

Quantifying the potential for sexual dimorphism using upper limits on Bateman gradients

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(Accepted: 27 August 2007)

Summary

Sex differences in the correlation between number of offspring and number of mates likely drive much of the evolution of morphological and behavioral sexual differences. These correlations have traditionally been represented by slopes from regressions of number of offspring on number of mates (Bateman gradients). Typically the Bateman gradient is assumed to be large for males and zero for females. However, five of nine examples where male and female gradients have been measured show appreciable gradients for females. Difference in these 'actual' gradients reflect sex differences in the force driving sexual selection. In the lab it is simple to estimate the 'upper limits' on these Bateman gradients. Differences between male and female upper limits can be used to quantify the potential for sexual dimorphism. We demonstrate how to estimate these upper limits in a katydid (*Conocephalus nigropleurum*) where males provide females with a large food gift (nuptial gift) during mating. By mating males and females to either one or two virgin mates, we estimated the way maximum fecundity increased with additional mates for each sex, giving an estimate of the upper limit of sexual selection on each sex. We compared these estimates to predictions based on the relative value of the nuptial gift and female pre-mating fecundity. Contrary to expectation, the male upper limit did not exceed the female upper limit. Both the fact that a male's second nuptial gift was smaller than his first and that many matings failed to transfer appreciable numbers of sperm seem to have contributed to the unexpected result.

Keywords: sexual selection, Bateman's principle, dimorphism, mating frequency, opportunity.

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Introduction

Males, more frequently than females, have unique structures, are more colorful, larger, louder, more mobile and more competitive for mates than females, indicating that sexual selection has typically been stronger on males (Darwin, 1871). Three aspects of current 'sexual difference theory' suggest that the potential for sexual dimorphism (in both morphology and behavior) depends on the difference in the strength of sexual selection acting on each sex, not just on the absolute strength of sexual selection on males. First, the relatively lower cost of producing sperm as compared to ova means males typically experience a strong positive correlation between the number of offspring they sire (their fecundity) and the number of different mates they obtain (their mating success; Bateman, 1948; Arnold & Duvall, 1994), while for most species it is generally assumed that there is no such correlation for females (based on only part of the data from the classic paper by Bateman, 1948; e.g., Trivers, 1972; Andersson, 1994, p. 147). Second, the asymmetry in this correlation for males compared to females is thought to be the cause of sexual selection (Bateman, 1948; Arnold & Duvall, 1994). This so-called 'causal sexual selection' is believed to adapt males to maximize mating success through mate competition, because they can gain more fecundity than females by remating. And third, males that mate most often are thought to be relatively more elaborate and competitive because this has allowed their male ancestors to attract or control access to more mates (Emlen & Oring, 1977). So sexual asymmetry in the correlation between fecundity and mating success drives much of sexual selection and the evolution of sex differences. (A fecundity by mate quality correlation could also drive sexual selection, but we follow the traditional emphasis on mating success and assume that this is the primary driver of sexual selection.) However, available evidence (reviewed below) does not support the assumption that there is no correlation for females in most species. This correlation can in fact be strong in females, and as a result, the asymmetry in this correlation between males and females can be smaller than expected. When this asymmetry is small the potential for sexual dimorphism can be less than what we would expect based on the estimates of the absolute strength of sexual selection on males alone.

When females can gain appreciable fitness by mating with multiple males, sexual selection can act on females. If the traits under sexual selection are the same in both sexes (e.g., body size, conspicuous coloration) then smaller

asymmetry in the fecundity by mating success correlation is predicted to result in less sexual dimorphism than expected based on the absolute strength of sexual selection acting on males. On the other hand, if the target of sexual selection is different in each sex, a small asymmetry may result in more overall sexual dimorphism. This makes it important to know not only the strength of sexual selection acting on males, but also the strength of sexual selection acting on females and the targets of selection in each sex. It also means that the asymmetry in the correlation between fecundity and mating success will be a better predictor of sexual dimorphism than the strength of sexual selection acting on males, whenever the targets of sexual selection are the same in both sexes.

