Ecological opportunities and individual condition as predictors of extra-pair paternity in a south-temperate swallow (*Tachycineta leucorrhoa*)

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ABSTRACT.   Ecological and physiological factors such as breeding density, breeding synchrony, and adult body condition can all affect extra-pair mating behavior, but the relative importance of these factors may vary among species. White-rumped Swallows (*Tachycineta leucorrhoa*) nesting in Buenos Aires Province, Argentina, exhibit high rates of extra-pair paternity, with 77% of nests having extra-pair young. Our objective was to determine the extent to which extra-pair paternity in this species is explained by breeding synchrony, breeding density, and adult body condition. Our study of a population of White-rumped Swallows breeding in nest boxes was conducted during two consecutive breeding seasons (September – early January 2006–2008). We found that neither breeding synchrony nor density of neighbors predicted levels of extra-pair paternity in our study population. Leaner females were more likely to engage in extra-pair behavior and fledged more nestlings, but did not differ in structural size from females that did not engage in extra-pair behavior, suggesting that female mass is an important predictor of mating decisions and fitness for these aerial insectivores. Male body condition was not related to male extra-pair behavior. The mass of female White-rumped Swallows may affect their flying ability such that, during their fertile period, they are exposed to more potential extra-pair mates during longer foraging flights. Being lighter may also improve the ability of females to provision nestlings later in the breeding cycle.

RESUMEN.   **Oportunidades ecológicas y condición física individual como predictores de paternidad extra pareja en una golondrina del sur templado (*Tachycineta leucorrhoa*)**
Factores ecológicos y fisiológicos, como ser la densidad y sincronía reproductiva, y la condición física de los adultos, pueden afectar el comportamiento de apareamiento extra pareja, pero la importancia relativa de estos factores puede variar entre especies. Las Golondrinas de Ceja Blanca (*Tachycineta leucorrhoa*) que nidifican en la Provincia de Buenos Aires, Argentina, tienen altas tasas de paternidad extra pareja, con un 77% de nidos con pichones extra pareja. Nuestro objetivo fue el de determinar en qué medida la paternidad extra pareja en esta especie puede ser explicada por la sincronía reproductiva, la densidad reproductiva y la condición física de los adultos. Nuestro estudio de una población de Golondrinas de Ceja Blanca nidificante en cajas nido fue llevado a cabo durante dos temporadas reproductivas consecutivas (Septiembre-a comienzos de Enero 2006–2008). Encontramos que ni la sincronía, ni la densidad de vecinos pueden predecir los niveles de paternidad extra pareja en nuestra población de estudio. Las hembras involucradas en comportamientos extra pareja fueron más delgadas, y éstas hembras tuvieron más volantones, pero no difirieron en tamaño corporal con aquellas hembras que no tuvieron comportamientos extra pareja, sugiriendo que la masa de las hembras es un predictor importante de las decisiones de apareamiento para estos insectívoros aéreos. No encontramos que la condición física de los machos estuviera relacionada con su comportamiento extra pareja. La masa de las hembras de Golondrina de Ceja Blanca puede afectar su habilidad de vuelo de modo que, durante su período fértil, podrían estar expuestas a un mayor número de parejas potenciales fuera de su pareja social durante los largos vuelos de forrajeo. Ser más livianas podría significar también un beneficio al mejorar la habilidad de las hembras para aprovisionar a sus polluelos más adelante en el ciclo reproductivo.

Key words: body condition, breeding density, breeding synchrony, extra-pair paternity, Hirundinidae, mating system

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Both the temporal and spatial distributions of mates have long been suggested to influence avian mating systems (Emlen and Oring 1977), but the expected effects of these variables on paternity vary across taxa and ecological settings (Bennett and Owens 2002, Griffith et al. 2002). For example, greater breeding synchrony may facilitate comparison of males by females and lead to higher rates of extra-pair paternity (EPP; Stutchbury and Morton 1995, Stutchbury 1998a,b, but see Weatherhead and Yezerinac 1998). In contrast, greater breeding synchrony might reduce opportunities for extra-pair copulations for males facing a trade-off between seeking extra-pair copulations and guarding their fertile mates (Birkhead and Biggins 1987, Westneat et al. 1990). This uncertainty in the direction of the expected relationship between breeding synchrony and rates of EPP is matched by variation across studies in empirical results. For example, investigators have reported a positive relationship between breeding synchrony and rates of EPP in Black-throated Blue Warblers (*Setophaga caerulescens*, Chuang et al. 1999) and Clay-colored Robins (*Turdus grayi*, Stutchbury et al. 1998), a negative relationship in Golden Whistlers (*Pachycephala pectoralis*, van Dongen and Mulder 2009) and Eastern Phoebes (*Sayornis phoebe*, Conrad et al. 1998), and no association in House Wrens (*Troglodytes aedon*, LaBarbera et al. 2010) and Sedge Warblers (*Acrocephalus schoenobaenus*, Langefors et al. 1998, see Griffith et al. 2002 for a more complete list of examples).

Variation across studies is also apparent in the relationship between breeding density and rates of EPP (Westneat and Sherman 1997, Griffith et al. 2002, Neudorf 2004). Greater breeding densities may provide more opportunities for extra-pair copulations (Bennett and Owens 2002), as found in Eastern Bluebirds (*Sialia sialis*, Gowaty and Bridges 1991), Yellow-billed Magpies (*Pica nutalli*, Birkhead et al. 1992), Barn Swallows (*Hirundo rustica*, Møller 1991), and Reed Buntings (*Emberiza shoeniclus*, Mayer and Pasinelli 2013). However, other investigators have found no relationship between breeding density and rates of EPP (see Appendix S2 in Mayer and Pasinelli 2013 for a comprehensive list of studies).

