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Combined influence of maternal and paternal quality on sex allocation in red-capped robins

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sex allocation;
sex ratios.

Abstract

Sex allocation theory predicts females will adaptively manipulate sex ratios to maximize their progeny's reproductive value. Recently, the generality of biased sex allocation in birds has been questioned by meta-analytic reviews, which demonstrate that many previously reported significant results may simply reflect sampling error. Here, we utilize a robust sample size and powerful statistical approach to determine whether parental quality is correlated with biased sex allocation in red-capped robins. Indices of maternal quality (including interactive effects of age and condition) were strongly related to sex allocation. These relationships were in the predicted directions, with larger effect sizes than those of previous studies in this field. There were also paternal correlates, involving age and the source of paternity. We propose that biased sex allocation occurs in this species, and is maintained by differing production costs of each sex and genetic benefits to females of producing sons when fertilized by high-quality males.

Introduction

When the fitness returns of producing sons and daughters differ, sex allocation theory predicts that females will manipulate offspring sex to maximize the reproductive value of their progeny (Trivers & Willard, 1973; Charnov, 1982). If so, then offspring sex allocation should be affected by a variety of factors. These include ecological parameters such as local resource enhancement and competition (Komdeur *et al.*, 1997), diet (Arnold *et al.*, 2003), habitat quality (Suorsa *et al.*, 2003) and laying date (Dijkstra *et al.*, 1990), as well as parental factors including maternal condition (Sheldon & West, 2004) and age (Côté & Festa-Bianchet, 2000), and measures of paternal quality such as plumage ornamentation and genetic quality (West & Sheldon, 2002).

When male variance in reproductive success exceeds female variance, and maternal investment has a strong effect on male quality, it is predicted that male offspring will gain greater fitness benefits from increased maternal care during the dependent period than female offspring (Trivers & Willard, 1973). Thus, mothers in high body condition (and thus capable of providing a high level of maternal care) should produce more sons, while those in poor condition should overproduce daughters (Trivers & Willard, 1973). Some empirical studies have provided convincing support for this prediction in ungulates and birds (Clutton-Brock *et al.*, 1984; Bradbury & Blakey, 1998; Nager *et al.*, 1999; Parker, 2002; Whittingham *et al.*, 2002; Sheldon & West, 2004), although some studies have failed to find the predicted patterns (Hewison & Gaillard, 1999; Ramsay *et al.*, 2003).

Male birds often possess elaborate ornamentation that provides an honest signal of their quality. In such cases, a male's reproductive success can be increased by enhanced expression of these ornaments (reviewed in Andersson, 1994). In terms of sex allocation theory, females can potentially use these ornaments to assess mate quality and thus the subsequent reproductive value of their offspring, and adjust nestling sex accordingly (Sheldon & Ellegren, 1996). Various studies have examined the effect of male ornamentation and quality on sex allocation. Some of these have reported effects on sex ratios (Ellegren *et al.*, 1996; Svensson & Nilsson, 1996; Sheldon *et al.*, 1999; Griffith *et al.*, 2003), while some have not (Saino *et al.*, 1999; Leech *et al.*, 2001).

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In addition, if it is costly for females to engage in extra-pair fertilizations (EPFs, Birkhead & Møller, 1992), then such fertilizations will only be beneficial if the resulting offspring are of higher reproductive value than within-pair offspring. Thus, it is predicted that extra-pair mates are better (higher quality or better genetic compatibility with the female) than within-pair mates. If adult males have higher variance in reproductive success than females, then females fertilized by extra-pair males should produce sons (Leech *et al.*, 2001). Kempenaers *et al.* (1997) reported that in nests with mixed paternity, blue tit offspring sired by extra-pair fathers were more often male than those sired by their social father. However, the sex of nestlings in that study was only assigned with limited accuracy (Kempenaers *et al.*, 1997) and a subsequent study of the same species, using molecular markers to assign sex (Leech *et al.*, 2001), found no such relationship between paternity source and sex ratio. Likewise, studies of other species in which the males gain EPFs have found no relationships between paternity source and offspring sex ratios (Westneat *et al.*, 1995; Sheldon & Ellegren, 1996; Westerdahl *et al.*, 1997; Saino *et al.*, 1999; Magrath *et al.*, 2002; Ramsay *et al.*, 2003).

The large number of published studies reporting facultative sex allocation recently inspired researchers to use meta-analytic techniques to confirm whether offspring sex ratios of birds do in fact consistently deviate from parity. West & Sheldon (2002) restricted their meta-analysis to a limited number of studies in which there was a clear theoretical prediction as to the direction of an effect, arguing that in most studies of offspring sex ratio variation the expected sex ratio shift depends on life-history details that are not known and thus cannot be predicted *a priori*. Their results suggested consistent facultative adjustment of sex ratios by birds in the direction predicted by theory. In contrast, Palmer (2000) and Ewen *et al.* (2004) have questioned the generality of facultative sex ratio adjustment. Ewen *et al.* (2004) argued that restricting analysis to studies with clear theoretical predictions violated the concepts of the meta-analytic approach. Instead, they included all studies examining biases in brood sex ratios published since the introduction of molecular techniques to assign offspring sex. Using this approach, they concluded that the published empirical evidence provides no consistent statistical pattern for facultative sex ratio manipulation according to temporal and biological parameters, with variability in effect sizes converging on a true effect of zero at larger sample sizes. Thus, it is currently unclear how general facultative sex allocation is in birds, with additional empirical research required to further test the conclusions of the meta-analyses. In doing so, researchers should negate the potential for spurious results, by collecting a larger number of samples and employing robust statistical techniques.

