

Sperm competition and the evolution of testes size in birds

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Abstract

Comparative analyses suggest that a variety of ecological and behavioural factors contribute to the tremendous variability in extrapair mating among birds. In an analysis of 1010 species of birds, we examined several ecological and behavioural factors in relation to testes size; an index of sperm competition and the extent of extrapair mating. In univariate and multivariate analyses, testes size was significantly larger in species that breed colonially than in species that breed solitarily, suggesting that higher breeding density is associated with greater sperm competition. After controlling for phylogenetic effects and other ecological variables, testes size was also larger in taxa that did not participate in feeding their offspring. In analyses of both the raw species data and phylogenetically independent contrasts, monogamous taxa had smaller testes than taxa with multiple social mates, and testes size tended to increase with clutch size, which suggests that sperm depletion may play a role in the evolution of testes size. Our results suggest that traditional ecological and behavioural variables, such as social mating system, breeding density and male parental care can account for a significant portion of the variation in sperm competition in birds.

Introduction

Molecular techniques have revealed multiple paternity in a broad range of species (reviewed in Birkhead & Møller, 1998), including species that were once thought to be both socially and genetically monogamous. Multiple mating has ramifications for sexual selection, as it provides a mechanism for sperm competition (e.g. Parker *et al.*, 1990; Parker, 1998) and, consequently, increased variance in male mating success (Webster *et al.*, 1995) and sexual conflict (e.g. Holland & Rice, 1998; Martin *et al.*, 2004). In monogamous birds, multiple mating typically occurs as a consequence of copulations between individuals located on nearby territories. These extrapair copulations often appear to be initiated by males that leave their territories to visit neighbouring females, but females in many populations also engage in extraterritorial forays (reviewed by Westneat & Stewart, 2003),

which suggests that females also benefit from extrapair matings.

The costs and benefits of extrapair mating appear to vary widely, as the incidence of extrapair paternity varies across species from 0 to ~70% of offspring in a nest (Griffith *et al.*, 2002). Ecological constraints are likely to limit the opportunity for both males and females to engage in extrapair mating. However, the extent to which ecological and behavioural factors account for the tremendous variability among birds in extrapair mating remains unclear. Attempts to explain interspecific variation in avian extrapair mating have been dominated by three factors: breeding synchrony, breeding density, and the extent of male participation in parental care (reviewed by Neudorf, 2004).

First, the synchrony of breeding females in a population may have positive or negative effects on the frequency of extrapair mating. For example, earlier studies suggested that greater breeding synchrony will reduce the ability of males to gain extrapair mates, simply because the ratio of fertilizable females to sexually active males (the operational sex ratio, Emlen & Oring, 1977) is closer to unity, and, thus, males have fewer mating opportunities with extrapair females (Westneat *et al.*,

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1990). On the contrary, it has been suggested that greater breeding synchrony facilitates extrapair mate choice by females, and, thus, extrapair mating will be greater when breeding is more synchronous (Stutchbury & Morton, 1995, 2001; Stutchbury, 1998). Greater breeding synchrony may facilitate extrapair mate choice if it increases male–male competition for extrapair matings and, thus, allows females to assess more easily the quality of potential extrapair mates (Stutchbury & Morton, 1995). Therefore, factors that influence the level of breeding synchrony in a population should be related to levels of extrapair mating. In particular, if breeding synchrony is related positively to extrapair mating, then we might expect species breeding at higher latitudes to have greater levels of extrapair mating than species breeding at lower latitudes because of the shorter, and thus more synchronous, breeding seasons at higher latitudes. Migratory species often breed at higher latitudes, so we might also predict that they will have greater levels of extrapair mating than nonmigratory species (see Pitcher & Stutchbury, 1998; Stutchbury *et al.*, 2004).

Secondly, the spatial distribution of breeding birds may also influence the rate of encounter between potential extrapair partners and, consequently, the level of extrapair mating. For example, greater breeding density may increase the availability of potential extrapair mates, and, indeed, extrapair copulations appear to be more common among colonially breeding species than among species with more dispersed nesting habits (e.g. Møller & Birkhead, 1993). However, for a number of reasons, frequent extrapair copulation does not necessarily result in high levels of extrapair paternity (Dunn & Lifjeld, 1994). Furthermore, greater breeding density may increase the risk of paternity loss at a male's own nest. Thus, it is not clear how breeding density should influence levels of extrapair paternity. Confounding factors such as breeding synchrony could also interact with breeding density to obscure any influence of density on levels of extrapair paternity (see Thusius *et al.*, 2001). Thus, it may not be surprising that comparative studies have generally found little evidence for a relationship between breeding density and extrapair paternity across species (reviewed by Griffith *et al.*, 2002). However, there is some evidence that breeding density influences variation in levels of extrapair paternity among populations (e.g. Richardson & Burke, 2001). This difference between inter- and intra-specific studies may simply reflect the poorer ability to control for confounding factors in inter-specific studies.

Thirdly, the level of male parental care may alter the costs and benefits of pursuing extrapair matings. Across species male parental care is generally expected to be related negatively to levels of extrapair paternity (reviewed by Whittingham & Dunn, 2001). This negative relationship could arise several ways. First, it is often thought that males provide less care when they have lower paternity because it reduces the cost of cuckoldry.

