

Offspring sex ratios in tree swallows: females in better condition produce more sons

LINDA A. WHITTINGHAM and PETER O. DUNN

Department of Biological Sciences, PO Box 413, University of Wisconsin–Milwaukee, Milwaukee, WI 53201, USA

Abstract

Organisms are expected to adjust the sex ratio of their offspring in relation to the relative fitness benefits of sons and daughters. We used a molecular sexing technique that amplifies an intron of the *CHD1* gene in birds to examine the sex ratio at egg-laying in socially monogamous tree swallows (*Tachycineta bicolor*). We examined all individuals in 40 broods (210 young), including all unhatched eggs and nestlings. Thus, the sex ratio we measured was the same as the sex ratio at laying. Overall, the mean sex ratio per brood (\pm SD) was biased significantly towards males ($57 \pm 2\%$ male). Within broods, male-biased sex ratios were associated with females in better body condition, and these females were more likely to produce sons in better condition. Tree swallows have one of the highest known levels of extra-pair paternity in birds (38–76% extra-pair young), and, as a consequence, variance in male reproductive success is greater than that of females. Thus, in tree swallows, investment in sons has the potential for higher fitness returns than investment in daughters, assuming that sons in better condition have greater reproductive success.

Keywords: CHD gene, condition, nestling mortality, *Tachycineta bicolor*, sex ratio, survival

Received 8 January 2000; revision received 18 March 2000; accepted 18 March 2000

Introduction

Individuals are expected to vary the sex ratio of their offspring in relation to the specific fitness benefits of sons and daughters (Hamilton 1967; Trivers & Willard 1973; Charnov 1982). These benefits may be influenced by a variety of social and ecological factors, including parental condition and variance in reproductive success between the sexes (Trivers & Willard 1973; Charnov 1982; Frank 1990). For example, in many organisms, males have greater variance in reproductive success than females, especially in polygynous species (Payne 1979; Arnold 1994), and thus a successful son has the potential to produce more offspring than a successful daughter. If a son's probability of successful reproduction is correlated positively with his mother's condition, and sons benefit relatively more than daughters from maternal investment, then a mother in good condition may achieve higher fitness if she produces a son rather than a daughter. Mothers in poor condition, on the other hand, have fewer resources to

produce a son in good condition, and thus should produce more daughters as they provide a less risky fitness return (Trivers & Willard 1973).

Although several studies have confirmed biased sex ratios at independence in vertebrates, these were often due to differential offspring mortality and not necessarily attributable to adaptive adjustments in the primary sex ratio (e.g. Clutton-Brock & Iason 1986; Clutton-Brock 1986). The recent availability of molecular sexing techniques in birds (Griffiths *et al.* 1996) has made it possible to obtain sufficient samples to determine primary sex ratios before substantial offspring mortality has occurred. This has led to a growing body of empirical evidence that birds make adjustments of primary sex ratios that appear to be adaptive (Dijkstra *et al.* 1990; Ellegren *et al.* 1996; Svensson & Nilsson 1996; Komdeur *et al.* 1997; Nager *et al.* 1999), although the mechanism remains unclear (Krackow 1995).

Sexually dimorphic species were the focus of early work on vertebrate sex ratios for two reasons: (1) they are often polygynous, and thus high-quality sons in these species are expected to be more valuable because of their potentially greater mating success, and (2) sons should be more costly to produce because of their larger size at the

Correspondence: Linda A. Whittingham. Fax: +1 414 2293926; E-mail: Whitting@uwm.edu

end of the period of parental investment (Trivers & Willard 1973). In birds, few studies had examined sex ratios in socially monogamous (and usually monomorphic) species until the advent of molecular techniques to determine sex. Socially monogamous species may be interesting for the study of sex ratios because extra-pair fertilizations are sometimes common and may result in much greater variance in male reproductive success than thought previously (Webster *et al.* 1995; Møller *et al.* 1998; Dunn & Cockburn 1999). This greater variance in male reproductive success occurs when a relatively small proportion of males monopolize most of the extra-pair matings. In fact, biased offspring sex ratios have been found in some monogamous species with moderate levels of extra-pair paternity (11–20% of young were extra-pair; Svensson & Nilsson 1996; Bradbury & Blakey 1998; Kölliker *et al.* 1999; but see Hartley *et al.* 1999).

