CHAPTER 4

THE RELATIVE IMPACT OF EXTRA-PAIR FERTILIZATION ON VARIATION IN MALE AND FEMALE REPRODUCTIVE SUCCESS IN DARK-EYED JUNCOS (JUNCO HYEMALIS)

ELLEN D. KETTERSON, PATRICIA G. PARKER, SAMRRAH A. RAOUF, VAL NOLAN JR., CHARLES ZIEGENFUS, AND C. RAY CHANDLER

1 Department of Biology and Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, Indiana 47405, USA;
2 Department of Zoology, The Ohio State University, Columbus, Ohio 43210, USA;
3 Department of Mathematics, James Madison University, Harrisonburg, Virginia 22801, USA; and
4 Department of Biology, Georgia Southern University, Statesboro, Georgia 30456, USA

ABSTRACT—Differences among species in breeding system and degree of sexual dimorphism are thought to be mirrored by species-level differences in paternity in present sexual selection. The greater the deviation from monogamy, the greater the potential influence of sexual selection on the sex that is more variable in reproductive success (RS). The recent discovery of extra-pair fertilizations (EPFs) in apparently (i.e., socially) monogamous bird species, and the fact that EPFs have the potential to increase variance in RS of such species, led us to quantify EPFs in Dark-eyed Juncos and to explore the relative impact of EPFs on male and female variation in RS. We obtained season-long measures of frequency of EPFs, numbers of males acquired, and apparent RS (young produced in nests of social mates) and genetic RS for 50 male and 45 female juncos studied during two breeding seasons in Virginia, USA. Forty-two of the 50 males produced offspring and 10 of the 42 (45.2%) lost paternity to EPFs. Thirty-eight of the 45 females produced offspring, and 13 of the 38 (34.2%) had at least one young sired by EPFs. There were no cases of conspecific brood parasitism. Of 172 young, 53 (28%) were sired by EPFs. Males that gained EPFs rarely suffered losses, and males that were victims of EPFs rarely gained by them. The result was that some males were more successful than others. We calculated variance in male and female apparent and genetic RS and mating success (MS: number of mates per season). We also calculated statistical dependence of RS on mating success. Among males, apparent reproductive success was less variable (standardized variance [SV] = 0.65) than genetic success (SV = 0.72), indicating that EPFs increased variance in male RS. The sex difference in variance in genetic success (male SV = 0.72, female SV = 0.51) was considerably less than the sex difference in variance in mating success (male SV = 0.65, female SV = 0.37). Interestingly, in both males and females, genetic RS increased with number of mates, suggesting that sexual selection, when defined as the statistical dependence of RS on MS, was acting on both sexes. To our knowledge this is the first study to report EPF-based sexual selection in a socially monogamous female songbird. Although an increase in RS with MS will clearly cause selection to favor attributes of individ-

81
Many species-level differences in degree of sexual dimorphism, broadly defined to include sexual dichromatism and sex differences in physiology and behavior, are thought to result from differences between the sexes in the intensity of sexual selection (Darwin 1871; Schaller 1972; Emlen and Oring 1977; Payne 1984; Gowaty 1985; Andersson 1994). Among bird species that are sexually monomorphic and monogamous and in which both parents care for young, the relationship between mating success (MS, number of mates) and fecundity or reproductive success (RS) is expected to be similar in males and females. As a consequence, variances in male and female MS and RS are also expected to be similar, as is the intensity of sexual selection acting on traits that affect mating success. At the other extreme, in highly dimorphic species with polygynous mating systems and little male parental care, fecundity is thought to depend more strongly on mating success in males than in females. As a consequence, variances in male mating success and RS are expected to be greater, and traits that enhance male mating success are expected to be under stronger selection.

Comparative and experimental tests of these generalizations require accurate measures of both MS and RS, and this was once thought to be a straightforward proposition (Clutton-Brock 1988). MS was determined by counting the number of all birds present in a marked territory, and to determine RS one needed only to find nests, associate them with a marked pair, quantify their contents, and observe their success or failure in producing fledglings or independent young. The success that was apparent to the field observer was believed to be true RS. Newly developed methods to determine genetic relatedness now allow more accurate measures of RS, and in many species the results have revealed discrepancies between apparent and actual mating systems and between apparent and genetic RS (e.g., Gowaty and Karth 1984; Gowaty 1985; Westneat 1987; Gáloš et al. 1990; Rabenold et al. 1996; for summaries see Westneat et al. 1990; Birkhead and Møller 1992; Westneat and Webster 1994).

As a consequence, we must reassess old generalizations and confront the paradox that new information has revealed. If extra-pair copulations (EPCs) are common in what are now called socially monogamous species and if these frequently result in extra-pair fertilizations (EPFs), we must ask whether males of such species are actually less subject to sexual selection than males of socially polygynous species. If not, why do we find greater phenotypic differences between the sexes in socially polygynous species? Similarly, if EPCs are common in monogamous species, then why is male parental care also so common, given that it is frequently directed toward unrelated young (Whittingham et al. 1992; Westneat and Sherman 1993; Møller and Birkhead 1993; Jeffersen and Nøst 1997)? Do certain forms of male parental care (e.g., incubation) appear only in truly monogamous species whereas other forms (e.g., feeding offspring) evolve
Despite deviations from true monogamy (Kettenring and Nolan 1994), we can give satisfactory answers to these questions. We need more information on the frequency of EPPs and their relative impact on variation in male and female RS from a wide variety of species.

