

LITTLE EFFECT OF EXTRAPAIR PATERNITY ON THE OPPORTUNITY FOR SEXUAL
SELECTION IN SAVANNAH SPARROWS (*PASSERCULUS SANDWICHENSIS*)

COREY R. FREEMAN

TABLE 1. Effect of extrapair paternity on the opportunity for sexual selection in passerine birds. Species arranged in order of percent extrapair young (%EPY) assigned.

Species	$I_{s,app}^1$	I_s^1	$I_s/I_{s,app}$	Mating system	%EPY	%EPY assigned to local males	Ref
<i>Passerculus sandwichensis</i>	0.27	0.48	1.8	Poly	47%	92.3%	this study (both years combined)
<i>Agelaius phoeniceus</i> (ON)	0.67	0.73	1.1	Poly	26%	78.5%	Weatherhead and Boag (1997)
<i>Parus caeruleus</i>	0.16	0.27	1.7	Mono	11%	72.3%	Kempnaers et al. (1992)
<i>Dendroica caerulescens</i>	0.49	0.71	1.4	Mono	21%	62.3%	Webster et al. (2001)
<i>Agelaius phoeniceus</i> (NY)	1.02	1.21	1.2	Poly	24%	60.0%	Westneat (1993)
<i>Junco hyemalis</i>	0.55	0.72	1.3	Mono	28%	54.7%	Ketterson et al. (1997)
<i>Wilsonia citrina</i>	0.18	0.46	2.6	Mono	27%	54.7%	Stutchbury et al. (1997)
<i>Ficedula albicollis</i>	0.03	0.14	4.7	Mono	16%	53.8%	Sheldon and Ellegren (1999)
<i>Progne subis</i>	0.05	0.33	6.6	Mono	19%	53.8%	Wagner et al. (1996) and Møller (1998)
<i>Tachycineta bicolor</i>	0.09	0.79	8.8	Poly ²	52%	47.2%	Kempnaers et al. (2001)
<i>Poecile atricapillus</i>	0.04	0.10	2.5	Mono	9%	46.9%	Otter et al. (1998)
<i>Icterus galbula</i>	0.07	0.17	2.4	Mono	32%	44.6%	Richardson and Burke (2001)
<i>Dendroica petechia</i>	0.04	0.53	13.3	Mono	37%	35.4%	Yezerinac et al. (1995)

¹ Standardized variance in realized fertilization success (I_s) and apparent fertilization success ($I_{s,app}$); $I_{s,app}$ assumes extrapair paternity is nonexistent and males sire all offspring on their territories.

² A total of 21.1% of males are socially polygynous.

competition and the opportunity for sexual selection in birds. The standardized variance in male reproductive success (I_s), an upper-bound estimate of the strength of sexual selection (Arnold and Wade 1984), is increased by EPP in all species examined to date, sometimes dramatically so (Table 1; Møller 1998; Møller and Ninni 1998). These estimates must be considered preliminary, however, because in most studies the sampling of extrapair sires is largely incomplete. While it is relatively easy to assess a male's success at siring young on his own territory, it is often difficult to tally the number of young he produces outside the pairbond either because the geographic scale of reproductive interactions exceeds the boundaries of the study site or because of methodological limitations associated with the molecular techniques used to include or exclude potential sires (Webster and Westneat 1998; Ligon 1999).

This sampling bias is problematic because it can potentially inflate I_s in two ways, one statistical (Møller and Ninni 1998)

TABLE 2. Microsatellite loci used to assign paternity in Savannah sparrows.

Locus	Repeat motif	T _a ¹	MgCl ₂ (mM)	Year	No. alleles	H _e	H _o	p ²
<i>Psa29</i> ³	(CA) ₇ CG(CA) ₈	60	7.7	2002	24	0.93	0.90	0.12
				2003	23	0.93	0.95	0.13
<i>Psa12</i>	(TG) ₇ TA(TG) ₃	60	8.3	2002	11	0.84	0.86	0.21
				2003	11	0.82	0.83	0.22
<i>Psap61</i>	(GT) ₁₇	52	3.3	2002	20	0.92	0.93	0.14
				2003	21	0.92	0.91	0.14
<i>Escu6</i>	(CA) ₁₅ CG(CA) ₁₀	59	5.7	2002	18	0.89	0.82	0.18
				2003	16	0.89	0.84	0.17
<i>Mme8</i>	(TG) ₃ TC(TG) ₁₃	65	4.0	2002	22	0.93	0.86	0.12
				2003	21	0.93	0.90	0.12
<i>Mme1</i>	(TG) ₃ TC(TG) ₁₃	57	5.0	2002	24	0.93	0.75	0.12
				2003	21	0.93	0.77	0.12

¹ Annealing temperature (8C).