Alternatively, high levels of extrapair paternity may make it more profitable for males to pursue extrapair matings than to care for their young. In this case, the costs and benefits of both parental care and extrapair mating need to be considered. Some forms of male parental care may be more costly than others, and, thus, species in which males perform more costly forms of parental care may be more constrained in their pursuit of extrapair matings than species in which males do not perform the same type of parental care. For example, Ketterson & Nolan (1994) argue that incubation restricts the ability of males to pursue extrapair mating more than other types of parental care. Incubation may be especially costly to males because it is more time consuming and less readily deferred than other forms of parental care. Furthermore, incubating males have lower levels of testosterone which may lead to a reduction in male mating behaviour.

Although several studies test some of these explanations for variation in extrapair paternity across species of birds (reviewed in Petrie & Kempenaers, 1998; Griffith *et al.*, 2002), further study is warranted for several reasons. First, to date only a small subset of bird species in terms of both phylogeny and geographical range have been examined. This may be skewing our understanding of avian promiscuity because of the reliance on paternity studies from primarily temperate zone passerine species, resulting in a 'temperate zone bias' (*sensu* Stutchbury & Morton, 2001). Secondly, there have been inconsistent results of interspecific tests; for example, some studies have shown that species with more synchronous breeding do indeed experience increased levels of extrapair mating (e.g. Stutchbury & Morton, 1995; Stutchbury, 1998) and others have found no such relationship (e.g. Westneat & Sherman, 1997). These inconsistencies may result from differences in sample size and in the phylogenetic and geographical span of the species included.

In this study we used variation in testes size (controlling for body size), as an index of extrapair mating in 1010 species of birds. We used testes size, rather than molecular parentage data, because testes size is correlated with population levels of extrapair paternity (see Møller & Briskie, 1995) and is available for a much broader range of species in terms of phylogeny and geography. Furthermore, it could be argued that testes size provides an excellent alternative estimate of sperm competition and extrapair mating, because testes size has been selected over evolutionary time and paternity analyses are often short-term and have small sample sizes. We compiled testes size data from species of birds from around the globe and used the comparative method to test for associations between variation in testes size and indices of breeding synchrony, breeding density and male parental care.

Materials and methods

Some of the data used in this study were accumulated for our earlier study of sexual dimorphism (Dunn *et al.*,

2001), which included data on testes mass, mating system, migratory behaviour, incubation behaviour and geographical region. For this study, additional data on breeding density, male provisioning of offspring, and clutch size were gathered from the literature (e.g. Cramp & Perrins, 1993, Birds of North America series, Reader's Digest, 1990). The entire dataset described here is available from the authors as an electronic appendix.

Testes mass

Testes mass (corrected for body mass) is considered to be a good measure of sperm competition and multiple mating in birds (Birkhead & Møller, 1992; Møller & Briskie, 1995; Dunn *et al.*, 2001) and many other taxa (e.g. Stockley *et al.*, 1997; Hosken & Ward, 2001; Byrne *et al.*, 2002). Males can increase their probability of fertilizing a female's eggs by producing large quantities of sperm and copulating frequently with a particular female (Birkhead & Møller, 1992; Birkhead, 1998). The ability to produce frequent ejaculates with a high volume of sperm is determined in large part by testes size (Møller, 1988), and is presumably advantageous when male–male competition occurs via sperm competition (see Møller & Briskie, 1995). Large testes allow males to inseminate their own mate(s) more frequently as a defense against extrapair mating and to copulate with more extrapair females. Testis size may also vary in relation to geography, social mating system and clutch size (see Cartar, 1985; Birkhead & Fletcher, 1995; Rising, 1996), and thus, we have also included these variables in our analyses (see below).

Testis mass for each species was obtained from the literature and museum specimens (see Dunn *et al.*, 2001 for details). Testis mass was estimated from museum specimen measurements (i.e. testis length and width) using the formula: testis mass (g) = $2 \times 1.087 \text{ g cm}^{-3} \times 1.33\pi[a(\text{cm})]^2b(\text{cm})$, where a and b are the width and length of each testis (see Pitcher & Stutchbury, 1998). Species from every continent were sampled, but most were from North America (36%), Australasia (36%), and South and Central America (15%). For each species, average testes mass was calculated as the mean for at least five breeding males, but typically testes mass was averaged for 10 or more breeding males (Dunn *et al.*, 2001). If multiple estimates of testes mass were available from the literature we used the mean of the different measures. Relative testes mass was estimated as the residuals from the regression of log testes mass on log body mass.

Migration

Our index of breeding synchrony was based on migratory behaviour. Each species was classified as either migratory (synchronous breeder) or nonmigratory (a less synchronous breeder). Species were considered migratory if they had largely nonoverlapping winter and summer ranges ($\leq 50\%$ overlap) and resident if there was little seasonal

change in distribution ($>50\%$ overlap in ranges; see Dunn *et al.*, 2001).

