Offspring sexual dimorphism and sex-allocation in relation to parental age and paternal ornamentation in the barn swallow

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Abstract

We analysed the morphology of nestling barn swallows (Hirundo rustica) in relation to their sex, and laying and hatching order. In addition, we studied sex-allocation in relation to parental age and expression of a secondary sexual character of fathers. Molecular sexing was conducted using the sex chromosome-linked avian CHD1 gene. Sex of the offspring was not associated with laying or hatching order. None of nine morphological, serological and immunological variables varied in relation to offspring sex. Sexual dimorphism did not vary in relation to parental age and expression of a paternal secondary sexual character. The proportion of sons declined with brood size. Individual males and females had a similar proportion of sons during consecutive breeding years. The proportion of sons of individual females declined with age, but increased with the expression of a secondary sexual character of their current mate. The generalized lack of variation in sexual dimorphism among nestlings may suggest that barn swallows do not differentially invest in sons vs. daughters. Alternatively, male offspring may require different parental effort compared to their female siblings in order to attain the same morphological state. The lack of variation in offspring sexual dimorphism with paternal ornamentation suggests no adjustment of overall parental effort in relation to reproductive value of the two sexes. However, male-biased sex ratio among offspring of highly ornamented males may represent an adaptive sex-allocation strategy because the expression of male ornaments is heritable and highly ornamented males are at a sexual selection advantage.

Keywords: cost of sons and daughters, Hirundo rustica, laying order, parental age, secondary sexual characters, sex ratio

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Introduction

Evolutionary theory of sex allocation predicts that parents should adjust their investment in sons and daughters according to the relative fitness benefits arising from producing offspring of either sex (Fisher 1930; Trivers & Willard 1973; Charnov 1982). Theoretical and empirical studies on the evolution and expression of sex-allocation strategies at the individual level (Charnov 1982; Godfray & Werren 1996; Hurst et al. 1996; Hardy 1997; Sheldon 1998; Hewison & Gaillard 1999) thus require knowledge of relative reproductive value and differential parental investment entailed by production of sons compared to daughters (Sheldon 1998; Hewison & Gaillard 1999). In addition, analysis of sex ratio data is fraught with technical difficulties owing, for example, to the nature of data which are typically clustered in broods or populations (see Krackow & Tkadlec 2001).

Variation of progeny sex ratio has been investigated in relation to a diverse set of extrinsic factors as well as parental phenotypic traits. Variation in relation to ecological factors can occur in species where large sexual dimorphism determines marked differential costs of rearing male vs. female offspring (e.g. Appleby et al. 1997; Oddie 2000). Sex ratio can be affected by social environment or maternal
condition (Lummaa et al. 1998; see also Clutton-Brock 1991; Hewison & Gaillard 1999). Ecological conditions, however, can interact with social effects in determining sex-allocation (Komdeur et al. 1997). Brood sex ratio variation has also been analysed in relation to year and seasonal effects (Radford & Blakey 2000; Grindstaff et al. 2001) and laying and hatching order (e.g. Clobetfeler 1996; Heg et al. 2000).

Besides maternal condition, other parental phenotypic traits may be expected to affect offspring sex ratio. For example, sex ratio may vary in relation to parental age (Blank & Nolan 1983; Heg et al. 2000; but see Grindstaff et al. 2001), if offspring of the two sexes entail parents with different costs and senescence processes reduce or, conversely, learning from previous breeding attempts enhance reproductive performance (e.g. Blank & Nolan 1983; Heinsohn et al. 1997; Westerdahl et al. 1997; Heg et al. 2000).

In species with heritable variation in sexually selected male epigamic characters, the proportion of sons may be predicted to covary positively with the expression of paternal ornaments because adult females mated to highly ornamented males will benefit from producing sons with large sexual ornaments. Empirical studies have provided support for this prediction, although not invariably so (Ellegren et al. 1996; Svensson & Nilsson 1996; Westerdahl et al. 1997; Koelliker et al. 1996; Saino et al. 1999a; Leech et al. 2001). In a recent meta-analysis, West & Sheldon (2002) found evidence for a general positive effect of paternal ornamentation on offspring sex in birds. However, no biased sex-allocation has been demonstrated in studies where the prediction of a male-biased sex ratio among offspring of extra-pair compared to within-pair fathers has been tested (e.g. Weston et al. 1995; Sheldon & Ellegren 1996; Westerdahl et al. 1997; Saino et al. 1999a; Leech et al. 2001).

