Original Article

Effects of known age on male paternity in a migratory songbird

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Many avian studies have shown that reproductive performance improves with age, but little is known about how key components of male fitness, extrapair and within pair paternity, vary across life spans. We tested for age effects on male paternity in purple martins (*Progne subis*) using cross-sectional analyses of known-aged males (1–9 years old) and longitudinal analyses of individuals sampled in 2 successive years. Microsatellite analyses found that 137 of 297 (46%) nests contained extrapair offspring and 273 of 1235 (22%) offspring were extrapair. Using a subsample of unique known-aged males ($n = 160$), we found significant linear and nonlinear effects of male age on the number of within pair offspring and, to a lesser extent, on the number of extrapair offspring sired. Male genetic reproductive success increased with age to 3 years and then leveled off. In longitudinal comparisons of known age males sampled in successive years ($n = 41$), within pair offspring increased with age, even for males ≥ 2 years old. Paired comparisons ($n = 74$) found that extrapair sires were older than the males they cuckolded, and that first-year males were significantly underrepresented as extrapair sires given the known age distribution in the population. Poor genetic reproductive performance in younger males is likely constrained through male–male competition during mate guarding and female choice for older males. *Key words:* age-related, extrapair paternity, genetic reproductive success, known age, life history strategies, sexual selection. *[Behav Ecol 23:313–321 (2012)]*

INTRODUCTION

Numerous studies on birds have shown that measures of individual reproductive performance (e.g., laying date, clutch size, egg volume, number of offspring produced, nesting success) improve with age (Robertson and Rendell 2001; van de Pol and Verhulst 2006; Brommer et al. 2007). Reproductive performance may also stabilize at mid-age and then decline in old individuals as a result of senescence (Bouwhuis et al. 2009; Ricklefs 2010). 3 main hypotheses explain why reproductive performance increases with age (reviewed in Forslund and Pärt 1995). 1) The selection hypothesis proposes that progressive mortality of lower quality individuals results in a population-level, but not individual-level, increase in performance among the remaining older birds (Perrins and Moss 1974). 2) The restraint hypothesis suggests that young birds face life-history trade-offs and withhold reproductive effort to improve future survival (Hamann and Cooke 1987; Lessels and Krebs 1989). 3) The constraint hypothesis suggests that young birds perform poorly due to lack of experience, lower foraging success, and/or lower competitive ability (Lack 1968).

Where females copulate with multiple partners a "hidden" component of male reproductive performance could include increased or decreased genetic reproductive success among older males. High fertilization success in older males could result from female choice (Morton et al. 1990) if females can use male age as a proxy for genetic quality (Kokko 1998, but see Beck and Promislow 2007). Low paternity by young males as a result of female choice would be a form of constraint on

performance. Age-related mating success could also occur via constraint if older males are more effective in male–male competition for females due to experience or larger size (Johnsen et al. 2003; Wagner et al. 1996). The oldest males may experience senescence in genetic reproductive success via reduced sperm competition (Raveh et al. 2010) or decline in male dominance and social status that limits mate access (Mainguy et al. 2009; Raveh et al. 2010). Older male feral fowl (*Gallus gallus domesticus*) experienced senescence in social dominance during intense male–male competition and older males were far less effective in sperm competition and fertilization (Dean et al. 2010). Many birds feature intense male–male competition for copulations via extrapair paternity in socially monogamous species (Westneat and Stewart 2003) but little is known about whether extrapair or within pair paternity varies with age among older males (Table 1).

Age class effects are common in avian paternity studies, with first-year males typically siring fewer within pair offspring (WPO) in their own nest (Griffith et al. 2002) or fewer extrapair offspring (EPO) in other nests compared with older males (Johnsen et al. 2001). Age class comparisons do not fully test for age effects in paternity because first-year versus older males may differ greatly in many other characteristics (e.g., experience, size, coloration) and one would expect age effects to continue beyond the second year. Few paternity studies have tested how components of genetic reproductive success vary among older males (Table 1) and most include individuals for whom only minimum age was known which may obscure subtle age effects. Some studies have found significant age effects among older males, but others have not and none have found that age affects both within pair and extrapair paternity.

Here, we investigate effects of known age on male paternity in purple martins (*Progne subis*), a short-lived migratory passerine for which age class has been previously shown to be

Table 1

DNA fingerprinting and microsatellite paternity studies on short-lived socially monogamous passerines that test for age effects among older males

Species	Age effect among older males?			Proposed mechanism	Reference
	WPO	EPO	Total		
Pied flycatcher *Ficedula hypoleuca*	Yes	—	—	Territory defense	Moreno et al. (2010)[1]
Blue tit *Cyanistes caeruleus*	No	—	—	—	Kempenaers et al. (1997)[1]
Coal tit *Periparus ater*	No	Yes	No	Unknown	Schmoll et al. (2007)[2]
Purple martin *Progne subis*	Yes	Yes	Yes	Mate guarding, female choice	This study[2]
American redstart *Setophaga ruticilla*	Yes	—	—	Male experience	Perreault et al. (1997)[1]
Hooded warbler *Wilsonia citrina*	No	No	—	—	Stutchbury et al. (1997)[1]
Red-winged blackbird *Agelaius phoeniceus*	No	Yes	No	Male experience, female choice	Weatherhead and Boag (1995)[1]

In all cases, significant effects were positive. Variables not tested indicated by '—'. Studies are based on minimum or known age birds (superscript 1 or 2, respectively, after reference) and report data for age effects among older males.

a powerful predictor of male genetic success (Morton et al. 1990; Wagner et al. 1996). Male age class is readily discernible in purple martins due to delayed plumage maturation; first-year males have dull female-like subadult plumage in contrast to the iridescent steel-blue coloration of older males (Brown 1997). We collaborated with the Purple Martin Conservation Association who has banded over 14 000 nestlings in northwestern Pennsylvania since 1994 resulting in known age martins up to 9 years old. Natal recruitment is relatively high in this region (26%, Tarof et al. 2011). We tested whether genetic reproductive success increased with age using 1) a cross-sectional analysis of known age males and 2) a longitudinal analysis of known age individuals sampled in 2 consecutive years. We also tested whether known age extrapair sires were older than the males whom they cuckolded using paired comparisons, and if first-year males were significantly underrepresented as extrapair sires given the known age distribution in the population. We demonstrate significant linear and nonlinear effects of known age on genetic reproductive success in purple martins.

MATERIALS AND METHODS

Study species and general field methods

Purple martins nest colonially in nest boxes in eastern North America and migrate to South America in winter (Brown 1997). From May to August 2006–2008, we studied purple martins at 2 breeding colonies in northwestern Pennsylvania, USA (42°08′N, 80°18′W) that were 19 km apart. The Edinboro colony averaged 60 pairs/year; Troyer colony 160 pairs/year. We visited colonies several times weekly and, using telescopes, read alphanumeric color bands of returning banded birds and identified social pairings. Social pairs were confirmed from observations of nest defense and nestling feeding. Males and females were assigned to age class (first-year versus older, i.e., ≥2 years old) using distinctive plumage characteristics (Brown 1997) and/or band records. Across all years, more first-year females paired to first-year males ($n = 48$) compared with older males ($n = 24$), whereas most older females paired with older males ($n = 184$) rather than with first-year males ($n = 41$).