In this study we examined offspring sex ratios in the tree swallow (*Tachycineta bicolor*), a monomorphic passerine. Although tree swallows are socially monogamous, they have extraordinarily high levels of extra-pair paternity (38–76% extra-pair young; Dunn *et al.* 1994; Barber *et al.* 1996). Most males have some extra-pair young in their nests, while relatively few males sire all of their nestlings. As a consequence, variance in male reproductive success is greater than that of females, which are almost always related to all nestlings (egg-dumping is rare; Lifjeld *et al.* 1993; Dunn *et al.* 1994). Thus, if there is a correlation between maternal condition and the condition and subsequent reproductive success of sons, then we would expect investment in sons to yield higher fitness returns than investment in daughters. In this study, we examined the following predictions: (1) females in better condition are more likely to produce male offspring, and (2) females in better condition produce sons in better condition.

Methods

Study area and species

We studied tree swallows in 1998 in a box-nesting population at the Field Station of the University of Wisconsin–Milwaukee near Saukville, Wisconsin, USA (43°23' N, 88°01' W). The study area contained 82 nest boxes in 6 hectares of field arranged as follows: (1) two grids with 28–30 nest boxes each, and (2) 24 additional nest boxes distributed throughout the rest of the study area. The nearest-neighbour distances between nest boxes at our study area (28 m in grids and 15–35 m elsewhere) were similar to those of tree swallows using natural cavities (27 m; Robertson & Rendell 1990) and nest boxes elsewhere (e.g. Barber *et al.* 1996). Seventy-one of the 82 boxes were occupied by tree swallows, and of these, 40 were chosen randomly for this study and 31 for other projects.

We checked nest boxes daily during the laying period, and each new egg was numbered with felt-tip marker on the day that it was laid. The incubation period in tree swallows is 14–15 days (Robertson *et al.* 1992). Most clutches hatch over 1–2 days and each egg takes 1–2 h to hatch (Robertson *et al.* 1992). We checked nests every 2 h between 06.00 and 20.00 h from the 12th day of incubation until all eggs in the clutch hatched. Eggs that did not hatch within 5 days of the first egg in the clutch were collected, and embryonic tissues, which were found in all eggs, were preserved in lysis buffer (Seutin *et al.* 1991). Newly hatched nestlings were marked with a unique colour combination using non-toxic felt-tip markers. If more than one egg hatched in the same 2 h interval, the eggs were assigned the same hatching order and the nestlings were given the same marking. Nestlings were remarked as necessary until they were 4-days-old when they were given a coloured plastic leg-band for individual identification. Nestlings were weighed to the nearest 0.1 g with an electronic balance when they were 4 and 12 days old. Nestlings reach their asymptotic body mass on days 12–14 (Robertson *et al.* 1992). We also measured tarsus length of nestlings on day 12 (to the nearest 0.1 mm with digital calipers). All breeding adults were captured in the nest box and marked individually with a numbered aluminium band and non-toxic colours on the breast (felt-tip markers), wings or tail (acrylic paint) (Dunn *et al.* 1994). For adults we measured body mass (to nearest 0.1 g) and tarsus length (to nearest 0.1 mm). As an index of parasites on adults, we also counted the number of holes in the primary, secondary (both wings) and tail feathers made by feather mites (Dunn *et al.* 1994). Adult body mass declines over the season (Robertson *et al.* 1992; this study), so to correct for seasonal changes we used the residuals from the regression of body mass on capture date. Body condition was estimated by the residuals of this seasonally corrected body mass regressed on tarsus length (a measure of structural size). Adult female swallows were classified as second-year (SY) or after second-year (ASY) on the basis of plumage coloration (Hussell 1983).

We determined the sex of 210 tree swallow eggs or nestlings from 40 broods using molecular sexing techniques that amplify an intron of the *CHD1* genes on the sex chromosomes (Griffiths *et al.* 1998). Blood samples (20–70 µL) were collected from 12-day-old nestlings from a puncture of the brachial vein and stored in Queen's lysis buffer (Seutin *et al.* 1991) at 4 °C. For nestlings that died before day 12 or as embryos, we used brain or muscle tissue as the source of DNA. DNA was extracted from blood samples using a 5 M salt solution (Miller *et al.* 1988) or from solid tissue samples using standard phenol and chloroform methods (Hillis *et al.* 1990). In birds, the female is the heterogametic sex, having one W and one Z