Studying adaptive sex-allocation implies a knowledge of parental effort required to produce a son vs. a daughter of a given quality. In fact, most studies of altricial birds have investigated sex allocation strategies in terms of sex ratio of the progeny (reviewed in Sheldon 1998) without providing information on the cost of producing sons vs. daughters, or offsetting sexual dimorphism (Droge et al. 1991; Slagsvold et al. 1992). Obtaining direct measures of per-offspring parental effort is problematic owing, for example, to the difficulty of accurately estimating food allocation. However, indirect measures of differential parental investment in the two sexes can be obtained by comparing size of offspring of the two sexes or phenotypic traits related to general state and, thus, viability (e.g. Diuz et al. 1996; Potti & Merino 1996; Dijkstra et al. 1998; Sheldon et al. 1998).

Hence, the difference in the expression of fitness-related traits between offspring of the two sexes is expected to increase with the difference in their reproductive value. Measures of fitness-related traits in the offspring can be assumed to reflect the reduction in residual reproductive value caused by parental effort when a negative causal association exists between the focal offspring trait and parental fitness. In the species we studied here, the barn swallow (Hirundo rustica), we have shown that a negative correlation exists between T cell-mediated immune competence of the nestlings produced by the same parents during consecutive breeding seasons (Saino et al. 2002). That study, together with the results of brood size manipulation experiments (Saino et al. 1997a, 1999b), suggests that both inter- and intragenerational trade-offs between fitness components exist in the barn swallow and that they are mediated by T cell-mediated immune response and body mass, two of the offspring phenotypic traits that we have considered in the present study. In addition, we also analyse a diverse set of phenotypic traits in relation to offspring sex, including measures of humoral immunity and serological variables, under the assumption that they also correlate with offspring condition and are affected by parental effort.

The first main aim of the present study was to compare nine phenotypical traits of morphology, physiology and immunity of nestlings while also considering the effect of laying and hatching order and to analyse offspring sexual dimorphism in these traits in relation to parental age and expression of a secondary sexual character in the father. Variation in sexual dimorphism would reflect differential overall parental investment in sons vs. daughters as parents aged or in relation to the level of paternal sexual ornamentation.

The second main aim was to analyse sex-allocation in relation to parental identity and age, brood size and parental ornamentation. Saino et al. (1999a) showed a lack of association between brood sex ratio and natural or experimentally manipulated ornamentation. However, the present study is based on a more than three times larger sample of broods with unmanipulated paternal fathers, and we analyse explicitly offspring phenotype in relation to sex, not analysed in the previous study.

We had no specific predictions on variation of laying and hatching order and phenotype of offspring in relation to sex. Because female barn swallows already appear to senesce after their first reproductive season (Saino et al. 2002), we predicted that the sex ratio should become female-biased with increasing maternal age. Finally, we predicted a positive relationship between the expression of a secondary sexual character of males and the proportion of sons. These effects may have gone undetected in a previous study owing to relatively small sample sizes and, thus, low power of statistical tests (see West & Sheldon 2002).

The barn swallow is a socially monogamous, biparental passerine. Females prefer males with large secondary sexual characters (length of outermost tail feathers) as social and extra-pair mates (e.g. Müller 1994; Saino et al. 1997b).
Natal philopatry is very low and male-biased. No evidence exists for helping at the nest or other forms of long-term social interactions with kin, suggesting that 'local resource competition' and 'local resource enhancement' (Clark 1978; Emlen et al. 1986; Lessells & Avery 1987) are irrelevant for interpretation of sex allocation in this species.

**Methods**

We studied barn swallows breeding in 11 colonies located in an agricultural area east of Milan (Northern Italy) during 1994–95 and 1997–2000. Adults were captured, sexed and subjected to standard morphological measurements (e.g. Saino & Möller 1994). Each individual was marked to allow assignment to pairs and nests by direct observation. Assignment of adults to sex was confirmed by observation of sexual behaviour and by inspection for presence (females) or absence (males) of an incubation patch at later recaptures.