We checked nest contents approximately every 5 days to determine first egg-laying date and nesting success. In a generalized linear model (GLM) involving unique females and taking year into account, first-year females laid smaller clutches (4.43 ± 0.10 eggs) than older females (4.92 ± 0.06 eggs) (whole model: $\chi^2_3 = 25.54$, $n = 262$, $P < 0.0001$; female age class: $\chi^2 =$

16.92, $P < 0.0001$; year: $\chi^2 = 8.04$, $P = 0.02$). Martins are single-brooded in our population and females rarely lay a replacement clutch following early nest failure. Across all years, older males paired to older females had earlier first egg dates (31 May, 30.86 ± 0.58 days [day 1 = 1 May]) than if paired to a first-year female (5 June, 34.69 ± 1.60 days; $F_{1,208} = 5.06$, $P = 0.03$). The same was true for first-year males (7 June, 38.58 ± 0.92 days versus 12 June, 42.11 ± 0.85 days; $F_{1,89} = 8.65$, $P = 0.004$).

In early July, once most eggs hatched, we caught adults at night using a trap that lowered doors simultaneously over all cavity entrances of a nest house. We returned before dawn to extract adults from compartments for banding and blood sampling. Daytime target trapping captured individuals not caught at night. We banded adults with a USGS numbered aluminum band and color auxiliary band with a unique alphanumeric code. We banded 2830 nestlings at 10–20 days old. Edinboro colony was blood sampled nearly completely in all 3 years of this study. Troyer colony was sampled partially in 2006 and nearly completely in 2007 but was not sampled in 2008 due to restrictions on access. Fieldwork and blood sampling were conducted under approval of the York University Animal Care Committee.

Known male age

Of all paired birds observed at the 2 breeding colonies, we had 89 first-year males and 71 older males (2–9 years old) of known age. We also had 137 other paired males for whom minimum age was known because they were first banded as an older individual. These males were included in general paternity patterns and age class comparisons but were excluded from paternity analyses involving known age birds.

Paternity methods

We used a panel of 4 hypervariable microsatellite-flanking polymerase chain reaction (PCR) primer sets (Stanley et al. 2011) to determine the frequency of extrapair fertilizations and assign parentage to 1235 nestlings based on the social parents of 297 families. Of the offspring sampled, 957 (77.5%) genotyped at 4 loci, 226 (18.3%) genotyped at 3 loci, 26 (2.1%) genotyped at 2 loci, and 26 (2.1%) genotyped at 1 locus. Offspring genotyped at less than 2 loci were excluded from further analysis. We only included families with known social parents and for which both attending parents and their offspring were sampled. In addition to family groups, 12 males were genotyped

as putative sires of EPO. In 2007, 37 males (14%) were also sampled in 2006 but only 1 paired with the same female both years. In 2008, 14 males (25%) were sampled in a previous year but all paired with different females.

We collected 10–50 µl blood samples from adults and nestlings and stored samples at 4 °C in 1 ml of Queen's lysis buffer until DNA extraction. Genomic DNA was amplified in 10 µl PCR reactions under the following conditions: initial denaturation at 94 °C for 3 min; 30 cycles consisting of 1 min at 94 °C, 1 min annealing (T_a: PUMA 19, 55.8 °C; PUMA 49, 50 °C; PUMA 74, 48.2 °C; PUMA 98, 55 °C), 45 s at 72 °C, followed by a final extension at 72 °C for 5 min. Each tube contained 5.7 µl water (PUMA 49, 4.8 µl; PUMA 19, 5.1 µl), 1.0 µl 10× TSG PCR buffer (Bio Basic, Markham, Canada), 0.8 µl 25 mM $MgCl_2$ (Fermentas, Burlington, Canada) (PUMA 19 and PUMA 49, 1.2 µl), 0.4 µl 10 mM deoxyribonucleotide triphosphates (PUMA 19, 0.6 µL; GE Healthcare, Baie d'Urfe, Quebec), 0.2 µl 10 µM fluorescently labeled Beckman Coulter WellRed forward primer (250 nm, HPLC purification; Integrated DNA Technologies, Coralville, Iowa), 0.2 µl 10 µM reverse primer, 0.2 µl 5 U/µl TSG DNA Polymerase, 1.5 µl undiluted DNA (212.5 ± 5.2 ng/ml) (PUMA 49, 2.0 µl; PUMA 74, 1.0 µl), and 0.5 µl 100% dimethyl sulfoxide (PUMA 74 only; BioShop, Burlington, Canada). Products were visualized on 1% agarose gels stained with 5 µl 5 mg/ml ethidium bromide (BioShop, Burlington, Canada) to confirm amplification. This protocol yielded dye-labeled amplicons for fragment analysis. Genotyping was conducted on the Beckman Coulter CEQ 8000. We genotyped individuals in 3-locus pool-plex reactions containing 1.5 µl PCR product mixture (4.4 µl PUMA 19, 4.3 µl PUMA 49, 1.3 µl PUMA 98) in 38.5 µl of CEQ cocktail (38 µl 99.5% deionized formamide [Sigma-Aldrich, St. Louis, MO] plus 0.5 µl size standard [Beckman Coulter, Mississauga, Canada]). We ran 1.0 µl PUMA 74 PCR product separately in 38.5 µl formamide plus 0.5 µl size standard. Genotypes were scored against a 400-bp size standard (600 bp for PUMA 74) with phosphoramidite dye mobility calibration and the "cubic" algorithm model ("quartic" for PUMA 74). Thirty random adults were genotyped twice at all loci to confirm repeatability; genotypes were 100% repeatable.

We defined WPO as nestlings that matched the genotypes of their social parents at all loci genotyped ($n = 102$ in 2006, $n = 404$ in 2007, $n = 219$ in 2008) or that mismatched with the social father at 1 locus and had low probability of resemblance by chance alone at the other matching loci (Ibarguchi et al. 2004). If mutation rate is high, single-locus mismatches can grossly overestimate extrapair paternity. Probability of resemblance (P_{Ra}), the likelihood that 2 particular individuals (e.g., offspring and social father) share at least 1 allele at a specific locus by chance alone, has high-resolving power for dealing with single-locus mismatches where genotypes for parents and offspring are known and microsatellite allele frequencies from the sampled population are available (Ibarguchi et al. 2004). For family groups containing a single-paternal mismatch, we calculated P_{Ra} using the formula $P_{Ra} = \left(2p_a - p_a^2\right)^2$, for a given allele (a) with allele frequency (p_a), at each locus other than the mismatching locus. The product of these values generated the cumulative probability of resemblance (P_{RaCum}) for the matching loci. Social males were considered to be the genetic sires if P_{RaCum} was ≤0.005 (after Ibarguchi et al. 2004). That is, offspring with a P_{RaCum} meeting this threshold had less than a 0.5% chance of sharing alleles at the other loci with the social male by chance ($n = 91$ in 2006, $n = 189$ in 2007, $n = 31$ in 2008) and thus were assigned as WPO (mean $P_{RaCum} = 0.0005 ± 0.006$). The mutation rate (# per meiotic event) of 1 of the loci, HrU10, among 6 species of swallows ranges from 0.6% to 10.8% and is relatively high for our purple martin population (5.6%, Anmarkrud et al. 2011). Allele size explained almost all the variance in mutation rate across species

and was relatively large (310 bp) in purple martins, which may explain why there were numerous cases of a single-locus mismatch, whereas the other microsatellite loci did match the social father.