Here, we examine the above predictions of sex allocation in the red-capped robin (*Petroica goodenovii*), a sexually dichromatic species. In this socially monogamous species there are high levels of EPFs (23% of nestlings, 37% of broods; D.K. Dowling and R.A. Mulder, unpublished) and thus males potentially have greater variance in reproductive output than females. The species is also strikingly sexually dichromatic (males are more colourful) and both sexes exhibit delayed plumage maturation, moulting into adult plumage at the end of their first year. It is not a cooperative breeder. These characteristics suggest potential differences in the costs and benefits of producing each sex under different conditions, and thus make the red-capped robin a good model to further test sex allocation theory, with the prediction that sons would be overproduced when either of the parents was in good condition/of high quality.

Methods

Study site

The study was conducted at Terrick Terrick National Park (36°10′S, 144°13′E), Vic., Australia. The park includes a woodland section of about 2500 ha dominated by white cypress pine (*Callitris glaucophylla*) and grey box (*Eucalyptus microcarpa*). White cypress pine woodland within the park appears to be saturated with red-capped robin breeding territories (Dowling *et al.*, 2003a).

Bird capture, measurement and observations

We monitored the nesting success and breeding behaviour of 50 breeding pairs of robins during the 2000/2001 breeding season and 60 pairs during the 2001/2002 season. Birds ($n = 158$) were caught in mist-nets and banded with a unique combination of colour rings to permit individual identification. A small blood sample (70 μL) was taken by brachial venipuncture and stored in 100% ethanol for subsequent DNA microsatellite parentage analysis. The length of each tarsus and the head-bill length was measured to 0.01 mm, and the length of the right wing to 0.5 mm. The mass of each bird was measured to 0.1 g.

Plumage ornamentation was scored for each captured bird. The species is sexually dichromatic, with birds moulting into adult plumage at the end of their first year. Adult males have black and white plumage with scarlet forehead and breast patches that consist primarily of two red keto-carotenoid pigments, canthaxanthin and adonirubin (S. Andersson, pers. comm.). Adult females are predominantly buff-grey, with rufous forehead patches. Yearling birds of both sexes resemble adult females, but yearling females do not possess forehead patches. The length and width of the red forehead and breast patches of adult males were measured using callipers to the nearest 0.01 mm. The length of adult female forehead caps was measured with callipers to the nearest 0.01 mm. Length measures were made along the central line of the patch (cranial-caudal axis), taking care that the feathers were aligned naturally. Width measures were made at the perpendicular angle to the length measures, across the widest section of the patch. Although patch areas were estimated by multiplying the length and width measures, patch lengths were ultimately used as the measure of plumage ornamentation because (i) repeated cap length measures of the same individual (within seasons) were more repeatable than repeated area measures (cap length $r_i = 0.82$ vs. cap area $r_i = 0.76$), and (ii) it was difficult to measure breast patch widths because the natural alignment of ornamental feathers was along the cranial-caudal axis (it was difficult to determine how the feathers sat naturally along the 'left-right' axis).

At the onset of the 2001/2002 breeding season, reflectance spectra of the red cap and breast were measured once for 35 adult males using an S2000 spectrometer, PX-2 pulse xenon light source, a fibreoptic reflectance probe and OOIBASE32 software (Ocean Optics, Inc., Dunedin, FL, USA). The end of the probe was cut at 45°, yielding a 4-mm-wide measuring spot, and mounted in a supporting brace on the same angle in a tripod. Four scans were taken from central areas of the cap and breast patch of each male. Reflectance spectra between 300 and 700 nm were measured relative to white Spectralon (Labsphere Inc., North Sutton, NH, USA) and dark references against which the spectrophotometer was calibrated between measurements of each new plumage patch.

Reflectance spectra were separated into three objective colour components: brightness (spectral intensity), hue (spectral location or colour), and chroma (spectral purity or saturation). Brightness was calculated as $R_{300-700}$, the sum of reflectances from 300 to 700 nm. Hue was calculated as λ(R50), the wavelength halfway between the minimum ($R_{\min}$) and maximum ($R_{\max}$) reflectance. Chroma was measured as an index of carotenoid saturation of the plumage, by dividing the reflectance of the red wavelengths of the spectrum by the total reflectance between 300 and 700 nm: $R_{450-700}/R_{300-700}$. Brightness and chroma measurements were calculated for each of the four scans taken per plumage patch (repeatabilities: breast brightness $r_i = 0.35$, $F_{34,105} = 3.258$, $P < 0.001$, cap brightness $r_i = 0.58$, $F_{35,108} = 6.587$, $P < 0.001$, breast chroma $r_i = 0.33$, $F_{34,105} = 2.927$, $P < 0.001$, cap chroma $r_i = 0.56$, $F_{35,108} = 4.358$, $P < 0.001$) and then averaged. Raw spectra were averaged for each patch per individual and hue measures were calculated from these averaged spectra.