To understand the importance of sexual asymmetry in the correlation between fecundity and mating success for explaining sexual dimorphism, what is needed are direct measurements of this asymmetry. However, it has been quantified for only nine species. We begin by reviewing this existing evidence. In six of the nine cases the evidence for sexual asymmetry in the correlation is not as strong as one might predict, assuming no correlation between fecundity and mating success in females. We then go on to suggest a way to quantify the potential for sexual dimorphism based on the sex difference in the upper limit of the correlation between fecundity and mating success (Lorch, 2005). These upper limits can be estimated by mating either males or females to either one or two virgin mates and measuring fecundity differences between single and double matings. This measure is not dependent on life history or environmental conditions. It can also be estimated with controlled matings in a lab setting, giving it advantages over methods requiring genetic parentage data. It may prove more useful in understanding the evolution of sexual dimorphism than measures based on the strength of sexual selection on males alone, specifically those measures based on variance in either mating success or reproductive success. As we explain below, these older measures are problematic for several reasons. Finally, using the proposed measures, we estimate the potential for sexual dimorphism in a katydid with nuptial gifts, where we might expect both males and females to gain fecundity from remating

Existing evidence

In the nine studies mentioned below and in our study, for a number of theoretical and practical reasons (Arnold & Duvall, 1994), the slope of the regression of fecundity on the number of mates (the ‘Bateman gradient’) was

used rather than a correlation coefficient. Fecundity is used in lieu of a measure of overall fitness, however, the approach can be generalized to include other fitness components and other fitness correlates like mate quality.

In his classic experiment on *Drosophila melanogaster*, designed to understand why sexual selection is generally stronger on males than females, Bateman (1948) split the results from six replicates into two groups. In one group (two replicates), the male gradient was 12.3 times greater than the female gradient (29.5/2.4, male/female gradients). However, in the other group the male gradient was only 1.6 times greater than the female gradient (23.3/14.7, in four replicates; based on a re-analysis of Bateman's 1948 data by Arnold & Duvall, 1994). The latter replicates do not show as strong evidence for sexual asymmetry in Bateman gradients as the former replicates do. Females in these latter replicates had a fairly high gradient and gained an average of 14.7 offspring with each additional mate. Apparently because they contradict expectation, this portion of Bateman's results (the majority of his data) are seldom cited (but see Cunningham & Birkhead, 1998; Arnold & Duvall, 1994). The bulk of Bateman's results are in direct conflict with recent evidence that multiple mating is costly for female fruitflies but not males (promiscuous females suffer reduced lifespan; Chapman et al., 1995). Under these circumstances, the male gradient should far exceed the female gradient. Bjork & Pitnick (2006) have repeated Bateman's 1948 experiment and added three new *Drosophila* species. For *D. melanogaster*, they found the male Bateman gradient was 10 times greater than the female gradient (28.1/2.8), which is comparable to the first part of Bateman's data. They also added three more species with increasing amounts of investment by males in gametes and gonads (*D. virilis*, *D. lummei*, and *D. bifurca*; Bjork & Pitnick, 2006). The male Bateman gradient was 4.7 (23.4/0.5), 1.5 (36.7/24.3) and 1.8 (19.5/10.7) times greater for these three species respectively (only the first of these is significant; from Bjork & Pitnick, 2006, Supplementary Table 1). *D. melanogaster* and *D. virilis* females have little or no Bateman gradient, but the female gradient for *D. lummei*, and *D. bifurca* is relatively large.

In studies on the dark-eyed junco (*Junco hyemalis*; Ketterson et al., 1998), the brown headed cowbird (*Molothrus ater*; Woolfenden et al., 2002) and the yellow-pine chipmunk (*Tamias amoenus*; Schulte-Hostedde et al., 2004), no significant difference was found between the positive Bateman gradients for males and females (juncos: $0.9 = 2.63/2.92$, cowbirds: $0.99 = 1.89/1.91$,