Breeding density

Our index of breeding density was based on the nesting dispersion of each species. Each species was classified as: (i) colonial, if nests were closely aggregated in particular breeding sites with inter-nest distance not usually exceeding nest diameters by one to two orders of magnitude, (ii) semi-colonial, if nests were built within close proximity to each other (inter-nest distance usually exceeding nest diameters by one to two orders of magnitude) and (iii) solitary, if nests were separated from each other by large, all-purpose breeding territories (see Siegel-Causey & Kharitonov, 1990).

Parental investment

Each species was classified according to male participation in incubation (yes or no) and feeding of offspring (yes or no). Both of these factors are likely to affect the ability of males and females to pursue matings outside of the pairbond (e.g. Schwagmeyer *et al.*, 1999; Pitcher & Stutchbury, 2000).

Potentially confounding variables

In order to account for geographical variation in testes size (see Stutchbury & Morton, 2001), for each species, we classified their primary breeding region as Eurasia, North America (north of Mexico), Africa, South and Central America, or Australasia (Australia, New Guinea and New Zealand). In cases where species overlapped two or more regions, we used the region from which we collected the most testes size data. To control for the potentially confounding effects of number of inseminations performed by males on the evolution of testes size we assigned species according to their social mating system and clutch size (see Birkhead & Fletcher, 1995). Following Dunn *et al.* (2001) we assigned species to one of six mating system categories: (1) monogamy ($<5\%$ polygyny); (2) mostly monogamy; but occasional polygyny ($5\text{--}15\%$ polygyny); (3) mostly polygyny ($>15\%$ polygyny); (4) cooperative breeding; (5) polyandry and (6) lek or promiscuous. For clutch size we used the midpoint of the range of clutch sizes given for each species using standard references (e.g. Cramp & Perrins, 1993, Birds of North America series). We included the breeding region, social mating system and clutch size in both univariate and multivariate (i.e. multiple regression) analyses to examine their potential effects on testes size.

Comparative methods

Comparisons across species can be confounded by common ancestry (Harvey & Pagel, 1991). However,

we analysed both raw species data and independent contrasts because differences between these types of analyses may provide biological insights (see Dunn *et al.*, 2001). To control for phylogeny, we calculated standardized linear contrasts (Harvey & Pagel, 1991) using Comparative Analysis of Independent Contrasts (CAIC) (Purvis & Rambaut, 1995). Contrasts were standardized assuming that lengths of branches in the phylogeny were: (1) equal in length which represents a punctuated model of evolution or (2) proportional to the number of taxa in each clade which is similar to a gradual model of evolution (Harvey & Pagel, 1991). These two models of evolutionary change produced qualitatively similar results in our study and thus we present only the results from the gradual model of evolution [i.e. (2), (c.f. Dunn *et al.*, 2001)]. Our phylogeny was based on the molecular phylogeny of Sibley & Ahlquist (1990), which provides a branching pattern to the level of family, subfamily or tribe, depending on the clade. When we had more than two species below the lowest level in Sibley and Ahlquist's phylogeny, we used recent phylogenetic analyses to complete the phylogeny to the species level, or, if there were not sufficient data to construct a fully bifurcating phylogeny, we formed polytomies (see Dunn *et al.*, 2001 for details).

Tests of association between traits were performed by comparing the contrasts of one trait against the contrasts of another trait. To control for the ambiguity associated with determining the sign of the independent contrasts, all regressions were forced through the origin, as suggested by Harvey & Pagel (1991). We used the CRUNCH procedure of CAIC to analyse both continuous and categorical variables. Categorical variables (geographical region, mating system, social dispersion, incubation and feeding young) were examined using dummy variables that were phylogenetically transformed (see Dunn *et al.*, 2001 for details).

Finally, we performed multivariate analyses to control for any potentially confounding relationships because of differences among species in geography, social mating system and clutch size. However, complete data were not available for all species. All seven independent variables (see above) were available for 598 of 1010 species. Thus, adding all variables to one regression model reduced our sample size to 362 phylogenetically independent contrasts. This change in sample size also changed the branches (and taxa) examined, and, thus, the differences we observed between univariate and multivariate analyses could result from species differences or sample size differences.

Results

Overall, testes mass averaged $1.03 \pm 0.03\%$ of total body mass (range: 0.01–9.8%) and testes mass was related positively to body mass [$F_{1,1008} = 892.5$, $r^2 = 0.47$, $P < 0.001$, $\log(\text{testes mass (g)}) = -1.56 + 0.61 \log(\text{body$

mass (g)); Fig. 1]. Below we examine variation in testes size in relation to migratory behaviour, social dispersion, two types of male parental care, geography, social mating system, and clutch size. We first present the univariate analyses and then the multivariate analyses of both the species data and independent contrasts.

Migratory taxa had larger testes than nonmigratory taxa (species data: t -test: $t_{1,1000} = 3.43$, $P < 0.001$; independent contrasts: t -test: $t_{1,457} = 3.90$, $P < 0.001$; Fig. 2a). Testes size was also related positively to coloniality (species data: ANOVA: $F_{2,895} = 4.15$, $P < 0.05$; independent contrasts: ANOVA: $F_{2,411} = 4.61$, $P < 0.05$; Fig. 2b); testes size was larger in taxa that bred colonially and semi-colonially and smaller in taxa that bred solitarily (Tukey–Kramer tests, $P < 0.05$).