Nesting

Breeding activity of birds was determined by following territorial females continuously for 20 min. This observation period was sufficient to determine whether birds had begun nesting. Each territory was checked at least every third day to determine the presence of nesting activity, stage of nesting and fate of nests. Each nestling was weighed once (between 3 and 14 days) to 0.1 g. Age was recorded, or calculated if unknown by comparing body traits such as body size, size of tarsi, presence of wing feather shafts, degree of body down and whether the eyes had opened with data for the same traits in nestlings of known hatching date. Nestlings ($n = 240$) were banded with a unique combination of three colour bands, usually when they were around 6–8 days old. A small blood sample (50 μL) was taken by brachial venipuncture and stored in 100% ethanol for subsequent molecular sex determination and paternity assignment.

Once fledged, colour-banded juveniles were monitored every 3–5 days in their natal territories until evicted by their parents. Juveniles reached nutritional independence 3–4 weeks after fledging, and 91% of all fledglings survived to independence (Dowling, 2003). At this stage, parents stopped feeding and began to actively chase away begging fledglings. If juveniles disappeared from their natal territories within 2 weeks of fledging (before they could forage independently), we assumed they had died. Juveniles that disappeared following parental aggression 3 or 4 weeks after fledging, and when they were no longer dependent on their parents to feed, were assumed to have reached nutritional independence and dispersed.

Nestling sex determination

DNA was extracted from blood samples using a salting-out procedure and stored in TE buffer (Bruford *et al.*, 1992). The sex of each nestling was determined by polymerase chain reaction (PCR) amplification of introns in two homologous genes (CHD-W and CHD-Z) using the P2/P8 primer pair (Griffiths *et al.*, 1998). PCRs consisted of about 40 ng DNA, 1.5 μL of each dNTP (2 mM, Promega, Madison, WI, USA), 1.5 μL of 10x PCR buffer (Promega), 3 μL $MgCl_2$ (25 mM), 1.6 μL each of P2 and P8 primers and 0.15 μL of *Taq* polymerase (5 U μL^{-1}, Promega) in a total volume of 15 μL. Each reaction was overlaid with a drop of mineral oil. PCR conditions were as follows: initial denaturation at 94 °C for 2 min, then 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis at 110 V for 1 h on 2% agarose gels stained with ethidium bromide and visualized by UV-trans-illumination. This technique was verified by testing it on adults of known sex (one male and one female; different adults used for each gel, 24 adults in total).

Microsatellite analysis and paternity assignment

All individuals were genotyped at six loci (*Pgm1–4*, *Escmu6*, *Pocc6*) to determine paternity (Hanotte *et al.*, 1994; Bensch *et al.*, 1997; Dowling *et al.*, 2003b). PCR conditions for each locus are described in Dowling *et al.* (2003b). The general exclusion probability, in the case where the mother is known, for all 398 genotyped individuals was 0.9877.

Two methods were used to assign paternity: exclusion and a likelihood-based approach in CERVUS 2.0 (Marshall *et al.*, 1998). First, putative paternal genotypes were identified by subtracting maternal alleles from the genotype of each nestling, and then this putative genotype was compared with that of the social father. In cases where the social male mismatched the nestling at one or more loci, the nestling was initially assumed to be extra-pair. A database containing the genotypes of potential fathers in the population was searched for males that possessed all the paternal alleles in the offspring. In cases where more than one potential extra-pair sire matched the genotype of the nestling, paternity was assigned based on parsimony; males in close proximity to the offspring's territory (usually the direct neighbour) were considered more likely to be the genetic sire than more distant males (Double & Cockburn, 2000).

To confirm that exclusion accurately identified the genetic sires, a likelihood-based approach was used to assign paternity which accounts for possible genotyping errors and mutations. The natural logarithm of the likelihood ratio is called the LOD score (Marshall *et al.*, 1998). The simulation program in CERVUS 2.0 was used to estimate the required critical difference in LOD scores between the first and second most likely candidate parent for assignment at a 95% (strict) and 80% (relaxed) confidence level. The number of candidate parents was set at 92 (estimate of number of possible fathers in the population = number of breeding males that were banded plus the number of males with territories directly on the perimeter of the study population), and the proportion of candidate parents sampled set at 0.93. The proportion of loci typed was 1, and the proportion mistyped set to 0.01 (which equals the proportion of offspring mismatching the mother at one locus; D.K. Dowling and R.A. Mulder, unpublished data). Nestling genotypes were compared with genotypes of all potential fathers in the population, using LOD scores to rank the likelihood of a given male being the genetic father, and assigning paternity at >80% confidence. In all cases but two where paternity had been assigned using exclusion, it was also assigned at >80% confidence in CERVUS. In these two cases, CERVUS produced similar LOD scores for two potential fathers that matched the offspring at all loci without the required 80% confidence separating one male from the other. However, one of the potential fathers held the territory next to that of the offspring, whereas the other resided more than four territories away. The neighbouring male was assigned as the father because most extra-pair fathers live in neighbouring territories to those of the offspring (D.K. Dowling and R.A. Mulder, unpublished). Thus, sires were identified for 44 of 53 assigned EPFs.