Nests were inspected regularly to record reproductive events. In a subset of nests in 1997 and 1999 we marked the eggs individually according to laying order. These nests were inspected on average every 4 h during the hatching period to mark the hatchlings individually in relation to laying and hatching order (Saino et al. 2001). In a subset of the nests we measured body mass on days 1, 4, 7 and 12, and tarsus length on days 7 and 12 after hatching. Body mass, tarsus length and left innermost rectrix were measured on day 12. A blood sample was taken from nestlings when 7–8 days old (1994–95) and from all nestlings when 12 days old. This allowed us to measure a number of serological and immunological variables. On day 12 we also performed an in vivo test of T cell-mediated immune response by measuring the response to a subcutaneous injection of a lectin, phytohaemagglutinin (PHA), which is mitogenic to T cells, as described in Lochmiller et al. (1993) and Saino et al. (1997a, 2001).

**Selection of broods**

We considered all broods that had already been included in a previous study and were fathered by males that received no manipulation of their tail length (N = 54; Saino et al. 1999a) and in a large sample of additional (N = 134) broods. However, in the present study morphological and immune response variables that were not included in the previous study were also considered. To test for the consistency of sex ratios of the offspring of the same individuals in consecutive years, we considered a sample of males and females that bred 2–4 consecutive years between 1997 and 2000. Some of these individuals mated with the same partner in consecutive years, whereas others mated with a different individual either because the previous mate had died or because the pair divorced. In the remainder of this paper we will refer to a ‘family’ as to all the offspring from different years that shared the same father (patrilineal families), mother (matrilineal families), or both (amphilineal families). A factor ‘family’ has therefore been entered into the analyses of the effect of sex on nestling phenotype. This factor allowed us to control partly for additive genetic effects on nestling phenotypic variation as well as for ecological factors such as breeding habitat, as swallows have extreme breeding philopatry (no individual bred in different colonies during this study). Only first broods have been considered throughout the study.

In the analysis of variation of offspring sex ratio between broods of the same family in consecutive years, ‘relative’ parental age was scored as ‘T’ for the first breeding year (‘first year’) and 1 + i for the following years, where i is the number of years elapsed from the first breeding year considered. Hence, relative age accounts for the effect of an increase of parental age by 1 or more years on offspring phenotype and sex ratio, but it does not account for any possible nonlinear variation of offspring sex ratio in relation to absolute parental age, i.e. the number of years since an individual was born.

Within-brood sex ratio was expressed as the proportion of sons relative to the total number of nestlings sexed (N = 744 nestlings = 98% of the total number of nestlings present at the time of blood sampling). In fact, some of the nestlings (N = 15) could not be sexed due to accidental reasons such as unavailability of a blood sample or breakage of capillaries during centrifugation. However, in the analysis of sex ratio in relation to brood size, we use the actual number of nestlings present in the brood.

In all (mixed-model) univariate analyses of variance, univariate analyse of variance for repeated measures and logistic regressions, brood, family or identity of the parent were considered as random effect factors, whereas the effect of sex, hatching order, offspring age and parental age was considered as fixed. Within-brood sex ratios were analysed in general linear models (GLM) models while using a quasi-likelihood estimation approach to control for possible over-dispersion of the data. In these analyses, to test for the statistical significance of the contribution of each factor or covariate to the model we used the change in deviance explained by the model after inclusion of the focal variable. Deviation of within-brood sex ratios from binomial random expectation was tested by analysing the deviance of the null model. To control for type I statistical errors in simultaneous tests on different variables of the same individual nestlings, we adjusted significance level downwards according to sequential Bonferroni correction. However, almost all tests were nonsignificant even without applying Bonferroni correction. Given the large number of analyses and the generally nonsignificant results, most analyses are not presented in detail. Statistical analyses were run with SPSS10,
Serological and immunological analyses

Full details of these analyses are given in Saino et al. (2001). Briefly, haematocrit was measured as the volume of packed blood cells after centrifugation (10 min, 11 500 r.p.m.) per unit blood volume. Absolute concentration of plasma proteins was measured by spectrophotometric analysis. Electrophoresis of plasma sample on agarose gels (Paragon SPE kit, Beckman Inc., Fullerton, CA, USA) and densitometric analysis of electrophoretic profiles allowed measurement of the concentration of albumin and immunoglobulins relative to total plasma proteins. Absolute concentration of albumin and immunoglobulins was then expressed as the product of their relative concentration and the absolute concentration of plasma proteins as determined by spectrophotometric analysis.