We identified potential EPO by evaluating genotypes of each offspring in a family against their social parents. EPO were defined as nestlings that mismatched the social male at ≥2 loci ($n = 68$ in 2006, $n = 130$ in 2007, $n = 42$ in 2008) or that had a P_{RaCum} value >0.005 with 1 paternal mismatch ($n = 18$ in 2006, $n = 21$ in 2007, $n = 1$ in 2008; average $P_{RaCum} = 0.076 ± 0.013$). Next, we used CERVUS 3.0.3 (Kalinowski et al. 2007) to identify candidates for true extrapair sires. Using genotype data, CERVUS calculates allele frequencies, deviations from Hardy–Weinberg equilibrium, polymorphic information content, null allele frequencies, and exclusion probabilities. A likelihood ratio approach determines true parentage of offspring from the pool of candidate sires by evaluating all offspring-sire genotype combinations (maternal genotype known). We used allele frequency data from our population in CERVUS' parentage analysis likelihood simulation to generate paternity assignments for individual EPO based on the following criteria. Both social parents needed to genotype at a minimum of 2 loci to be included in parentage analyses. Cases of putative intraspecific brood parasitism (<4.7%) were excluded. We included the putative social male and all other sampled males each year in a colony to identify the most likely and second most likely candidate extrapair sires. In simulations, we used 100 000 permutation cycles, 80% (2006), 95% (2007), and 80% (2008) of candidate sires in our sampled population, a minimum of 2 loci genotyped per individual, 95.6% of loci genotyped (from allele frequencies), and 1% genotyping error rate. Determination of the extrapair sire for EPO was based on delta scores calculated by CERVUS, defined as the difference in LOD scores between the most likely and second most likely candidate sire, at either the 80% ($n = 68$ EPO) or 95% confidence level ($n = 64$ EPO). Because we sampled 80–95% of males per colony, we assumed that unassigned offspring were likely sired by males outside the colony.

We determined the probability of alleles matching the candidate genetic male by chance alone based on nonexclusion probabilities calculated by CERVUS. Nonexclusion probability (1-exclusion probability) was the likelihood of not excluding a candidate male that was unrelated genetically to an offspring. The mean (±standard deviation) nonexclusion probability for EPO with both social parents known was 0.017 ± 0.002 (median = 0.007). The average for all offspring (WPO and EPO) was 0.016 ± 0.001 (median = 0.006). Combined exclusion probability (P_E) with both social parents known was 99.998%.

Statistical analysis

Analyses were performed using JMP 9.0.2 for Macintosh. We verified parametric assumptions and evaluated outliers using Cook's D Influence tests and Mahalanobis plots. Equal variance–covariance between groups was tested using Bartlett's test (all Bartlett's $F < 0.87$, all $P > 0.35$). Pearson chi-square Goodness-of-fit analysis tested whether the distribution of EPO in nests was random among brood sizes. Unless otherwise indicated, we used the proportion of WPO instead of the number of WPO in analyses to control for variation in brood size. Means are presented ± standard error of the mean (SEM) and significance level was set at 0.05.

We used maximum likelihood (χ^2) GLMs to test for population-level (e.g., cross-sectional analysis) effects of male known age on genetic reproductive success (McCullagh and Nelder 1989). We ran GLMs on 160 independent males

of known age (1–9 years old). In the 41 cases where we sampled the same individual in multiple years, we used only the latest year. Response variables (WPO, EPO, total number of offspring) were nontransformed. Independent variables used to examine age-related patterns of male success included known age (fixed continuous effect), female age class, and year (fixed categorical effects). We included the quadratic age^2 term because male success could vary nonlinearly with age, and quadratic curves were fit onto model-generated GLM estimates (Schmoll et al. 2007; Mainguy et al. 2009; Raveh et al. 2010). For the number of WPO, we used a binomial distribution with logit links. Clutch size was set as the binomial denominator to account for variation in within pair paternity due to older females laying larger clutches. We used the Poisson distribution function with log links for number of EPO and total number of offspring sired (Everitt 2010).

We used paired t-tests to examine longitudinal variation in genetic reproductive success within unique repeat-sampled individuals as they aged (year x, $x + 1$; $n = 41$) (Schmoll et al. 2007). Longitudinal analysis over the full range of ages was not possible as this was a 3-year study and only 12 males were sampled in 3 consecutive years. We compared the known age of each cuckolded male with the age of the identified extrapair sire using a Wilcoxon paired test. Because old birds are less prevalent in the population, we also tested whether the overall known age distribution of extrapair sires differed significantly from the population distribution of known age males using a heterogeneity chi-square test.

We used a GLM with Poisson distribution and log links to investigate if clutch size (response) differed for first-year versus older females. Female age class and year were fixed effects. To investigate possible correlates of known age among older males (other than genetic reproductive success) while controlling for multicollinearity, we performed a partial correlation analysis. Variables included male body mass, wing chord length, tarsus length, and tail length. Finally, to determine if first egg date was related to the known age of older males, we performed a GLM with a Poisson distribution and log links. In this analysis, we set Julian first egg date as the response and male age and year as fixed effects.

RESULTS

Extrapair paternity

For the 297 families genotyped from 2006 to 2008 (minimum and known age males combined), 137 nests (46%) contained EPO and 273 of 1235 offspring (22%) were extrapair. Brood size averaged 4.24 ± 0.07 offspring per brood, and for nests containing at least 1 EPO, the proportion of EPO per brood averaged 0.53 ± 0.03 ($n = 137$ nests). We identified extrapair sires for 132 of 273 (48%) EPO and for nests containing ≥ 2 EPO, 46% (32 of 70) had multiple extrapair sires.

Older males sired 83% of offspring in their own nests and produced a total (WPO + EPO) of 4.0 offspring annually compared with only 63% WPO and 2.5 offspring for first-year males. Because purple martins feature strong assortative mating by age class, we examined if the age class of a male's mate influences paternity. For both first-year and older males, the proportion of WPO in males' nests was similar irrespective of the age class of their social mates (Figure 1a), whereas individuals paired to older females tended to sire more EPO compared with males paired to first-year females (Figure 1b). An overall effect of female age class was observed in the total number of offspring sired for older ($F_{1,208} = 4.06$, $P = 0.045$) but not first-year males ($F_{1,89} = 0.61$, $P = 0.44$) (Figure 1c).