Differences in body condition may also explain some of the variation in rates of EPP through two different, non-mutually exclusive processes. Female body condition might influence extra-pair behavior because females in poor condition might be constrained to trade male help for paternity assurance given that females presumably risk losing their social partner when engaging in extra-pair behavior, and only those in good condition will be able to raise a brood without male parental care, i.e., the female constraint hypothesis (Gowaty 1996a,b). For example, clutch initiation dates of Tree Swallows (*Tachycineta bicolor*) are tightly linked to individual quality/condition and reproductive success (Stutchbury and Robertson 1988, Winkler and Allen 1996, Hasselquist et al. 2001, Ardia 2005), so females that lay earlier in the season might be higher quality individuals, leading to higher rates of EPP in nests initiated earlier. Alternatively, females could choose to mate with extra-pair males in better condition or larger than their mates, a process explained by indirect-benefits, female-choice sexual selection (Andersson 1994). Moreover, body condition may also influence the distances birds fly to forage or find mates. For example, after experimental manipulation that increased the cost of flying for female Tree Swallows, handicapped females mated with extra-pair sires closer to their own nests, whereas control females sought copulations farther away (Dunn and Whittingham 2007). Thus, adult body condition and relative mass could potentially influence rates of EPP.

White-rumped Swallows (*Tachycineta leucorrhoa*) breeding at south temperate latitudes have high rates of EPP (77% of nests, 56% of young; Ferretti et al. 2011), and studies of other species in the genus *Tachycineta* have revealed considerable variation in rates of EPP (ranging from 13 to 89% of nests with at least one extra-pair young, and 7 to 69% of nestlings being extra-pair; e.g., Dunn et al. 1994, 2009, Barber et al. 1996, Kempenaers et al. 1999, Moore et al. 1999, Whittingham and Dunn 2001, Whittingham et al. 2006, O'Brien and Dawson 2007, Stapleton et al. 2007, Crowe et al. 2009, Ferretti et al. 2016). Previous studies at our study site have revealed that a relative high percentage of extra-pair young are sired by local males (47%, Ferretti et al. 2011), consequently local

breeding density and synchrony might be important determinants of EPP rates. Our objectives were to examine (i) the relationship between EPP and increased opportunities to find extra-pair mates due to variation in nest density and breeding synchrony; and (ii) the possible effects of body size, mass, and body condition on rates of EPP. We predicted that increased breeding synchrony and density would lead to increased extra-pair mating because White-rumped Swallows appear to have little mate-guarding (VF, unpubl. data), hence a negative relationship between paternity and breeding synchrony is not expected. In addition, female condition should be positively related to extra-pair mating because females in better condition can risk losing male parental care when engaging in extra-pair behavior (i.e., female constraint hypothesis), and females should choose to mate with extra-pair males in better condition or larger than their social partners. Although there is no evidence that the female constraint hypothesis applies to White-rumped Swallows or other species in the genus *Tachycineta*, comparative studies have provided support for this hypothesis (Arnold and Owens 2002, Bennett and Owens 2002). Finally, for females that engage in extra-pair behavior, we expected that female condition would be positively related to the distance between their nests and nests of extra-pair males because females in better condition may be able to move over a larger area to forage and find extra-pair mates.

METHODS

We studied a population of White-rumped Swallows breeding in nest boxes in Buenos Aires province, Argentina (35°34′S, 58°01′W), during two consecutive breeding seasons (September – early January 2006–2008). At our study site, 136 nest boxes placed 15–35 m apart were available for breeding swallows. For more details about the site, see Ferretti et al. (2011) and Bulit et al. (2014). For each nest box, we recorded latitude and longitude (± 3 m) using a GPS unit (Garmin 76, Garman International, Olathe, KS).

Measures of body condition. White-rumped Swallows are socially monogamous, only females incubate the eggs, and both adults care for the young (Bulit et al. 2008). When visiting nests, adults were captured in nest boxes using wig-wag traps (information about these traps is provided at http://golondrinas.cornell.edu/Data_and_Proto col/WigwagAdultTrap.html), then measured and banded with uniquely numbered aluminum bands. Females were captured during the incubation period, 8 or 9 d after clutch completion. Males were captured while feeding nestlings that were 8 or 9 d old. Captured adults were weighed with a 30-g Pesola spring scale (± 0.25 g). In addition, we measured the length of the head plus bill and the length of the tarsus using a dial caliper (± 0.1 mm), and wing length (both flat and straightened) using a wing ruler (± 1 mm).

Using the morphological measures, we generated a body size index (BSI) by running a principal component analysis (PCA) on wing length, tarsus, and head plus bill length, and extracting the first principal component (PC1) from that analysis. We subsequently calculated a body condition index for each bird derived from the residuals of mass regressed against BSI using an orthogonal fit with equal variance ratio (reduced major axis regression; Green 2001). To avoid handling birds for longer than necessary during bad weather conditions, we did not take body measurements during periods of inclement weather (i.e., rain and wind gusts and low ambient temperatures).

Blood or tissue samples. We banded 7- to 9-d-old nestlings with uniquely numbered aluminum bands and took a blood sample from each. Adults were sampled for blood at the time of capture. We took 20–70 μl of blood from brachial veins using heparinized capillary tubes. Whole blood was stored in Queen's lysis buffer (Seutin et al. 1991). For nestlings that died in nests, we collected a tissue sample from their pectoral muscle and stored it in 96% ethanol.

Microsatellite amplification for paternity exclusions and assignments. Details on the paternity analysis protocol used in our study were described in Ferretti et al. (2011). In short, we extracted DNA from blood and muscle samples using DNA purification kits by Eppendorf (Perfect gDNA blood mini isolation kit, Hamburg, Germany) and Qiagen (DNeasy blood and tissue kit, Valencia, CA). Extracted DNA was diluted 1:10 in ultra-purified H_2O

and then amplified at 12 highly polymorphic microsatellite regions (Table S1) through polymerase chain reactions in a DYAD thermal cycler (Bio-Rad, Hercules, CA) following the conditions in Makarewich et al. (2009). PCR products were then genotyped on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA), and the sizes of the microsatellite alleles were estimated using GeneScan-500 LIZ size standard (Applied Biosystems) and the software GeneMapper (v3.7 Applied Biosystems). We analyzed only families where we had DNA samples of females, their social mate, and the nestlings. We genotyped 342 nestlings and their social parents and assigned paternity using the maximum likelihood approach in the program Cervus 3.0 (Marshall et al. 1998, Kalinowski et al. 2007, 2010). The exclusion probability for the 12 loci combined was 0.9999. We compared genotypes of nestlings to that of the male attending their nest. If nestlings mismatched their social father's genotype at two or more loci, we considered them extra-pair young. Additionally, we compared the genotypes of nestlings to those of all males sampled in our population to assign potential extra-pair sires.