and chipmunk: approx. 1.3 = approx. 1.7/approx. 0.8; male/female gradient; chipmunk gradients are approximate due to estimation from Figure 2 in Schulte-Hostedde et al., 2004). Behavioral observations had indicated that both the birds were socially monogamous (pair bonds form and both sexes care for young) and that yellow-pine chipmunks were polygynous. It was, therefore, surprising to find that females gained as many additional offspring with additional mates as males, and so might be experiencing as much sexual selection as males. Males might have been expected to have a positive Bateman gradient, even in a socially monogamous mating system (with relatively equal male and female parental care), due to the relatively lower cost of producing gametes (Bateman, 1948; Trivers, 1972) and, thus, the potential for males to engage in extra-pair copulations and increase their fecundity. However, since females seem to have a similar Bateman gradient to males, there was no detectable sexual asymmetry in gradients in these species. So, in five of the seven species considered so far, there is no evidence for sexual asymmetry in Bateman gradients.

In a study on a pipefish (*Syngnathus typhle*) with exclusive male parental care, we might expect sexual selection to be stronger on females than males (Jones et al., 2000). This study provides evidence that in some cases the direction of sexual asymmetry in Bateman gradients can be reversed, so that females can gain more fitness by remating than males can (in the form of additional goods and services from males, e.g., male care). Females experienced roughly twice as much selection to remate as males ($0.50 = 12.1/24.1$, male/female gradient). In this study the evidence for sexual asymmetry in Bateman gradients is strong (male and female gradients were significantly different from zero and female gradient significantly exceeded male gradient). However, both sexes gain fecundity from remating (Cunningham & Birkhead, 1998).

Finally, a study on a newt (*Taricha granulosa*), finds support for the entrenched idea that males gain more from remating than females (Jones et al., 2002). It was the first study to show this with respect to Bateman's original correlation (rather than demonstrating that variance in male reproductive success exceeds that of females). In this case males experienced just over three times more selection to remate than females experienced ($3.36 = 74.6/22.2$, male/female gradient). Therefore, in this newt study, there is also strong evidence for sexual asymmetry in Bateman gradients,

with males selected for higher remating frequency (male and female gradients were different from each other, and the male gradient but not the female gradient was different from zero).

Five of these nine examples taken together show that, in very disparate taxa, there is potential for sexual selection on females. This conclusion agrees with the results of a meta-analysis of 122 studies in insects showing that females, in both gift giving and non-gift giving species, gain fecundity by remating (Arnqvist & Nilsson, 2000). These five examples have important implications for sexual selection theory. As Arnold & Duvall (1994) have pointed out, such studies are changing how we understand the evolution of mating systems, and they may also affect our understanding of how sexual dimorphism evolves. The five studies suggest that sexual selection on females may be stronger than predicted by existing sexual difference theory. These studies also make it clear that, rather than focusing on the strength of sexual selection acting on males alone to understand how sexual dimorphism evolves, we must also consider the difference in the strength of sexual selection acting on males and females.

Potential for sexual dimorphism

Actual sex differences in the Bateman gradient described above depend on environmental conditions and life history details (Gowaty, 2004; Lorch, 2002), making it difficult to gauge the overall importance of the sex difference (e.g., between species or populations in different environments). In the past this difficulty has been dealt with by using sex differences in the standardized variance in reproductive success to estimate the opportunity or potential for sexual selection (Wade, 1979; Shuster & Wade, 2003). These variance-based methods assume the existence of a correlation between fecundity and mating success. Without such an assumption, the variance in reproductive success tells us nothing about the strength of sexual selection. This assumption is not necessary when Bateman gradients are used because the gradient is an actual estimate of the correlation (Lorch, 2005). Violations of these assumptions and assumptions about differences between males and females in the correlation between fecundity and mating success can cause overestimation of the opportunity for sexual selection and the potential for sexual dimorphism (Lorch, 2005).

A better estimate of the potential for sexual dimorphism than the variance based methods may be the difference between the upper limits of male and

female Bateman gradients (Lorch, 2005). The upper limit on the Bateman gradient is the upper limit on sexual selection for a given sex, defined as the maximum rate of gain in fecundity with increasing numbers of ‘ideal’ mates (Lorch, 2005). By ideal mates we mean for males, virgin fully-fecund females that never mate again, and for females, virgin males with a full complement of sperm and paternal investment (nuptial gift or paternal care). To measure the upper limit on sexual selection in each sex, we will use regression estimates of the relationship between maximum fecundity (with ideal mates) and numbers of mates (for detailed justification of this method see Lorch, 2005). The potential for sexual dimorphism is then measured as the ratio of these upper limits for each sex.