First-year males who sired EPO had higher total genetic reproductive success compared with other first-year males who
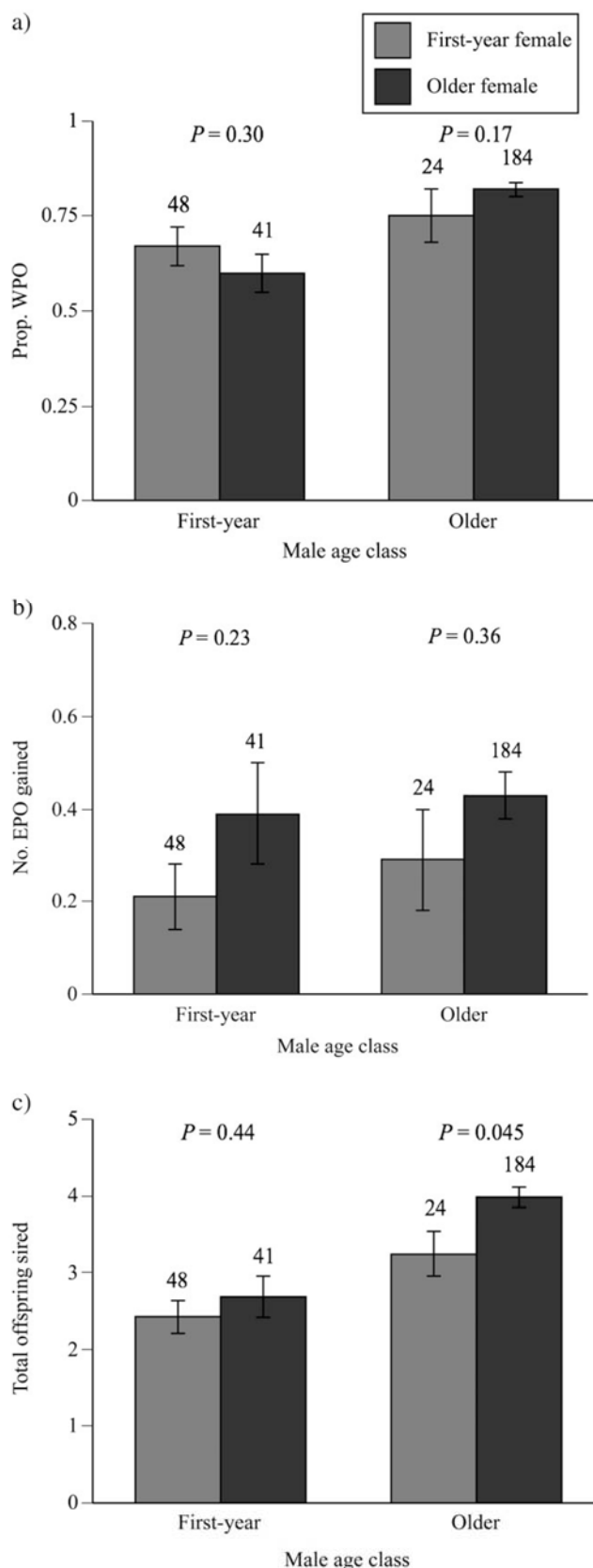

Figure 1
Genetic reproductive success of first-year and older males paired to first-year versus older females in terms of the (a) proportion of WPO, (b) number of EPO gained, and (c) total number of offspring sired. Values are mean $\pm$ SEM with sample sizes above bars.

Table 2

GLM models testing the effects of male age, male age^2, female age class, and year on the number of WPO, number of EPO gained, and total number of offspring sired for unique known age purple martins ($n = 160$) breeding in Pennsylvania

	Est. ± SEM	Lower, upper	χ^2_5	P
No. of WPO				
Male age	0.47 ± 0.24	0.004, 0.93	3.80	0.05
Male age^2	−0.04 ± 0.03	−0.09, 0.02	1.44	0.23
Female age class	0.03 ± 0.12	−0.21, 0.27	0.06	0.81
Year	−0.39 ± 0.19	−0.76, −0.03	11.07	0.004
No. of EPO				
Male age	−0.08 ± 0.32	−0.72, 0.57	0	1.00
Male age^2	0.01 ± 0.04	−0.07, 0.08	0	1.00
Female age class	0.26 ± 0.19	−0.09, 0.66	2.15	0.14
Year	0.29 ± 0.22	−0.14, 0.74	2.04	0.36
Total RS				
Male age	0.22 ± 0.08	0.06, 0.38	7.28	0.007
Male age^2	−0.02 ± 0.01	−0.04, 0.003	3.07	0.08
Female age class	0.06 ± 0.05	−0.03, 0.15	1.61	0.20
Year	−0.17 ± 0.08	−0.32, −0.02	7.74	0.02

Functions based on a binomial distribution (binomial denominator = clutch size) with logit links (WPO) and Poisson distributions with log links (number of EPO, total number of offspring). Parameter estimates (Est.) with SEMs, lower and upper confidence intervals, maximum-likelihood χ^2 values, and P-values reported. See text for whole model statistics.

did not gain extrapair fertilizations (gained EPO: 3.39 ± 0.37, $n = 18$; no EPO: 2.32 ± 0.18, $n = 71$; analysis of variance (ANOVA), $F_{1,89} = 6.75$, $P = 0.01$). We found a similar pattern among older males as individuals who gained EPO produced 39% more offspring annually than older males who sired no EPO (gained EPO: 4.79 ± 0.19, $n = 72$; no EPO: 3.44 ± 0.14, $n = 136$; ANOVA, $F_{1,208} = 33.79$, $P < 0.0001$).

