Reinterpreting Bateman gradients: multiple mating and selection in both sexes of a songbird species

N.M. Gerlach, J.W. McGlothlin, P.G. Parker, and E.D. Ketterson

Department of Biology, Indiana University, 1001 E. 3rd St., Bloomington, IN 47405, USA, Department of Biological Sciences, Virginia Tech, 2125 Derring Hall, Blacksburg, VA 24061, USA, Department of Biology, University of Missouri—St. Louis, One University Blvd, St. Louis, MO, 63121, USA

INTRODUCTION

The idea that males are eager to seek multiple mates, whereas females are resistant and prefer to mate with only a single male, has been a pervasive notion since Darwin (1859) published his theory of sexual selection. However, this idea was first discussed in quantitative terms by Bateman (1948) in a landmark paper relating sex differences in mate acquisition to offspring production in Drosophila. Bateman’s results generated three main predictions regarding sexual selection (Arnold 1994): males should display greater variance in offspring number than females (Bateman 1948), the correlation between number of mates and number of offspring should be stronger in males than in females (Bateman 1948), and the term “Bateman’s principle,” which was originally limited to the first of Bateman’s three predictions (Dewsbury 2005), whereas the term “Bateman gradient” refers specifically to the statistical relationship between mating success (number of mates with which an individual produces offspring) and reproductive success described in (3), above (Arnold and Duvall 1994; Andresson and Iwasa 1996; Jones et al. 2002). Although theorists have attempted to formalize the relationships among Bateman’s principles and the evolution of sex differences (e.g., Wade and Arnold 1980; Arnold and Wade 1984; Arnold and Duvall 1994; Shuster and Wade 2003) and others have addressed potential conceptual problems in the application of Bateman’s ideas (Gowaty 1997; Parker and Tang-Martinez 2005; Klug et al. 2010; Krakauer et al. 2011), empirical tests of Bateman’s predictions have been rare until relatively recently (Tang-Martinez 2010).

A number of studies testing Bateman’s first principle, that variance in reproductive success should be greater in males than in females, has confirmed its applicability to a variety of species (Clutton-Brock 1988; Le Boeuf and Reiter 1988; McLain 1991; Oring et al. 1991; Jones et al. 2002). However, a number of other studies from an equally broad taxonomic distribution have found that males and females do not differ in variance in reproductive success, including studies from birds (Scott 1988; Marti 1997; Weatherhead and Boag 1997; Thomas and Coulson 1998; Webster et al. 2001; Jensen et al. 2004), mammals (Ribble 1992; Topping and Millar 1998; Bartmann and Gerlach 2001), and invertebrates (Hauber and Lacey 2005). Determining the factors that contribute to variance in reproductive success in both sexes, and thus to the potential strength of selection, remains a major challenge.
challenge for behavioral ecologists and evolutionary biologists (e.g., Jones et al. 2000; Webster et al. 2007).

Bateman’s third principle, that the relationship between mating success and offspring number should be strong and positive in males but not in females, has received mixed support. It has been supported by studies in taxa as diverse as moths (Bezzerides et al. 2008), water striders (Ronkainen et al. 2010), spiders (Maklakov and Lubin 2004), and Columbia ground squirrels (Murie 1995), adders (Capula and Luiselli 1994), crayfish (Yue et al. 2010), and rough-skinned newts (Jones 2009). However, the universal applicability of Bateman’s principle has been called into question by an increasing number of empirical studies. Indeed, even in some of Bateman’s own replicates, females showed a positive relationship between mate number and offspring production, although with a shallower slope than males (Bateman 1948; Snyder and Gowaty 2007). A positive relationship between multiple mating and reproductive success has also been demonstrated in females of a variety of species across a wide taxonomic range (Table 1).

Despite increasing evidence of the prevalence of multiple mating by females (Ridley 1988; Griffith et al. 2002; Ivarson and Clutton-Brock 2007; Uller and Olsson 2008), a generalized version of Bateman’s third principle—that females do not benefit directly from such matings—still holds sway in many areas of behavioral research. This is particularly true with respect to extra-pair mating, the term used to refer to multiple mating in species in which individuals form pair bonds to rear offspring but also copulate with individuals other than their pair-bonded partner. Most reviews of the subject have concluded that extra-pair mating rarely leads to increased individual reproductive success for the female, often by implicitly or explicitly citing Bateman’s third principle as rationale, and that the benefits of extra-pair mating for females must arise primarily from indirect benefits such as greater offspring quality or numbers of grand-offspring (Westmeat et al. 1990; Kempenaers and Dhondt 1993; Jennions and Petrie 2000; Griffith et al. 2002) although tests of this hypothesis have yielded mixed support (Aյ jelly and Roughgarden 2007; but see Gerlach et al. 2012).