A primary objective of this paper is to make such comparisons using genetic data from the Dart-eyed Junco (Junco hyemalis). Juices are socially monogamous, weakly dimorphic songbirds in which both males and females care for young. In asking whether sexual selection is acting on this species, and, if so, whether it is acting more strongly on males or females, we employ the approach of Arnold (1994, after Bateman 1948) and Arnold and Duvall (1994). The approach distinguishes between selective and response to selection, and it asserts, like Bateman (1948), that the cause of sexual selection is the dependence of fecundity on MS. To characterize sexual selection, the argument proceeds, comparisons of males to females must be made along three key parameters: (1) variance in RS, (2) variance in MS, and most fundamentally, (3) the relationship between MS and RS (Arnold 1994; Arnold and Duvall 1994).

When such comparisons are made on a socially monogamous bird such as the Junco, three outcomes are possible (Fig. 1). First, if a species is truly monogamous, we would expect all males and females either to be unmated or to have only one mate. If the sexes are equally likely to acquire mates, they would be equally dependent on MS for RS and would exhibit equal variance in RS (Fig. 1a). Sexual selection on both sexes would be weak. Second, if EPPs occur, and if multiple mating is beneficial only to males, RS in males should increase with number of mates. If female reproductive potential is saturated by a single mate, female RS would level off with MS (Fig. 1b). Variance in MS and RS would be greater in males than females, and male RS would be more dependent on MS than female RS would be, with the result that sexual selection would act more strongly on males than females. Third, if both males and females benefit from multiple mating via EPPs, then RS in both sexes would increase with MS. As with true monogamy, variance in MS and RS would again be similar for males and females (Fig. 1c). However, in this case, instead of the relatively low magnitudes of variance in MS and RS that are associated with a true monogamy, the small intersexual differences in variance in MS and RS could be accompanied by relatively large within-sex variation. Sexual selection would be acting strongly on both sexes in the sense that individuals that acquired more mates would produce a disproportionately larger number of the next generation’s offspring.

If EPPs enhance variation in RS of one sex more than the other, it is likely over evolutionary time that EPPs will have a greater impact on the phenotype of the more affected sex, leading to greater sexual dimorphism (Moller and Firthhead 1994). To determine whether EPPs differentially affect variance in RS of male and female juncos, we measured apparent and actual genetic success in a free-living population studied for 2 years and for which we determined both the frequency of EPPs and the identities of the sizes of RS young. We then compared means, variances, and standardized variances of male and female RS and MS.

To investigate the existence of sexual selection, season Arnold (1994) and Arnold and Duvall (1994), we used regression analysis to determine and compare the extent to which RS is affected by number of mates in both males and females.
SPECIFIC OBJECTIVES

1. How frequently do EPFs occur in juncos? What proportion of males loses paternity to EPFs? What proportion of offspring is produced by EPFs? Is the number of individuals acquiring parentage as the result of EPFs equal to the number losing parentage in this way?

2. Are EPFs important to males? Is male RS enhanced by an increase in number of mates, defined as the number of females successfully inseminated? Does RS achieved by EPFs come instead of, or in addition to, apparent RS with social mates? Are EPFs important to females? Is female RS enhanced by an increase in the number of mates, defined as the number of males that fertilizes a female's eggs? Does the proportion of young sired by EPFs increase as an individual's RS increases, as would be expected if females with greater success attracted more mates?
(3) When males and females are compared, what are the relative magnitudes of variance in RS, variance in number of mates, and degree of dependence of RS on number of mates?

THE SYSTEM

Background: We studied a population of Carolina Dark-eyed Juncos (J. h. carolinensis) that breeds at or near Mount Lake Biological Station, Pembroke, Giles County, Virginia, USA (see Chandler et al. 1994 for description of the study area) and that has been under study since 1983 (e.g., Wolf et al. 1988, 1991; Ketterson et al. 1991b, 1996). Juncos are widely distributed, abundant, and geographically variable, the Carolina race breeds at higher elevations (~1,000 m and above) in the mountains of southeastern North America. Males and females exhibit moderate dimorphism (Fretwell 1972). They differ slightly in body mass (e.g., at the time young leave the nest, breeding males average 21.0 g, breeding females 20.0 g [Wolf et al. 1991; Ketterson et al. 1992]) and flattened wing length (male mean is 82 mm, female mean is 78 mm [Ketterson et al. 1991b]). The sexes are similar in coloration, although the predominant color, gray, is darker in males and males have more white in their rectrices. Juncos form socially monogamous pairs, females build the nest and perform all incubation, and both sexes help protect and feed nestlings and dependent fledglings. Most pair bonds are season-long (greater than 90% of cases in our population), and year-to-year fidelity to breeding sites is very high. The annual rate of return of breeders from the preceding season is approximately 50% (Ketterson et al. 1996; Nolan et al., unpubl. data). If both members of a pair return to breed next season, the bond is usually reformed (approximately 80% of cases [Ketterson et al. 1995]). Both sexes breed in their first year of life (Nolan et al., unpubl. data). If young juncos survive until about the middle of their first winter, further expression of life is about 2 years.

