, *

☒ dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

☒ dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

☒ dass ich mich mit Erfolg der Doktorprüfung im Hauptfach ..Naturwissenschaften……
und in den Nebenfächern Biowissenschaften.....a
bei der Fakultät für ..Biologie…….. der ..Ludwig-Maximilians-Universitat Munchen
(Hochschule/Universität)
unterzogen habe.

☐ dass ich ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

München, den 11/10/2022 Luisana Carballo González

(Unterschrift)

*) Nichtzutreffendes streichen