Breeding synchrony and time of breeding. We characterized each female's fertile period as spanning 6 d prior to the date the first egg was laid (Ardia et al. 2006) through the lay date of the penultimate egg (Moore et al. 1999). White-rumped Swallows in our population lay on average 4.92 eggs (Massoni et al. 2007). We calculated a female synchrony index using the formula:

$$SI_p = \left[\frac{\sum_{i=1}^{t_p} f_{i,p}}{t_p(F-1)} \right] \times 100,$$

where SI_p is the synchrony index for each female p in the population, $f_{i,p}$ the number of fertile females, excluding female p, on day I, F the number of total females breeding in the population, and t_p the number of fertile days for female p (Kempenaers 1993, Stutchbury and Morton 1995). This index is a measure of the overlap of the fertile periods of each female with respect to those of the rest of the breeding females in the population. The average SI of the colony was calculated as the mean of the SI_p for all females in the colony. For our measure of timing of breeding (lay date), we considered day 1 the day the first egg was laid in our colony in that particular year (i.e., the first egg for that breeding season), and we numbered subsequent clutch initiation dates from that point onward.

Breeding density. We calculated distances between nest boxes, using their geographic coordinates, with the program Geographic Distance Matrix Generator (Ersts 2016). We did this for all active nest boxes in each year (i.e., nest boxes where a pair of adults defended the box and nested in it). We used two measures of nest density: distance to the nearest neighbor, and number of active nests within a given radius. We calculated the nearest neighbor distance (i.e., the nearest active nest box) for each active nest using the distance data as described above. For each active nest, we also counted the number of other nests within a set radius that were active during the fertile period of females at focal nests, and used this number as our measure of density. We thereby took into account the density of active nests only during female fertile periods when they might engage in extra-pair copulations. We repeated this procedure for 100-, 200-, and 300-m radii. In addition, when we identified extra-pair genetic fathers, we determined the distance between that male's nest box and the box where he fathered extra-pair young.

Statistical analyses. We first analyzed which explanatory variables mentioned above had an effect on the dependent variable (EPP status). We ran three logistic regressions to test for the ability of the two measures of nest density, breeding synchrony, lay date, and social male and female condition indices, BSI, or mass (we used only one of these parameters in each logistic regression) to predict the binary outcome of EPP status (presence or absence of extra-pair young in a nest). In this analysis, we only considered the presence/absence of extra-pair young in broods and not the proportion of extra-pair young. We did this because female birds can store sperm from different males (Birkhead 1998), so the proportion of extra-pair young in a brood (i.e., number of extra-pair offspring in a given brood/total number of offspring in that same brood) is not a good measure of extra-pair behavior because sperm introduced in one

extra-pair copulation during the fertile period could fertilize one or more eggs. Instead, we believe that, for the purpose of our analyses, presence/absence of extra-pair young in a nest is a better measure of extra-pair mating behavior (Stutchbury and Morton 1995, Arnold and Owens 2002). Moreover, extra-pair young do not seem to be randomly distributed among broods in our population, with some broods consisting entirely of extra-pair offspring and some having all within-pair offspring (Ferretti et al. 2011). Therefore, to best capture the variables that affect the extra-pair mating decisions of females we used the binary outcome of extra-pair mating behavior. We refer to females with at least one extra-pair young as EPY females. We used only information from first broods during each breeding season. For each predictor variable, we calculated the power of the parameter a posteriori (i.e., observed power) using the effect size as the difference observed between means in that predictor variable for each EPP status. In addition, we evaluated the magnitude of the difference in the predictors that were significant in the abovementioned logistic regressions by conducting post hoc one-way ANOVAs to analyze differences in mass and condition index for females with all within-pair young (NO EPY), those with at least one extra-pair young and the genetic father was breeding in our colony (KNOWN EPY), and those with at least one extra-pair young and the genetic father was not breeding in our colony (UNKN EPY). We subsequently compared each pair of means with a Student's *t*-test.

We examined how condition indices and other morphological parameters (BSI and mass) of females and their social mates varied with timing of breeding (synchrony index and lay date) because the relationship between these parameters could confound our results, e.g., if females in better condition started breeding early and were asynchronous with the rest of the colony, they might not have many opportunities for extra-pair mating. We therefore used a linear regression analysis to examine how variation in breeding synchrony and lay date were related to variation in condition indices and body measures, respectively, for females and their social mates. We did this for a subset of individuals with complete information on mass, lay date, and all body measures used in the calculation of the BSI. We also conducted a reduced major axis regression test of the distance between nest boxes (i.e., distance between the focal nest and the nest where the sire of the extra-pair offspring nested) regressed against the female condition index and mass to analyze the relationship between female breeding condition and distances traveled by females to obtain extra-pair fertilizations. We did this only when we identified a sire in our breeding colony.

When we were able to identify extra-pair sires and had all the morphological measures, we tested whether females with extra-pair young mated with extra-pair males either in better condition or larger than their social mates. We conducted three Wilcoxon signed-rank matched-pairs tests, with one using the male BSI, one using male mass, and one using our measure of body condition, condition index, as the dependent variables. All statistical analyses were conducted using JMP 10 (SAS Institute Inc. 2012). Values are presented as means $\pm$ SE.

RESULTS

We sampled 171 adults (87 females and 84 males) across both years of our study. This represented 90% of the breeding females in our population, and 75% of the socially paired males. We genotyped 342 nestlings in 78 broods of 55 breeding pairs. We also genotyped an additional 22 resident males whose nests were excluded from the paternity analyses (e.g., nests used in other experimental protocols) to help identify genetic fathers. Considering only birds with all body measures, we had 47 observations for female condition index and BSI, 54 for female mass, 50 observations for male condition index, 51 for male BSI, and 52 for male mass.

Rates of EPP in our population for the same years studied here, and the same birds, have been reported previously (Ferretti et al. 2011; Table S2). Median distance between nests of extra-pair males and nests where they fathered young was 165 m ($\bar{x} = 262.3 \pm 39.6$ m, $N = 30$ males, range = 23.9–798.4 m). Mean SI for all nests with complete clutches was $23.08 + 0.92\%$ ($N = 192$, Upper 95% = 24. 90%, Lower 95% = 21.26%).