Next prediction is common, reaching 75% of all attempts in some years, with 30% of these losses occurring between clutch completion and hatching (Ketterson et al. 1996). Many predators contribute to this mortality, but eastern chipmunks (Tamias striatus) are believed to be most important (Ketterson et al. 1996; Cav- thorn et al., in prep.). Juncos in this population renest, sometimes repeatedly, after nest failure, and if successful they produce second and even third broods.

METHODS

Data collection in the field: Data on mating success and reproductive success were collected during two breeding seasons, 1990–1991. All adults were individually color-marked, most of them as juveniles in the year they hatched and the first early in the breeding seasons in which they were first studied as adults. As part of an ongoing study, a subset of the males (approximately one half) was treated with testosterone by placing hormone-packed silastic implants beneath the skin (see Ketterson et al. 1992). Control males were treated similarly in all respects except that their implants were empty. Implant status was assigned at random after blocking for sex (first-year adults or older adults) and capture site within the study area. Males that were discovered breeding on the study area after a predetermined date in spring were not implanted, but because they were treated similarly in all other ways (e.g., caught, bled, weighed, and so on), we also
considered them to be controls. This group comprised 1 of the total of 13 control males in 1990 and 3 of 13 in 1991.

Early in each season we mapped the locations of males and females and determined the identities of pair members on the basis of color bands. Adults seen together repeatedly during the initial nest-building and laying period were considered paired; if and when the eggs hatched, all such conclusions were confirmed. If we found nests only after hatching, we treated the adults that fed the nestlings as the social parents of those young. We maintained a season-long effort (from late April to early August) to monitor the reproductive status of all nesting pairs. Exceptions to social monogamy were rare. Over 14 years, <2% of pairings have involved simultaneous associations of more than one female with a single male, and <1% have involved the association of multiple males with the same female (Nolan et al., unpubl. data). Males that remained unmated for an entire season were also rare but detected by their constant advertising, and we also mapped their locations. Some pairs failed to leave any offspring because their nests were repeatedly raided by predators.

We bled adults when we captured them in the spring or at the time young left the nest, taking approximately 100 μl of whole blood from the alar vein. Blood was held on ice and returned to the laboratory where it was preserved in phosphate-buffered saline (3 mM KCl, 3 mM Na2HPO4, 2 mM KH2PO4, 0.14 M NaCl, 6 mM ethylenediaminetetraacetic acid 0.2% sodium azide) (see Rabenold et al. 1990). Nestlings were counted, weighed, and bled on day 6 (hatching of the first egg = day 0), and their blood was treated identically to that of adults.

Laboratory work: Genetic relatedness was determined by Parker and T. Peare using multilocus minisatellite DNA fingerprinting (Rabenold et al. 1990). We extracted DNA with phenol:chloroform, purified it with diethyl, cut it with the restriction enzyme HaeIII, and transferred the digested fragments from agarose gels to nylon membranes. We probed the membranes with one to three radioactive-labeled sequences, Jeffreys' 33.6, Jeffreys' 33.15, or M13 (Rabenold et al. 1992).

Gels were arranged so that DNA of offspring and social parents were in adjacent lanes, and when there was sufficient room on the gel, we ran potential extra-pair sires (neighbors) on the same gel. Young that could not be assigned to their putative (social) sire or to other males represented on the original gel were run again on new gels until a match was obtained. On these subsequent gels we ran the female, the unassigned offspring, and additional neighboring males. We scored (using both Jeffreys' probes) an average of 413 bands for each individual, and a frequency distribution of bands shared between putative parents and offspring was distinctly bimodal. Young in the first mode were considered to have been produced by the putative sire and his social mate; young in the second mode were considered to have been sired by an extra-pair male. Young and assigned genetic parents differed by an average of 0.231 bands. Excluded young assigned to a male other than their social father differed from the social father by an average of 10 bands (extremes, 5–16) (Parker et al. in prep.).

Terminology: We define annual separate reproductive success (AARS) of adults as the number of nestlings reared to the age of 6 days summing over all nests of the social pair during one breeding season. Day 6, the age at which we bleed young, represents survival of about one half of the nesting stage and see
quarter of the period of dependence after hatching (Wolf et al. 1988). Because genetic analysis revealed no cases of conspecific brood parasitism of females by other females, to female AARS equals annual gain or loss success (AGS). This was not true for males, and for them we calculated the number of young lost to EPPS (EPP losses) and subtracted that number from their apparent reproductive success; we refer to the difference as home success (AARS = EPP losses − home success).

We also determined the number of young these males sired with females other than their special mates, and we call these EPP gains or away success. Thus for males, AGS was the sum of home success and away success (home success + away success = annual genetic success).

For members of both sexes we calculated the number of individuals with which they produced at least one genetic offspring that survived to day 6, that is, mating success (MS; number of mates per season). For a female this was the number of different males that sired her offspring, and for a male it was the number of different females whose eggs he was known to have fertilized. Mates that were strictly social did not contribute to an individual’s MS. Thus, for example, if a male with a social mate failed to sire any offspring with her or with any other female, he was classified as having had zero mates, and if all of a female’s young were sired by a single extra-pair sire she was classified as having had only one mate.