Species-level analyses revealed that testes size was significantly larger in species in which males do not participate in incubation (t -test: $t_{1,671} = 4.67$, $P < 0.001$; Fig. 3a) relative to species in which males do incubate. However, independent contrast analyses showed that there was no difference in testes size among species in relation to male incubation (t -test: $t_{1,363} = 0.49$, n.s.; Fig. 3a). Testes size did not differ among species in relation to male provisioning of the young (species data: t -test: $t_{1,930} = 1.58$, $P = 0.11$; independent contrasts data: t -test: $t_{1,426} = 0.66$, n.s.; Fig. 3b).

Testes size was related to the geographical range for breeding (species data: ANOVA: $F_{4,1005} = 9.58$, $P < 0.001$; independent contrasts: ANOVA: $F_{4,458} = 4.61$, $P < 0.05$; Fig. 4a). Testes were larger in taxa that bred in Eurasia and smaller in other regions, particularly South and Central America and Australasia (Tukey–Kramer tests, $P < 0.05$; see Fig. 4a). Testes size varied among social mating systems (species data: ANOVA: $F_{5,996} = 7.13$, $P < 0.001$; independent contrasts: ANOVA: $F_{5,454} = 6.44$, $P < 0.001$; Fig. 4b). Testes size was significantly larger in

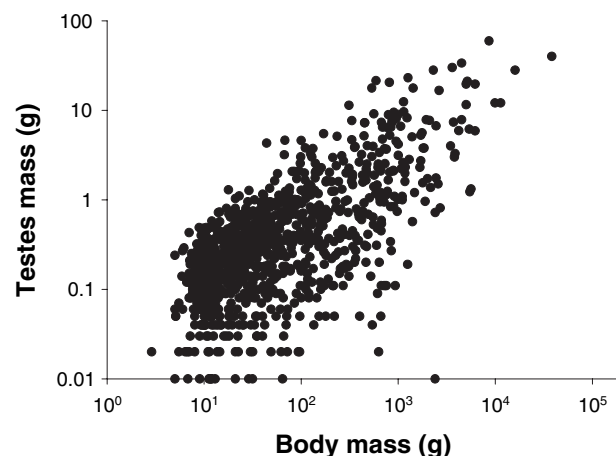


Fig. 1 Relationship between the logs of testes mass and body mass [$\log(\text{testes mass (g)}) = -1.56 + 0.61 \log(\text{body mass (g)})$].