In this study, we examine whether extra-pair mating results in direct fitness benefits by examining the relationships among mating success, reproductive success, and extra-pair behavior in both female and male dark-eyed juncos (Junco hyemalis), a North American songbird. Juncos are a mildly sexually dimorphic and socially monogamous sparrow, with appreciable rates of extra-pair offspring (EPO) production (~27% of offspring; this study). A previous study of this species that reported a positive relationship between mate number and offspring number in both sexes was based on only two years of data (Ketterson et al. 1998; this study). A previous study of this species that reported a positive relationship between mate number and offspring number in both sexes was based on only two years of data (Ketterson et al. 1998; this study). Although tests of this hypothesis have yielded mixed support (Aж и Roughgarden 2007; but see Gerlach et al. 2012).

### Materials and Methods

**Field methods**

This study was conducted on a population of free-living juncos breeding at and around Mountain Lake Biological Station in Giles Co., Virginia (Chandler et al. 1994). This population has been monitored during the breeding seasons continuously since 1983. Data reported in this study are from 1990 to 2007. During each year’s adult census (typically 15 April–15 May), juncos were individually color banded, measured, and checked for age, sex, and reproductive success on offspring production in both males and females.

### Table 1

<table>
<thead>
<tr>
<th>Species with a positive relationship between mate number and reproductive success in females</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td>Nematodes (Caenorhabditis spp.)</td>
</tr>
<tr>
<td>Promethea moth (Callosamia promethea)</td>
</tr>
<tr>
<td>Leaf beetle (Chrysocoris coebulatus)</td>
</tr>
<tr>
<td>Katydid (Lygophila nigropalearum)</td>
</tr>
<tr>
<td>Pseudoscorpion (Cordylochernes scorpioides)</td>
</tr>
<tr>
<td>Fly (Drosophila simulans)</td>
</tr>
<tr>
<td>Polychaete worm (Galeolaria caespitosa)</td>
</tr>
<tr>
<td>Cricket (Gryllus bimaculatus)</td>
</tr>
<tr>
<td>Sea urchin (Heliocidaris erythrogramma)</td>
</tr>
<tr>
<td>Moth (Heliceverpa armigera)</td>
</tr>
<tr>
<td>Redback spider (Larutatus hasselti)</td>
</tr>
<tr>
<td>Fireflies (Photinus spp.)</td>
</tr>
<tr>
<td>Seed beetle (Stator limbatus)</td>
</tr>
<tr>
<td>Sea urchins (Strongylocentrotus spp.)</td>
</tr>
<tr>
<td>Grain beetle (Tribolium molitor)</td>
</tr>
<tr>
<td>Red flour beetle (Tribolium castaneum)</td>
</tr>
<tr>
<td>Arctic moth (Ulethesia ornatrix)</td>
</tr>
<tr>
<td><strong>Birds</strong></td>
</tr>
<tr>
<td>Tengmalm’s owls (Aegolius funereus)</td>
</tr>
<tr>
<td>Northern flicker (Colaptes auratus)</td>
</tr>
<tr>
<td>Dark-eyed junco (Junco hyemalis)</td>
</tr>
<tr>
<td>Brown headed cowbird (Molothrus ater)</td>
</tr>
<tr>
<td><strong>Fish</strong></td>
</tr>
<tr>
<td>Cichlid (Neolamprologus pulcher)</td>
</tr>
<tr>
<td>Guppy (Poecilia reticulata)</td>
</tr>
<tr>
<td>Atlantic salmon (Salmo salar)</td>
</tr>
<tr>
<td>Brown trout (Salmo trutta)</td>
</tr>
<tr>
<td><strong>Amphibians</strong></td>
</tr>
<tr>
<td>Small-mouthed salamander (Ambystoma texanum)</td>
</tr>
<tr>
<td>Tiger salamander (Ambystoma t. tigrinum)</td>
</tr>
<tr>
<td>Foam-nesting treefrog (Chromantis xenopomphila)</td>
</tr>
<tr>
<td>Montandon’s newt (Triturus montandoni)</td>
</tr>
<tr>
<td><strong>Reptiles</strong></td>
</tr>
<tr>
<td>Black ratsnake (Elaphe obsoleta)</td>
</tr>
<tr>
<td>Leopard gecko (Eublepharis macularius)</td>
</tr>
<tr>
<td>Sand lizard (Lacerta agilis)</td>
</tr>
<tr>
<td>Common lizard (Lacerta vivipara)</td>
</tr>
<tr>
<td>Water python (Lialis fascius)</td>
</tr>
<tr>
<td>Adder (Vipera berus)</td>
</tr>
<tr>
<td><strong>Mammals</strong></td>
</tr>
<tr>
<td>Agile antechinus (Antechinus agilis)</td>
</tr>
<tr>
<td>Gunnnison’s prairie dog (Cynomys gunnisoni)</td>
</tr>
<tr>
<td>Yellow-toothed cavy (Cavia sp.)</td>
</tr>
<tr>
<td>Yellow-pine chipmunk (Tamias amoenus)</td>
</tr>
</tbody>
</table>
condition (Reed et al. 2006). Beginning in 1990, all adults also had a small blood sample drawn via wing vein puncture for later DNA analysis (Ketterson et al. 1998).