Statistical analyses and methodological considerations: All statistical analyses were done using SYSTAT 5 for the Macintosh (SYSTAT Inc., Evanston, IL). One of our first methodological concerns was how to treat individuals that were present in more than 1 year. Of the 50 males and 45 females studied in 1990–1991, 6 males and 6 females were present in both years, providing data from 56 male-years and 51 female-years.

We considered three methods for dealing with replication: one was to select at random 1 year to represent each individual that was present in more than 1 year, another was to average the data across years to represent each bird (e.g., Whittingham and Lifjeld 1995), and the third was to treat each year as an independent data point (e.g., Weatherhead and Boag 1995). We found no between-year correlation in RS of individuals present in more than 1 year (in these data and also in a larger set of data covering additional years), so it is arguable that we might have treated as independent the data collected on the same individual in more than 1 year. Nevertheless, we elected to use the first method as the most conservative, and we performed the random selections independently on females and males, so that all pair members entered the sample as pairs.

A second concern was that males treated with testosterone might have increased the population-level frequency of EPPs and increased variance in male and female RS. However, because male treatment had no significant effects on AARS, EPP losses, EPP gains, or AGS of this sample of males (Ketterson et al. 1996), we elected to pool males across treatments for all comparisons. Although legitimate statistically, we note that patterns described here should be verified from unmanipulated populations. Such a population is currently under investigation (Rowe et al., in prep.).

A third concern relates to the difficulty in obtaining accurate measures of RS in open-nesting species that can rear more than one brood and that are subject to heavy nest predation. Junco nests can be difficult to find in mountainous terrain,
Fig. 2. Limits to accuracy when comparing reproductive success (RS) in the field for males and females of usually monogamous bird species in which extra-pair fertilizations (EPF) occur and where specific brood parasites or exotic individuals for females will typically be more complete than for males. Circles represent RS of a set of males and females (left circle females, right circle males) occupying a study area. Offspring of females will have been reared by some combination of males entering the study area from outside its boundaries to engage in EPFs (region A), by social males (region D), or by social males of other females in the sample (region C). Males will probably have left the study area to rear offspring by females not co-occupying (region D). But the magnitude of D is often unmeasurable.

and, despite our efforts to locate all of them for each pair throughout the season, we almost inevitably missed some. Pairs believed to have reared only one brood may actually have reared more than one, and pairs classified as having achieved zero apparent success (because all their known broods were lost to predators) might actually have reared a brood we missed. Both facts may have caused us to underestimate mean RS. Despite these limitations, we do not think this tendency to underestimate RS would have a differential effect on comparative estimates of male and female RS.

A final concern relates to the relative accuracy of measures of male and female RS (Fig. 2). In open populations in which there is no brood parasitism, data for females can be complete if they remain socially mated to the same male all season; that is, if all their nests are found, then all their eggs, regardless of how many males sired them, can be accounted for. Males, on the other hand, regardless of presence of their social mates on the study area, may sire young in nests located off the study area, and if they do those young will not be considered in calculations of male RS. To illustrate this point as applied to our sample, the adults that we used to compare males to females consisted of (1) individuals belonging to local pairs that tended the offspring that we banded (2) individuals belonging to pairs that left prior to offspring because of nest predation, and (3) unmated males (no evidence indicated that any females were unmated). Some of the young in the sample were sired by the males that tended them (region B, Fig. 2), and some were sired by males socially mated to other females on the study area (region C, Fig. 2). Thus, the sample of males used to characterize male RS did not include all males whose territories lay outside the study area but that entered the area and sired young as the result of EPFs (region A, Fig. 2). These males were excluded because their social mates, assuming they had mates, lived off the study area and had young we therefore did not sample.

In this study of 50 males, 45 females, and 187 nestlings, 24 nestlings (12.8%)
were sired by 7 males that were not among the 50 males in the study sample (region A, Fig. 2). Although we might have assumed that the number of young in the study population sired by males outside is was equal to the number of young sired outside the area by males from within it (i.e., region A and region D were equal, Fig. 2), we opted not to do this because we could not know which males increased their RS via EPFs and which did not.

RESULTS

Frequency of EPFs: Of the 50 males, 42 had some degree of apparent success, 5 lost all broods to predators, and 3 were unmated. A total of 37 males had some genetic success, that is, sired at least one offspring (37 of 50, 74.9%; 37 of 42, 88%), and 5 males that were apparently successful had no genetic success. Nineteen of the 42 males with some apparent success lost paternity to EPFs (45.5%). Of 187 young, 53 were sired by extra-pair males, that is, 28.3% were produced by EPFs. Of the 45 females, 25 produced only young sired by social mates, 13 produced at least one young sired by an extra-pair male, and 7 had zero apparent and genetic success because of repeated losses to predators. Thus 34% of 38 successful females had multiple mates. As stated earlier, no young were found that could not be assigned to the females that tended them.

Variation in AGS is a males: From the 50 males in the sample, we identified 9 that had EPF gains. An additional 7 males from outside the sample were sires of EPF young that were in the sample (see above), so the total number of sizes that gained paternity on the study area via EPFs was 16. Comparing this number to the 19 males that lost paternity to EPFs indicates that some males sired EPF young with more than one female.