Genetic reproductive success and male known age

For unique males of known age, we found significant effects of male age on the number of WPO in a male's own nest (GLM, whole model: $\chi^2_5 = 23.74$, $n = 160$, $P = 0.0002$, Table 2). There was also a significant effect of year ($P = 0.004$) but not of female age class ($P = 0.81$) (Table 2). Male within pair reproductive success increased up to 3 years old and then leveled off (Figure 2a). The influence of male age on within pair reproductive success was captured by the linear (age) and quadratic (age^2) variables (age: $r^2 = 0.41$, $n = 160$, $P < 0.0001$, $\beta = 0.34 \pm 0.03$; age^2: $r^2 = 0.53$, $n = 160$, $P < 0.0001$, $\beta = -0.07 \pm 0.01$; Figure 2a, Table 2). Modeling variation in the number of EPO sired by males in relation to male age, male age^2, age class of the social mate, and year revealed a non-significant whole model fit (GLM, $\chi^2_5 = 4.26$, $n = 160$, $P = 0.51$; Table 2). Number of EPO increased from 1 to 2 years of age but remained relatively constant among older males (age: $r^2 = 0.10$, $n = 160$, $P < 0.0001$, $\beta = 0.02 \pm 0.004$; age^2: $r^2 = 0.10$, $n = 160$, $P < 0.0001$, $\beta = -0.003 \pm 0.002$; Figure 2b, Table 2). In a GLM analysis of the total number of offspring sired (WPO + EPO), male age and year were significant ($\chi^2_5 = 34.31$, $n = 160$, $P < 0.0001$, Table 2). Total number of offspring sired annually increased between 1 and 3 years old, but did not continue to increase after 3 years of age (age: $r^2 = 0.58$, $n = 160$, $P < 0.0001$, $\beta = 0.37 \pm 0.02$; age^2: $r^2 = 0.73$, $n = 160$, $P < 0.0001$, $\beta = -0.08 \pm 0.008$; Figure 2c, Table 2). Quadratic effects for the 3 measures of genetic reproductive success had shallow negative slopes and were driven primarily by unavoidably small sample sizes for the oldest males.
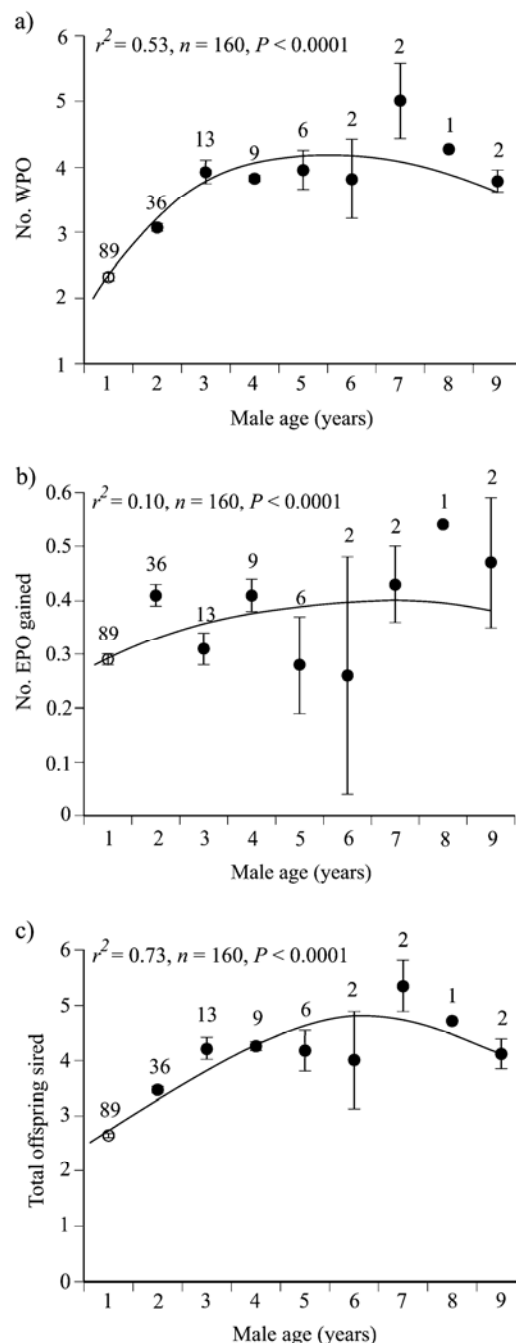

Figure 2
Quadratic curve fits of male known age (1–9 years old) versus the (a) number of WPO, (b) number of EPO, and (c) total number of offspring sired. Values are mean ± SEM GLM estimates from Table 2. See text for complete regression results.

In longitudinal analyses of individuals sampled in 2 successive years, the proportion of WPO and total number of offspring sired increased from 1 to 2 years old (Table 3). This pattern was also true for older males between year x and $x + 1$. Most (19/22) older males in this analysis were <5 years old when first sampled.

An alternative approach for assessing the effect of known age on extrapair mating is to compare directly the ages of the extrapair sire and cuckolded male for EPO where both male identities are known. Extrapair males were significantly older

Table 3

Longitudinal comparisons of genetic reproductive success for male purple martins sampled in successive years

	Year x Mean ± SEM	Year $x + 1$ Mean ± SEM	t	P
1- to 2-year-old males				
Proportion of WPO	0.63 ± 0.09	0.82 ± 0.06	1.67	0.056
No. of EPO gained	0.29 ± 0.10	0.35 ± 0.11	0.44	0.33
Total offspring sired	2.48 ± 0.31	3.85 ± 0.38	2.79	0.01
Older males				
Proportion of WPO	0.74 ± 0.06	0.86 ± 0.05	2.19	0.02
No. of EPO gained	0.48 ± 0.13	0.42 ± 0.15	−0.54	0.70
Total offspring sired	3.45 ± 0.32	4.45 ± 0.36	2.37	0.01

Analyses (paired t-tests) based on within-individual success for males at 1 year old versus 2 years old ($n = 19$) and for older males sampled in 2 consecutive breeding seasons ($n = 22$).

than males they cuckolded (Wilcoxon, $W = -179.5$, $n = 74$, $P = 0.02$, Figure 3a). Only 5 of 15 2-year-old males and 3 of 18 males ≥ 3 years old were cuckolded by a first-year extrapair male, despite the fact that 55% of known age males in the population were first-year. Most (59%, 24 of 41) first-year males were cuckolded by older extrapair males rather than by other first-year extrapair males. If extrapair success is random with respect to male age then the age distribution of extrapair sires should not be significantly different from the age distribution of all known-aged males in the population. The age distribution of extrapair sires that cuckolded first-year (heterogeneity chi-square test, $\chi^2_4 = 11.78$, $n = 41$, $P < 0.05$) and older males ($\chi^2_4 = 10.15$, $n = 33$, $P < 0.05$) differed from that expected under random mating (Figure 3b). First-year males were underrepresented as extrapair sires, whereas males ≥ 3 years old were extrapair sires more often than expected by chance. Distributions of ages of extrapair sires that cuckolded first-year versus older social males did not differ from each other ($\chi^2_{4hetero} = 2.97$, $P > 0.05$).

Known age and male size, fertility, and timing of breeding

To explore factors that might contribute to higher paternity by older males, we looked for other correlates of age among known age older males. In a multivariate analysis age (2–9 years) was not correlated with body mass, wing chord length, tarsus length, or tail length (partial correlation: $-0.11 < r < 0.18$, $n = 68$, all $P > 0.05$). The proportion of infertile eggs was also not correlated with male age ($r = -0.12$, $n = 71$, $P = 0.30$). In a GLM analysis examining the relationship between first egg date and male age, we found that older males tended to have earlier first egg dates (whole model: $\chi^2_3 = 23.73$, $n = 71$, $P < 0.0001$; age: $\chi^2 = 3.05$, $n = 71$, $P = 0.08$; year: $\chi^2 = 20.62$, $n = 71$, $P < 0.0001$) after controlling for year. Older pairs also tended to mate assortatively by known age (2–9 years old; $r = 0.25$, $n = 49$, $P = 0.087$).