During each breeding season, we located nests throughout the study area. The female and the social male associated with each nest were identified by behavioral observations of marked birds. Six days after hatching (day 6), nestlings were individually sexed and blood samples were drawn for DNA analysis. We continued to monitor nests until the offspring left the nest on day 11–12 post-hatching. Only offspring seen on or after nest-leaving day were considered to have successfully fledged. Juncos in our population re-nest throughout the breeding season (late April–early August) and can produce 2–3 successful broods during this time (Nolan et al. 2002).

Genotyping and paternity analyses

Blood samples were stored in Longmire’s solution, a lysis buffer, between collection and later analysis (Longmire et al. 1988). All samples were extracted using a standard phenol–chloroform procedure and then genotyped either by minisatellites (1990–1996) or by microsatellites (1997–2007) (for microsatellite details, see Gerlach et al. 2012). Nestling paternity for individuals prior to 1997 was determined by band-sharing analyses (Raouf et al. 1997). For nestlings from 1997–2007, paternity was assigned using the program CERVUS (Marshall et al. 1998; Kalinowski et al. 2007). Conspecific brood parasitism is extremely rare in the junco; only one nestling out of 2182 sampled offspring did not match the female associated with its nest (Ketterson et al. 1998; this study).

Of the 2182 sampled offspring, 1563 were determined to be sired by their social father (within-pair offspring, WPO), and 585 were sired by an extra-pair male (EPO). The remaining 34 nestlings were assigned a genetic sire but could not be classified as either WPO or EPO because their social father was not identified in the field. For 142 of the 585 EPO, either band-sharing analyses or CERVUS eliminated the social father as a putative genetic sire (indicating the nestling was an EPO), but these methods were unable to assign a genetic sire (indicating the true sire was not among the sampled males). In cases in which there was more than one “excluded but unassigned” offspring in a brood, we used the program COLONY (Jones and Wang 2010) to estimate full and half-sibships among nestlings with known maternity, and thus the minimum number of sires that contributed to the brood.

Quantifying offspring and mate numbers

To obtain the most comparable measures of offspring numbers for males and females, we considered only those offspring for which we had at least partial information about their genetic paternity (n = 2182) and only those adults that could be associated with these offspring (n = 436 females/435 males). Thus, every female in our sample produced at least one genotyped offspring, and every male in our sample was either the social father or the genetic sire of at least one offspring. By limiting our sample in this way, we avoided falsely inflating female reproductive success relative to that of males and were able to examine both apparent and actual reproductive success for the males in our sample. As a consequence of this sampling technique, the numbers presented represent minimum values for each parent’s total number of offspring. Similarly, because we excluded females and most males with zero reproductive success, our estimates of variance in these measures are almost certainly underestimates of the true level of variance in the population at large (Shuster 2009).

This sampling method may affect estimates of mate number as well; uncounted offspring sired off the study site by a male in our sample also represent an uncounted mate for that male. For females, mating with a male from off the study site would produce an offspring that was known to be an EPO, but for which no genetic sire could be assigned. We were able to partially address this issue by using the sibship analyses in the program COLONY (Jones and Wang 2010) to estimate the number of unknown sires. For broods in which neither sire identity nor sire number could be determined (i.e., broods prior to 1997 for which microsatellite genotypes were unavailable), all “excluded but unassigned” offspring were conservatively considered to have had a single sire. Thus, the number of mates acquired by both males and females is also a minimum estimate of the true value. For all analyses of mating success, only genetic mates were considered; social partners with which an individual never produced offspring were not included as part of that individual’s number of mates. In this study, we used the mean annual number of mates for each individual as its measure of mating success; using total lifetime number of mates instead did not affect the significance of the relationships between mating success and reproductive success in any of our analyses.

Breeding years

Number of sampled breeding years indicates the number of years between 1990 and 2007 in which an adult was known to be present on our study site, and so equals the number of years in which they could have potentially contributed to the sample of genotyped offspring. For the majority of birds sampled (391M/400F), this was equivalent to longevity (Table 2); 80 birds (44M/36F) had at least 1 breeding year (average = 2.0 years) either before or after our sampling period of 1990–2007.

Statistics

As part of an ongoing study, during some years some individuals in our population were implanted with exogenous testosterone (males: 1994–2000; females: 2001–2002 and 2005–2007). As testosterone affects extra-pair behavior in male juncos (Raouf et al. 1997; Reed et al. 2006) and nest success in female juncos (O’Neal et al. 2008), we accounted for these implants by calculating a score (“% T years”) that represents the number of years an individual was implanted with testosterone divided by the number of years they were present in our sample. Although this technique does not account for every potential effect of testosterone manipulations (e.g., number of implanted neighbors influencing mating success), it does address the individual contributions of implanted individuals to the Bateman gradient. Further, the slopes of Bateman gradients for years in which implants were given are comparable to years in which they were not (Supplementary Figure S1).