Sex determination

Molecular sexing of nestlings from 1994 to 1995 was run according to the procedure described in Saino et al. (1999a). Samples 1997–2000 were sexed using P2/P8 primers (Griffiths et al. 1998). Polymerase chain reaction (PCR) amplifications were carried out in a total volume of 20 µL. Conditions were as follows: 50 mM KCl; 10 mM Tris-HCl pH 9 (25 °C); 1.5 mM MgCl₂; 0.1% Triton X-100; 200 µM of each dNTP; 200 ng of each primer and 0.9 units of Taq polymerase (Promega, Waltham, MA, USA). PCR was performed in a PTC-100 programmable thermal controller (MJ Research Inc.). An initial denaturing step at 94 °C (for 1 min 30 s) was followed by 30 cycles of 48 °C for 45 s, 72 °C for 45 s and 94 °C for 30 s. A final run of 48 °C for 1 min and 72 °C for 5 min completed the programme. PCR products were separated by electrophoresis for 45–60 min at 7–10 V cm in a 3% agarose gel stained ethidium bromide.

Results

Offspring phenotype in relation to sex

We analysed nine offspring morphological, serological and immunological characters measured at age 12 days in relation to sex in mixed-model univariate analyses of variance with brood as a factor. This allowed us to partition out the effect of interannual, seasonal and microgeographical variation in ecological conditions on nestling phenotype. We found no significant effect of sex on any phenotypical trait (F-values associated to P > 0.05 in all cases; see Fig. 1 for mean within-sex phenotypic values, and brood and ‘family’ sample sizes). Some broods from different years had the same mother and/or father. Mixed-model analyses of variance of which we included offspring sex and family (see Methods), rather than brood, as factors showed consistently a general lack of effect of sex on offspring phenotype (F-values associated with P > 0.05 in all cases). In addition, in univariate mixed-model analyses of variance
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Offspring sexual dimorphism in relation to parental age and paternal ornamentation

To test for variation of sexual dimorphism in body mass, tarsus and rectrix length, index of T cell-mediated immune response and haematocrit in relation to relative parental age or paternal ornamentation we adopted two different approaches. First, the difference between mean within-brood values of males and females was subjected to analyses of covariance where family and tail length of the father were included as covariates (N = 134 broods for morphological variables, 111 for immunocompetence index and 93 for haematocrit, where between-sexes difference could be computed). Second, we ran repeated-measures analyses of covariance on mean within-brood and sex phenotypic values with family as between-subjects factor, sex as within-subject effect and relative parental age or father tail length as covariates. We found no significant interaction between sex and the covariates in any of the analyses, indicating that sexual dimorphism of the offspring did not vary in relation to parental age or the size of paternal ornaments (P > 0.05 in all cases).

The analyses of the other immunological variables and of serological variables in relation to paternal ornamentation were carried out using the difference between mean within-brood and sex phenotypic values using Pearson’s correlation coefficient because only one brood per parent existed in the data set (50 broods for protein, albumin and immunoglobulin concentration). Again, no significant relationships between sexual dimorphism and paternal tail length were found (P > 0.05 in all cases).

Offspring sex in relation to laying and hatching order

Nestlings could be assigned to the egg from which they hatched in 27 broods (N = 118 nestlings), all from different parents. Sex of the offspring was not predicted by laying order in a logistic mixed model analysis of brood as a factor (effect of laying order: log-likelihood = −80.19, Z = 1.10, P = 0.27; mean (SE) laying order of males: 2.71 (0.18); females: 2.81 (0.17)).