DISCUSSION

4 main results emerged from our analysis of age-related patterns of paternity in purple martins. 1) First-year males experienced lower within pair paternity and were underrepresented as extrapair sires. 2) There were significant linear and nonlinear effects of known age on the number of WPO and total reproductive success of males, with paternity increasing to 3 years of age and then leveling off. 3) For individual males, the number of WPO increased between successive years both for 1- to 2-year-old comparisons and for males ≥ 2 years old. 4) In paired tests, extrapair sires were older than the males they cuckolded.
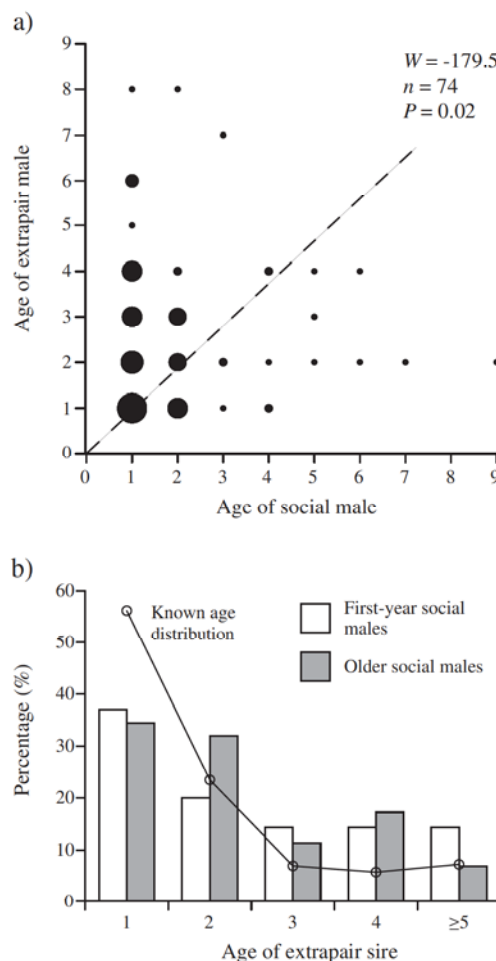

Figure 3
(a) Paired comparison of the age of the identified extrapair sire versus the social male that lost paternity ($n = 74$ EPO). In cases where multiple EPO in a social male's nest had the same extrapair sire, only 1 was included in the analysis. Diagonal is the line of equality. Dot size reflects the number of entries with equal value (range = 1–13). (b) Age distribution of extrapair sires of offspring in nests of first-year ($n = 41$, white bars) and older ($n = 33$, gray bars) social males in relation to the population distribution of all known age males ($n = 160$, solid line with open symbols).

Older males sired 83% of offspring in their own nests compared with only 63% WPO for first-year males, similar to a different population of purple martins (96% and 57%, respectively; Wagner et al. 1996). We also showed that within pair mating success increased among older males (Figure 2) in both cross-sectional and longitudinal comparisons. In pied flycatchers (*Ficedula hypoleuca*), within pair fertilization success increased with age among older males and was greater for males that exhibited more aggressive territorial defense (Moreno et al. 2010). In American redstarts (*Setophaga ruticilla*), within pair success increased among older males, and Perreault et al. (1997) suggested that experience allows males to better prevent extrapair copulations from occurring through more effective territory defense. Purple martins defend nesting compartments rather than large feeding territories and copulations (within pair or extrapair) do not occur at the nest site. Nevertheless, male defense of the mate while females are away from the colony could determine within pair paternity and drive age-related patterns. Wagner et al. (1996) found that first-year males guard their fertile mates more intensely than older males

suggesting that restraint in time or energy invested in mate defense does not explain the poor performance of young males. Despite high guarding effort, first-year males may be constrained to be less effective in mate guarding as a result of inexperience or smaller size (Wagner et al. 1996; Johnsen et al. 2003). It is not known how mate guarding effectiveness or effort varies among older male martins.

Older males may have an advantage in gaining access to extrapair partners due to age-dependent spring arrival schedules in purple martins. First-year males arrive several weeks after older males, and their mates lay eggs a week or more later than do the social mates of older males (Morton and Derrickson 1990; Wagner et al. 1996). Older males are therefore emancipated from guarding their own mate at the same time as most first-year females are egg laying, giving older males a competitive edge in terms of the time and energy available to pursue extrapair matings. Similarly, among older males, there was a strong negative trend between first egg date and age which could explain why 3-year-old males performed better in siring young in their own nest than did 2-year-old males. Extrapair success may be also be driven by timing of breeding and create pronounced variation among males in opportunity to seek extrapair matings, similar to the "mating opportunity" hypothesis for protandrous emergence in insects (Wiklund and Fagerström 1977).

Morton et al. (1990) proposed that extrapair choice of female martins is based on male age because older males have "good genes" or greater heterozygosity as a result of differential mortality of poor quality males at a younger age (Davidar and Morton 1993). If male paternity is strongly influenced by female choice, and females use male age as a proxy for male genetic quality, then 1) older males should gain more EPO as well as lose less WPO and 2) females would be expected to have an open-ended mating preference resulting in the oldest males having the highest genetic reproductive success (Johnsen et al. 2001; Schmoll et al. 2007). In coal tits (*Periparus ater*), Schmoll et al. (2007) found that EPO but not WPO increased among older males, up to 3 years of age. In purple martins, WPO increased up to 3 years of age, but there was not as strong an age effect on EPO, which increased from 1 to 2 years of age and remained relatively constant among older males. In a paired comparison, extrapair sires were significantly older than the males they cuckolded (Figure 3a) and fewer first-year males achieved EPO than expected by chance, similar to several other age-related studies (Weatherhead and Boag 1995; Schmoll et al. 2007). The oldest males in our population were extrapair sires surprisingly often. Although only 12% of known age males in our sample were ≥4 years old, these males sired approximately 30% of EPO in nests of first-year males and 23% of EPO in the nests of older males (Figure 3b). These results are consistent with female preference for the oldest males; however, we caution that we do not demonstrate female choice. Direct observation of female choice for particular males (e.g., Chiver et al. 2008) is difficult to obtain for purple martins because they travel far from their nest on a daily basis and within pair copulations are rarely observed at the colony (Tarof SA, personal observation).

If females prefer the oldest males as extrapair mates, it remains unclear how females might discern age among older males and whether females assess age directly or indirectly (Brooks and Kemp 2001). Male age is often correlated with body size, competitive ability, ornament size, color, display, or song making it difficult to separate age effects from age-trait relationships (Freeman-Gallant et al. 2009). Several studies have shown that age, rather than color ornaments, predict paternity among older males despite the presence of a correlation between age and ornament (Weatherhead and Boag 1995; Moreno et al. 2010). In our study, body size was inde-

pendent of known age among older males, leaving ornament size, color, or song and other behaviors as potential phenotypic mechanisms for females to assess male age. Annual survival probability in our study population is relatively high (0.48–0.62, Stutchbury et al. 2009) and older pairs tended to mate assortatively by age, making individual recognition of males by returning females another possible mechanism.