Male and female mean mating and reproductive success were compared using t-tests, and male and female variances in mating and reproductive success were compared using Levene’s F-test. Bateman gradients were calculated using a generalized linear model (GLM) with a Poisson error distribution, including number of mates, number of sampled breeding years, and % T years as covariates. Each model was run for the sexes separately, and then with the sexes combined, with sex added to the model as a fixed factor, and the interaction terms sex × number of mates, sex × breeding years, and sex × % T years included in the model, but removed if they were not significant (Engqvist 2005).
RESULTS

General distribution of paternity

Of 2182 offspring for which we had at least partial knowledge of their genetic relationships (paternity and/or sibships), 1563 were WPO and 585 were EPO (27% EPO). A total of 436 females produced the 2173 offspring of known maternity (4.98 offspring/female). Regarding males, 401 social males were associated with 2163 offspring (5.39 apparent offspring/social father), and 390 males sired the 2019 offspring that had identifiable genetic fathers (5.18 offspring/genetic sire). Although the sets of social males and genetic sires were mostly overlapping, there were some EP sires without a known social mate and some social males that did not sire any genetic offspring, such that our data set included 435 males in total.

Opportunity for selection

As expected based on the adult sex ratio, the sexes did not differ significantly in mean total number of offspring, mean number of WPO, or mean number of any type of offspring at fledging (Table 2). Females had significantly higher mean annual total and extra-pair mating success, as well as higher numbers of EPO than did males (Table 2), although this is likely due to the fact that in our sample, females but not males could produce offspring with extra-pair partners of unknown identity. Females also had significantly greater mean annual within-pair mating success than males (Table 2); the mean lifetime number of within-pair mates is the same for both sexes (1.02 ± 0.03 in females, 1.02 ± 0.04 in males), so this difference in mean annual success may reflect the fact that males in our sample had significantly longer life spans than females.

Number of mates were significantly greater than variances for males in number of total offspring, number of EPO both overall and at fledging, and mean annual number of within-pair and extra-pair mates. Males and females did not differ significantly in any other variance component (Table 2).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>t-test</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>i</td>
<td>df</td>
</tr>
<tr>
<td>Previous study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3.73 ± 0.11</td>
<td>3.26 ± 0.20</td>
<td>-0.02</td>
<td>849.7</td>
</tr>
<tr>
<td>No. of mates</td>
<td>436</td>
<td>436</td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of sampled yrs</td>
<td>2.01 ± 0.06</td>
<td>2.476 ± 0.07</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
<tr>
<td>No. of offspring</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.98 ± 0.19</td>
<td>4.64 ± 0.22</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
<tr>
<td>At fledging</td>
<td>3.10 ± 0.16</td>
<td>2.89 ± 0.16</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
<tr>
<td>No. of mates</td>
<td>1.33 ± 0.05</td>
<td>1.04 ± 0.02</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
<tr>
<td>No. of EPO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.33 ± 0.10</td>
<td>1.00 ± 0.09</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
<tr>
<td>At fledging</td>
<td>0.82 ± 0.07</td>
<td>0.63 ± 0.07</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
<tr>
<td>No. of WP mates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.84 ± 0.02</td>
<td>0.74 ± 0.02</td>
<td>-5.128</td>
<td>841.8</td>
</tr>
</tbody>
</table>

Number of mates are measured as mean annual mating success. $I_{\text{females}}$ and $I_{\text{males}}$ are calculated as the relative variance, i.e., variance divided by the mean squared. The t-test compares male and female mean values; the F-test compares male and female variances. Historical data (referred to in Table as "previous study") are from Ketterson et al. (1998). Bold values are significant at $P<0.05$.

Figure 1

Bateman gradients for male (black) and female (gray) juncos, depicting the relationship between the mean number of annual genetic mates and the lifetime number of offspring (A) at genotyping and (B) at fledging. The diameter of the circle represents the number of individuals at that point, with the smallest circles in each graph representing one individual.
However, because we restricted our sample to only those adults that produced at least one genotyped offspring, the true difference in variances between the sexes will likely depend on the number of individuals with zero reproductive success in each sex.

Bateman gradients—overall

Both the annual number of genetic mates and the number of sampled breeding years strongly predicted the number of offspring surviving to day 6 in both sexes (Table 3, Figure 1A). When the sexes were considered together, there was a significant interaction effect of sex and number of sampled breeding years such that a longer breeding tenure increased reproductive success more in males than in females, and a significant interaction between sex and the number of sampled breeding years such that a longer breeding tenure increased reproductive success more in females than in males (Table 3).