We found no effect of either breeding synchrony (power = 0.71) or nest density (power = 0.05) on rates of EPP (Table 1). However, our power to detect differences, given the observed effect size for nest density, was limited. In addition, neither distance to the nearest neighbor (power = 0.96) nor nest density within a given radius was found to influence the EPP status of female White-rumped Swallows (power for detecting between-group differences in density at 100 m = 0.93, 200 m = 0.23, and

Table 1. Logistic regression models for the effects of different predictor variables on EPP status (no epy, known epy, and unkn epy) of female White-rumped Swallows.

Predictor variables[a]	Chi-square	Prob > Chi-sq[b]
(A)		
Synchrony	0.8	0.37
Lay date	0.2	0.68
Nearest neighbor	1.0	0.33
Density at 100 m	2.1	0.15
Density at 200 m	0.1	0.80
Density at 300 m	0.1	0.71
Female CI	4.7	0.03*
Male CI	1.1	0.29
(B)		
Synchrony	0.04	0.84
Lay date	0.2	0.62
Nearest neighbor	1.4	0.24
Density at 100 m	3.1	0.078
Density at 200 m	0.3	0.60
Density at 300 m	0.1	0.81
Female mass	8.1	0.004*
Male mass	0.7	0.39
(C)		
Synchrony	< 0.001	0.99
Lay date	0.1	0.81
Nearest neighbor	2.5	0.11
Density at 100 m	0.7	0.41
Density at 200 m	0.02	0.87
Density at 300 m	0.01	0.90
Female BSI	0.01	0.91
Male BSI	0.1	0.81

[a]Synchrony: female breeding synchrony index; Lay date: clutch initiation date; Nearest neighbor: distance to the nearest neighbor; Density 100–300: number of active nests within a radius of 100, 200 and 300 m, respectively; Female CI, female condition index; Male CI, male condition index.
[b]Asterisk denotes $P < 0.05$.

300 m = 0.08). The only variables found to influence EPP status in our models were female condition index (Table 1A) and female mass (Table 1B); we found no effect of female BSI (Table 1C, power = 0.05), male condition, male mass, or male BSI on female EPP status (power male condition index = 0.21, power male mass = 0.61, and power male BSI = 0.08).

We found no difference in the condition indices of NO EPY, KNOWN EPY, and UNKN EPY females (Fig. 1A, $F_{2, 45} = 2.4$, $P = 0.10$), but did find a difference in mass (Fig. 1B, $F_{2, 52} = 5.4$, $P < 0.01$), with UNKN EPY females being the leanest ($\bar{x} = 23.02 \pm 0.25$ g), followed by KNOWN EPY females ($\bar{x} = 23.84 \pm 0.35$ g) and NO EPY females ($\bar{x} = 24.40 \pm 0.36$ g). Comparison of mean condition indices of females revealed significant differences between NO EPY ($\bar{x} = 0.32 \pm 0.18$) and UNKN EPY ($\bar{x} = -0.16 \pm 0.12$) females, but no difference between either of these two and KNOWN EPY females ($\bar{x} = 0.01 \pm 0.19$; Table 2A). We obtained similar between-group results for female mass (Table 2B).

Both BSI and mass of female White-rumped Swallows increased with increasing breeding synchrony (Fig. 2A, $R^2 = 0.18$, $F_{1, 45} = 10.0$, $P < 0.01$ for BSI; Fig. 2B, $R^2 = 0.10$, $F_{1, 52} = 5.9$, $P = 0.02$ for female mass). However, we found no relationship between female condition indices and either breeding synchrony (Fig. 2C, $R^2 = 0.02$, $F_{1, 45} = 0.8$, $P = 0.36$) or lay date (Fig. 2D, $R^2 = 0.02$, $F_{1, 45} = 1.0$, $P = 0.33$). We also found no relationship between female condition indices and distance to extra-pair mates ($R^2 < 0.09$, $F_{1, 21} = 2.1$, $P = 0.16$).

For males, we found no significant relationships between breeding synchrony and BSI ($R^2 = 0.05$, $F_{1, 49} = 2.7$, $P = 0.11$), mass ($R^2 < 0.001$, $F_{1, 50} = 0.04$, $P = 0.84$), or condition index ($R^2 = 0.03$, $F_{1, 48} = 1.5$, $P = 0.23$). However, male condition index decreased with lay date ($R^2 = 0.11$, $F_{1, 48} = 6.1$, $P = 0.02$). We found no differences in the size ($|Z| = 42.5$, $P = 0.29$, $N = 26$), mass ($|Z| = -29.0$, $P = 0.50$, $N = 28$), or condition index ($|Z| = -40.5$, $P = 0.28$, $N = 25$) of social males and extra-pair males.