² Probability of false inclusion (Jamieson 1994).

³ Z-linked; observed (H_o) and expected heterozygosities (H_e) calculated for males only.

nestlings for survival on day 7 (hatching occurs on day 1), and on day 8, we measured each offspring's weight and wing chord and obtained a sample of blood from the brachial vein. Young found dead in the nest on day 7 or 8 were also sampled. Blood was stored in a lysis buffer (Seutin et al. 1991) at 5

attempt is the sum of his within-pair (W) and extrapair (E) fertilization success,

$$\text{var}(T) = \text{var}(W) + \text{var}(E) + 2 \text{cov}(W, E). \quad (2)$$

Both W and E can be partitioned further into components describing the number of mates a male attracts (M), the number of young produced per mate (N), and the percentage of these young he sires (P). The variance and covariance terms, then, can be parsed into components that address the individual contributions of M, N, and P as well as their respective interactions.

To avoid conflating variance estimates across fitness components, individuals that had zero success in one component were not included in the analysis of subsequent components (after Webster et al. 1995). For example, if a male remained unpaired ($M_w = 0$), he was included only in the variance estimates for M_w and not N_w or P_w .

All variance estimates are expressed as standardized variances (variance divided by mean reproductive success squared; Arnold and Wade 1984). To assess the influence of EPP on the opportunity for sexual selection, we compared the realized variance in male success (I_s) to the variance in apparent success ($I_{s,\text{app}}$).

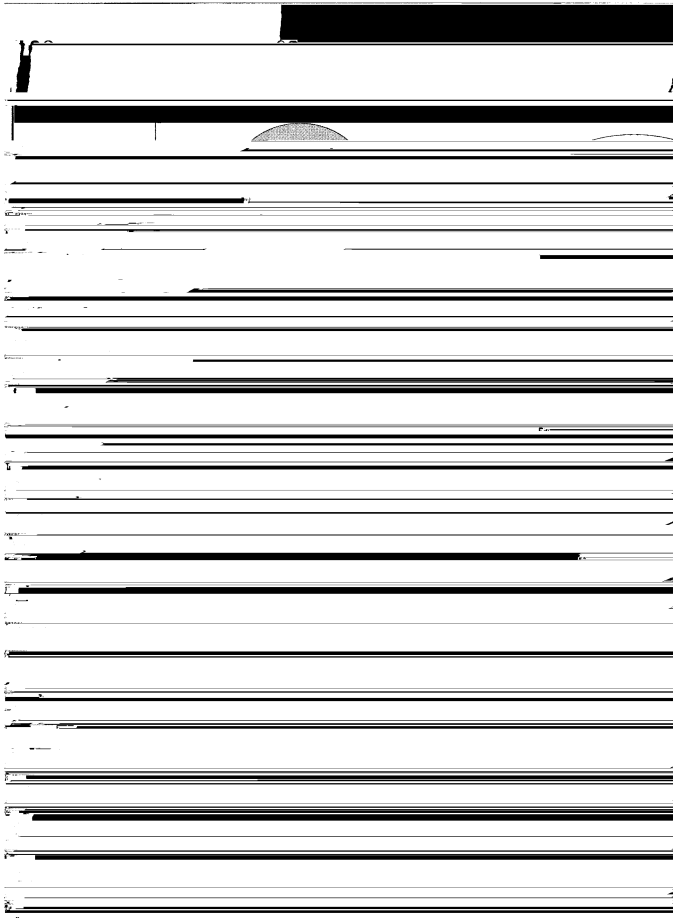


FIG. 1. Geography of extrapair paternity in Savannah sparrows for a 130 \times 130-m area in 2002 and 2003. Size of arrows indicates number of extrapair young (EPY); opposing arrows and shaded territories show cases of reciprocal cuckoldry.

Psa29 and *Psap61* and then compared the full complement of six paternal alleles to the genotypes of all males captured on Kent Island. In seven cases, a close neighbor matched at five of the six loci, and these offspring were assigned to those males. We were unable to assign paternity to only 15 of the 194 extrapair young, all distributed in nests at the periphery of our study site. Overall, we were able to assign parentage to 396 of 411 offspring (96.4%).

Geography of Extrapair Paternity

Our assignments can be used to describe the spatial scale at which reproductive interactions occurred. Pooling over 2002 and 2003, 95% of the extrapair young with known parentage were sired by males whose own social mates nested no more than 87.1 m away. Because the average territory diameter in Savannah sparrows is approximately 38 m (Wheelwright and Mauck 1998), this result suggests that local interactions are important in determining patterns of EPP. Indeed, 154 of the 169 extrapair young with unambiguous assignments were sired by males occupying an adjacent territory (for an example, see Fig. 1).