Many offspring were lost to predators between day 6 and the age of nest-leaving (fledging), so the mean number of genotyped young at fledging was smaller, but the relationships between mating success and reproductive success remained significant when the number of fledged offspring was considered as the measure of reproductive success (Table 3; Figure 1B). Similarly, the number of breeding years was again a significant predictor of the number of fledglings in both sexes, and mating success had a stronger effect on reproductive success in males than in females, whereas number of breeding years had a stronger effect on reproductive success in females than in males (Table 3).

Bateman gradients—EP vs. WP mating

When an individual’s overall annual mating success was partitioned into extra-pair and within-pair mates, both of these variables significantly predicted total reproductive success in both sexes, with number of within-pair mates having the stronger effect on total offspring number. Again, number of breeding years also significantly predicted reproductive success in both sexes. When the sexes were considered together, there was a significant interaction effect of sex and both within-pair and extra-pair mating success, with males having a greater slope than females for both measures (Table 4).

In both males and females, both extra-pair mating success and the number of breeding years significantly predicted the number of EPO produced. When the sexes were considered together, there was a significant interaction effect between sex and extra-pair mating success such that increased extra-pair mating success had a larger effect on EPO production in males than in females (Table 4; Figure 2A). However, there was no interaction of sex and the number of breeding years, indicating that the number of breeding years affects EPO production similarly in males and females (Table 4).

Although the number of within-pair mates significantly predicted the number of WPO produced by both males and females, there was no interaction of sex and mating success, indicating that increased within-pair mating success had a similar effect on WPO production in both males and females (Table 4; Figure 2B). As in analyses of total reproductive success, the number of breeding years predicted WPO production in both sexes, but had a larger effect in females (Table 4).

DISCUSSION

Although Bateman’s assertion that multiple mating should not lead to increased offspring production by females has until relatively recently been widely accepted in behavioral ecology, empirical tests to support this prediction have been surprisingly rare (Tang-Martinez 2010). In this study, we have shown that both male and female dark-eyed juncos vary in the number of mates with which they produced offspring and that this variation strongly predicts reproductive success in both sexes. This confirms the findings of a previous study in this species (Ketterson et al. 1998) and extends its perspective from annual to lifetime reproductive success; even when the number of active breeding years was taken into account, both sexes had significant positive Bateman gradients. These results suggest that multiple mating appears to have direct fitness benefits in both sexes. For males, this matches findings from many other species, and the causal mechanisms by which this pattern likely arises are well established (Trivers 1972; Westneat et al. 1990; Webster et al. 1995). As we discuss below, however, the relationship between mating

Table 3

Bateman gradients in dark-eyed juncos: reproductive success and overall mating success

<table>
<thead>
<tr>
<th>Factor</th>
<th>Both Sexes, n = 871</th>
<th>Females, n = 436</th>
<th>Males, n = 435</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>Wald χ²</td>
<td>P</td>
</tr>
<tr>
<td>Total offspring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.332</td>
<td>23.452</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean annual mates</td>
<td>0.863</td>
<td>409.990</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of sampled years</td>
<td>0.498</td>
<td>733.427</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% T years</td>
<td>-0.095</td>
<td>3.088</td>
<td>0.079</td>
</tr>
<tr>
<td>Sex</td>
<td>0.713</td>
<td>56.335</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex × Mean annual mates</td>
<td>-0.616</td>
<td>119.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex × Sampled years</td>
<td>0.097</td>
<td>12.670</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex × % T years</td>
<td>Removed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fledged offspring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.801</td>
<td>84.313</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean annual mates</td>
<td>0.856</td>
<td>296.312</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of sampled years</td>
<td>0.516</td>
<td>498.433</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% T years</td>
<td>-0.148</td>
<td>3.024</td>
<td>0.082</td>
</tr>
<tr>
<td>Sex</td>
<td>0.667</td>
<td>30.204</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex × Mean annual mates</td>
<td>-0.623</td>
<td>73.444</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex × Sampled years</td>
<td>0.147</td>
<td>18.136</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex × % T years</td>
<td>-0.554</td>
<td>9.844</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Bold values are significant at $P < 0.05$. $β$s given are for a GLM with a Poisson error distribution. For analyses with sexes combined, $β$s are for male $= 0$ and female $= 1$. 

Behavioral Ecology
success and offspring production need not be causal in either sex, and particularly for females, a positive Bateman gradient may arise through other means than sexual selection.

What do Bateman gradients say about selection in juncos?

The similar variances in mate numbers and the strong positive slopes of the Bateman gradients for male and female juncos suggest that both sexes have the potential to experience similarly strong sexual selection. These variances include only individuals that bred successfully; including nonbreeding individuals would likely increase the variance in both mating success and reproductive success. However, although a positive Bateman gradient allows us to estimate an upper limit of the strength of sexual selection experienced by a population (Arnold and Duvall 1983), which is often much weaker in females than in males (for juncos, see McGlothlin et al. 2004; Whittingham and Dunn 2005).