Analysis of genetic diversity and pairwise relatedness

To assess individual genetic diversity at the six microsatellite loci, we calculated individual heterozygosity (number of heterozygous loci/total number of typed loci). For broods of mixed paternity, heterozygosity of extra-pair sires was compared with their within-pair counterparts. Pairwise relatedness values were calculated between breeding birds in the population (Queller & Goodnight, 1989) using RELATEDNESS 5.0 (http://www.gsoftnet.us/Gsoft.html). In cases where females engaged in EPFs, the relatedness between the female and her social partner was compared with the relatedness between the female and her extra-pair mate using a paired *t*-test.

Data analysis

Estimating body condition

The average length of both tarsi, the length of the right wing, and head-bill length were entered as variables into a principal components analysis, and the first principal component (PC1) was used as an index of body size. Separate analyses were conducted for each sex because tarsus, wing and head-bill lengths all differ between the sexes (Dowling, 2003). There were no differences in the body size index between adults and yearlings for either sex (males $t_{108} = -1.659$, $P = 0.10$; females $t_{69} = -0.584$, $P = 0.561$). PC1 accounted for 52% of the variance in males and 44% of the variance in females.

A body condition index (relative mass) was calculated separately for each sex by taking the residuals of linear mixed model (REML) regressions of body mass (g) on body size (PC1). Body mass of individuals does not change throughout the breeding season (Dowling, 2003), and it does not vary according to the different reproductive stages (no nesting, nest building or nestling/fledgling provisioning) for either females ($F_{2,64} = 1.75$, $P = 0.182$) or males ($F_{2,92} = 1.308$, $P = 0.275$). However, male mass varies according to time of day (Dowling, 2003). Thus, time of day was included as a fixed effect in the male body condition model. Residuals were calculated from mixed models, with individual identity as a random effect to account for individuals that were measured in both breeding seasons. These models were performed using the statistical package JMP 5 (2002, SAS Institute Inc., Cary, NC, USA).

The use of such residuals is appropriate only if certain assumptions are satisfied (Green, 2001; Schulte-Hostedde *et al.*, 2005), and therefore the residuals were carefully examined to validate if they were an adequate index of relative mass. LOWESS smoothing on the scatterplots of body weight and PC1 for both sexes revealed that the relationship between the two variables was linear. The residuals were also unrelated to PC1 scores. Furthermore, reanalysis of the sex allocation data using maternal mass, with maternal size as a covariate, does not change the results of this manuscript (D.K. Dowling and R.A. Mulder, unpublished).

Overall nestling sex ratio

The observed number of male and female nestlings ($n = 240$) was compared with parity using a *G*-test with William's correction factor (Fowler *et al.*, 1998). The proportion of males per brood within the population was compared with a value of 0.5 using a Wilcoxon signed rank test.

Generalized linear mixed models

Generalized linear mixed models (GLMMs) were used to investigate patterns of sex allocation. These models allow patterns to be explored at the level of the individual after accounting for variation attributable to differences between broods and mothers. Nestling sex was fitted as the response variable and analysed with a binomial error structure (female = 0, male = 1) and logit link function. We only included nestlings that were sampled together with all other siblings from their clutch. The sample of nestlings was reduced for these analyses ($n = 168$–171) because many nestlings were sampled from broods in which neither of the social parents were sampled. In some cases, nestlings were sampled from nests in which only one of the social parents was sampled, which results in slight differences in sample sizes between analyses. Breeding females may produce multiple broods per season (Dowling, 2003). Thus brood identity nested within female identity was fitted as a random effect. The dispersion parameter was fixed at 1 because the response variable was 'ungrouped binary' data; thus the residual deviance does not have a χ^2 distribution and over- and under-dispersion cannot be estimated from the data (G. Hepworth, pers. comm.). The influence of a number of predictor variables was then tested as fixed effects. Statistical significance of the fixed effects was assessed using Wald statistics, which approximately follow a χ^2 distribution on the respective degrees of freedom. When using the χ^2 distribution, all the statistics are correct asymptotically, and when the dispersion parameter is fixed at 1, it is preferable to use the χ^2 rather than the *F* distribution (G. Hepworth, pers. comm.). Probabilities are estimated here using a χ^2 distribution and an *a priori* significance criterion (α) of 0.05. GLMMs were performed in GENSTAT 6.0 (2002, The Numerical Algorithms Group Ltd, Oxford, UK). Additionally, correlation coefficients of each parameter were calculated, using conversion equations provided in Rosenthal (1994) and treating the Wald statistic as a χ^2 statistic, and then converted into Fisher's transformation, Z_r, so that effect sizes in our study can be readily compared with those of other studies.