Estimates of the potential for sexual selection based on upper limits of Bateman gradients should not be confused with estimates of actual levels of sexual selection based on Bateman gradients. There might be a large difference between the upper limits and the actual Bateman gradients in some species due to sperm competition or female behaviors that reduce how much males gain from additional matings. For example sperm competition can reduce how much fecundity males gain from remating, preventing them from reaching their maximum rate of gain. Since sperm competition is not likely to reduce female fecundity gains, actual estimates of sexual selection intensity may be closer to potential levels for females than for males. The upper limits on Bateman gradients can only be measured in situations where maximum fecundity can be estimated using controlled matings between virgins, whereas the actual Bateman gradients will most commonly be measured using genetic markers in natural populations (Ketterson et al., 1998; Woolfenden et al., 2002) or populations where mating is not controlled (Bateman, 1948; Jones et al., 2000). One important strength of using upper limits to quantify the potential for sexual selection is that genetic parentage data are not needed. We will focus on estimating the potential for sexual selection and, therefore, dimorphism, in a species where males provide females with nuptial gifts that can be converted into additional eggs so that both females and males gain by multiple mating. One important target of sexual selection is also likely to be the same in both males and females, large body size (see below).

The purposes of the remainder of the paper are to demonstrate that there is the potential for significant sexual selection on females and to estimate the upper limit of sexual selection acting on males and females, thereby

quantifying the potential for sexual dimorphism. We will also discuss the importance of this quantity, difficulties in estimating it, and how viewing the potential for sexual selection in the way we propose affects existing sexual selection theory. Finally, our results lead us to propose a new hypothesis for how ‘failed matings’ influence the upper limits on the sexual selection.

Background and study system

Nuptial gifts are a widespread type of ‘goods’ that males of many insects provide to their mates (many of which appear to be subtle sources of nutrition passed in the ejaculate). These mating meals often increase the number of offspring females produce (Boggs, 1995; Vahed, 1998; Arnqvist & Nilsson, 2000). Therefore, we would expect females to have a significant positive correlation between fecundity and numbers of mates, reducing the extent of sexual asymmetry in Bateman gradients and their upper limits. Such correlations have been reported for many insects (Ridley, 1988), including gift-giving katydids (Orthoptera: Tettigoniidae) in which males feed their mates with a large nutritious spermatophylax attached to the spermatophore (Simmons, 1990; Gwynne, 2001). In some species the spermatophylax nutrients are so important to females that females compete with one another for access to matings (leading to a reversal in the typical mating roles so that females compete for mates and males are choosy; Gwynne, 1981, 1985; Simmons & Bailey, 1990). Unfortunately, no estimates of the correlation between fecundity and numbers of mates have been obtained for male katydids, making it difficult to assess the relationship between actual male and female Bateman gradients in this group.

In this paper we focus on a nuptial gift giving katydid, *Conocephalus nigropleurum*. A single nuptial gift in this species represents roughly 10% of the males’ body weight (Gwynne, 1982). Females prefer male vibratory signals that are correlated with larger male body size (De Luca & Morris, 1998). This would lead females to mate with males that donate larger spermatophores (Gwynne, 1982), indicating that females place a premium on nuptial gifts. Nevertheless, unlike some other katydids, *C. nigropleurum* and other members of the Conocephalinae exhibit typical sex roles where males compete for mates and females choose between potential mates based on male display (Gwynne, 1982, 2001).

Methods

Rearing

In late April and early May 1996 we collected *Conocephalus nigropleurum* eggs laid in the 'pine-cone' galls formed on willow buds by midges (*Rhabdophaga strobiloides*; Diptera: Cecidomyiidae). These galls were found along the Credit River in Erindale Park, Mississauga, Ontario, Canada.