The greater effect of within-pair vs. extra-pair mating on reproductive success in juncos indicates that producing broods with multiple sequential social mates, rather than extra-pair mating, is most related to increased offspring production. This suggests that acquiring and breeding with multiple social mates during a breeding season, either by facultative mate switching or via the ability to acquire a replacement mate following mate desertion or death, may be an important component of reproductive success in both sexes.

Probability of detection

A Bateman gradient with a positive slope may indicate a statistical artifact rather than a biological signature of sexual selection. Rare events are more likely to be detected in a larger sample; thus, multiple matings are more likely to be detected for individuals with greater numbers of offspring (Burley and Parker 1998). Most Bateman gradients based on data from natural populations (including ours) do not use mating success per se, but rather the number of successfully fertilized females (for males) or the number of males that successfully fertilized an egg (for females) as detected from the surviving offspring (Dewsbury 2005). Thus, the data points used to estimate these gradients necessarily have a lower limit of $y = 0$; e.g., it is impossible to detect 6 mates for an individual that produced only 4 offspring. This lower bound tends to bias gradients

\begin{table}
\centering
\caption{Bateman gradients in dark-eyed juncos: reproductive success and within-pair (WP) and extra-pair (EP) mating success}\\
\begin{tabular}{llllllllllll}
\hline
Factor & \multicolumn{3}{c}{Both sexes, $n = 871$} & \multicolumn{3}{c}{Females, $n = 436$} & \multicolumn{3}{c}{Males, $n = 435$} \\
 & $\beta$ & Wald $X^2$ & $P$ & $\beta$ & Wald $X^2$ & $P$ & $\beta$ & Wald $X^2$ & $P$ \\
\hline
Total offspring Intercept & \textbf{-0.543} & 46.141 & $<$0.001 & & & & & & & \\
Mean annual EP mates & \textbf{0.708} & 205.439 & $<$0.001 & & & & & & & \\
Mean annual WP mates & 1.198 & 294.517 & $<$0.001 & & & & & & & \\
No. of sampled years & \textbf{0.495} & 74.899 & $<$0.001 & & & & & & & \\
% T years & \textbf{-0.120} & 4.892 & 0.027 & & & & & & & \\
Sex & \textbf{0.877} & 60.619 & $<$0.001 & & & & & & & \\
Sex $\times$ EP mates & -0.504 & 68.625 & $<$0.001 & & & & & & & \\
Sex $\times$ WP mates & -0.853 & 72.928 & $<$0.001 & & & & & & & \\
Sex $\times$ sampled years & 0.094 & 11.936 & 0.001 & & & & & & & \\
\hline
EP offspring Intercept & \textbf{-2.348} & 425.577 & $<$0.001 & \textbf{-1.576} & 237.412 & $<$0.001 & \textbf{-2.391} & 300.762 & $<$0.001 & \\
Mean annual EP mates & 2.069 & 576.982 & $<$0.001 & 1.144 & 631.612 & $<$0.001 & 2.066 & 549.950 & $<$0.001 & \\
No. of sampled years & 0.637 & 563.900 & $<$0.001 & 0.630 & 261.192 & $<$0.001 & \textbf{0.686} & 308.816 & $<$0.001 & \\
% T years & 0.021 & 0.034 & 0.855 & 0.155 & 0.961 & 0.327 & -0.117 & 0.484 & 0.487 & \\
Sex & \textbf{0.731} & 43.172 & $<$0.001 & & & & & & & \\
Sex $\times$ EP mates & -0.921 & 91.151 & $<$0.001 & & & & & & & \\
Sex $\times$ sampled yrs & & Removed & & & & & & & & \\
\hline
WP offspring Intercept & \textbf{-0.998} & 159.844 & $<$0.001 & \textbf{-1.141} & 102.340 & $<$0.001 & \textbf{-1.026} & 116.183 & $<$0.001 & \\
Mean annual WP mates & 1.645 & 672.355 & $<$0.001 & 1.608 & 262.421 & $<$0.001 & 1.675 & 399.402 & $<$0.001 & \\
No. of sampled years & 0.514 & 642.730 & $<$0.001 & 0.617 & 657.365 & $<$0.001 & \textbf{0.514} & 641.354 & $<$0.001 & \\
% T years & \textbf{-0.156} & 6.422 & 0.011 & -0.128 & 1.048 & 0.306 & \textbf{-0.172} & 5.711 & 0.017 & \\
Sex & \textbf{-0.180} & 6.804 & 0.009 & & & & & & & \\
Sex $\times$ WP mates & & Removed & & & & & & & & \\
Sex $\times$ sampled yrs & 0.104 & 11.151 & 0.001 & & & & & & & \\
\hline
\end{tabular}
\end{table}
Bateman gradients is the conflation of correlation and causation. In studies—including this one—that use Bateman gradients to discuss the direction and force of sexual selection, the slope of the gradient is frequently described as “number of offspring gained for each additional mate” (e.g., Krakauer 2008), a phrasing that is certainly suggested by the standard orientation of the axes of mating success and reproductive success. However, the relationship created by reversing the axes has an equally valid causal interpretation: a positive slope would suggest that individuals have more mates because they have more offspring. The slope of this “inverse Bateman gradient” could be interpreted as “number of additional mates detected for each offspring sampled.” Although no such relationship between large broods and increased incidence of multiple mating was found across the mean values for viviparous species in a recent meta-analysis (Avise and Liu 2011), such a pattern may still hold within a single species. In fact, a positive relationship between offspring number and mate number can be generated by repeated subsampling of a single large brood (Panova et al. 2010), demonstrating that a positive Bateman gradient may sometimes have statistical but not biological significance. The fact that the relationship between mate number and offspring number may be probabilistic rather than predictive is particularly problematic for females. If selection is acting primarily on traits that affect fecundity rather than those that affect mating behavior, then the acquisition of multiple mates may be a passive effect of increasing offspring number, rather than its cause.