Reciprocal cuckoldry was common in both 2002 and 2003 (Fig. 1) and always occurred between neighbors. In 2002, 12

of 34 males (35.3%) who sired at least one extrapair young were involved in reciprocal cuckoldry; in 2003, 11 of 21 extrapair sires (52.4%) traded extrapair young with neighboring males. The exchange of extrapair young also occurred across years. In at least one case, a female nesting in 2003 left her social mate from 2002 and paired with the extrapair sire of her 2002 young only to cuckold him in favor of her original social mate. Both males were thus the source and recipient of each other's extrapair young over the two years of the study.

Variance in Male Reproductive Success

The standardized variance in reproductive success (I_s) among males was 0.58 in 2002. This is 3.6 times the variance in reproductive success observed among females at the same stage of the reproductive cycle, and 2.1 times the variance in apparent reproductive success among males ($I_{s,app}$). In 2003, the standardized variance in reproductive success among males was 0.37, 8.2 times the variance in reproductive success observed among females but only 1.5 times the variance in apparent reproductive success observed among males.

Compared to 2003, relatively few males were polygynously mated in 2002. Only 13 of 57 males (22.8%) attracted multiple mates, and variation among males in number of social mates (M_w) was relatively unimportant, accounting for only 10.4% of the overall variance in male reproductive success (Table 3). Among the 52 males whose social mates fledged at least one offspring, 41 (78.8%) lost paternity in their own nests. Differences among males in their ability to protect their within-pair paternity (P_w) accounted for 29.6% of I_s (Table 3), and the total number of fertilizations (T) increased with increasing within-pair paternity (Spearman rank, $r_s = 0.66$, $P = 0.0001$). Elements of a male's extrapair success were even more influential. Many males (59.6%) sired young outside the pairbond, and differences in the number of extrapair mates (M_e) alone accounted for 56.6% of I_s . Overall fertilization success (T) increased significantly with increasing number of extrapair mates (Spearman rank, $r_s = 0.75$, $P = 0.0001$).

In 2003, 15 of 33 males (45.5%) attracted more than one social mate, and differences in the number of social mates accounted for 22.4% of the total variance in male reproductive success (Table 3). Unlike in 2002, variance among males in their ability to sire young on their own territories (P_w) was more important than their ability to acquire extrapair mates (M_e). Nevertheless, many males (70.0%) sired young outside the pairbond, and the number of extrapair mates was positively correlated with a male's total reproductive success (T ; Spearman rank, $r_s = 0.40$, $P = 0.04$). Overall, extrapair components of male reproductive success contributed 65.3% of I_s in 2002 and 36.9% of I_s in 2003 (Table 3).

There was little positive covariance between the within-pair and extrapair components of male reproductive success in either year (Table 3). In 2002, males that sired young outside the pairbond were just as likely to be cuckolded as males that failed to produce extrapair young (Fisher's exact, $P = 0.17$), and no element of a male's within-pair success predicted any element of his extrapair success in a series of

TABLE 3. Partitioning of variation in male reproductive success (I_s) into within-pair (WP) and extrapair (EP) components. On Kent Island, the standardized variance in reproductive success among males was 0.58 in 2002 and 0.37 in 2003.

Source	2002		2003	
	Value	% Total	Value	% Total
Total WP variance due to	0.199	34.6%	0.277	75.1%
var no. social mates (M_w)	0.060	10.4%	0.083	22.4%
var female fecundity (N_w)	0.025	4.4%	0.010	2.8%
var % young sired (P_w)	0.172	29.6%	0.126	34.0%
covar (M_w, N_w)	20.014	22.5%	0.010	2.8%
covar (M_w, P_w)	0.008	1.3%	0.035	9.5%
covar (N_w, P_w)	20.021	23.6%	0.007	1.9%
Total extrapair variance due to	0.375	65.3%	0.136	36.9%
var no. EP mates (M_e)	0.325	56.6%	0.087	23.6%
var EP female fecundity (N_e)	0.008	1.4%	0.001	0.3%
var % young sired (P_e)	0.057	10%	0.023	6.2%
covar (M_e, N_e)	20.033	25.8%	0.002	0.5%
covar (M_e, P_e)	0.034	5.9%	0.001	0.3%
covar (N_e, P_e)	0	0%	20.002	20.5%
Total within-pair and extrapair covariance due to	20.004	20.6%	20.044	211.9%
cov between no. social mates and				
no. EP mates	0.012	2.1%	0.033	9.0%
fecundity of EP females	20.012	22.1%	0	0%
% young sired with EP females	20.025	24.4%	20.028	27.6%
cov between WP female fecundity and				
no. EP mates	20.013	22.3%	20.011	23.0%
fecundity of EP females	20.004	20.8%	0.002	0.5%
% young sired with EP females	0.012	2.1%	20.008	22.1%
cov between % WP young and				
no. EP mates	0.073	12.6%		