Two clear a priori alternatives describe how EPFs might affect variation in male RS. If EPFs involve a trade-off such that the greater the number of EPF-young a male sires, the greater his loss of paternity at home, then gains and losses would be positively correlated and the impact of EPFs on variance in male RS would be small (trade-off relationship). Alternatively, if some males gain RS by EPFs without losing paternity to other males as the result of EPFs, and if others show the opposite pattern, then gains and losses could be negatively correlated and the impact on variance would be high (winners and losers relationship).

Figure 3 plots individuals according to the number of EPF gains and losses they accumulated and appears to describe three types of males: one group near the origin that neither gained nor lost RS by means of EPFs (n = 25), a second group in the lower right that suffered losses and had no gains (n = 16), and a third group in the upper left that accumulated gains and suffered few (n = 3) or no (n = 6) losses. When we considered either all males or all males with apparent success, fertilizations gained and lost by EPFs were neither positively nor negatively correlated (r = 0.03, P = 0.84, n = 50; r = -0.08, P = 0.61, n = 42). This result supports neither the trade-off nor the winners and losers relationship. However, when we considered only those males affected by EPFs, that is, males that experienced either losses or gains, there was a negative correlation between gains and losses (r = -0.375, P = 0.06, r = 25) and a significant negative correlation between gains and proportion of young lost to EPFs (r = -0.453, P = 0.02, n = 25). These results provide no support for the trade-off relationship and limited support for the winners and losers relationship.
We next considered possible associations between a male’s RS at home and his RS away from home (Fig. 4). A positive correlation would indicate that males successful in gaining EPF were also successful both at preventing losses at home and in being socially mated to females with high success. We found no such correlation either among all males (home success vs. EPF gains) \( r = 0.07, P = 0.629, n = 50 \) or among males with an apparent success of at least one young \( r = -0.026, P = 0.870, n = 42 \) (Fig. 4). Visual inspection of the pattern (Fig. 4) suggested that males with lowest and highest home success had few EPF gains, whereas those with low to intermediate home success were the ones that acquired EPF gains. To test this possibility that a nonlinear relationship existed between home success and EPF gains, we divided observed values of home success into thirds: home success of zero to three offspring \( n = 20, \text{mean gain} = 0.536, \text{SE} = 0.251 \), four to six offspring \( n = 18, \text{mean gain} = 0.667, \text{SE} = 0.412 \), and
Table 1. Comparing variance in annual apparent reproductive success (AARS) and annual genetic reproductive success (AGS) in male and female dual-eyed lizards.

<table>
<thead>
<tr>
<th>Measure</th>
<th>AGS (n = 13)</th>
<th>AARS (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>standard deviation</td>
<td>2.52</td>
<td>2.76</td>
</tr>
<tr>
<td>variance</td>
<td>7.73</td>
<td>7.16</td>
</tr>
</tbody>
</table>

seven to eight offspring (n = 3, mean gains = 0). When we compared the three classes for AGS, we found no significant differences (F = 0.261, P = 0.771). To determine the impact of EPF on variation in male RS, we compared variance in AARS to variance in AGS (Table 1, Wade and Arnold 1980), employing the standardized variance (SV, variance divided by the square of the mean [Wade and Arnold 1980]). AARS was somewhat less variable among males (SV = 0.54) than was AGS (SV = 0.72), suggesting that EPFs enhanced variance in male RS (ratio of standardized variances equaled 1.3, Table 1). We used F ratios to compare the nonstandardized variances and found no significant differences. No test of significance was applied to the standardized variances.

Mean AGS of males was 1.28 young (SV = 0.467) (Table 1). Analysis of the degree of dependence of RS on mating success (Table 2; Fig. 5; figure patterned after Arnold 1984. Fig. 1) showed that male RS increased with mating success. Males with zero known mating partners (n = 13) left no offspring. AGS of males with one mate averaged 1.65 young (n = 26, SE = 0.39); with two mates 5.73 young (n = 8, SE = 0.41), and with three mates 7.33 young (n = 3, SE = 2.03) (RS = 2.65 MS = 0.58) (Table 2). RS also increased with MS when number of young per mate was held constant (partial regression coefficients significantly different from zero) (Table 2).

Variance in AGS in females. Mean AGS of females was 3.73 young (SV = 0.513), and mean number of mates acquired was 1.36 (SV = 0.373) (Table 1). Because we found no conspecific brood parasitism, AARS was the same as genetic success. AGS according to number of mates (Fig. 6) revealed that mean AGS of females with one mate was 3.85 young (n = 25, SE = 0.17), whereas females with two mates averaged 5.83 young (n = 12, SE = 0.88), and the single female with three mates produced 10 young. The genetic success of the seven females that produced no offspring was, of course, zero. RS increased significantly with MS (RS = 2.96 MS = 0.37) (Table 2; Fig. 6), and the effect was significant when number of young per mate was held constant (partial regression coefficients significantly different from zero) (Table 2).

Because the relationship between AGS and number of mates was surprising to us, we refer to a second female with three mates whose RS we determined. She was excluded from the sample by our method of randomly eliminating females present during both years of the study to avoid pseudoreplication (see section.
Statistical analyses and methodological considerations: During the year for which this female was included, she had two mates and six young. During the year for which she was excluded, she had three mates and seven young. Thus high RS of females with three mates may occur more commonly than the single observation in Figure 6 would suggest.