We stored eggs on wet filter paper in petri dishes that were placed in an environmental chamber at 25°C, 80% humidity, and a 12:12 light:dark photoperiod. Upon hatching, larvae were treated in two ways. Larvae hatched from eggs collected in April were transferred to fiberglass screen cages measuring 30 × 30 × 30 cm, with initial densities of first instar nymphs up to 60 per cage, and fed a diet of apple and a specially made cake (a high-protein mixture of rolled oats, millet seed, bee pollen and commercial fish flakes) three times weekly. Larvae hatched from eggs collected in May were reared in plastic cages measuring 13 × 11 × 18 cm (BioQuip®), up to densities of 12 individuals per cage. The katydids hatched from eggs collected in April experienced slightly lower densities than those collected in May (due to an attempt to rear a large number of long-winged individuals from the eggs collected in May for another study). Preliminary analyses taking these rearing treatments into consideration revealed that they did not significantly contribute to any of the analyses, so animals from both treatments were pooled in all analyses presented here. Only short-winged males and females were used in this study since rare long-winged individuals can have reduced fecundity. Three times weekly, we fed katydids a diet of apple, beef-based canned cat food, and a mix of seeds, oatmeal, pollen and tropical fish food. To obtain virgin adults for matings, we isolated penultimate instar individuals from both treatments into separate cylindrical Plexiglas jars (7 cm diameter and 7.5 cm in height) with fiberglass screen tops.

Data collection

We kept newly molted adults of both treatments isolated for a minimum of six days before pairing them with a member of the opposite sex. We paired individuals of similar age haphazardly, noting the duration of copulation, and the time taken by the female to remove and consume both the spermatophyllax (the 'meal') and the attached sperm ampulla (eaten by the female when

empty of sperm). Only matings that resulted in successful spermatophore transfer were analyzed. We also recorded male weight loss during mating (assumed to be equivalent to combined weight of the nuptial gift (spermatophylax) plus sperm ampulla).

After mating, females were isolated and given a small (5-10 cm) section of grass stem (reed canary grass, *Phalaris arundinacea*) in which to oviposit. They were fed as described earlier. Leaf sheaths of this grass were examined weekly for eggs, at which time we provided fresh stems to females.

The opportunity for sexual selection can be estimated by measuring how maximum fecundity increases with each additional 'ideal' mate (Lorch, 2005). In other words, if each additional mate were virgin and never mated again, how much fecundity could be gained (Lorch, 2005)? This measure sets and upper limit on Bateman gradients and tells us how strong sexual selection can be. We estimated this upper limit by comparing the fecundity resulting from single and double matings with virgin mates. Only two matings were necessary because we are estimating how maximum fecundity changes with additional ideal mates. We assume that a third ideal mate would bring the same increase in fecundity as the second, making it unnecessary to conduct three or more matings (Lorch, 2005). So, for example, the female upper limit was estimated by allowing 15 virgin females to mate with virgin males individually. Eight of these females were arbitrarily chosen to mate with a second virgin male on a subsequent day. If this second pairing was unsuccessful (after one hour) one day after the first, another virgin partner was presented on the following day and subsequent days until the focal individual had mated twice. We recorded the interval between matings in days. This mating protocol potentially confounds fecundity increases due to remating with simple differences in the size of single and double-mated females (or other aspects of condition related to fecundity). We attempted to remove any potential confounding effects by correcting for female size (see Analysis section below), however, there may be other variables correlated with fecundity that we could not control. To estimate male upper limits, 15 males were mated with individual virgin females. Eleven of these males were mated with a second virgin female on a subsequent day (except for two males that were mated twice in one day; four males failed to remate).

Male and female maximum fecundities were estimated differently. Fecundity for a female who had mated either once or twice was simply the total number of eggs laid in her lifetime. Male fecundity was estimated only for

twice mated males, and for each male a separate estimate of fecundity from each of his two mates was obtained. Because of a shortage of insects, time and space, egg counts from some females were used both to estimate the fecundity of once mated females and the fecundity of once mated males. Fecundities measured in the way we have just described give an estimate of an animal's maximum fecundity with one or two virgin mates, rather than the lower fecundity it would likely have with one or two matings under natural conditions. This is because in nature, females may encounter mates that have fewer sperm or smaller gifts due to recent mating. Males in nature might encounter females that had already mated, resulting in competition for fertilizations among the stored ejaculates of a female's mates.