chromosome, whereas males have two Z chromosomes. We used PCR primers P8 and P2 which anneal to conserved exonic regions and amplify across an intron in both *CHD1-W* and *CHD1-Z* genes (Griffiths *et al.* 1998). The length of the non-coding introns usually differs between the *CHD1-W* and *CHD1-Z* genes with the result that PCR products from males (one band) and females (two bands) are easily distinguished on a 3% agarose gel (Griffiths *et al.* 1998). However, in tree swallows, the PCR products were similar in size and could not be distinguished on an agarose gel. Fortunately, a *HaeIII* cutting site is present in the *CHD1-Z* gene (near the 3' end of the fragment amplified using the P8 and P2 primers), but not on the female-specific *CHD1-W* gene (Griffiths *et al.* 1996, 1998). Thus, digestion of the PCR products with *HaeIII* cuts about 45 bp off the *CHD1-Z* fragment which results in W and Z fragments of different lengths. For tree swallows, this procedure resulted in one band for males and two bands for females which were easily differentiated on an agarose gel.

PCR was carried out in a total volume of 20 μ L with the following final reaction conditions: 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 200 μ M of each dNTP, 200 ng of each primer, 0.5 U of *Taq* polymerase (AmpliTaq DNA polymerase, Perkin-Elmer) and 50–200 ng of genomic DNA. PCR amplifications were performed under the following thermal cycling conditions: an initial denaturing step at 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 47 °C for 45 s and 72 °C for 45 s. The programme concluded with a final cycle of 48 °C for 1 min and 72 °C for 5 min. A 5 μ L aliquot of the PCR product was digested with 10 U *HaeIII* and 1 μ L 10 \times buffer (Promega) in a total reaction volume of 12 μ L. Digested PCR products were separated by electrophoresis for 45–60 min at 10 V/cm in a 2% NuSieve 3:1 agarose (FMC Corp.) gel stained with ethidium bromide. Digested PCR products were visualized under UV light and scored one band as male and two bands as female. The DNA of 20 different known-sex adults (10 males and 10 females) was amplified, digested and run on each of the first 10 gels (two adults per gel), and in all 20 cases the molecular method correctly identified the sex of these adults. We also replicated the PCR amplification and restriction digests for 14 randomly chosen individuals (7 males, 7 females) and the nine embryos (which are often more difficult to amplify), and our results were the same in all cases ($n = 23$).

Data analysis

We used goodness-of-fit tests to examine departures from 1:1 in the sex ratio of all individuals (eggs and nestlings) in the population, and a *t* test to examine departures from 1:1 of the mean percentage of males per brood. Sex ratio is distributed binomially, so we used logistic regression to

examine the sex of individuals in relation to within-brood correlates, such as laying order. To examine female condition in relation to sex ratio in each brood, we used generalized logistic models with binomial errors and logit links (McCullagh & Nelder 1983) as implemented in the Macintosh computer package GLMstat (Beath 1997). This analysis used the number of males in each brood as the dependent (response) variable and brood size as the binomial denominator (all eggs or nestlings in a brood were sexed). The significance of predictor variables was tested by the change in deviance of the model with and without these terms. The change in deviance has a distribution similar to χ^2 (McCullagh & Nelder 1983). Sample sizes vary among analyses because it was not always possible to measure every variable for each brood.

Results

Among all nestlings in the study population, 58% (121/210 eggs or nestlings) were male (likelihood $\chi^2 = 4.89$, d.f. = 1, $P = 0.027$). The 210 eggs and nestlings sampled represent every individual from 40 broods in 1998, including nine unhatched eggs (from eight nests) and six nestlings that died before fledging (from four nests). Thus, the sex ratio we measured was the same as the sex ratio at laying, as the sex of every egg laid was determined. Unhatched eggs and nestlings that died were mostly male (12/15; $\chi^2 = 5.8$, d.f. = 1, $P = 0.016$). The overall sex ratio at fledging also tended towards males (56%, 109/195 young), but it was not significant (likelihood $\chi^2 = 2.7$, d.f. = 1, $P = 0.099$). Sex of individuals at laying was not related to laying order ($\chi^2 = 0.1$, d.f. = 1, $P = 0.75$) or clutch size ($\chi^2 = 0.8$, d.f. = 1, $P = 0.37$) in a logistic regression with both variables as predictors ($n = 140$ young with known laying order).