Do females that are inherently more fecund attract more mates? If the null hypothesis is that male effort to sire young by EPFs is independent of a target female’s potential RS, we would predict no relationship between AGS and the proportion of a female’s young sired by EPFs (and thus potentially number of mates). We found instead that the proportion of young sired by EPFs increased with RS ($r = 0.475$, $P < 0.001$, $n = 45$) (Fig. 7). That correlation might have been driven by including in the analysis females that had zero RS. Therefore, we performed the same calculation limited to females with greater than zero RS ($n = 38$) and again found a positive correlation ($r = 0.423$, $P < 0.008$) (Fig. 7).

Comparing males to females: To ask whether the existence of EPFs generates stronger selection in males than in females, we compared means, variances, and standardized variances of key reproductive parameters of males and females (Table 1). Mean genetic success of males was 14% lower than that of females (3.36 vs. 3.75) (Table 1). This is consistent with the caveat that female RS can be
known more accurately than male RS (see Methods), because females in the sample can be inseminated by males from outside the study population, whereas we would not detect cases in which males inseminate females not part of the study population. When we compared variance in genetic success, we found that it was 1.07 times greater in males than in females (7.67/7.16, Table 1; right-hand histograms in Figs. 5, 6); the standardized variance in genetic RS was 1.41 times greater in males than females (0.722/0.513) (Table 1). We used F ratios to compare the nonstandardized variances and found no significant differences. No test of significance was applied to the standardized variances.

Variance in number of mates obtained was also greater in males; the ratio of male to female variance in this measure was 1.34 (0.65/0.50) (Table 1; top histograms in Figs. 5, 6), and when variances were standardized, the ratio was 1.73 (0.647/0.373) (Table 1). F ratios revealed no significant differences in the nonstandardized variances, and no test of significance was applied to the standardized variances.

Consideration of the regression lines relating genetic RS to MS provides a comparison of the sexual selection gradient (Arnold 1994) in males and females (Figs. 5, 6; Table 2). The slopes did not differ.
Table 2. Regression analyses (simple and multiple) of the dependence of annual genetic reproductive success (AGS) on number of mates of males and females. For multiple regressions, second independent variable was number of young per young.

<table>
<thead>
<tr>
<th></th>
<th>Males: dependent variable = AGS, $n = 50$</th>
<th>Females: dependent variable = AGS, $n = 46$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficients</td>
<td>Regression coefficients</td>
</tr>
<tr>
<td></td>
<td>constant</td>
<td>constant</td>
</tr>
<tr>
<td>Simple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>regression</td>
<td>Constant = 0.58, 0.40</td>
<td>Constant = 0.37, 0.30</td>
</tr>
<tr>
<td></td>
<td>Number of mates = 2.63, 0.30</td>
<td>Number of mates = 1.79, 0.16</td>
</tr>
<tr>
<td>Multiple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>regression</td>
<td>Constant = -0.64, 0.21</td>
<td>Constant = -1.06, 0.34</td>
</tr>
<tr>
<td></td>
<td>Number of mates = 1.79, 0.16</td>
<td>Number of mates = 2.04, 0.24</td>
</tr>
<tr>
<td></td>
<td>Young per mate = 0.79, 0.06</td>
<td>Young per mate = 0.87, 0.02</td>
</tr>
</tbody>
</table>

Discussion

Frequency of EPFs: As in many songbirds studied to date (reviews in Westneat et al. 1990; Birkhead and Møller 1992; authors in this volume), EPFs are common in Dark-eyed Junco: 43.2% of 42 males that had positive apparent success lost at least one offspring to an extra-pair sire, and the percentage of young sired by EPFs was 28%. No female laid an egg in the nest of another female. Interestingly, in 7 years of intensive field study prior to 1990, we had had no conclusive observational evidence of EPFs in Junco.

Fig. 7. Proportion of a female's young sired by extra-pair fertilization in relation to annual genetic success of females (size of circle proportional to number of observations, $n = 45$).
EPFs and variation in male RS: We had anticipated that if success at acquisition of EPFs was balanced by losses, EPFs might affect males equally. For example, when females begin to build nests synchronously and therefore are forced to synchronize, the frequency of such behavior varies in some species (Stutchbury and Morton 1995; Stutchbury and Neufeld, Chapter 5). In that case, a male's time spent off-territory inseminating other females might be at the cost of preventing his own social mate's access to EPFs. Under that hypothesis, the net effect of EPFs on variance in genetic success might be negligible, and a plot of EPF gains versus EPF losses would have a positive slope (trade-off relationship). Alternatively, if some males gain in genetic success via EPFs and are also successful at maintaining paternity at home, whereas other males are relatively unsuccessful both at home and away, the result would be that some males were highly successful, others were notable losers (Westneat 1992; Sorenson 1994), and still others were intermediate (winners and losers relationship).