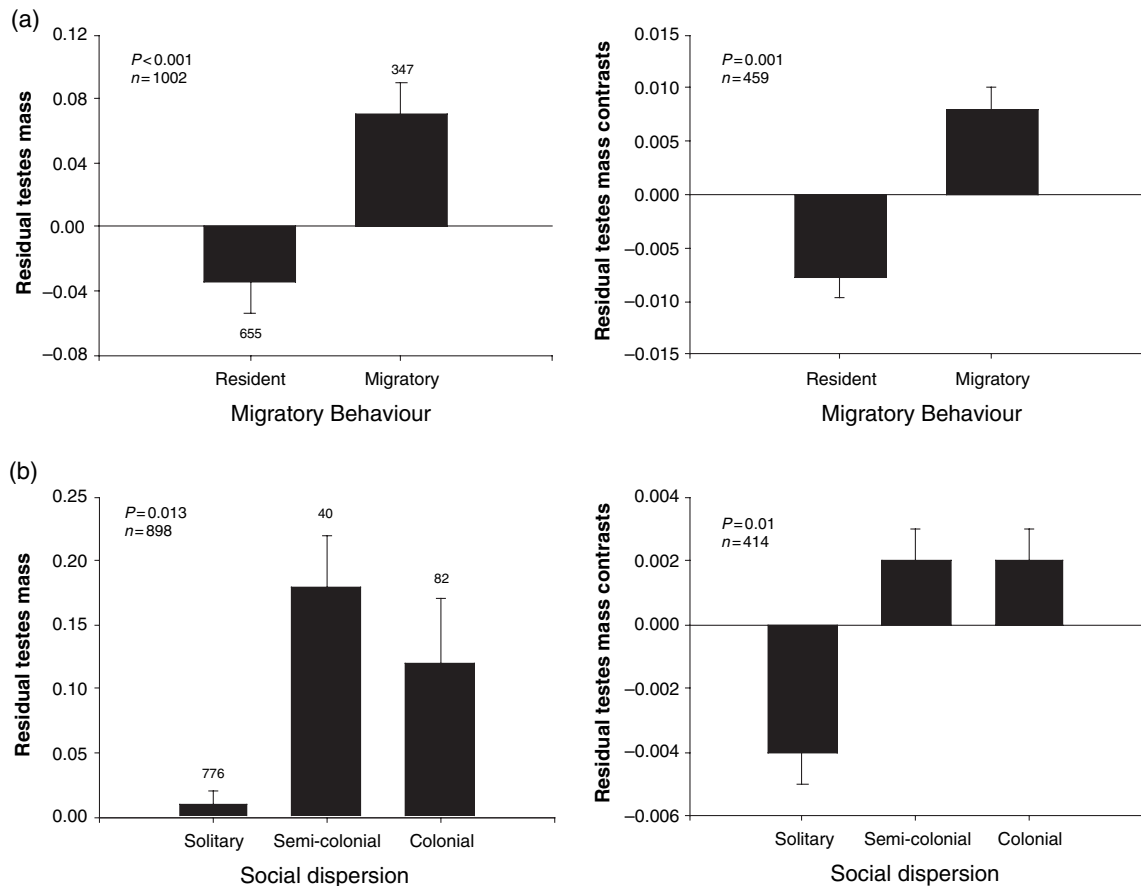


Fig. 2 Relative testes size in birds in relation to (a) migration and (b) breeding density. Panels on the left are based on bivariate analyses of species data, whereas panels on the right are based on analyses of phylogenetically independent contrasts. Mean and 1 SE are shown for each variable (see Methods for calculations).

social mating systems with multiple mates (polyandrous, polygynous and cooperative breeding) relative to monogamous taxa (Tukey–Kramer tests, $P < 0.05$, Fig. 4b). Testes size was related positively to clutch size, using both raw species data ($F_{1,932} = 9.35$, $r^2 = 0.02$, $P < 0.001$; Fig. 4c) and independent contrasts ($P < 0.05$; Fig. 4c).

Multivariate analyses

Multiple regression analysis of the species data generally revealed relationships similar to those of the univariate species-level analyses (see Table 1). The most prominent exceptions involved migration and clutch size, which were nonsignificant in the multiple regression analysis but significant in the univariate tests. When we performed a univariate analysis of just the species used in the multiple regression (i.e. species with data for all seven variables), the results were significant for both migration and clutch size ($P = 0.028$ and 0.018 , respectively). This suggests that the different results between the univariate and multivariate analyses were not due to using different

species, but rather the effect of confounding factors that were controlled in the multiple regression model. Geographical region had the strongest association (log-likelihood chi-square = 294, d.f. = 4, $P < 0.001$) with migratory behaviour in a logistic regression that also included mating system (log-likelihood chi-square = 26.7, d.f. = 5, $P < 0.001$), clutch size (log-likelihood chi-square = 16.3, d.f. = 1, $P < 0.001$) and male participation in provisioning offspring (log-likelihood chi-square = 10.8, d.f. = 1, $P < 0.001$) and incubation (log-likelihood chi-square = 3.3, d.f. = 1, $P = 0.07$). Thus, in the analysis of species, the effect of migratory behaviour on testes size was primarily due to geographical region, mating system, clutch size and male provisioning.

In the analysis of independent contrasts, differences between the univariate and multivariate analyses were most pronounced for migration and male provisioning. Again, we performed the univariate analyses using just the taxa that were used in the multivariate analysis to determine whether differences were because of using different taxa or the effects of confounding variables. In