Sex of the offspring could affect the order of hatching in the case that embryos of the two sexes develop at different rates. We therefore analysed hatching order (a dichotomous variable accounting for early (= 1) vs. late hatching (= 2)) in a larger sample of broods compared to that used in analyses of laying order in relation to sex in a logistic regression for mixed effects with brood as a factor. In 53 broods with 223 sexed nestlings, sex did not predict hatching order (log-likelihood = −153.5, Z = 0.36, P = 0.72; males: 1.45 (0.05); females: 1.47 (0.05).
Offspring sex ratio in relation to year, colony and brood size

In the 188 broods there were 759 nestlings (mean brood size = 4.04 (0.07 SE)), the sex of 744 of which (98%) could be identified. The proportion of male offspring was 0.492, with no significant deviation from 0.5 ($\chi^2 = 0.19$, d.f. = 1, NS). We found no evidence of significant variation in sex ratio among study years (GLMs, see Methods; deviance of the null model = 8.701, d.f. = 5, $P = 0.12$) or colonies (deviance = 8.085, d.f. = 10, $P = 0.62$). In addition, calendar date when laying started did not affect within-brood sex ratio (deviance = 0.856, d.f. = 1, $P = 0.35$). Qualitatively similar results were obtained when only first year broods of focal individuals were considered (details not shown).

Frequency of within-brood sex ratios significantly deviated from that expected according to a binominal distribution (deviance = 281.85, d.f. = 187, $P < 0.001$), with a marked proportional excess of unisexual families among very small broods and of all-female broods among broods with six sexed offspring (Table 2). However, a significant deviation also persisted when broods with less than three sexed nestlings and the single all-female brood with six nestlings were excluded from the analysis (deviance = 227.91, d.f. = 169, $P < 0.001$).

The proportion of male offspring did not vary with clutch size (deviance = 0.65, d.f. = 1, $P = 0.75$) but declined significantly with brood size (deviance = 6.84, d.f. = 1, $P = 0.009$; Fig. 2). When we removed from the analyses the broods with extreme size (i.e. those with one and six nestlings) that had relatively small sample sizes, the association of sex ratio with brood size was still significant (deviance = 6.15, d.f. = 1, $P = 0.013$).

Broods from different years that shared one or both parents might not be considered independent if individual parents tend to produce offspring of the same sex during their lifetime. Therefore, we included only one brood per parent, by considering the brood from the first year of the focal parent. Brood sex ratio did not significantly vary in relation to clutch size (deviance = 6.94, d.f. = 1, $P = 0.009$; Fig. 2). When we removed from the analyses the broods with extreme size (i.e. those with one and six nestlings) that had relatively small sample sizes, the association of sex ratio with brood size was still significant (deviance = 6.15, d.f. = 1, $P = 0.013$).

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Table 1 Multivariate analysis of variance of offspring phenotypical variables at age 12 days in relation to sex while controlling for the effect of brood. This analysis is based on 64 males and 84 female nestlings from 37 broods. Analyses where we considered only morphological variables, based on a larger sample, showed no effect of offspring sex

<table>
<thead>
<tr>
<th>Factor</th>
<th>Dependent variable</th>
<th>MS</th>
<th>d.f.</th>
<th>$F$</th>
<th>$P$</th>
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<td>Brood</td>
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<td></td>
<td>Albumin concentration</td>
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<td>6.68</td>
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<tr>
<td></td>
<td>Relative immunoglobulin concentration</td>
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Offspring mortality in relation to sex

Sex ratio was measured when nestlings were 7 or more days old. Of 881 eggs, 122 (13.8%) failed to produce a nestling 7 or more days old. However, a large proportion of these failures can be attributed to egg sterility or embryo mortality, as mortality of hatchlings has been found consistently to be very low (2–3% of the eggs) in our study population. In order to test whether mortality differed between the sexes, we regressed within-brood sex ratio on the number or the proportion of eggs that did not produce a nestling. The proportion of males increased significantly with the number and the proportion of eggs that failed to produce a nestling (number of eggs: deviance = 7.572, d.f. = 1, \( P = 0.006 \); proportion of eggs: deviance = 7.56, d.f. = 1, \( P = 0.006 \)). In addition, the proportion of males was found to increase with the number or, respectively, the proportion of eggs that failed when we simultaneously entered into the GLM model original clutch size (number of egg failures: deviance = 9.134, d.f. = 1, \( P = 0.003 \); proportion of egg failures: deviance = 7.070, d.f. = 1, \( P = 0.008 \)). Hence, relatively more males were found in clutches with larger early offspring mortality (at the egg or hatchling stage) or egg sterility also when controlling for a possible covariation of sex ratio with original clutch size, thus suggesting that female offspring were more likely to die than males. The sex ratio in nests where all eggs generated a surviving nestling was not significantly different from 1:1 (220 males to 255 females; sex ratio = 0.463, \( \chi^2 = 2.57, P > 0.1 \)).