variance in male reproductive success. Like many passerines (Stutchbury et al. 1997; Webster et al. 2001; but see Foerster et al. 2003), most extrapair sires were within two territories of the focal nest and reciprocal cuckoldry was common (Fig. 1), making it impossible for a small subset of males to account for a disproportionate number of fertilizations. The factors that constrained females to acquire extrapair mates so close to their nesting site are unknown. The fact that only local males sired extrapair young suggests that females may prefer to mate with males with whom they are familiar (see Slagsvold and Lifjeld 1997; Slagsvold et al. 2001).

Despite the local scale of interaction, variation in extrapair success was relatively substantial, and we emphasize that the components of male fitness that best reflect the occurrence of EPP (P_w , M_e) together accounted for much of I_s (Table 3). Indeed, it was the lack of a strongly positive covariance between within-pair and extrapair success—and not the absence of variation in any one component—that limited the influence of EPP on the opportunity for sexual selection. For example, the male with the greatest extrapair fertilization success in 2002 (siring 10 extrapair young with four different females) was cuckolded completely by two neighboring males, while the two males with the most within-pair success

between EPP and testis size among birds showing fewer than 30% extrapair young (Møller and Briskie 1995); more than 80% of all passerines fall in this category (Griffith et al. 2002). Likewise, early reports that sexual dimorphism is more a consequence of sexual selection arising from sperm competition (and EPP) than number of social mates (Møller and Birkhead 1994; Owens and Hartley 1998) were challenged by Dunn et al. (2001), who found that social mating system was a better and more consistent predictor of dimorphism than testis mass in a study of more than 1000 species of birds. Interestingly, while I_s shows little covariance with the extent of EPP in our small sample, I_s averages higher for socially polygynous species than for socially monogamous species (Mann-Whitney, $P = 0.021$) despite a relatively complete accounting of EPP in three of four studies focusing on polygynous systems (Table 1; including Hasselquist et al. [1995] and only one of the two blackbird studies). The percentage of males attracting more than one social mate might be a more informative index of sexual selection than the extent of EPP in comparative studies (Dunn et al. 2001).

We cannot assume that the relative importance of M_w and other within-pair fitness components covaries with the incidence of polygyny, however. Even in socially polygynous species, fitness components related to EPP might still generate the bulk of I_s . In Savannah sparrows, for example, variation in number of extrapair mates is of overwhelming importance, suggesting that the strongest sexual selection is on traits that affect extrapair fertilization success, at least in some years (Kempnaers et al. 2001). By contrast, in the socially monogamous black-throated blue warbler (*Dendroica caerulescens*), variation in within-pair success accounts for more than 75% of I_s , which emphasizes the importance of male phenotypes that influence the number, quality, and fidelity of social mates (Webster et al. 2001).

In conclusion, we emphasize two important caveats in the study of EPP, sperm competition and sexual selection in birds. First, if the effect of EPP on I_s is much less than commonly appreciated, other sources of variance in male fitness cannot be ignored. This is especially important in the study of socially monogamous species where EPP has routinely been invoked to explain the evolution of sexual dimorphism and more traditional explanations involving the number and quality of social mates (Darwin 1871) are currently out of favor. Second, even if EPP only minimally increases I_s and is a poor predictor of the opportunity for sexual selection in interspecific comparisons, fitness components related to EPP could still generate more sexual selection than fitness components related to within-pair paternity in any one species. This caveat is especially relevant to the study of socially polygynous species where variation in within-pair fertilization success—and in particular, variation in M_w —might seem most important. Clearly, it will take increased sampling effort in additional species before the influence of EPP can be characterized with confidence.

ACKNOWLEDGMENTS

We thank M. Butler, I. Levin, V. Marzot, K. Oh, T. Tsunekage, and R. Zaino for assistance in the field and laboratory. R. Mauck analyzed the relationship between EPP and

distance, and M. Webster, J. Karubian, and an anonymous reviewer provided helpful comments on earlier versions of the manuscript. This work was supported by the National Science Foundation (IBN-0116139) and represents contribution number 166 from the Bowdoin Scientific Station.