We found a low assignment rate (48%) for EPO despite sampling 80–95% of the candidate sires in our population. First-year males spend from several days up to several weeks "floating" while they compete with older males for nesting sites (Stutchbury 1991). Consequently, it is possible that some unassigned extrapair sires were non-territorial floaters that gained extrapair copulations before obtaining a nest site in a different colony. We cannot distinguish between floaters siring EPO versus females leaving the colony to mate with breeding males at other colonies. In purple martins, adults often leave the breeding colony for extended periods of time during the day and therefore have opportunities to mate with distant individuals. Similarly, in tree swallows, most extrapair sires originate from outside the immediate population (Kempenaers et al. 2001) and breeding females travel far from their nest site when fertile (Dunn and Whittingham 2005).

Interpreting patterns of female choice based on male fertilization success may fail to detect age effects if older males are less fertile due to senescence (Hansen and Price 1995; Brooks and Kemp 2001; Dean et al. 2010). Male reproductive senescence could generate sexual conflict in age-structured populations if older males are more likely to achieve extrapair copulations with females but have low fertility, resulting in lower than expected female reproductive success (Gasparini et al. 2009; Dean et al. 2010). Passerines are expected to exhibit senescence in male genetic mating success because they typically have a "fast" life-history strategy (Jones et al. 2008). Senescence is best tested longitudinally by examining genetic mating success over an individual's lifetime, but for paternity studies, this is logistically difficult. In purple martins, we found no correlation between egg infertility and age among older males, implying that the lack of increase in total fertilization success among males ≥3 years old was not simply a result of low fertility. A robust test of the hypothesis that females choose older males in order to benefit by producing higher genetic quality EPO could compare fitness-related traits of half-siblings and differences in offspring quality should correspond to the age difference of the cuckolded versus extrapair male (Schmoll et al. 2007).

Predicting and interpreting age-related patterns in paternity among species (Table 1) are difficult because the underlying behavioral mechanisms that determine paternity are often not well understood. In hooded warblers (*Wilsonia citrina*), for example, paternity is not male age-related (Stutchbury et al. 1997) and their extrapair behavior, studied via radiotelemetry, provides insights as to why. Males make little attempt to mate guard (Fedy et al. 2002) because females make frequent off-territory forays to neighbouring males and most intrusions onto the territory by neighbouring males go undetected (Stutchbury 1998). Extrapair paternity is determined primarily via female choice. Females made more frequent off-territory forays if their social mates had a low song rate, and in paired comparisons extrapair sires sang more than the males whom they cuckolded (Chiver et al. 2008).

For species where mate guarding and territory defense are effective for increasing within pair paternity, age effects on within pair success would be expected if older males are competitively superior as a result of aggression (Moreno et al. 2010), earlier breeding (Wagner et al. 1996, this study), or experience in territory defense (Perreault et al. 1997). Similar traits may also influence extrapair paternity depending on the mating system. In some species, neighboring females

eavesdrop on male–male territorial disputes to assess male quality for extrapair matings (Mennill et al. 2002). Weatherhead and Boag (1995) suggested that older male Red-winged blackbirds (*Agelaius phoeniceus*) may be more successful in gaining extrapair fertilizations due to experience, but it is not known whether there are age-related differences in male off-territory foray frequency, duration, or strategy.

If paternity is determined primarily by female choice, then within pair and extrapair fertilization success should be the result of a male's intrinsic quality, as assessed indirectly by females, and preferred males should both gain WPO and EPO. In purple martins, we found evidence for age-related increases in WPO and, to a lesser extent, EPO. 2 other studies found that males gained more EPO but not more WPO as they aged (Weatherhead and Boag 1995; Schmoll et al. 2007) but it is not clear why. Extrapair copulations occur in the context of a complex social environment of male–male competition during defense and acquisition of mates, as well as female choice of extrapair mates. Although patterns of age class effects on paternity are well documented (Griffith et al. 2002), we suggest that the subtle age effects among older males will be far more variable as a result of the details of how and why extrapair copulations are achieved.

CONCLUSIONS

Our results, along with studies of other species (Table 1), are consistent with the constraint hypothesis for age-related male performance (reviewed in Forslund and Pärt 1995). WPO increased for male martins sampled in consecutive years, suggesting that the cross-sectional age-related patterns were not simply a result of mortality of lower quality individuals (see also Weatherhead and Boag 1995; Perreault et al. 1997; Schmoll et al. 2007). Young martins have lower competitive ability in mate guarding (Wagner et al. 1996), later initiation of egg-laying by their mates (Morton et al. 1990, this study), and are disadvantaged as a result of female preference for older males (Wagner et al. 1996). In purple martins, the relatively high level of mate guarding shown by first-year males (Wagner et al. 1996) is not consistent with the restraint hypothesis. Furthermore, first-year males who sired EPO had 46% higher total genetic reproductive success compared with first-year males who did not gain extrapair fertilizations. Among older males, individuals who gained EPO produced 39% more offspring. Even given the comparatively short life span of this species (Stutchbury et al. 2009), the high fitness gains of extrapair fertilizations would presumably be sufficient to offset life-history trade-offs. If so, the survival probability of males who sired EPO should be similar to same-aged males that did not sire EPO. Studies of other species showing age effects among older males (Table 1) also suggest that poor paternity performance in younger males is constrained via male–male competition and female choice.

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REFERENCES

Anmarkrud JA, Kleven O, Augustin J, Bentz KH, Blomqvist D, Fernie KJ, Magrath MJL, Pärn H, Quinn JS, Robertson RJ, et al. 2011. Factors affecting germline mutations in a hypervariable microsatellite: a comparative analysis of six species of swallows (Aves: Hirundinidae). Mutat Res. 708:37–43.

Beck CW, Promislow DEL. 2007. Evolution of female preference for younger males. PLoS ONE. 2(9):e939.

Bouwhuis S, Sheldon BC, Verhulst S, Charmantier A. 2009. Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. Proc R Soc Lond B Biol Ser. 276:2769–2777.

Brommer JE, Wilson AJ, Gustafsson L. 2007. Exploring the genetics of aging in a wild passerine bird. Am Nat. 170:643–650.

Brooks R, Kemp DJ. 2001. Can older males deliver the good genes? Trends Ecol. 16:308–313.

Brown CR. 1997. Purple Martin (*Progne subis*). In: Poole A, editor. The birds of North America online. Ithaca (NY): Cornell Lab of Ornithology. Available from: http://bna.birds.cornell.edu/bna/species/287

Chiver I, Stutchbury BJM, Morton ES. 2008. Do male plumage and song characteristics influence female off-territory forays and paternity in the hooded warbler? Behav Ecol Sociobiol. 62:1981–1990.

Davidar P, Morton ES. 1993. Living with parasites: prevalence of a blood parasite and its effect on survivorship in the purple martin. Auk. 110:109–116.

Dean R, Cornwallis CK, Løvlie H, Worley K, Richardson DS, Pizzari T. 2010. Male reproductive senescence causes potential for sexual conflict over mating. Curr Biol. 20:1192–1196.

Dunn PO, Whittingham LA. 2005. Radio-tracking of female tree swallows prior to egg-laying. J Field Ornithol. 76:259–263.

Everitt BS. 2010. Multivariate modeling and multivariate analysis for the behavioural sciences. Boca Raton (FL): Taylor and Francis.