We froze all males after their final mating and all females after their death. We recorded female lifespan after mating. All morphometric measurements were made using a microscope fitted with a digital video camera connected to a Power Macintosh. NIH image (version 1.61), a digital imaging program, was used to compute the following five morphometric measurements: pronotum length, average of left and right front femur, average of left and right hind femur, average of left and right front wing, and average of left and right hind wing. After all measurements had been made, we dried the specimens (minus the gut) to a constant weight and recorded the dry mass of all individuals.

Analysis

In order to obtain a single index of body size for females, we conducted a principal components analysis (PCA; based on the correlation matrix) on the five morphological measures obtained plus the dry weight. The first principal component (PC1; unrotated) from this analysis was used to generate a PC score for each individual from their six size measurements.

Since we want to quantify the potential for sexual dimorphism using sex differences in the upper limit on sexual selection for males and females (Lorch, 2005), we estimated the slope of the regression of maximum fecundity on numbers of mates for each sex separately (while controlling for the effects of body size, where needed, so that body size effects are not confounded with the effects of multiple mating; Ketterson et al., 1998). This was done by mating a focal animal and either one or two virgins of the opposite sex (as described above). For females, we then computed a multiple

regression where the dependent variable was the mean of maximum fecundity across females, and the independent variables were numbers of mates and PC1 (for female size). This was done to reduce variance in estimates of upper limits due to female size variation. The upper limit of sexual selection for females was estimated as the partial regression coefficient corresponding to numbers of mates (with the sums of squared error term used as the variance around this estimate). PC1 was also included in order to try to control for the possibility that larger females were more willing to mate twice (potentially confounding fecundity increases due to number of mates with increase due to female size). If this confound is a problem, female willingness to remate should increase with size. To test this prediction, we reported the Spearman rank correlation between female PC1 and the time interval between first and second mates. We also tested whether females who mated twice were larger.

To see whether a second mating affected a female's lifespan (through the acquisition of additional male-derived nutrients; Arnqvist & Nilsson, 2000; Brown, 1997; Wagner Jr. et al., 2001) or egg laying rate (Gwynne, 1988; Simmons, 1993), we compared the number of days a female lived after mating and the number of eggs laid per day over this period for once and twice mated females (excluding females who did not lay any eggs). Errors are reported as \pm one standard error unless otherwise indicated.

For males, the fecundity of the first and second mate of a double-mated male can be estimated separately, so we estimated the upper limit of sexual selection for males more simply and with less error than was possible in the case of females. The upper limit of sexual selection or the maximum fecundity gained by remating for males is simply the average number of eggs laid by second mates. A *t*-test can then be used to ask whether the male upper limit estimate is greater than zero. In a preliminary analysis of the male upper limit we used an analysis equivalent to the one used to estimate the female upper limit (multiple regression of fecundity on number of mates and male PC1 for the same six size measures). Male PC1 contributed <0.0001 to the of the model (relative to the simple linear regression model of fecundity on numbers of mates) and reduced the value of the male upper limit estimate by only 0.028. For this reason we did not correct the male upper limit estimate for male size, and we could use the simpler method of estimating male upper limits. We then compared upper limits for males and females by using the upper limit estimates (average second mate fecundity for males, and partial

regression coefficient due to number of mates for females) and their variance estimates to compute a *t*-test (for unequal variances; Sokal & Rohlf, 1981).