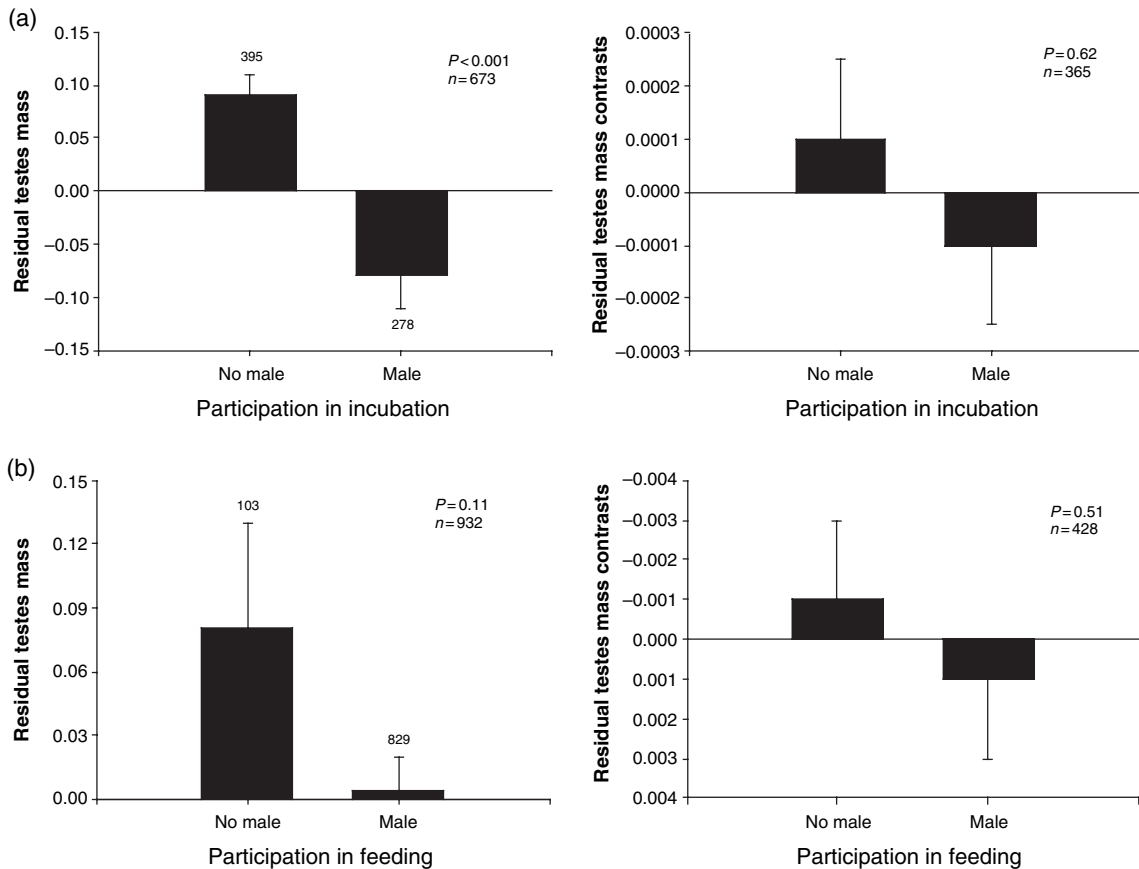


Fig. 3 Relative testes size in birds in relation to male participation in (a) incubation, and (b) feeding. Panels on the left are based on bivariate analyses of species data, whereas panels on the right are based on analyses of phylogenetically independent contrasts. Means and 1 SE are shown for each variable (see Methods for calculations).

	Univariate				Multivariate*	
	Species		Contrasts		Species	Contrasts
	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>P</i>
Migration	<0.001	1002	<0.001	459	0.46	0.91
Breeding density	0.01	898	0.01	414	0.056	0.064
Male incubation	<0.001	673	0.62	365	0.001	0.61
Male provisioning	0.11	932	0.51	428	0.94	<0.001
Geographical region	<0.001	1010	0.013	463	0.07	0.001
Social mating system	<0.001	1002	<0.001	460	0.007	0.007
Clutch size	<0.001	934	0.014	422	0.47	0.058

*Species-level model: d.f. = 1, 596, $r^2 = 0.10$, $P < 0.0001$. Contrast level model: d.f. = 1, 327, $r^2 = 0.12$, $P = 0.0002$.

the univariate analysis of the taxa with data for all seven variables there was no effect of migratory behaviour on testes size (n.s.), similar to the multivariate analysis (n.s.; Table 1), which suggests that the different results were due to using different taxa in the univariate and multivariate analyses in Table 1. In the univariate analysis of male provisioning there was a tendency for testes

to be smaller in taxa with male provisioning ($P = 0.08$), which was closer to the significant results of the multivariate analysis ($P < 0.001$; Table 1). Again, this tends to suggest that the different results were because of using different taxa, although the other variables included in the multivariate analysis probably contributed to the difference in results.

Table 1 Residual testes size of birds in relation to ecological and behavioural factors. Probability values (from *t*-tests and regression) are given for univariate analyses of testes size in relation to each of the seven predictors on the left for both the raw species data and phylogenetically independent contrasts. On the right are multiple regression analyses of species and independent contrasts with all seven predictor variables.