We also analysed the data using nests as the unit of analysis, rather than individuals. Here the mean sex ratio was 56.8% males per brood (± 2.0 SD, $n = 40$ broods), which was significantly different from 50% ($t = 2.09$, $P = 0.04$). We also found that females in better body condition had a greater proportion of male young in their nest (Fig. 1; change in deviance = 4.59, d.f. = 1, $P = 0.032$). In contrast, females with more mite holes in their feathers tended to have a lower percentage of male young in their nest (Fig. 2; change in deviance = 3.8, d.f. = 1, $P = 0.051$). Female condition did not differ between yearling (SY) and older (ASY) birds ($t = 0.23$, $P = 0.82$); however, yearlings had significantly more mite holes (mean \pm SE = 14.4 \pm 3.5, $n = 14$) than older females (3.1 \pm 0.9, $n = 21$; $t = 3.8$, $P = 0.0007$). Thus, we examined other variables that may have confounded the relationship between sex ratio and female condition in a multivariate analysis that included female age and condition, number of mite holes, laying date and male condition as predictors ($n = 28$ broods).

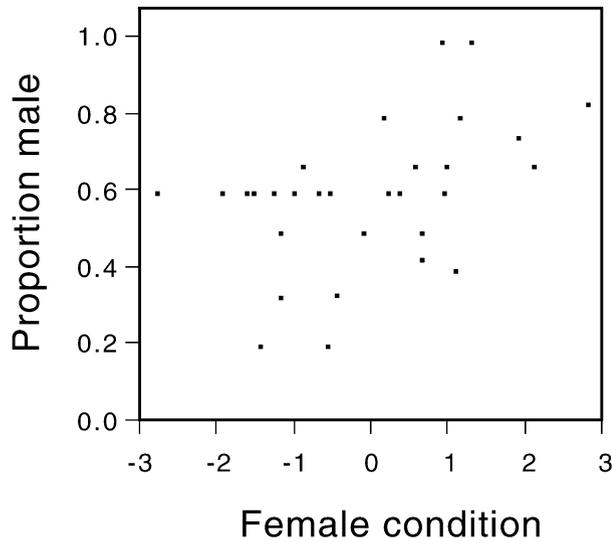


Fig. 1 Relationship between sex ratio (proportion of males) at egg-laying and female condition ($P = 0.032$, $n = 31$ broods; see text for details). Female condition was the residual from the regression of seasonally corrected body mass on tarsus length (see Methods).

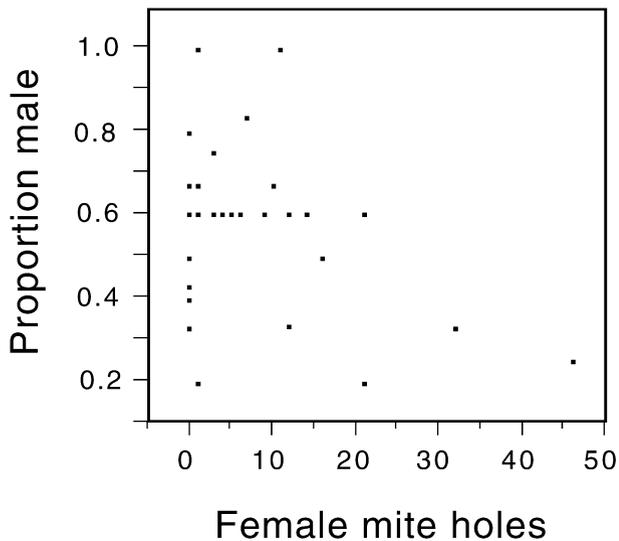


Fig. 2 Relationship between sex ratio (proportion of males) at egg-laying and number of feather mite holes ($P = 0.051$, $n = 34$ broods; see text for details).

The only significant variable in the model was female condition (change in deviance = 4.65, d.f. = 1, $P = 0.031$); all others were non-significant (all P values > 0.59).