Several factors (other than female body size) could have affected our estimates of the upper limit of sexual selection on the two sexes (and our estimates of the potential for sexual dimorphism). We focus on two factors in particular: (1) a possible reduction in the size of the nuptial gift in second matings relative to the first (as in Simmons, 1995; Reinhold & von Helverson, 1997) and (2) a failure to successfully transfer sperm even though spermatophore transfer appeared to be normal. With regard to the first factor, double mated males that give smaller nuptial gifts to their second mates are expected to have maximum fecundities that are less than twice the fecundity of single mated males. If males give less to second mates (see Gwynne, 2001) because they remate before they have fully replenished spermatophore glands from their first mating (see Gwynne, 1990), this behavior will reduce our estimate of the upper limit of sexual selection on males (without affecting estimates for females who get two virgin mates). We tested for differences in the weight of first and second spermatophores of double-mated males (using a Wilcoxon signed-rank test and reporting medians and inter-quartile ranges (IQR)). We then tested the hypothesis that males have not fully recovered between matings by testing for correlations between the inter-mating interval and the weight difference between first and second spermatophores (using Spearman rank correlations).

The second factor that may affect our estimates of the potential for sexual dimorphism is unsuccessful sperm transfer. We developed a simple model to explain how this factor can affect our estimates of the upper limits on sexual selection for males and females. We then used this model to describe how failures to transfer sperm may have affected our estimates of the potential for sexual dimorphism.

Results

Female body size

The first principal component (PC1) explains 47% of the variation in female size measurements. Table 1 shows the mean and standard errors for each of the six measurements along with how each character loaded in the principal components analysis. Variation in the length of the two wings was mostly

Table 1. Female body size measurement (with standard error, SE) and coefficients of first principal component. All lengths are in mm and weight is in grams. Coefficients are unrotated and for first eigenvector from the principal components analysis of the correlation matrix of female size measures.

Factor	n	Mean	SE	Coefficient
Pronotum length	23	2.94	0.038	0.542
Front femur length	23	3.41	0.047	0.532
Hind femur length	23	11.73	0.307	0.502
Front wing length	23	8.74	0.254	0.059
Hind wing length	23	6.05	0.185	-0.113
Dry weight	23	0.04	0.0025	0.394

independent of other size measures (see low wing coefficients in Table 1). The wing lengths loaded strongly onto PC2 (not shown). Female PC1 was significantly correlated with the total number of eggs laid (for once and twice mated females together, $r_s = 0.49$, $N = 23$, $p = 0.02$). The correlation between female size and the interval between matings was positive but not significant ($r_s = 0.2717$, $N = 18$, $p = 0.2754$). Females who mated twice were also slightly larger but not significantly so (based on dry weight; $t' = -1728$, $df = 11.2398$, $p = 0.1113$). These two results indicate that increases in fecundity due to remating are unlikely to be due to more fecund females being more likely to remate. Including female PC1 in the analysis of female upper limits should make this even less likely.

Upper limits on sexual selection

The upper limit of sexual selection acting on females (i.e., the partial regression coefficient for number of mates, controlling for the effects of female body size) is 31.0 ± 12.82 (see Figure 1). This upper limit is significantly different from zero ($MS_{\text{number of mates}} = 4098.89$, $F_{1,22} = 5.85$, $p = 0.03$) indicating that females have the potential to gain fecundity by mating multiple times (similar results have been found in a beetle Worden & Parker, 2001). Multiple mating significantly increased female post-mating lifespan (from 28.83 ± 4.79 days for one mating to 52.75 ± 7.18 days for two; $t = 2.89$, $N = 12$ and 8 , $p = 0.01$) without increasing egg laying rate (over a female's mated life; from 0.85 ± 0.15 eggs per day for one mating to 1.11 ± 0.19 for two; $t = 1.04$, $N = 12$ and 8 , $p = 0.31$). This indicates fecundity gains due to remating are primarily the result of increases in female post-mating

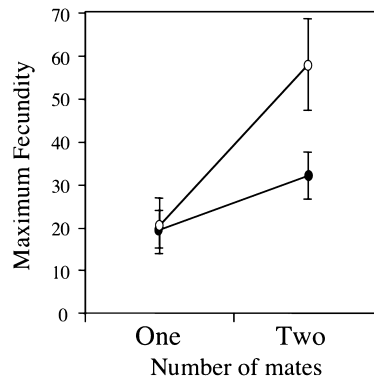


Figure 1. Estimates of the maximum fecundities of once and twice mated females (open circles) and males (closed circles). The upper limit on sexual selection (or the rate of gain in maximum fecundity with increased numbers of mates) is calculated from these data (as described in the text). The effects of body size were removed from female upper limits using partial regression (see text).

lifespan as reported by Brown (1997) for female tree crickets (*Oecanthus nigricornis*) that receive larger gifts, as well as in most other nuptial feeding insects (Arnqvist & Nilsson, 2000).