LITERATURE CITED

- Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.
- Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: Theory. *Evolution* 38:709–719.
- Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* 60: 145–164.
- Birkhead, T. R., and A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press, London.
- Blomqvist, D., M. Andersson, C. Küpper, I. C. Cuthill, J. Kis, R. B. Lanctot, B. K. Sandercock, T. Székely, J. Wallander, and B. Kempnaers. 2002. Genetic similarity between mates and extrapair parentage in three species of shorebirds. *Nature* 419: 613–615.
- 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.
- Darwin, C. 1871. *The descent of man, and selection in relation to sex*. Murray, London.
- Dixon, A., D. Ross, S. L. C. O'Malley, and T. Burke. 1994. Paternal investment inversely related to degree of extrapair paternity in the reed bunting. *Nature* 371:698–700.
- Dunn, P. O., L. A. Whittingham, and T. E. Pitcher. 2001. Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution* 55:161–175.
- Eberhard, W. G. 1996. *Female control: sexual selection by cryptic female choice*. Princeton Univ. Press, Princeton, NJ.
- Endler, J. A. 1986. *Natural selection in the wild*. Princeton Univ. Press, Princeton, NJ.
- Foerster, K., K. Delhey, A. Johnsen, J. T. Lifjeld, and B. Kempnaers. 2003. Females increase offspring heterozygosity and fitness through extrapair mating. *Nature* 425:714–717.
- Freeman-Gallant, C. R., E. M. Johnson, F. Saponara, and M. Stanger. 2002. Variation at the MHC in Savannah sparrows. *Mol. Ecol.* 11:1125–1130.
- Freeman-Gallant, C. R., M. Meguerdichian, N. T. Wheelwright, and S. V. Sollecito. 2003. Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. *Mol. Ecol.* 12:3077–3083.
- Forstmeier, W. 2002. Factors contributing to male mating success in the polygynous dusky warbler (*Phylloscopus fuscatus*). *Behaviour* 139:1361–1381.
- Forstmeier, W., B. Kempnaers, A. Meyer, and B. Leisler. 2002. A novel song parameter correlates with extrapair paternity and reflects male longevity. *Proc. R. Soc. Lond. B* 269:1479–1485.
- Garamszegi, L. Z., and A. P. Møller. 2004. Extrapair paternity and the evolution of bird song. *Behav. Ecol.* 15:508–519.
- Gray, E. M. 1997. Female red-winged blackbirds accrue material benefits from copulating with extra-pair males. *Anim. Behav.* 53:625–639.
- Griffith, S. C. 2000. High fidelity on islands: a comparative study of extrapair paternity in passerine birds. *Behav. Ecol.* 11: 265–273.
- Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extrapair paternity in birds: a review of interspecific variation and adaptive function. *Mol. Ecol.* 11:2195–2212.
- Hanotte, O., C. Zanon, A. Pugh, C. Greig, A. Dixon, and T. Burke. 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. *Mol. Ecol.* 3:529–530.
- Hasselquist, D., and P. W. Sherman. 2001. Social mating systems and extrapair fertilizations in passerine birds. *Behav. Ecol.* 12: 457–466.

- Hasselquist, D., S. Bensch, and T. von Schantz. 1995. Low frequency of extrapair paternity in the polygynous great reed warbler, *Acrocephalus arundinaceus*. *Behav. Ecol.* 6:27–38.
- Hasselquist, D., S. Bensch, and T. von Schantz. 1996. Correlation between male song repertoire, extra-pair paternity, and offspring survival in the great reed warbler. *Nature* 381:229–232.
- Jamieson, A. 1994. The effectiveness of using co-dominant polymorphic allelic series for (1) checking pedigrees and (2) distinguishing full-sib pair members. *Anim. Gen.* 25(Suppl.) 1:37–44.
- Jeffery, K. L., L. F. Keller, P. Arcese, and M. W. Bruford. 2001. The development of microsatellite loci in the song sparrow, *Melospiza melodia* (Aves) and genotyping errors associated with good quality DNA. *Mol. Ecol. Notes* 1:11–13.
- Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* 75:21–64.
- Jones, A. G., D. Walker, C. Kvarnemo, K. Lindström, and J. C. Avise. 2001. How cuckoldry can decrease the opportunity for sexual selection: data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. *Proc. Natl. Acad. Sci. USA* 98:9151–9156.
- Kempenaers, B., G. R. Verheyen, M. van den Broeck, T. Burkey, C. van Vroeckhoven, and A. A. Dhondt. 1992. Extra-pair pa-