Females that produced fewer male young were less likely to return to breed the next year (1999). Just one of

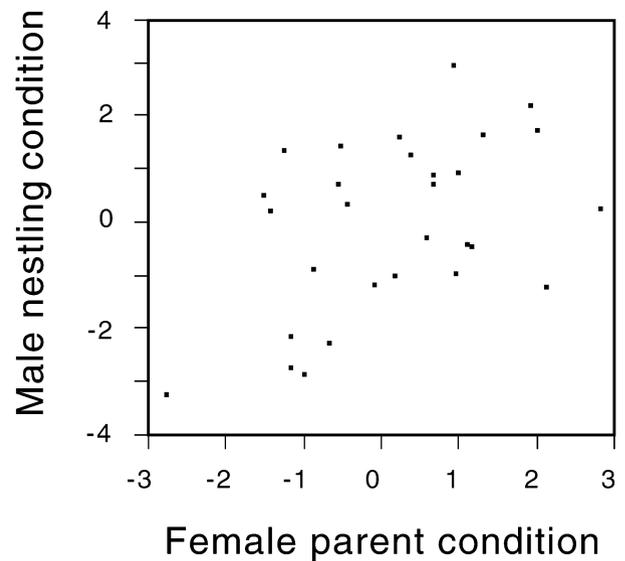


Fig. 3 Relationship between condition of sons and their mother ($r^2 = 0.22$, $P = 0.01$, $n = 29$ broods). Means were used when there were multiple sons in a nest.

10 females with mostly daughters in the nest (< 50% male) returned to breed, whereas females with mostly sons ($\geq 50\%$ male) were equally likely to return or not return (15 returned and 15 did not return; likelihood $\chi^2 = 5.75$, d.f. = 1, $P = 0.02$). Based on the results above, we might expect females in better condition to be more likely to return to breed the following year, but there was no significant difference ($t = 0.18$, $P = 0.86$). There are several possible reasons for this lack of effect, including small sample sizes for returning birds and other factors that influence return rate besides body condition.

Females in better condition may be more likely to produce male offspring if males are more costly to produce and thus only females in better condition can afford to produce more males. Male offspring might be more costly to produce than females if males fledge at a larger body size, and, as a consequence, require more parental care. Indeed, the mass (\pm SE) of male nestlings at 12 days of age (21.1 ± 0.2 g, $n = 79$ nestlings) was greater than that of female nestlings (20.1 ± 0.3 g, $n = 59$ nestlings; $t = 2.9$, $P = 0.004$). This comparison only used nests without nestling mortality to avoid any differences in size due to differential mortality of one sex or the other. A paired comparison of male and female nestlings within the same brood also revealed that mean mass of males was greater than that of females at 12 days of age (mean difference = 0.82 ± 0.2 g; paired $t = 3.9$, $P < 0.0005$, $n = 33$ broods without nestling mortality). Furthermore, females in better condition produced male offspring that were also in better condition (Fig. 3; $r^2 = 0.22$, $P = 0.01$, $n = 29$ broods).

Discussion

In tree swallows, the mean sex ratio per brood was biased significantly towards males (57%). The sex ratio we measured was probably close to the primary sex ratio because we sampled all eggs or nestlings in the nest (i.e. including unhatched eggs). Within broods, male-biased sex ratios were associated with females in better body condition and possibly lower parasite burdens. Females that produced fewer sons in their nests were less likely to return to breed the next year. Furthermore, females in better condition produced sons in better condition. Sons may have been more costly to produce because their asymptotic nestling mass and presumably energetic demands (e.g. Fiala 1981) were greater than those of daughters. Below, we discuss how these results relate to previous studies of avian sex ratios and some of the factors that appear to influence offspring sex ratio in birds.

The fitness returns to females from sons and daughters may be influenced by sex-specific differences in the variance in reproductive success. Recent paternity studies show that some males are better at gaining fertilizations (within-pair and extra-pair) than others (Kempnaers *et al.* 1992; Whittingham & Lifjeld 1995; Dunn & Cockburn 1999), and this may lead to differential benefits of producing sons or daughters. Tree swallows have one of the highest rates of extra-pair paternity found in any bird (Dunn *et al.* 1994; Barber *et al.* 1996), and, as a result, reproductive success may be more variable in males than females (Webster *et al.* 1995). Thus, females may benefit from producing sons in good condition, if condition influences the subsequent reproductive success of sons.