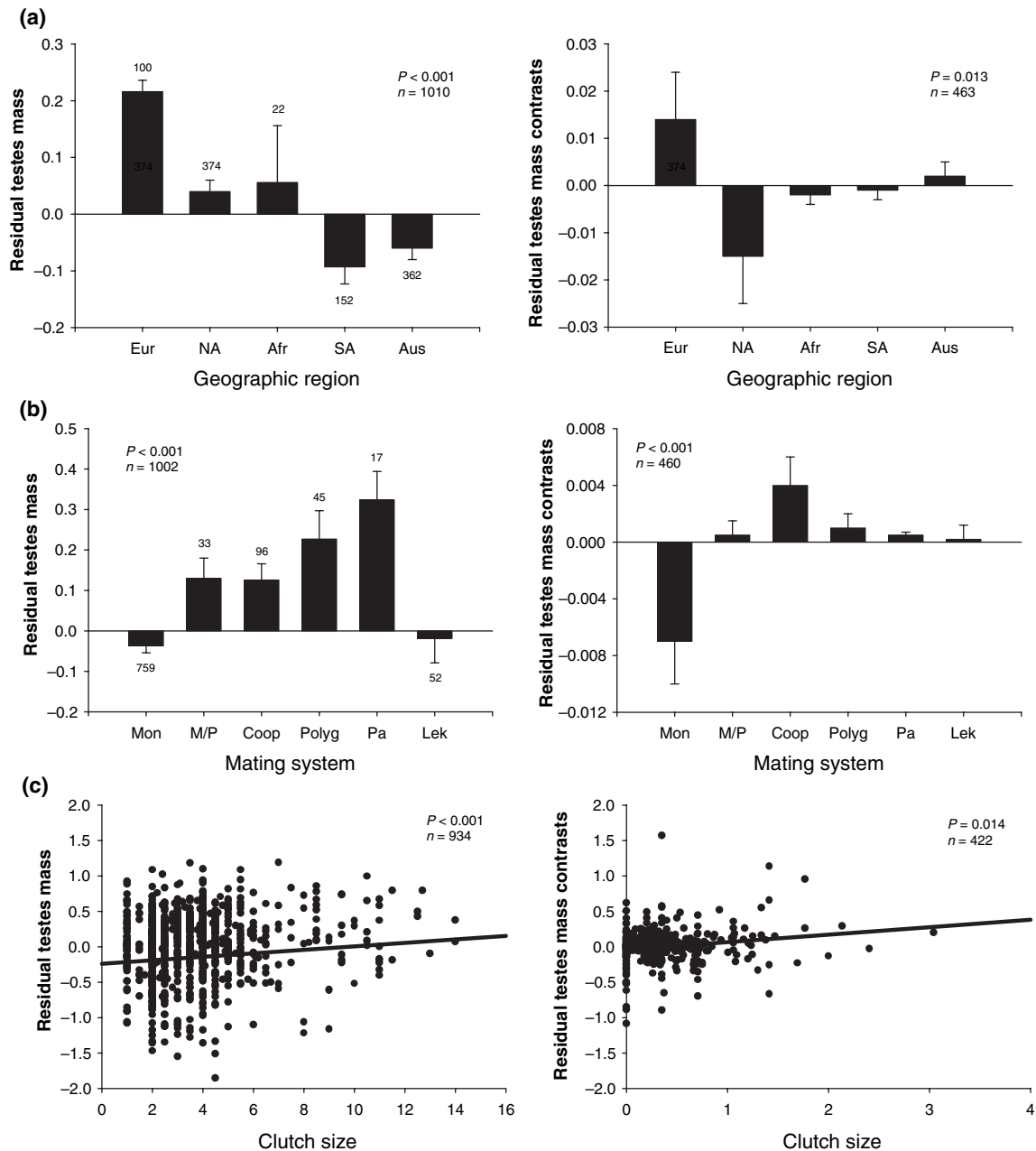


Fig. 4 Relative testes size in birds in relation to (a) geography, (b) social mating system and (c) clutch size. Geographical regions are abbreviated by; Eur = Eurasia, NA = North America, Afr = Africa, SA = South and Central America and Aus = Australasia (see Methods for details). Mating systems are abbreviated by; Mon = monogamous, M/P = mostly monogamous; but occasional polygyny, Polyg = polygynous, Pa = polyandry, Coop = cooperative breeding and Lek = lek or promiscuous. Panels on the left are based on analyses of species data, whereas panels on the right are based on analyses of phylogenetically independent contrasts. Mean and 1 SE are shown for each variable (see Methods for calculations).

In summary, the analyses of migratory behaviour, our index of breeding synchrony, revealed that migratory species generally had larger testes, as was predicted. However, it is likely that this relationship was due primarily to confounding factors such as geographical

region and mating system. Testes size was greater in taxa breeding at higher density in analyses of both species and independent contrasts, although the trend was not as strong in multivariate analyses. Testes were smaller in taxa in which males provisioned offspring, but this was

only revealed in the multivariate analysis of independent contrasts. Lastly, testes were smaller in taxa in which males participated in incubation, but this difference was only found in the analysis of species and not in the analysis of independent contrasts.

Discussion

In this large-scale study we used testes size data and the comparative method to examine the relative influences of ecological and behavioural factors on sperm competition and extrapair mating in birds. We asked whether interspecific variation in testes size was associated with our indices of: (1) breeding synchrony (i.e. migration), (2) breeding density, or (3) male parental care. All of these variables explained some variation in testes size, although the results differed between univariate and multivariate analyses and between the analyses of species and independent contrasts. These differences likely reflect the effects of confounding variables and phylogeny.

Migration and breeding density

In a comparative study, Stutchbury & Morton (1995) found a positive relationship between the degree of breeding synchrony and extrapair paternity (also see Stutchbury, 1998). However, as in most comparative analyses, it is difficult to assign cause and effect, particularly without controlling for other variables that may also be associated with extrapair mating or sperm competition. In our study we used migratory behaviour as an index of breeding synchrony. Migratory taxa had larger testes in univariate analyses, but not in multivariate analyses (Table 1). Further analysis suggested that the difference between the univariate and multivariate analyses was because of the effects of other variables in the multivariate analysis. Thus, it appears that variation in testes size is only indirectly associated with migration. The factor actually related to testes size covaries with migration. In our data set migration was most strongly associated with geographical region, followed by mating system and clutch size. Thus, the relationship between testes size and migration was probably due to the large proportion of migratory species in North America (72%, 214 of 298) and Eurasia (61%, 54 of 88), and in mostly polygynous (79%, 26 of 33) and polyandrous (53%, nine of 17) mating systems, which also have taxa with relatively larger testes, rather than the effect of migration *per se*. Our index of synchrony, migratory behaviour, was not significantly related to testes size after accounting for geography and mating system, so it may not be a good index. Future studies may benefit from more direct measures of synchrony.

We found that testes were larger in species that bred at higher density (i.e. in colonies; see Fig. 2b), although the effect was borderline in significance after controlling for phylogeny and other variables ($P = 0.064$, Table 1). This

supports previous studies that suggest testes size is larger in more social taxa, presumably as a direct result of sperm competition because of matings outside the social pair bond (see Møller & Birkhead, 1993; Brown & Brown, 2003). Sperm competition could be higher in colonial species because of the increased opportunity afforded by high nesting densities or because mate-guarding may not be feasible due to ecological constraints (see Birkhead & Møller, 1992; Wagner, 1993). In contrast, Westneat & Sherman (1997) concluded that there was little evidence that interspecific variation in the level of extrapair paternity was related to breeding density. One explanation for this discrepancy between studies of testes size and extrapair paternity is that larger testes in colonial species provide a more effective defense against extrapair fertilizations, because they allow for more frequent within-pair copulations. As a consequence, rates of extrapair paternity may remain low despite intense sperm competition (Brown & Brown, 2003).