Therefore, although a positive covariance between offspring number and mate number indicates the potential for sexual selection on females (i.e., reproductive success as a function of mate number), selection may often be acting more strongly on traits that increase fecundity rather than mate number. Males have been shown to prefer females with traits that signal high fecundity in a variety of taxa, including insects (Bonduriansky 2001), fish (Herdman et al. 2004), and birds (Griggio et al. 2005). If this increased male preference correlates with increased likelihood of copulation/fertilization, then more fecund females will have greater numbers of realized mates, and thus females will tend to display a positive Bateman gradient. It is unclear whether male juncos display such a preference; if males were directing the majority of their courtship effort toward highly fecund females, then the females with the highest reproductive success should also have the highest percent of EPO, a pattern found in a previous study of the junco (Ketterson et al. 1998) but not in our larger data set (r = 0.035, n = 429, P = 0.476). However, as we have argued above, nonrandom mating by males is not necessary; higher fecundity may lead to a larger number of realized mates by chance as well.

One example of this interdependence of fecundity, female mating success, and positive Bateman gradients comes from northern water snakes (Nerodia sipedon). Prosser and colleagues (2002) found a strong positive Bateman gradient in female water snakes. However, when they accounted for female body size, the positive relationship between mating success and reproductive success disappeared. A positive correlation between female body size and offspring number is common in ectotherms (Vitt and Congdon 1978; Sargent et al. 1986; Shine 1992; Kraak and Bakker 1998). In the water snake, larger females also have more males that sire their clutches (Prosser et al. 2002), although whether this is due to active male choice on female size or an increased likelihood of detection of multiple sires with increased sample size is unclear. In cases such as this, although the Bateman gradients may not indicate a strong potential for sexual selection, the increased mate numbers are most likely a result of increased female body size rather than its cause, and thus the actual...
selection experienced by females is more properly classified as fecundity selection (Clutton-Brock 2009).

**Life history**

The extent to which a positive Bateman gradient reveals something about sexual selection may also depend in large part on the life history of the organism in question. In organisms in which females are semelparous and each large clutch of eggs is fertilized by an even mixture of sperm from all the males with which a female has mated, we would indeed predict that females should have shallow Bateman gradients. However, in iteroparous species, each clutch of eggs represents more offspring and a new chance to acquire more mates, which may lead to a positive association between mating success and reproductive success by random chance. In the extreme case, females sample from a large pool of males each time an egg needs to be fertilized. The more times a female samples, the more unique males she is likely to encounter, which is likely to lead to an even stronger positive Bateman gradient by random sampling. Thus, in iteroparous species, positive Bateman gradients in females may arise from a real biological phenomenon distinct from sexual selection: the need to mate anew each time an egg or a clutch is laid.

Extreme iteroparity may also tend to reduce Bateman gradients in males. If a female must acquire fresh sperm for each egg she produces, a male can no longer monopolize all of a female’s offspring by mating with her once. As a consequence, males will likely face a tradeoff between mating with the same female repeatedly and searching for new females. In such a tradeoff, there may be multiple equivalent strategies. For example, if a single mating event tends to lead a single fertilized egg, it does not matter from a male’s point of view whether he mates with the same female four times in a row or with four different females over the same time period. Although these two strategies have equivalent reproductive success, they have very different mating success, which creates variation in the relationship between these two variables, thus reducing the Bateman gradient. Although this example is highly simplified, the same general principle—that variation in male mating strategy may reduce the male Bateman gradient—should apply to real-world systems as well. When we consider life history, it should not be surprising for Bateman gradients to be similar in males and females, even in species with typical sex roles.