DISCUSSION

Although about half of the extra-pair nestlings in our study were sired by males known to be breeding concurrently in our study area (Ferretti et al. 2011), we found that breeding synchrony, lay date, distance to the nearest neighbor, and nest density were not good predictors of the EPP status of female White-rumped Swallows. These variables might be important predictors of extra-pair behavior for other species, particularly territorial species, e.g., Clay-colored Robins (Stutchbury et al. 1998) and Yellow-billed Magpies
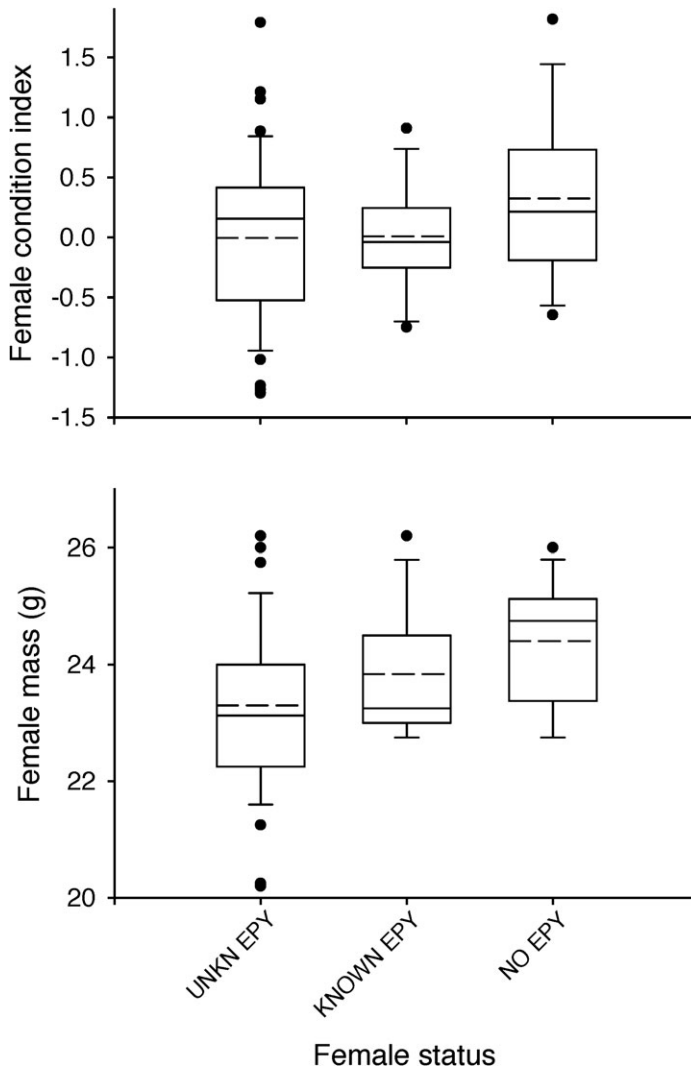
Fig. 1. Comparison of condition indices (A) and mass (B) of female White-rumped Swallows that differed in extra-pair paternity status. NO EPY: females with all within-pair young; KNOWN EPY: females with at least one extra-pair young and the genetic father was breeding in our study area; and UNKN EPY: females with least one extra-pair young and the genetic father was not breeding in our study area. Conditions indices of females with different extra-pair paternity status did not differ, but differences in mass between NO EPY and UNKN EPY females were significant (Table 2). Box plots show mean (dashed line), median (solid line), 25th–75th (box line) percentiles, 5th–95th percentiles (whisker caps), and outliers (solid circles).

Table 2. Between-category comparisons of female White-rumped Swallows that differed in extra-pair paternity status. (A) Comparison of condition indices for each pair of female categories using Student's *t*-test. (B) Comparison of mass for each pair of categories using Student's *t*-test.

Females compared[a]	Difference	SE of difference	P value[b]
(A)			
NO EPY – UNKN EPY	0.48	0.22	0.03*
NO EPY – KNOWN EPY	0.32	0.26	0.23
KNOWN EPY – UNKN EPY	0.17	0.23	0.46
(B)			
NO EPY – UNKN EPY	1.39	0.44	0.003*
NO EPY – KNOWN EPY	0.82	0.43	0.06
KNOWN EPY – UNKN EPY	0.57	0.51	0.27

[a]NO EPY: females with all within-pair young, KNOWN EPY: females with at least one extra-pair young and the genetic father was breeding in our study area, and UNKN EPY: females with at least one extra-pair young and the genetic father was not breeding in our study area.
[b]Asterisk denotes $P < 0.05$.

(Birkhead et al. 1992), but White-rumped Swallows do not have territories where males can attempt to control access to their mates (Massoni et al. 2007). White-rumped Swallows are secondary cavity nesters that depend on natural cavities or cavities excavated by other species of birds (e.g., woodpeckers) and, during the breeding season, they defend these cavities against other birds, both conspecifics and heterospecifics (Massoni et al. 2007). This competition for nest sites results in little or no mate-guarding by males during female fertile periods because pairs often alternate guarding nest boxes and foraging, i.e., while one adult remains at the nest box, the other forages (VF, unpubl. data). Similar behavior has been reported in Tree Swallows (Leffelaar and Robertson 1984). Because female White-rumped Swallows forage at variable distances from their nest boxes and social mates during their fertile period, local nest density and synchrony have less influence on their mating strategies. Density may still influence opportunities for extra-pair matings by White-rumped Swallows, but the density of individuals at foraging sites rather than density of nest sites. We did not, however, measure densities of birds at a larger geographic scale that would include possible foraging areas.

The breeding synchrony hypothesis, as proposed by Stutchbury and Morton (1995), is based on the premise that female birds can better assess the quality of males displaying synchronously (e.g., singing, building nests, or any other reproductive behavior).

However, female White-rumped Swallows may be constrained by the need to quickly find a nest cavity to breed, so assessment of male quality may not be as important (Weatherhead and Yezerinac 1998, Spottiswoode and Møller 2004), and this may result in females benefiting from engaging in extra-pair behaviors through a mechanism different than that originally proposed by Stutchbury and Morton (1995). In Tree Swallows, for example, females compete intensely for nest boxes (Berzins and Dawson 2018), with reports of nest usurpations by females (Leffelaar and Robertson 1985) highlighting the possible limited availability of nest sites and the importance of arriving early in breeding areas. In White-rumped Swallows, Miño and Massoni (2017) found that females in the same study area where we conducted our study re-used nest boxes based on previous breeding performance, i.e., they breed in the same box if they had previously fledged young, with retention of mates not as important and not related to breeding performance. Therefore, for females at our study site, nest-box quality may be more important than male quality; females paired with lower quality males may seek extra-pair copulations after procuring a nest site, eliminating any relationship between EPP and breeding synchrony at the population level.