Male participation in parental care

Male participation (yes/no) in provisioning of offspring was the most important predictor of testes size, after controlling for phylogeny and other ecological variables (Table 1) (also see Birkhead & Møller, 1996; Arnold & Owens, 2002). However, there was no significant relationship in the bivariate analyses or the multivariate analyses of species, which suggests that both confounding effects of other variables and phylogeny may obscure the relationship. The relationship between male parental care and extrapair paternity has been widely debated, but there is relatively little evidence for an interspecific relationship (reviewed by Whittingham & Dunn, 2001). As noted above, this discrepancy between our study of testes size and those of extrapair paternity may be due to the fact that copulation rates do not correlate with rates of extrapair paternity (Dunn & Lifjeld, 1994). In contrast, after controlling for phylogeny and other factors, there was little evidence that testes size was smaller in taxa in which males incubate (Table 1). There was a significant relationship between testes size and male incubation in the analysis of species, but not in the analysis of independent contrasts, which suggests that there may be large numbers of related species with both male incubation and small testes that is driving the relationship.

Geography, social mating system and clutch size

Species-level data and independent contrasts revealed that Eurasian taxa have larger testes than other geographical regions (see Fig. 4a). However, we found no general pattern of greater testes size in northern latitudes which suggests that there may be no general latitudinal pattern for levels of multiple mating as suggested by Stutchbury & Morton (1995).

We also examined social mating system and clutch size, which are likely related to sperm depletion (e.g. Cartar, 1985; Birkhead & Fletcher, 1995) and, thus, may also affect avian testes size. Although the inclusion of these variables did not alter our main conclusions (see Table 1), they did provide some interesting insights into the evolution of interspecific variation in avian testes size. First, there was considerable variation in testes size across social mating systems (Fig. 4b). Testes size was larger in taxa with multiple mating partners (polygyny and lekking) than in monogamous taxa (Fig. 4b, independent contrasts). Polyandrous taxa also had relatively large testes, which may seem like an exception because males seem to pair with one female, but often in these species males will compete for matings with the same female (e.g., Davies, 1992; Whittingham *et al.*, 1997; Emlen *et al.*, 1998). A similar explanation may also explain the large testes in cooperatively breeding taxa (Fig. 4b). Recent molecular analyses have revealed that paternity is often shared (albeit unequally) among males living together in social groups (see Table 3 in Cockburn, 1998). In an analysis of species data, Birkhead & Møller (1992, pp 30–31) reported similar patterns of testes size variation in relation to mating system. It is unclear whether selection for larger testes is because of the need for more sperm for many social pair bond matings or because of higher levels of sperm competition in taxa with mating systems in which there are several social mates.

Secondly, testes size was related positively to clutch size, although clutch size only explained 2% of the variation in testes size (Fig. 3b). Larger testes could be selected for in species with large clutches for many reasons. One reason might be that species copulate more often for larger clutches and, thus, require relatively more sperm. The relationship between clutch size and testes size could also be spurious because of the fact that larger clutches are generally found in northern temperate zone species compared to more southern tropical species. Indeed, a *post hoc* analysis of clutch sizes revealed a significant effect of the geographical breeding region (ANOVA: $F_{4,929} = 83.4$, $P < 0.0001$). Clutch sizes were larger in taxa that bred in Eurasia and North America and relatively smaller in other regions (i.e. Africa, South and Central America and Australasia, Tukey–Kramer tests, $P < 0.05$). Therefore, differences in clutch size do not appear to play a major role in the evolution of testes size in birds.

Conclusions

Although there is currently much interest in finding new variables that may account for interspecific variation in avian promiscuity (e.g. genetic variation – Petrie *et al.*, 1998), our results suggest that traditional ecological and behavioural variables associated with variation in social mating systems can account for a significant portion of the variation in extrapair mating.

Our results support previous studies that suggest that species breeding at greater density (particularly colonial species) have higher levels of extrapair mating and, consequently, larger testes. One interesting finding is that although some factors, such as migration and male incubation, appeared to be related to sperm competition in univariate analyses or analyses of species, these variables were not related to sperm competition in multivariate analyses that controlled for other ecological variables and phylogeny. These differences highlight the importance of controlling for a variety of ecological and behavioural variables in comparative studies. We also found that testes size was larger in taxa with multiple social mates, which suggests that sperm depletion may play a role in the evolution of testes size.

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Supplementary Material

A PDF file with more information on this subject is available to download from the following website: <http://www.blackwellpublishing.com/products/journals/suppmat/JEB/JEB874/JEB874sm.htm>

Appendix A1. List of species with information on residual testes mass, migratory behaviour, breeding density, male participation in incubation and feeding, geographic region of the breeding grounds, social mating system and clutch size.

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