Fedy BC, Norris DR, Stutchbury BJM. 2002. Do male Hooded Warblers guard their mates when their paternity is most at risk? J Field Ornithol. 173:420–426.

Freeman-Gallant CR, Taff CC, Morin DF, Dunn PO, Whittingham LA, Tsang . Sexual selection, multiple male ornaments, and age- and condition-dependent signaling in the common yellowthroat. Evolution. 64:1007–1017.

Forslund P, Pärt T. 1995. Age and reproduction in birds-hypotheses and tests. Trends Ecol Evol. 10:374–378.

Gasparini C, Marino IAM, Boschetto C, Pilastro A. 2009. Effect of male age on sperm traits and sperm competition success in the guppy (*Poecilia reticulata*). J Evol Biol. 23:124–135.

Griffith SC, Owens IPF, Thuman KA. 2002. Extrapair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol. 11:2195–2212.

Hamann J, Cooke F. 1987. Age effects on clutch size and laying dates of individual female lesser snow geese (*Anser caerulescens*). Ibis. 129:527–532.

Hansen TF, Price DK. 1995. Good genes and old age: do old mates provide superior genes? J Evol Biol. 8:759–778.

Ibarguchi G, Gissing GJ, Gaston AJ, Boag PT, Friesen VL. 2004. Male-biased mutation rates and the overestimation of extrapair paternity: problem, solution, and illustration using thick-billed murres (*Uria lomvia*, Alcidae). J Hered. 95:209–216.

Johnsen A, Lifjeld JT, Andersson S, Örnborg J, Amundsen T. 2001. Male characteristics and fertilisation success in bluethroats. Behaviour. 138:1371–1390.

Johnsen A, Lifjeld JT, Krokene C. 2003. Age-related variation in mate guarding intensity in the bluethroat (*Luscinia s. svecica*). Ethology. 109:147–158.

Jones OR, Gaillard J-M, Tuljapurkar S, Alho JS, Armitage KB, Becker PH, Bize P, Brommer J, Charmantier A, Charpentier M, et al. 2008. Senescence rates are determined by ranking on the fast–slow life-history continuum. Ecol Lett. 11:664–673.

Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol. 16:1099–1106.

Kempenaers B, Everding S, Bishop C, Boag P, Robertson RJ. 2001. Extrapair paternity and the reproductive role of male floaters in the tree swallow (*Tachycineta bicolor*). Behav Ecol Sociobiol. 49:251–259.

Kempenaers B, Verheyen GR, Dhondt AA. 1997. Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behav. Ecol. 8:481–492.

Kokko H. 1998. Good genes, old age, and life-history trade-offs. Evol Ecol. 12:739–750.

Lack D. 1968. Ecological adaptations for breeding in birds. Methuen: London.

Lessels CM, Krebs JR. 1989. Age and breeding performance of European bee-eaters. Auk. 106:375–382.

Mainguy J, Côté SD, Festa-Bianchet M, Coltman DW. 2009. Father-offspring phenotypic correlations suggest intralocus sexual conflict for a fitness-linked trait in a wild sexually dimorphic mammal. Proc R Soc Lond B Biol Ser. 276:4067–4075.

McCullagh P, Nelder JA. 1989. Generalized linear models. London: Chapman and Hall.

Mennill DJ, Ratcliffe LM, Boag PT. 2002. Female eavesdropping on male song contests in songbirds. Science. 296:873–873.

Moreno J, Martínez J-G, Morales J, Lobato E, Merino S, Tomás G, Vàsquez RA, Mõstl E, Osorno JL. 2010. Paternity loss in relation to male age, territorial behaviour and stress in the pied flycatcher. Ethology. 116:76–84.

Morton ES, Derrickson KC. 1990. The biological significance of age-specific return schedules in breeding purple martins. Condor. 92:1040–1050.

Morton ES, Forman L, Braun M. 1990. Extrapair fertilizations and the evolution of colonial breeding in purple martins. Auk. 107:275–283.

Perreault S, Lemon RE, Kuhnlein U. 1997. Patterns and correlates of extrapair paternity in American redstarts (*Setophaga ruticilla*). Behav Ecol. 8:612–621.

Perrins CM, Moss D. 1974. Survival of young Great tits in relation to age of female parent. Ibis. 116:220–224.

Raveh S, Heg D, Dobson FS, Coltman DW, Gorrell JC, Balmer A, Neuhaus P. 2010. Mating order and reproductive success in male Columbian ground squirrels (*Urocitellus columbianus*). Behav Ecol. 21:537–547.

Ricklefs RE. 2010. Life-history connections to rates of aging in terrestrial vertebrates. Proc Natl Acad Sci U S A. 107:10314–10319.

Robertson RJ, Rendell WB. 2001. A long-term study of reproductive performance in tree swallows: the influence of age and senescence on output. J Anim Ecol. 70:1014–1031.

Schmoll T, Schurr FM, Winkel W, Epplen JT, Lubjuhn T. 2007. Male age predicts extrapair and total fertilization success in the socially monogamous coal tit. Proc R Soc Lond B Biol Ser. 276: 337–345.

Stanley AA, Graham AM, Wagner RH, Morton ES, Schug MD. 2011. Characterization of 10 polymorphic microsatellite markers for the purple martin, *Progne subis*. Conserv Genet Resour. 3:577–579.

Stutchbury BJ. 1991. Floater behavior and territory acquisition in male purple martins. Anim Behav. 42:435–443.

Stutchbury BJM. 1998. Extra-pair mating effort of male Hooded Warblers. Anim Behav. 55:553–561.

Stutchbury BJM, Hill JR 3rd, Kramer PM, Rush SA, Tarof SA. 2009. Sex and age-specific annual survival in a Neotropical migratory songbird, the purple martin (*Progne subis*). Auk. 126:278–287.

Stutchbury BJM, Piper WH, Neudorf DL, Tarof SA, Rhymer JM, Fuller G, Fleischer RC. 1997. Correlates of extra-pair fertilization success in hooded warblers. Behav Ecol Sociobiol. 40:119–126.

Tarof SA, Kramer PM, Hill JR 3rd, Tautin J, Stutchbury BJM. 2011. Brood size and late breeding are negatively related to juvenile survival in a Neotropical migratory songbird. Auk. 128(4):1–10.

van de Pol M, Verhurst S. 2006. Age-dependent traits: a new statistical model to separate within- and between-individual effects. Am Nat. 167:766–773.

Wagner RH, Schug MD, Morton ES. 1996. Condition-dependent control of paternity by female purple martins. Behav Ecol Sociobiol. 38:379–389.

Weatherhead PJ, Boag PT. 1995. Pair and extra-pair mating success relative to male quality in red-winged blackbirds. Behav Ecol Sociobiol. 37:81–91.

Westneat DF, Stewart IRK. 2003. Extrapair paternity in birds: causes, correlates, and conflict. Annu Rev Ecol Evol Syst. 34:365–396.

Wiklund C, Fagerström T. 1977. Why do males emerge before females? A hypothesis to explain incidence of protandry in butterflies. Oecologia. 31:153–158.