In the juncos reported on here, EPFs did not affect all males equally (Fig. 3). Among those that either gained or lost paternity via EPFs, the two measures, gains and losses, were negatively correlated, significantly so when losses were expressed as a percentage of apparent success. Thus, male juncos tend either to gain or lose RS via EPFs but infrequently do both. This is an argument for the existence of differences in "male quality" in a free-living population of a monogamous species. Still, we saw more than one path to high genetic RS (home success + EPF gains) (Fig. 4).

Studies of patterns of EPF gains and losses across males in other species have shown them to be complex and varied (Gibbs et al. 1990; Westneat 1992; Dunn et al. 1994; Whittingham and Lifjeld 1995; Yezersinac et al. 1991; Dunn and Cobbourn, Chapter 7). Juncos appear to resemble the pattern in Red-winged Blackbirds (Agelaius phoeniceus (Westneat 1993)) and Purple Martins (Progne subis (Morton et al. 1990)), in which males that gain from EPFs tend not to suffer EPF losses. In other species, such as Superb Fairy-wrens (Malurus cyaneus (Dunn and Cobbourn, Chapter 7)), Yellow Warblers (Dendroica petechia (Yezersinac et al. 1995)), and House Martins (Delichon urbica (Whittingham and Lifjeld 1995)), males tend to experience both gains and some offsetting losses. In general, however, for males that acquire EPFs, the net effect on genetic RS is positive.

A related question is whether EPFs increase the opportunity for sexual selection (Wade and Arnold 1980). One way to answer this question is to compare variance in apparent success to that in genetic success, after standardizing the variances (Gowaty 1982; Westneat 1987; Arnold 1994; Whittingham and Lifjeld 1995). In male juncos, these variances were 0.545 versus 0.721 for apparent and genetic success, respectively, and the ratio of larger over smaller was 1.32. When these calculations were made on only those males that acquired a social mate and raised young (i.e., whose apparent success was greater than zero), the standardized variances were lower overall (0.296 vs. 0.444), and the ratio was actually greater (1.50).

These ratios in juncos are similar to or smaller than those reported from other species, for example, Red-winged Blackbirds (0.25 vs. 0.90, ratio = 1.50 (Gibbs et al. 1990)), Blue Tits (Parus caeruleus; 0.16 vs. 0.27, ratio = 1.69)
[Kempner et al. 1992], and House Martins (0.66 vs. 0.31, ratio = 2.17 [Whittingham and Lifjeld 1995]). Yezierinac et al. (1995) estimate that EPFs increased variance in RS in male Yellow Warblers 3- to 15-fold. Only Hill et al. (1994) do not report an increase in variance after considering the effects of EPFs on house finches (Carpodacus mexicanus). Comparatively, the impact of EPFs on variance in male genetic RS in juncos is relatively low.

**MS and RS in males and females:** A priori, theory predicts that EPFs should increase variance in male RS more than in female RS (Trivers 1972; Wade and Arnold 1980; Andersson 1994). Female RS is usually thought to be limited by the number of eggs females can lay, not by access to extra-pair sires, whereas each offspring a male sires with a female mated to another male increases his EPF gains as the cost of the home success of the other male.

Nevertheless, of the three possible relationships between MS and RS outlined in the Introduction (Fig. 1), juncos appear to resemble most closely the third outcome, in which variance in both male and female RS is increased by EPFs, because genetic success increases with MS. This EPF-related increase in variance suggests that sexual selection (as defined here, dependence of RS on MS; individuals with more mates leave disproportionately larger numbers of offspring) is acting on both sexes. Thus, attributes that enhance multiple mating should be favored in both sexes.

Studies of males of other species are still relatively few and were summarized above. In general, male RS increases with MS (Webster et al. 1995). A few reports have also been made of enhanced RS in female birds that mate with more than one male (e.g., Westneat 1992; Watton and Parkin 1991). Although EPFs are potentially costly, both for males (e.g., loss of opportunity to inseminate social mate, sexually transmitted disease) (Sorensen 1994; Stutchbury and Morton 1995; Stutchbury and Neudorf, Chapter 4) and for females (e.g., loss of paternal care, sexually transmitted disease) (Davies 1992; Whittingham et al. 1992; Westneat and Sherman 1993; Davies et al. 1996), most data seem to indicate that EPFs are either beneficial or neutral for the individuals involved.

How did males with multiple mates achieve greater RS? One explanation, that males that gain EPFs are socially mated to more fecund females, seems unlikely because our regression analysis showed that MS was a significant predictor of RS even when young produced per mate was held constant. Rather, some males apparently possess attributes that allow them to accomplish EPF gains without suffering EPF losses at home (Fig. 3). Our data say nothing about whether the attributes are associated with intersexual or intrasexual dynamics, or both.

To answer whether the lifetime fitness (survival and reproduction) of male juncos with high MS is also greater, we need to relate survival to EPFs. Whittingham and Lifjeld (1995) found no relationship between annual survival and success at EPFs in House Martins, although they did find that high total success (herein AGS) was positively related to annual survival, as did Kempner et al. (1992) for the Blue Tit. A similar analysis for juncos is currently underway.

There are several possible answers to the question why females with more than one mate might produce more young: they may produce larger clutches,
have greater hatching success, or produce more broods per season. These questions as well as whether females with extra-pair mating partners are larger, older or otherwise more experienced will be addressed in a future paper (Park, et al., in prep.). Regardless of the reason(s), our results suggest multiple mating is beneficial to female juncos.