It was necessary to fit three models because of the structure of the data. Most variables were collected over two breeding seasons, but male plumage colour was measured only in 2001/2002. The data collected over two seasons were unbalanced. There was only one case where a yearling male gained an EPF and, therefore, 'age of sire' and 'paternity source' (i.e. whether the sire was within-pair or extra-pair) were analysed in separate models.

The first model contained fixed effects for which data had been collected during both breeding seasons: paternity source (within- or extra-pair), age of the mother (yearling, adult), breeding season (2000/2001, 2001/2002), date the egg was laid, maternal and paternal body size and condition, brood size, relative nestling mass (residuals of logarithmic regression of nestling mass on age, $y = 2.331x^{0.572}$, $r^2 = 0.731$, $n = 193$, see Dowling *et al.*, 2003a), whether the nestling fledged and survived to nutritional independence (termed 'independence'), the length of the mother's forehead cap, and the lengths of the genetic sire's forehead and breast patch. Second-order interactions between terms were also tested. The second model was identical except 'paternity source' was replaced with 'age of sire' (yearling, adult).

In the third model, all the above variables were tested as fixed effects, as well as measures of the spectral components of male plumage collected in 2001/2002. These were brightness, hue and chroma of the red forehead cap, and brightness and hue of the breast patch (breast chroma was omitted from the analysis because it was highly correlated with breast hue). None of these spectral variables were related to sex allocation and, in fact, none of the other variables that had been related to nestling sex in the previous models were related to nestling sex allocation in this model. This suggests low statistical power of this model, and thus only the models representing data from both seasons are presented in the results.

In each model, nonsignificant terms were dropped one at a time from the model, starting with the least significant interactions, followed by the least significant main effects. The model was checked by adding these effects back into the final model, one at a time, to confirm that they had no effect on the model. For simplicity, only the final model is presented. Standardized effect sizes were calculated, but not Bonferroni corrections, for reasons outlined in Nakagawa (2004).

Results

Male robins had higher variance in reproductive success (coefficient of variation = 92.5), measured as the total number of offspring surviving to nutritional independence per individual, than females (coefficient of variation = 76.5). The overall sex ratio of nestlings did not differ from parity (females = 126, males = 114, $G_{\mathrm{adj}} = 0.6$, d.f. = 1, n.s.). The mean proportion of males per brood was 0.477 (lower 95% CI = 0.406, upper 95% CI = 0.548), which did not differ from 0.5 ($z_{108} = 0.539$, $P = 0.590$).

Correlates of nestling sex

Both maternal and paternal factors were related to nestling sex. There was a significant interaction between maternal age and maternal body condition on offspring sex (Tables 1 and 2, Fig. 1a). To further explore this interaction, separate GLMMs (containing the same random effect as the above models detailed in Tables 1 and 2, and using the same approach described in the methods to drop nonsignificant terms) were conducted for yearling and adult females. Yearling females in high condition produced more sons than those in poor condition (estimate = 2.767, lower 95% CI = 0.47, upper 95% CI = 5.07, Wald = 5.56, $P < 0.05$, $Z_r = 0.367$), but there was no statistical difference in sex allocation between adult females in high and low condition (estimate = −0.814, lower 95% CI = −1.897, upper 95% CI = 0.2689, Wald = 2.17, $P = 0.14$, $Z_r = 0.135$).

There was also an interaction between maternal age and whether the offspring reached nutritional independence on sex allocation (Table 1, Fig. 1b). Sons of yearling females tended to be less likely to reach nutritional independence than daughters (estimate = −1.388, lower 95% CI = −3.027, upper 95% CI = 0.252, Wald = 2.75, $P = 0.097$, $Z_r = 0.252$). Conversely, among adult females, sons were more likely to reach independence than daughters (estimate = 1.169, lower 95% CI = 0.377, upper 95% CI = 1.96, Wald = 8.37, $P < 0.01$, $Z_r = 0.269$).

The age of the genetic sire was related to offspring sex; yearling males were more likely to produce sons than adult males (Table 2, Fig. 1c). Additionally, nestlings resulting from EPFs tended to be male more often than those resulting from within-pair fertilizations (Table 1, Fig. 1d), with this result approaching statistical significance. This result may occur if females willing to engage in extra-pair copulations inherently produce more sons than those females which only copulate with their social mate. We tested this, but found no difference in sex allocation between such females within this data set (GLM, binomial error, logit link, response variable = number of sons, binomial denominator = total number of offspring, fixed effect = occurrence of extra-pair paternity among offspring – yes/no, $t_{54} = 0.93$, $P = 0.356$). Finally, there was an effect of year, with more males produced than expected in the 2001/2002 breeding season than in 2000/2001 (Table 2).