Female condition index and mass were the only variables that helped explain variation in EPP status in our study, with EPY females lighter on average for their size than NO EPY
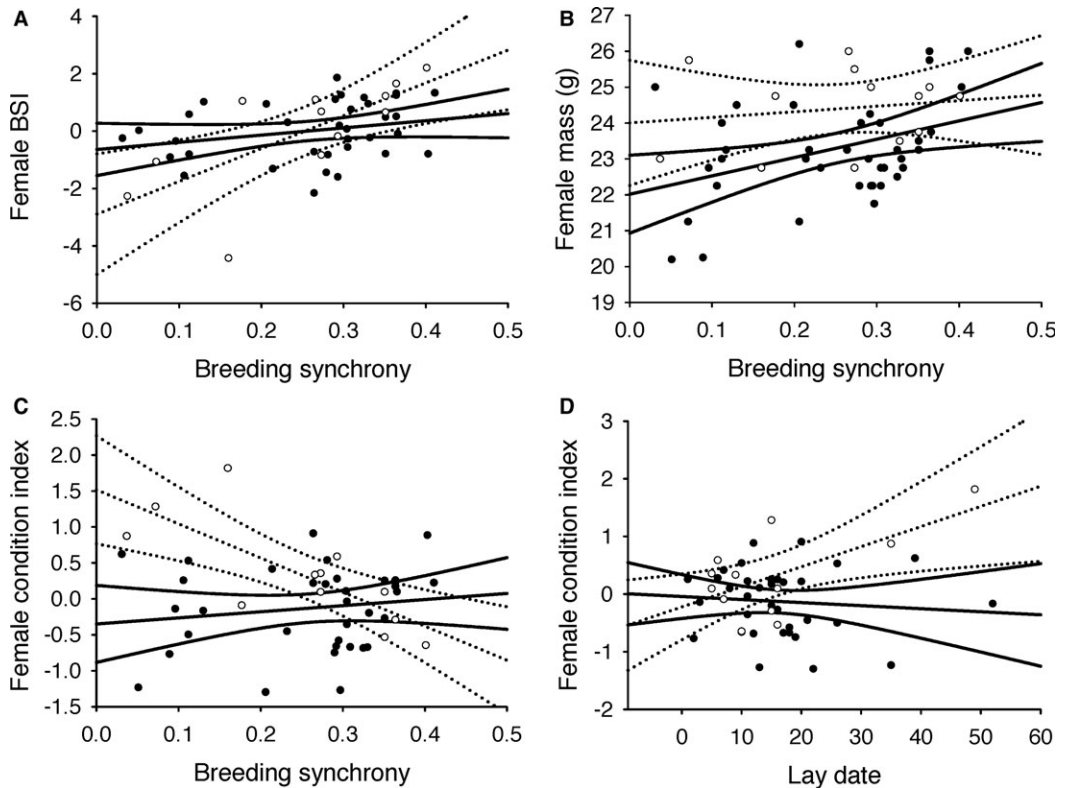
Fig. 2. Relationships between breeding synchrony and lay date and body parameters of female White-rumped Swallows with (filled circles and solid lines) and without (open circles and dotted lines) extra-pair young. (A) Relationship between female body size index and breeding synchrony index, (B) Relationship between female mass (g) and breeding synchrony index, (C) Relationship between body condition index of females and breeding synchrony index, and (D) Relationship between body condition index of females and lay date. Regression lines and 95% confidence intervals are shown.

females. A fundamental indicator of condition for aerial insectivores like swallows is body mass because lighter birds can be more efficient flyers (Norberg 1981). The flight-efficiency hypothesis states that lighter birds might have an adaptive advantage, with energy saved during flight potentially translating into more food for nestlings. In fact, Boyle et al. (2012) found that female Tree Swallows lost mass prior to the nestling period, with small losses of lean mass during incubation and steep losses of fat prior to eggs hatching. Their results are consistent with the flight-efficiency hypothesis, with adult birds able to adjust their energetic stores to maximize flight efficiency when feeding nestlings. For White-rumped Swallows, energy conserved by having a lower body mass and a higher lean mass index might

allow them to provide more food for their young, increasing their chances of survival. In fact, Ferretti et al. (2011) found that, in the same study area where we conducted our study, EPY female White-rumped Swallows fledged more young than NO EPY females even though females in these two categories had, on average, clutches of similar size. In addition, however, lighter female White-rumped Swallows might be less constrained by the need to trade paternity assurance for male assistance with parental care, and thus might be more likely to engage in extra-pair behavior (Gowaty 1996a,b), resulting in a negative relationship between residual body mass (corrected for structural size) and EPP. Furthermore, assuming that the measurements of females in our study that we obtained during incubation were comparable to those

during their fertile periods, more energy-efficient flight of lighter females might have also allowed them to forage further from nest sites, providing access to more potential extra-pair mates.

Although we found no relationship between female condition and distance to extra-pair males, our results were limited to cases where we were able to identify the sire of extra-pair offspring. We did find, however, that females that sired offspring of unidentified males (presumably from outside the limits of our study area where we did not sample adults) were lighter and leaner than females that sired young with males in our study area (both social males and extra-pair males breeding in boxes in our study area). This suggests that female mass might have had an effect on the distance from nest boxes where extra-pair copulations occurred. Supporting evidence for this comes from a study of congeneric Tree Swallows by Dunn and Whittingham (2007). They experimentally manipulated the cost of flying for females and found that the spatial distribution of extra-pair fertilizations changed, with handicapped females obtaining extra-pair copulations closer to their nests than control females.

We found that female BSI and mass increased with increasing breeding synchrony, i.e., females that bred more synchronously later in the breeding season were larger and weighed more than those that bred earlier. Female condition indices, however, did not vary with either synchrony or lay date. Massoni et al. (2007) found that White-rumped Swallows breeding in the same study area experienced a decline in clutch size and reproductive output with lay date, with late-season breeders laying fewer eggs and having nestlings that weighed less at fledging than those in early-season nests. Therefore, lean mass of female White-rumped Swallows is likely a better indicator of reproductive success, and the likelihood of engaging in EPP, than other ecological variables.

We also found that extra-pair and social males did not differ in size, mass, or body condition. In addition, unlike females, these variables did not vary with breeding synchrony and lay date. Similarly, extra-pair and social male Tree Swallows did not differ in plumage coloration and brightness, or any other morphological traits measured (Dunn

et al. 1994, Bitton et al. 2007). However, males that sired extra-pair offspring had brighter plumage than those that only sired within-pair offspring (Bitton et al. 2007). Although not examined in our study, differences in plumage coloration and brightness of males might be used by female White-rumped Swallows to assess male quality.

In sum, White-rumped Swallows have one of the highest rates of EPP reported for socially monogamous passerines (Ferretti et al. 2011). Variables that could affect opportunities for successful mating encounters, such as timing of breeding and nest density, had no apparent effect on the genetic mating system of this species, but leaner and lighter females had more extra-pair offspring, fledged more young, and tended to sire offspring with males not socially paired to other females in the same breeding area. These results suggest that female White-rumped Swallows with extrapair young may be more efficient foragers and flyers during periods of high-energy demand (i.e., feeding nestlings). The mating system of White-rumped Swallows may derive from a combination of pressure to secure nest sites (Miño and Massoni 2017) and their foraging ecology, mediated through female body mass and condition. The high rate of EPP for female White-rumped Swallows in our study appears to be due to a combination of opportunities for mating with local neighbors and with males encountered in foraging and roosting areas. Female body condition and flight ability are therefore among the factors that appear to influence extra-pair behavior.