Breeding success expressed as the size of the brood when nestlings were 12 days old was correlated negatively with egg or nestling mortality (\( r = -0.61, N = 188, P < 0.001 \)).
Offspring sex ratio in relation to parentage, parental age and paternal ornamentation

We tested for an effect of parentage on offspring sex ratio in separate analyses for patrilineal or matrilineal families, respectively. Amphilineal families were included in analyses of both patri- and matrilineal families. We found a significant effect of family on offspring sex ratio among matrilineal families (85 broods of 40 different females in 2–4 years; deviance = 58.076, d.f. = 39, P = 0.025), as well as among patrilineal families (76 broods of 34 different males in 2–4 years; deviance = 64.788, d.f. = 33, P = 0.025). Hence, parents of both sexes tended to produce broods with similar sex ratio in consecutive breeding years.

The proportion of male offspring covaried negatively with relative maternal age in an analysis of covariance where we controlled for the effect of identity of the mother (85 broods of 40 different females in 2–4 years; deviance = 7.041, d.f. = 1, P = 0.008; Fig. 3). Relative paternal age, however, did not affect offspring sex ratio significantly (76 broods of 34 different males in 2–4 years; deviance = 6.640, d.f. = 1, P = 0.013). These results imply that individual females produced more daughters late compared to early in their life.

An analysis of covariance where we controlled for the effect of identity of the father of broods from consecutive years showed a significant positive effect of father’s tail ornament size on offspring sex ratio (deviance = 6.780, d.f. = 1, P = 0.009). This effect was still statistically significant when brood size was included in the model as a covariate (deviance = 6.640, d.f. = 1, P = 0.01). In this analysis, the effect of identity of the father on offspring sex ratio was significant after controlling for the effect of tail ornamentation (deviance = 181.3, d.f. = 64, P < 0.001), indicating that males had broods of similar sex ratio during consecutive breeding seasons independently of their level of sexual ornamentation, relative to the other males, that is known to be significantly repeatable among breeding seasons. In addition, in the subset of matrilineal families, the proportion of sons was also associated positively with paternal tail length after controlling for the effect of maternal relative age, which could have confounded the analysis due to its negative association with proportion of sons (see above; effect of paternal tail length: deviance = 7.611, d.f. = 1, P = 0.006).

We also analysed the difference in sex ratio of the offspring produced by females that mated with different males between year i and year i + 1 in relation to the difference in tail length between their mates, and found a statistically significant positive correlation (r = 0.49, d.f. = 19, P = 0.025; Fig. 4). Hence, females increased the proportion of sons in their progeny when they were mated to a male with relatively long tail feathers. Individual females that changed mate in two consecutive years (N = 21) had mates with similar tail length, as indicated by an analysis of variance of tail length of consecutive mates where identity of the female was entered as a factor (F21,45 = 2.75, P = 0.013). Consistency of tail length of male mates from consecutive breeding seasons was larger when females in the analysis that retained the same mate in consecutive years were included (F20,40 = 4.35, P < 0.001), as could be predicted from the high repeatability of tail length of individual males in consecutive breeding seasons, i.e. after winter moult of tail ornaments, as demonstrated by an analysis of variance with male identity as a factor (F33,42 = 21.56, P < 0.001).

Discussion

Sexual dimorphism in relation to parentage age and paternal ornamentation

In this study we have shown that no sexual dimorphism in morphological, physiological and immunological characters exists among barn swallow nestlings and growth trajectories of the two sexes are similar. This is the first
study where sexual dimorphism in physiological and immunological characters has been investigated in any free-living bird species and among the first investigating dimorphism in morphological characters.