Table 1 Influence of paternal, maternal and environmental variables on sex allocation; model 1: with paternity source ($n = 171$). Generalized Linear Mixed Model (binomial error, logit link) describing final model of variables (fixed) related to nestling sex allocation in the red-capped robin. Random effect: female ID ⁄ brood = 0.494 (SE = 0.716). 'Maternal age' is categorized into two classes (yearling, adult), 'maternal BC' denotes maternal body condition, 'independence' denotes whether the nestling survived to nutritional independence, 'paternity source' denotes whether the nestling was sired by a within- or extra-pair sire and 'breeding season' denotes whether the data were collected in the 2000/2001 or 2001/2002 season.

Term	Effect	SE	W	Z_r	P
Maternal age × maternal BC	−2.400	1.103	4.72	0.168	0.03
Maternal age × independence	2.639	0.872	9.16	0.235	0.002
Paternity source	0.882	0.467	3.56	0.145	0.059
Breeding season	0.771	0.417	3.41	0.142	0.065

Table 2 Influence of paternal, maternal and environmental variables on sex allocation; model 2: with age of sire ($n = 168$). Generalized Linear Mixed Model (binomial error, logit link) describing final model of variables (fixed) related to nestling sex allocation in the red-capped robin. Random effect: female ID ⁄ brood = 0.454 (SE = 0.656). 'Age of sire' is categorized into two classes (yearling and adult).

Term	Effect	SE	W	Z_r	P
Maternal age × maternal BC	−3.181	1.076	8.75	0.232	0.003
Maternal age	0.859	0.465	3.42	0.144	0.064
Age of sire	−1.633	0.687	5.65	0.185	0.017
Breeding season	0.830	0.402	4.27	0.160	0.039

Comparison of social males to their extra-pair counterparts

For broods with mixed paternity, the paternal characteristics of the within- and extra-pair sires were compared using paired t-tests. The sires of extra-pair nestlings did not have more colourful plumage than the sires of the within-pair nestlings of the same brood, and nor were they in better body condition. However, extra-pair males were more heterozygous than their within-pair counterparts ($t_{32} = -2.316$, $P < 0.05$; extra-pair males: mean = 0.6, SE = 0.03, within-pair males: mean = 0.51, SE = 0.03), and tended to be more distantly related to the breeding female than did the social male ($t_{31} = 1.915$, $P = 0.065$; extra-pair males: mean = −0.08, SE = 0.04, within-pair males: mean = 0.04, SE = 0.05).

Comparison of yearling to adult males: recruitment, morphometrics and mating success

We compared various quality-related indices between yearling and adult males to explore the underlying factors that may determine why yearling males sired sons more often than adult males. At the onset of, and during, the 2000/2001 breeding season, nine yearling males were recruited into the study site, all of which retained these territories as first-year adults for the subsequent 2001/2002 season. We were unable to determine how many adult males were newly recruited into the site at the onset of 2000/2001; however, during this season four incoming adults established territories. None of these adults retained their territories for the subsequent 2001/2002 season. At the onset of the