In general, young in better condition are more likely to fledge and return as breeders (e.g. Nur 1984; Perrins 1988; Tinbergen & Boerlijst 1990). Low recruitment rates prevented us from analysing the subsequent reproductive success of sons from the nests in this study. However, nestlings with greater body mass, which are presumably in better condition, are more likely to return to the study area in subsequent years (McCarty 2000). We do not know whether young in better condition are also more successful at gaining mates as adults. Even for males that gain mates, estimates of reproductive success are incomplete because recent studies have been unable to find the sires of most extra-pair young (Dunn *et al.* 1994; Kempnaers *et al.* 1999). Thus, we do not yet know the reproductive benefits of producing a son in good condition, although the benefits could be great.

In a few monogamous species with extra-pair paternity, specific male traits have been identified that are related to both male fitness and male-biased sex ratios. In blue tits (*Parus caeruleus*, Svensson & Nilsson 1996), great tits (*Parus major*, Kölliker *et al.* 1999) and collared flycatchers (*Ficedula albicollis*, Ellegren *et al.* 1996), specific traits indicating

male quality are heritable and related positively to male fitness. As predicted, females mated to more attractive males produce more sons. Female barn swallows preferentially chose males with longer tails, but they did not bias the sex ratio of their brood when mated to males with experimentally elongated tails (Saino *et al.* 1999). Traits preferred by females have not been identified in tree swallows, so it is not clear how the quality of a female's social mate should be related to offspring sex ratio.

Other studies have found that offspring sex ratios are associated with variation in female condition. In birds, experimental studies in which researchers manipulated food abundance or diet quality of zebra finches (Bradbury & Blakey 1998; Kilner 1998) and lesser black-backed gulls (*Larus fuscus*, Nager *et al.* 1999) showed that improving food supply or diet resulted in females in good condition, and these females subsequently produced more male offspring. Similarly, non-experimental studies of sexually dimorphic raptors have found that offspring sex ratio varies between years in relation to resource abundance (e.g. Weibe & Bortolotti 1992; Appleby *et al.* 1997). Tree swallows feed almost exclusively on aerial insects, which vary in abundance between populations and years (Dunn & Hannon 1992; Dunn & Robertson 1992). If variation in food abundance has a strong influence on female condition, then we might expect to see differences in offspring sex ratio between populations and years that are related to food abundance. Further studies that examine the variation in both offspring sex ratio and food abundance across years will allow this idea to be tested.

The adaptive benefits of manipulating sex ratio may be influenced by time of the season or position in the laying sequence in which sons and daughters are produced. This occurs because one sex, usually the larger sex, is expected to benefit differentially from hatching earlier in the season or earlier in the laying sequence, and thus have relatively more time to mature than later-hatched young. Several studies, particularly of sexually dimorphic species, have focused on laying date and sequence. Many species of raptors produce more daughters than sons, and daughters often occur earlier in the season and earlier in the laying sequence (e.g. Bednarz & Hayden 1991; Olsen & Cockburn 1991; Zijlstra *et al.* 1992; but see Dijkstra *et al.* 1990). Offspring sex ratio did not vary in relation to laying date or laying sequence in tree swallows, possibly because the breeding season is relatively short and synchronous.

This study, along with other recent investigations, suggests that females can bias the sex ratio of their brood in a way that can potentially influence their fitness. It is clear that the important selective forces on sex ratio differ among species (e.g. the influence of season, position in the laying sequence). Until recently, studies of the relationship between variation in male reproductive success and sex ratio focused mainly on polygynous species;

however, our results suggest that adaptive adjustment of sex ratio may also occur in socially monogamous species if extra-pair paternity results in greater variance in male reproductive success. Since extra-pair paternity is common in many socially monogamous birds, adaptive adjustment of sex ratio may be more widespread than thought previously.

Acknowledgements

We thank Lisa Belli, Ethan Clotfelter and Kevin Thusius for assistance during field work, and Stacy Valkenaar for excellent work in the lab. We are grateful to the University of Wisconsin–Milwaukee Field Station staff, especially Lou Nelson for building the nest boxes. This research was supported in part by a University of Wisconsin–Milwaukee Graduate School Research Award and National Science Foundation grant IBN-98-05973.

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This work is part of a large-scale study of extra-pair mating and parental investment in tree swallows. The authors have wide-ranging interests in avian ecology and evolution. Current studies focus on swallows, warblers, wrens and grouse, and use a variety of molecular genetic techniques to study mating systems, population genetics and systematics.