One possible implication of present findings is that no differential costs of producing male vs. female offspring exist in the barn swallow. However, an alternative interpretation is that differential investment in relation to sex does indeed occur but the benefits, in terms of offspring condition, per unit of parental effort differ between the sexes because metabolism or susceptibility to parasites may differ between male and female nestlings (e.g. Clotfelter 1996; Dijkstra et al. 1998), and this study

Variation in the difference in phenotypic state between siblings of the two sexes in relation to parental age or paternal ornamentation could suggest differential investment according to parental experience or relative reproductive value of sons and daughters. However, we found no evidence for offspring sexual dimorphism to vary in relation to parental age and paternal ornamentation. Hence, sons of highly ornamented males did not attain better general state compared to their sisters as a result of differential parental investment in male offspring that will inherit secondary sexual characters preferred by females. Some other studies of altricial species with little size dimorphism have compared morphology of male vs. female offspring (e.g. Nishiumi et al. 1996; Dijkstra et al. 1998; Sheldon et al. 1998), but none has addressed the issue of sex-related difference in offspring phenotype in relation to paternal ornamentation.

First eggs laid had no significantly different chances of being males relative to the last eggs in a clutch, and sex of the egg was not related to hatching order. No phenotypic trait of male offspring was differentially affected by hatching order compared to their female siblings. Similarly, hatching order did not affect somatic growth trajectories of sons vs. daughters differentially. Sex of the offspring has been shown to vary with laying order of eggs in some species (e.g. Clotfelter 1996; Dzun et al. 1996), and this study suggests therefore that this pattern is not general (Heg et al. 2000).

Sex ratio in relation to brood size and breeding success

Overall sex ratio among nestling barn swallows did not deviate from 1:1, and no deviation from an even sex ratio was found even in broods where all laid eggs produced a nestling. This result is consistent with most studies on birds. However, frequency distribution of sex ratios strongly deviated from that expected according to a binomial distribution, due mainly to an excess of unisexual broods of either sex among small broods. Brood size at blood sampling, which reflects fledging success because mortality of relatively small nestlings is invariably very low (e.g. Saino et al. 1999a; unpublished data), was correlated negatively with the number of egg and young nestling failures, suggesting that sex ratio may become skewed in either direction as a consequence of mortality, and/or zygote sex ratio is more skewed in nests that will subsequently experience larger mortality.

The proportion of sons was larger in small compared to large broods but no association between proportion of sons among fledged offspring and clutch size existed. In addition, the proportion of sons increased with the number of egg failures and this was also the case when we controlled for original clutch size. Our results might suggest therefore that male offspring were more likely to survive compared to their sisters.

Differential mortality of the two sexes at an early stage after hatching can hardly account for sex ratio variation in relation to fledging success, as only a very small fraction of nestlings died in our population of barn swallows. Some studies of passerines have shown that last-laid eggs are smaller and produce less viable offspring (e.g. Schifferli 1973; Bolton 1991). However, in the present study laying order did not predict sex, indicating that laying order cannot account for differential mortality of the two sexes.

Another possible interpretation of our results is that mortality is dependent of primary sex ratio, and clutches where egg failures occurred had a male-biased primary sex ratio. This might be the case if egg quality is correlated with their sex. If male eggs are more costly to produce, average egg quality in male-biased clutches may be lower and these clutches may experience more failures. We have no information for the barn swallow on relative male vs. female egg quality. However, egg quality has been shown to vary with the sex of the zygote (Anderson et al. 1997; Cordero et al. 2000). For example, androgen concentration has been shown to be larger in male eggs in at least one bird species (Petrie et al. 2001). Because androgens in the eggs are of maternal origin, and because females may have to increase their own circulating levels of hormones in order to pass more androgens to their eggs, females producing male eggs may be more exposed to the detrimental effects of circulating androgens on immunity (Grossman 1988).
Sex ratio in relation to parentage, parental age and paternal ornamentation