Although it is beyond the scope of the current paper to analyze in detail the life histories of the species in which females have positive Bateman gradients (Table 1), a few broad trends can be observed. In general, these species can be classified by their degree of iteroparity and their offspring output per reproductive event. In species in which females produce one (or a few) very large broods of offspring, such as many of the invertebrates and fish, a positive Bateman gradient may result from the increased detection probability of rare events. Species with several large broods may primarily be experiencing male preference for highly fecund females. On the other hand, highly iteroparous species, such as the junco, may show positive female Bateman gradients primarily due to the increased opportunity for re-sampling available males at each reproductive event. Although we do not have any direct evidence regarding sperm storage and usage in the junco, remating for each clutch of eggs is probably a necessity, and it is likely that females also often recoupulate to fertilize each egg within a clutch (Nolan et al. 2002).

The effects of detection probability, fecundity selection, and iteroparous resampling, which are likely to interact and correlate with one another, may all contribute to positive Bateman gradients across species. The relative importance of these factors within a species is probably dependent on that species’ life-history characteristics. Teasing apart these effects should be a major goal of future research in the evolution of mating systems.

**Why do females mate multiply?**

Although we now have numerous examples of taxa in which females that mate multiply have increased reproductive success (see Table 1), there is still a lack of widespread acceptance of the idea that such promiscuity may be the result of direct selection on females (but see Rosvall 2011). This is perhaps due to the lack of any obvious and universal cause for why mating with multiple males would increase female reproductive success. Offspring production by females is believed to be limited primarily by available resources or time rather than by number of mates, particularly in species in which females invest more in individual offspring than do males (Trivers 1972). If females are limited by their access to nutrients, parental care, or viable sperm, then an extra-pair mating that provides these resources will indeed increase a female’s reproductive success (Stacey 1982; Davies 1985; Ridley 1988; Fox 1993; Hunter et al. 1993; Sheldon 1994; Levitan and Petersen 1995; Reynolds 1996; Arnquist and Nilsson 2000). However, in many species, females are not sperm limited, and extra-pair males do not provide any resources other than genetic material. In these cases, the mechanism that underlies any positive relationship between female mating success and offspring number is rarely clear.

In our population of dark-eyed juncos, there is certainly a strong relationship between female mate number and reproductive success; females that mate multiply have more offspring. However, referring to this phenomenon as a “direct benefit” of multiple mating may infer a degree of causality that is not supported empirically. The evidence that multiple mating directly causes female juncos to produce more offspring is scant at best. Rather, female juncos may vary intrinsically in their ability to produce and rear offspring in ways that are independent of the number of males with which they mate. The sources of such variation are as yet unknown but may include variation in heterozygosity, health, territory quality, nest site selection, or ability to defend against predators (Nagy and Holmes 2004; Clotfelter et al. 2007; Ortego et al. 2007; Goodenough et al. 2008). If these highly fecund females acquire more mates than less fecund females, either by active male mate choice for some trait that signals fecundity or as an effect of increased detection of rare events, then a positive Bateman gradient would emerge.

To conclude, Bateman gradients serve to describe the relationship between numbers of mates and reproductive success. When the relationship is positive in females, the gradients reveal situations in which females may benefit from multiple matings, but it is critical to recall that multiple mating may not be the cause of increased fitness, but rather a consequence of increased fecundity. Thus, mating behavior per se may not be the trait under selection, but rather an epiphenomenon of selection on other traits that lead to increased reproductive success in females. Although there are a number of clear cases of sexual selection acting directly on female traits (Rosvall 2011), positive Bateman gradients may arise from many other mechanisms. Future studies should exercise caution in interpreting such gradients as evidence for sexual selection in females and instead focus on the causal mechanisms that lead to this statistical pattern.

**SUPPLEMENTARY MATERIAL**

Supplementary Material can be found at http://www.beheco. oxfordjournals.org/.
FUNDING
This work was supported by the National Science Foundation (grant numbers BSR 91-114980, IBN 94-08061, IBN 97-28384, IBN 02-16091, BSC-05-19211, and IOS-0820055 to E.D.K., DDIG 0808051 to N.M.G., and DDIG 0508692 to J.W.M.).

Thanks to members of the E. Brodie III and Ketterson research groups and M. Webster for comments on the manuscript; M. Wade for helpful discussion; Mountain Lake Biological Station and the Mountain Lake Hotel for facilitating fieldwork on their properties; the Center for the Integrative Study of Animal Behavior for assistance in the laboratory; B. Harr for providing primers; E. Snajdr for help with data compilation; and the many people who contributed to collecting the long-term field data.

REFERENCES


Bezczereidt A, Yogevar VK, Esner T. 2008. Female promiscuity does not lead to increased fertility or fecundity in an arctic moth (Uleidea arnottii). J Insect Behav. 21:213–221.


Enquist L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. Anim Behav. 70:967–971.


Downloaded from http://beheco.oxfordjournals.org/ at Virginia Tech on September 11, 2012