LITERATURE CITED

ARDIA, D. R. 2005. Tree Swallows trade off immune function and reproductive effort differently across their range. Ecology 86: 2040–2046.

———, M. F. WASSON, AND D. W. WINKLER. 2006. Individual quality and food availability determine yolk and egg mass and egg composition in Tree Swallows *Tachycineta bicolor*. Journal of Avian Biology 37: 252–259.

ARNOLD, K. E., AND I. P. F. OWENS. 2002. Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. Proceedings of the Royal Society B 269: 1263–1269.

BARBER, C. A., R. J. ROBERTSON, AND P. T. BOAG. 1996. The high frequency of extra-pair paternity in Tree Swallows is not an artifact of nestboxes. Behavioral Ecology and Sociobiology 38: 425–430.

BENNETT, P. M., AND I. P. F. OWENS. 2002. Evolutionary ecology of birds: life histories, mating systems and extinction. Oxford University Press, Oxford, UK.

BERZINS, L. L., AND R. D. DAWSON. 2018. Experimentally altered plumage brightness of female Tree Swallows (*Tachycineta bicolor*) influences nest site retention and reproductive success. Canadian Journal of Zoology 96: 600–607.

BIRKHEAD, T. R. 1998. Sperm competition in birds. Reviews of Reproduction 3: 123–129.

———, AND J. D. BIGGINS. 1987. Reproductive synchrony and extra-pair copulation in birds. Ethology 74: 320–334.

———, K. CLARKSON, M. D. REYNOLDS, AND W. D. KOENIG. 1992. Copulation and mate guarding in the Yellow-billed Magpie *Pica nuttalli* and a comparison with the Black-billed Magpie *P. pica*. Behaviour 121: 110–130.

BITTON, P.-P., E. L. O'BRIEN, AND R. D. DAWSON. 2007. Plumage brightness and age predict extrapair fertilization success of male Tree Swallows, *Tachycineta bicolor*. Animal Behaviour 74: 1777–1784.

BOYLE, W. A., D. W. WINKLER, AND C. G. GUGLIELMO. 2012. Rapid loss of fat but not lean mass prior to chick provisioning supports the flight efficiency hypothesis in Tree Swallows. Functional Ecology 26: 895–903.

BULIT, F., M. BARRIONUEVO, AND V. MASSONI. 2014. Insights into life history theory: a brood size manipulation on a southern hemisphere species, *Tachycineta leucorrhoa*, reveals a fast pace of life. Journal of Avian Biology 45: 225–234.

———, A. G. PALMERIO, AND V. MASSONI. 2008. Differences in rates of nest-visitation and removal of faecal sacs by male and female White-rumped Swallows. Emu 108: 181–185.

CHUANG, H. C., M. S. WEBSTER, AND R. T. HOLMES. 1999. Extrapair paternity and local synchrony in the Black-throated Blue Warbler. Auk 116: 726–736.

CONRAD, K. F., R. J. ROBERTSON, AND P. T. BOAG. 1998. Frequency of extrapair young increases in second broods of Eastern Phoebes. Auk 115: 497–502.

CROWE, S. A., O. KLEVEN, K. E. DELMORE, T. LASKEMOEN, J. J. NOCERA, J. T. LIFJELD, AND R. J. ROBERTSON. 2009. Paternity assurance through frequent copulations in a wild passerine with intense sperm competition. Animal Behaviour 77: 183–187.

DUNN, P. O., J. T. LIFJELD, AND L. A. WHITTINGHAM. 2009. Multiple paternity and offspring quality in Tree Swallows. Behavioral Ecology and Sociobiology 63: 911–922.

———, R. J. ROBERTSON, D. MICHAUD-FREEMAN, AND P. T. BOAG. 1994. Extra-pair paternity in Tree Swallows: why do females mate with more than one male? Behavioral Ecology and Sociobiology 35: 273–281.

———, AND L. A. WHITTINGHAM. 2007. Search costs influence the spatial distribution, but not the level, of extra-pair mating in Tree Swallows. Behavioral Ecology and Sociobiology 61: 449–454.

EMLEN, S. T., AND L. W. ORING. 1977. Ecology, sexual selection and the evolution of mating systems. Science 197: 215–223.

ERSTS, P. J. [online]. 2016. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. http://biodiversityinformatics.amnh.org/open_source/gdmg (Accessed 10 April 2018).

FERRETTI, V., M. LILJESTHRÖM, A. S. LÓPEZ, I. J. LOVETTE, AND D. W. WINKLER. 2016. Extra-pair paternity in a population of Chilean Swallows breeding at 54 degrees south. Journal of Field Ornithology 87: 155–161.

———, V. MASSONI, F. BULIT, D. W. WINKLER, AND I. J. LOVETTE. 2011. Heterozygosity and fitness benefits of extrapair mate choice in White-rumped Swallows (*Tachycineta leucorrhoa*). Behavioral Ecology 22: 1178–1186.

GOWATY, P. A. 1996a. Battles of the sexes and origins of monogamy. In: Partnerships in birds: the study of monogamy (J. M. Black, ed.), pp. 21–52. Oxford University Press, Oxford, UK.

———. 1996b. Field studies of parental care in birds: new data focus questions on variation among females. In: Parental care: evolution, mechanisms, and adaptive significance (J. S. Rosenblatt and C. T. Snowdon, eds.), pp. 477–531. Academic Press, London, UK.

———, AND W. C. BRIDGES. 1991. Nestbox availability affects extra-pair fertilizations and conspecific nest parasitism in Eastern Bluebirds *Sialia sialis*. Animal Behaviour 41: 661–676.

GREEN, A. J. 2001. Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82: 1473–1483.

GRIFFITH, S. C., I. P. F. OWENS, AND K. A. THUMAN. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11: 2195–2212.

HASSELQUIST, D., M. F. WASSON, AND D. W. WINKLER. 2001. Humoral immunocompetence correlates with date of egg-laying and reflects work load in female Tree Swallows. Behavioral Ecology 12: 93–97.

KALINOWSKI, S. T., M. L. TAPER, AND T. C. MARSHALL. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16: 1099–1106.

———, ———, AND ———. 2010. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 19: 1512.