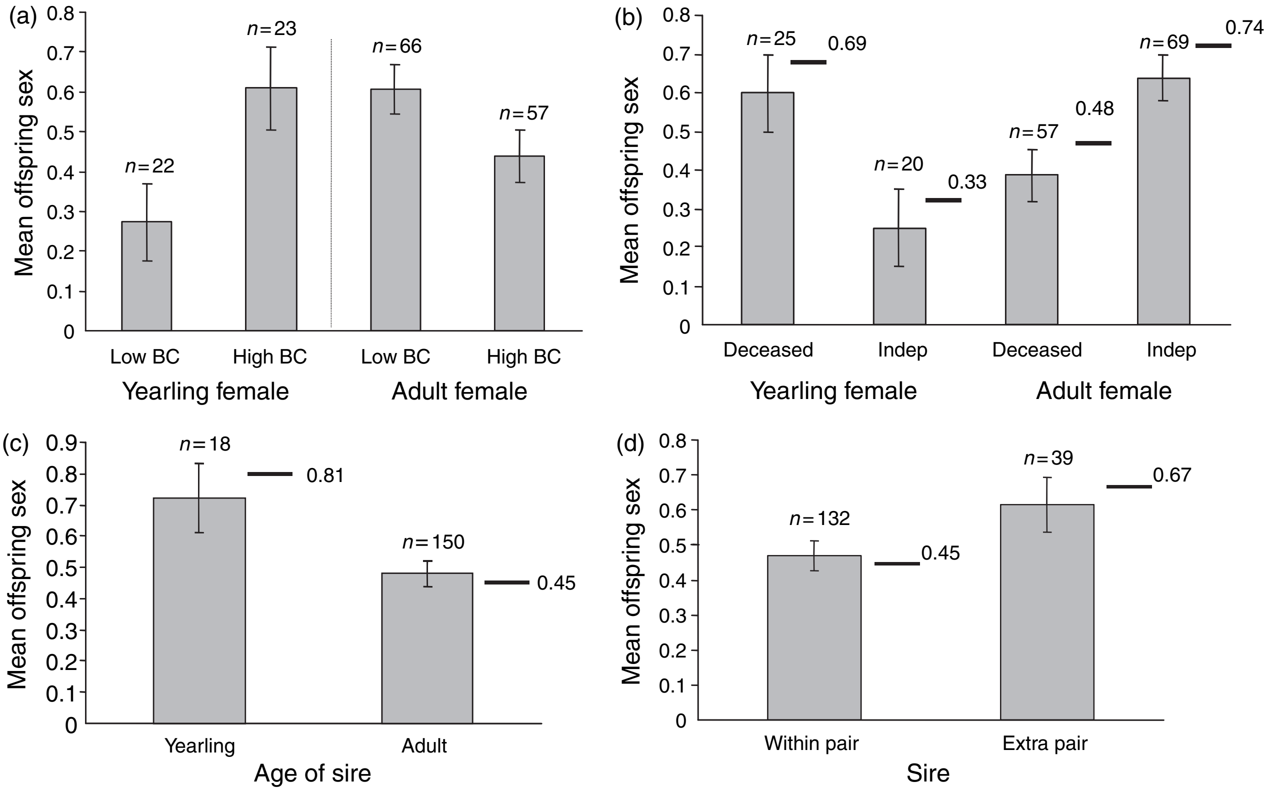

Fig. 1 (a) Mean offspring sex (±1 SE) for nestlings produced by yearling (<1 year old) and adult (>1 year old) females in low and high body condition. Sample sizes (number of individual nestlings) are indicated above each column. Mean offspring sex ranges from 0 (0% males produced) to 1 (100% males produced). Body condition in the analysis was a continuous variable, but was categorized in this figure to show the trend. Females with body condition values >0 (heavier than expected for their mass) were classified as having high body condition (High BC), and those with negative values (lighter than expected for their mass) classified as having low body condition (Low BC). The probability values were derived from the Generalized Linear Mixed Models (GLMMs). (b) Mean offspring sex (±1 SE) for nestlings produced by yearling (<1 year old) and adult (>1 year old) females that disappeared (assumed Deceased) before reaching nutritional independence and survived to independence (Indep). Columns denote mean values (±1 SE) of raw data and sample sizes (number of individual nestlings) are indicated above each column. Horizontal lines beside each column, with values, denote GLMM predictions. (c) Offspring sex for nestlings sired by yearling (<1 year old) and adult (>1 year old) males. Columns denote mean values (±1 SE) of raw data and sample sizes (number of individual nestlings) are indicated above each column. Horizontal lines beside each column, with values, denote GLMM predictions. (d) Offspring sex for nestlings sired by their social father (within-pair) and those sired by extra-pair males. Columns denote mean values (±1 SE) of raw data and sample sizes (number of individual nestlings) are indicated above each column. Horizontal lines beside each column, with values, denote GLMM predictions.

2001/2002 breeding season, and during this season, nine yearling males were newly recruited into the study area compared with 10 adult males. Five of the yearlings retained their territories for the 2002/2003 season compared with three of the adults. During the 2002/2003 season, 12 incoming adult males recruited into the study area compared with six yearlings. Overall, incoming yearlings were more likely to retain their territories for the subsequent breeding season than incoming adult males (GLM, response = retained territory [binary], $t_{30} = -2.96$, $P < 0.01$).

Yearling males tended to be in poorer body condition [GLMM with binomial error, logit link, response variable = male age, random effect: male identity = 0.298 (SE = 1.002), dispersion fixed at 1, fixed effect: male body condition: Wald = 2.91, $P = 0.088$] and smaller (Wald = 5.32, $P < 0.05$) than males in adult plumage. We monitored yearling males from 2000/2001 that remained in the study site the next season as first-year adults. There was a positive relationship between body condition of these males as yearlings and as first-year adults ($r^2 = 0.627$, $n = 6$, $P < 0.05$), and size as yearlings and first-year adults ($r^2 = 0.644$, $n = 6$, $P < 0.05$). First-year adult males had smaller forehead caps (separate variance t-test: $t_{22} = -3.158$, $P < 0.01$) and breast patches ($t_{22} = -3.444$, $P < 0.01$) than older adults (≥2 years old). However, these first-year adults produced more offspring in their own territories (within-pair offspring) that reached independence than older males ($t_{27} = 2.088$, $P = 0.053$).

Discussion

Both maternal and paternal factors were related to sex allocation in the red-capped robin, which suggests that females may manipulate offspring sex according to their own and the genetic sire's quality. Importantly, effect sizes in this study (especially those for maternal factors) are generally typical of those found across all ecological and evolutionary biological literature (Møller & Jennions, 2002), but atypical of those found in the sex allocation literature at larger ($n > 100$) sample sizes (Ewen *et al.*, 2004). Moreover, the statistical approach we employ here is more powerful than that traditionally used (where sex allocation is modelled at the brood-level), and the size of our sample ($n = 168$–171) is relatively large. We thus propose that it is more likely that the results presented here best reflect genuine biological phenomena rather than sampling error.