Relative intensity of sexual selection in male and female juncos: Whether our results should be taken to indicate that sexual selection acts on male and female Dark-eyed Juncos, with similar intensity depends in part on how one defines sexual selection (Arnold 1994). If one defines it as the degree of dependence of RS on number of mates as we have done here, then, because the slopes of the lines relating RS to MS were nearly identical in male and female juncos, sexual selection can be said to be equally strong on both sexes. However, we are aware of two potential objections to this interpretation.

The first objection is that the relationship between RS and number of mates might arise purely as an artifact of sampling. If EPPs are visited randomly on females (in Poisson fashion), then females that achieve high RS may be more exposed to EPPs, and EPPs of such females would be more readily detected. Thus, if the probability of an EPP is 0.32 per egg laid, then a female that produces nine eggs and young should produce three by EPPs, whereas for a female that produces only one egg and young, in approximately two out of three cases it would not be EPP-sired. Under this hypothesis, EPPs, and thus potentially number of mates would increase with RS, but the reason would be simply that such fertilizations would be more detectable in more fecund females (see also Johnson and Barley, Chapter 2). However, our finding that the proportion of young sired by EPPs increases with AGS in female juncos (Fig. 7) makes it unlikely that the sole explanation for the observed relationship between RS and MS in the juncos was an artifact of sampling.

A second objection addresses whether a statistical demonstration of dependence of RS or MS is sufficient to demonstrate sexual selection, particularly in females. This question challenges the definition of sexual selection used thus far in this paper and raises the issue of whether our results are interpretable as natural selection or sexual selection on females (compare McCauley 1983). Should we reserve the term sexual selection for situations in which female RS is enhanced directly or indirectly by additional mates, or should the term be extended to cover situations in which inherently more fecund females are more attractive to males and thus accumulate more matings?

It seems probable that some female juncos would have had higher RS than others, regardless of the number of males siring their offspring, because they were older or in better condition or possessed some other trait associated with fecundity. If we then suppose that males have means of assessing potential fecundity and more fecund females are more attractive to males, we might expect such females to have acquired more mates. The greater MS among females with higher RS would reflect male preference for females that would have been favored by natural selection, whether they had one mate or three. Greater mating success would be the result, not the cause, of higher RS, and, for those who hold that selection can be sexual only if mate acquisition causes differential RS, the situation in the juncos would not suffice.

Stating this position in another way, in order for selection to be sexual, many
would require not only evidence that RS increases with MS but also evidence that RS decreases in the absence of access to additional mates. The female that could leave equal numbers of young with or without access to extra males does not benefit from extra males. Her situation may be seen as fundamentally different from that of the male that can achieve higher RS only if he gains access to more females. Defined in this way, demonstration of sexual selection on females requires evidence of (1) direct enhancement of RS as the result of multiple matings, such as by greater fertility (percentage of eggs fertilized) (e.g., Wetton and Parkin 1991), protection from predators, or access to food (see Gray, Chapter 3) or (2) indirect enhancement such as by acquiring 'better genes' for her offspring (Eberlova et al. 1992; Houman 1992).

Sexual selection as it is defined by Arnold (1994a) and Arnold and Davall (1994) requires only demonstrated statistical dependence of RS on MS because when this condition holds—genetic RS increases with MS—it follows that the offspring of individuals that acquire more mates will be overrepresented in the next generation. Thus, phenotypic selection will have favored the attributes of parents that mate multiply. If the traits that made males or females more attractive or competitive or subject to chance-based advantages are heritable, then those traits too will be overrepresented in the next generation. But whether or not the traits are heritable, selection has occurred, and because that selection relates to mating success, it is in nature.

Regardless of one's definition of sexual selection, most would probably agree that variation in RS that is related to variation in MS is the 'raw material for sexual selection,' because without such variation sexual selection cannot occur. In nature, selection on females is likely to be a blend of natural and sexual, and separating the quantitative effects of the two forms of selection poses a challenge (Webster and Westneat 1994; Webster et al. 1995). In the meantime, determining the degree of correlation of MS and RS should provide a useful comparative index of the potential for sexual selection, however it is defined.

As authors we do not fully agree as to which definition should be employed. We do note that if the standard for sexual selection is that access to multiple mates must be the cause of higher RS in the sense that RS would be diminished if that access were denied, then we are almost certain to conclude that sexual selection is generally less important in females than in males. If we continue to contemplate how female fecundity might serve as an attractant to males and have accompanying consequences for evolution of the female (and male) phenotype, our understanding of the role of mate choice is evolution as well as our understanding of differences between the sexes should be enhanced.

Acknowledgments

E.D.K., P.G.P., and V.N. Jr. thank the National Science Foundation (NSF) for financial support (NSF grants BSR 91-111498, BSR 91-11148 and IBN 94-09053), the reviewers who helped to improve this manuscript (Kris Johnson and Steve Arnold), and our editor Nancy Burley. We also gratefully acknowledge excellent suggestions made by others who read the manuscript (Lynda Delph, Peter Dunn, Dave Enstrom, and Dave McCauley), and we heartily thank Tigirn Peare for her role in the typesetting. E.D.K. and V.N. Jr. appreciate