KEMPENAERS, B. 1993. The use of a breeding synchrony index. Ornis Scandinavica 24: 84.

———, B. CONGDON, P. BOAG, AND R. J. ROBERTSON. 1999. Extrapair paternity and egg hatchability in Tree Swallows: evidence for the genetic compatibility hypothesis? Behavioral Ecology 10: 304–311.

LABARBERA, K., P. E. LLAMBIAS, E. R. A. CRAMER, T. D. SCHAMING, AND I. J. LOVETTE. 2010. Synchrony does not explain extrapair paternity rate variation in northern or southern House Wrens. Behavioral Ecology 21: 773–780.

LANGEFORS, A., D. HASSELQUIST, AND T. VON SCHANTZ. 1998. Extra-pair fertilizations in the Sedge Warbler. Journal of Avian Biology 29: 134–144.

LEFFELAAR, D., AND R. J. ROBERTSON. 1984. Do male Tree Swallows guard their mates? Behavioral Ecology and Sociobiology 16: 73–79.

———, AND ———. 1985. Nest usurpation and female competition for breeding opportunities by Tree Swallows. Wilson Bulletin 97: 221–224.

MAKAREWICH, C. A., L. M. STENZLER, V. FERRETTI, D. W. WINKLER, AND I. J. LOVETTE. 2009. Isolation and characterization of microsatellite markers from three species of swallows in the genus *Tachycineta*: *T. albilinea*, *T. bicolor* and *T. leucorrhoa*. Molecular Ecology Resources 9: 631–635.

MARSHALL, T. C., J. SLATE, L. E. B. KRUUK, AND J. M. PEMBERTON. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7: 639–655.

MASSONI, V., F. BULIT, AND J. C. REBOREDA. 2007. Breeding biology of the White-rumped Swallow *Tachycineta leucorrhoa* in Buenos Aires Province, Argentina. Ibis 149: 10–17.

MAYER, C., AND G. PASINELLI. 2013. New support for an old hypothesis: density affects extra-pair paternity. Ecology and Evolution 3: 694–705.

MIÑO, C. I., AND V. MASSONI. 2017. Sexual differences in the effect of previous breeding performance on nest-box reuse and mate retention

in White-rumped Swallows (*Tachycineta leucorrhoa*). Emu 117: 130–140.

MØLLER, A. P. 1991. Density-dependent extra-pair copulations in the swallow *Hirundo rustica*. Ethology 87: 316–329.

MOORE, O. R., B. J. M. STUTCHBURY, AND J. S. QUINN. 1999. Extrapair mating system of an asynchronously breeding tropical songbird: the Mangrove Swallow. Auk 116: 1039–1046.

NEUDORF, D. L. H. 2004. Extrapair paternity in birds: understanding variation among species. Auk 121: 302–307.

NORBERG, R. A. 1981. Temporary weight decrease in breeding birds may result in more fledged young. American Naturalist 118: 838–850.

O'BRIEN, E. L., AND R. D. DAWSON. 2007. Context-dependent genetic benefits of extra-pair mate choice in a socially monogamous passerine. Behavioral Ecology and Sociobiology 61: 775–782.

SAS INSTITUTE INC. 2012. JMP® 10. Cary, NC, USA.

SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82–90.

SPOTTISWOODE, C., AND A. P. MØLLER. 2004. Extrapair paternity, migration and breeding synchrony in birds. Behavioral Ecology 15: 41–57.

STAPLETON, M. K., O. KLEVEN, J. T. LIFJELD, AND R. J. ROBERTSON. 2007. Female Tree Swallows (*Tachycineta bicolor*) increase offspring heterozygosity through extrapair mating. Behavioral Ecology and Sociobiology 61: 1725–1733.

STUTCHBURY, B. J. M. 1998a. Breeding synchrony best explains variation in extra-pair mating system among avian species. Behavioral Ecology and Sociobiology 43: 221–222.

———. 1998b. Female mate choice of extra-pair males: breeding synchrony is important. Behavioral Ecology and Sociobiology 43: 213–215.

STUTCHBURY, B. J., AND E. S. MORTON. 1995. The effect of breeding synchrony on extra-pair mating systems in songbirds. Behaviour 132: 675–690.

———, AND R. J. ROBERTSON. 1988. Within-season and age-related patterns of reproductive performance in female Tree Swallows (*Tachycineta bicolor*). Canadian Journal of Zoology 66: 827–834.

STUTCHBURY, B. J. M., E. S. MORTON, AND W. H. PIPER. 1998. Extra-pair mating system of a synchronously breeding tropical songbird. Journal of Avian Biology 29: 72–78.

VAN DONGEN, W. F. D., AND R. A. MULDER. 2009. Multiple ornamentation, female breeding synchrony, and extra-pair mating success of Golden Whistlers (*Pachycephala pectoralis*). Journal of Ornithology 150: 607–620.

WEATHERHEAD, P. J., AND S. M. YEZERINAC. 1998. Breeding synchrony and extra-pair mating in

birds. Behavioral Ecology and Sociobiology 43: 217–219.

WESTNEAT, D. F., AND P. W. SHERMAN. 1997. Density and extra-pair fertilizations in birds: a comparative analysis. Behavioral Ecology and Sociobiology 41: 205–215.

———, ———, AND M. L. MORTON. 1990. The ecology and evolution of extra-pair copulations in birds. In: Current ornithology (D. M. Power, ed.), pp. 331–369. Plenum Press, London, UK.

WHITTINGHAM, L. A., AND P. O. DUNN. 2001. Survival of extrapair and within-pair young in Tree Swallows. Behavioral Ecology 12: 496–500.

———, ———, AND M. K. STAPLETON. 2006. Repeatability of extra-pair mating in Tree Swallows. Molecular Ecology 15: 841–849.

WINKLER, D. W., AND P. E. ALLEN. 1996. The seasonal decline in Tree Swallow clutch size: physiological constraint or strategic adjustment. Ecology 77: 922–932.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Microsatellite loci used for paternity assessment and their characteristics. N: number of unrelated individuals genotyped, N_A: number of alleles, H_O: observed heterozygosity, H_E, expected heterozygosity.

Table S2. Summary of extra-pair paternity rates for White-rumped Swallows (from Ferretti et al. 2011). Exclusion probability for loci used > 0.9999.