Maternal quality

The maternal condition hypothesis (Trivers & Willard, 1973) predicts that females should allocate offspring sex according to their own body condition, assuming one sex has higher variance in reproductive success and is thus more costly to produce than the other. Initial studies of ungulate species revealed maternal condition can affect sex allocation, with mothers in good condition producing male offspring (e.g. Clutton-Brock *et al.*, 1984; Kruuk *et al.*, 1999) and it was recently demonstrated that maternal age can also influence sex allocation (Côté & Festa-Bianchet, 2000). Studies have also revealed that maternal condition can be an important factor in sex allocation in avian species, with the most costly sex overproduced when mothers are in high condition (Bradbury & Blakey, 1998; Nager *et al.*, 1999; Parker, 2002; Velando, 2002; Whittingham *et al.*, 2002).

In this study, maternal characteristics were related to sex allocation and patterns of offspring survival. Adult females (regardless of condition) and yearling females in high body condition produced more sons than yearling females in low condition. Yearling females were relatively unsuccessful at raising sons to nutritional independence; most of their sons perished while their daughters survived to independence. In contrast, the sons produced by adult females were more likely to survive to independence than daughters.

Yearling females in low condition presumably benefit from producing fewer sons because variance in reproductive success is potentially lower for daughters than for sons – their daughters will, on average, enjoy higher future fitness than the sons these females may be capable of producing. In contrast, adult mothers were more successful at raising sons than daughters, and presumably stand to gain higher fitness benefits by investing in successful sons (Trivers & Willard, 1973).

Combined, these results suggest that sons may be more costly to produce than daughters. As yearling females are inexperienced breeders, decreases in condition may be more physically costly to yearlings than to experienced breeders. This seems likely because among yearlings, generally only those in high condition produced sons. A relationship between body condition and sex allocation was absent in adults, but here substantial effects of experience may mask more subtle relationships between condition and sex allocation in this group.

Paternal quality

The theoretical prediction that paternity source should affect offspring sex has been tested in a variety of species. However, with the exception of some preliminary results on blue tits (Kempenaers *et al.*, 1997), which were left unconfirmed by the results of another study on the same species (Leech *et al.*, 2001), no study has found that paternity source can influence sex allocation (Westneat *et al.*, 1995; Sheldon & Ellegren, 1996; Westerdahl *et al.*, 1997; Saino *et al.*, 1999; Magrath *et al.*, 2002).

In this study, we found that nestlings fathered by extra-pair sires tended to be male more often than those fathered by within-pair sires. Our results suggest that this pattern was not a corollary of those females willing to engage in extra-pair paternity being inherently more likely to produce sons. To our knowledge this is the first study to employ robust (molecular) methods to assign offspring sex and find that sex allocation may be related to the source of paternity. We also found that extra-pair fathers were more heterozygous (at six microsatellite loci measured) and tended to be more distantly related to social females than their within-pair counterparts. Similar relationships involving extra-pair paternity, genetic similarity and heterozygosity have been demonstrated in blue tits and shorebirds (Blomqvist *et al.*, 2002; Foerster *et al.*, 2003). Thus, females may mate with extra-pair males because such males are more genetically diverse (see Griffith *et al.*, 2002), and when fertilized by such males, overproduce sons. This possibility requires further testing, especially considering this interpretation assumes that genetically diverse genotypes equal 'good genotypes', and that microsatellite heterozygosity is an adequate proxy of genome-wide genetic diversity, an assumption that is essentially unfounded (Balloux *et al.*, 2004).

Surprisingly, yearling males (which resemble females) were more likely to produce sons than adult males. This appears to be inconsistent with the idea that females adjust sex allocation according to the genetic quality of the sire. However, females could assess the genetic quality of yearling males on the basis of their ability to procure breeding territories during their first year as breeders. Most territory vacancies were filled by incoming adult males. Only relatively heavy nestlings survive to nutritional independence (Dowling *et al.*, 2003a) and with few available territory vacancies for incoming juveniles, presumably only the highest quality yearlings can successfully compete with adult males for these territories. Yearling males were more successful at retaining their breeding territories between seasons than newly recruited adult males. In particular, all of the yearlings recruited in 2000/2001 successfully defended their territories for the subsequent breeding season, when they were in adult plumage (i.e. as first-year adults). Furthermore, these first-year adults produced more within-pair offspring that reached nutritional independence than older adults, suggesting they were indeed high quality. Nevertheless, yearling males were generally phenotypically inferior to adult males; they were smaller and tended to be in poorer body condition, and once they moulted into adult plumage in 2001/2002 they had shorter forehead caps and breast patches than older adult males. Thus, overall, yearling males were phenotypically inferior to older males, but more successful at retaining newly recruited territories and had higher within-pair success as first-year adults than older males. These two factors suggest these yearling males may have been high quality.

Conclusions

Biased sex allocation in this species may be maintained by two factors; differing production costs for sons vs. daughters, and benefits to females of producing sons when fertilized by high-quality males. Several relationships involving maternal age and body condition suggest that sons are more costly to produce than daughters, and good quality mothers may be investing more into production of sons than daughters, possibly to increase the reproductive value of their male offspring relative to other males. This study provides tentative suggestion that nestlings sired by extra-pair fathers are more likely to be sons, and also found that offspring sired by yearling males were more often sons. Both of these findings suggest that females may acquire genetic benefits for their offspring by producing sons when mating with such males.

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