Individual females produced more daughters, relative to sons, as they aged. The effect of age was measured in terms of change in sex ratio during up to 4 consecutive years, rather than as absolute maternal age. However, barn swallows are short-lived, and only 10% of first-year breeders survive until their third breeding season. Hence, in most cases the change in offspring sex ratio was measured for females sampled at absolute ages of 1 and 2 years. In a previous study we have shown that breeding performance is influenced by parental age, and we provided evidence that offspring quality declines already in the second compared to the first breeding season (Saino et al. 2002). This result could be expected because large mortality of adult barn swallows due to extrinsic factors, resulting in short longevity, may have selected for large parental effort during the first breeding season. Female barn swallows may thus have to trade parental performance in the first year against that in subsequent years or already undergo the senescence processes after their first breeding season, and this translates into poorer parental performance when 2 or more years old. Mothers may thus produce more female eggs when relatively old, if these are less costly than male eggs.

Males with large secondary sexual characters currently under directional intersexual selection (e.g. Møller 1994; Saino et al. 1997b), had relatively more sons compared to those with small sexual ornaments. This was shown consistently in analyses where we controlled for the potentially confounding effects of brood size and relative maternal age, and confirmed when we analysed the effect of the difference in tail length between mates of individual females from consecutive years on sex ratio of the offspring of these females. These results contradict those of a previous study where we found no effect of paternal ornamentation on offspring sex ratio were found (Saino et al. 1999a). However, previous results were based on a much smaller sample of broods. The difference in sample size and the adoption, in the previous study, of nonparametric analyses could have reduced the power of the statistical tests.

Sex ratio of the offspring of individual male barn swallows was consistent in up to four consecutive breeding seasons. This result is a novel one, as in some previous studies consistency of offspring sex ratio could be demonstrated for mothers, rather than fathers (e.g. Westerdahl et al. 1997; Heinschon et al. 1997). The mechanism leading to consistency of offspring sex ratio in patrilineal families could have been mediated by paternal ornamentation because barn swallows have one moult per year, but individual males have highly repeatable levels of ornamentation in consecutive breeding seasons (Møller 1994). However, consistency of offspring sex ratio within patrilineal families was found consistently after also controlling for the effect of male ornamentation.

Birds have chromosomal sex determination and females are the heterogametic sex, thus reducing the scope of paternal influence on the sex of the zygote (but see Sheldon 1998). The effect of ornamentation on sex of their offspring could thus be mediated by their mate, although the physiological mechanism allowing adjustment of sex-allocation in birds remains obscure.

Offspring sex ratio was also consistent among broods of the same female from consecutive years, although the proportion of sons declined as mothers aged. Females that changed mate tended to have mates with similar tail length in consecutive breeding seasons. Hence, consistency in phenotypic quality of mates could have resulted in consistency of the sex ratio of the offspring of females in consecutive breeding seasons.

Increase in the proportion of sons in relation to the level of sexual ornamentation of the father can be interpreted as a form of adaptive sex-allocation by parents producing more offspring of the sex with the largest reproductive value. Ornamental tail length has a component of additive genetic variation in the population of the barn swallow studied (unpublished results). Since males with a long tail are preferred both as social and extra-pair mates (e.g. Møller 1994; Saino et al. 1997b), a male bias in the sex ratio of offspring sired by highly ornamented males may result in a fitness benefit for their parents. In the barn swallow, approximately 35% of offspring are extra-pair (Møller & Tegelström 1997; Saino et al. 1997b). However, frequent extra-pair paternity probably did not bias our results, as in a previous study we showed no effect of paternity on offspring sex (Saino et al. 1999a), similar to all studies on other bird species (e.g. Westneat et al. 1995; Sheldon & Ellegren 1996; Westerdahl et al. 1997).

In conclusion, no sexual dimorphism exists among barn swallow nestlings and no adjustment of relative quality of nestlings of the two sexes, as reflected by a diverse set of phenotypic variables, occurred in relation to parental age or paternal phenotypic quality. Mothers were more likely to produce daughters rather than sons as they aged, but they increased the proportion of sons when mated to a male with large secondary sexual characters. Hence, we found no evidence for sex-allocation strategies that affect the quality of sons vs. daughters. However, more sons were produced when their father had large sexual ornamentation, suggesting that barn swallows adjust the sex ratio of their offspring in relation to paternal sexual attractiveness.

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References


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