The upper limit on males (estimated as the average fecundity of second mates) is 12.55 ± 3.84 (Figure 1) which is also significantly greater than zero (t -test: $t_{\text{one-tailed}} = 3.26$, $N = 11$, $p = 0.004$), indicating that males also gain fecundity by remating. They do not gain as much as theory would predict; the male upper limit on sexual selection should equal the fecundity of single mated females (Lorch, 2002, 2005) which in our data is 19.64 ± 4.30 . However, though the female upper limit is more than twice as large as for males, the two are not significantly different (Figure 1; t -test for unequal variances: $t' = 1.15$, $N = 11$ and 23 , $p > 0.2$). Therefore, we cannot detect statistically significant potential for sexual dimorphism. However, as the variance about the two estimates (especially for the females) is large and the sample sizes fairly small, we have low power to detect a significant difference between slopes.

Spermatophore weight

If the difference between the male and female upper limits is real, the difference may be, in part, a result of a decrease in spermatophore size in second matings (see Davies & Dadour, 1989; Gwynne, 2001) resulting in a smaller

gift in the second mating and consequently a smaller fecundity gain. This was the case. There was a significant decrease in spermatophore weight between a male's first and second matings (0.013 ± 0.008 g (median \pm IQR) for one and 0.008 ± 0.004 g (median \pm IQR) for two mates; Wilcoxon $Z = -2.31$, $N = 11$, $p = 0.02$). There was no equivalent difference between the weights of first and second spermatophores given to females by their two virgin mates (0.017 ± 0.004 g (median \pm IQR) for the first mating and 0.015 ± 0.006 g (median \pm IQR) for the second mating; Wilcoxon $Z = -1.18$, $N = 8$, $p = 0.24$). If males are providing smaller second nuptial gifts because they have not fully replenished spermatophore glands, there should be a negative correlation between the difference in spermatophore weight (first minus second) and inter-mating interval. We found a negative correlation, although it was not significantly less than zero ($r_s = -0.37$, $N = 11$, one-tailed $p = 0.12$). Spermatophore weight (for single virgin male, virgin female matings) had no detectable effect on number of eggs produced (even if matings that produced no eggs were excluded: Eggs = $(27.38-142.82) \times$ spermatophore weight, $R^2 = 0.0001$, $F_{1,10} = 0.001$, $p = 0.92$) over the range of spermatophore weights seen (0.006 to 0.02 g). This reduction in second spermatophore weight may be an artifact of the lab protocol we used. Nonetheless, it is still a serious problem to be aware of in estimating upper limits on sexual selection.

Failure of sperm transfer

The lower male estimate for the upper limit on sexual selection might also be due, in part, to what appears to be the failure of sperm transfer in some matings in which spermatophores were transferred; 12% (4 of 34) first matings produced no eggs and 35% (12 of 34) produced <10 eggs. Among females that mated with previously mated males 8% (1 of 13) produced no eggs and 54% (7 of 13) produced <10 eggs. These failures do not appear to be due to male sterility because, of the four males who failed to transfer sperm during their first mating, three mated a second time and all of these mates laid >10 eggs. Failure to transfer sperm may be due to improper insertion of the sperm ampulla. The failure of sperm transfer can have different effects on the upper limit of sexual selection for males as compared to females. We illustrate this more clearly with a simple model for understanding how failures to transfer sperm affect male and female maximum fecundities and the resulting upper limits on sexual selection.

In mating systems in which males supply goods and services to females, when sperm transfer is successful, the maximum fecundity of males will always equal or exceed female maximum fecundity (Lorch, 2005). This occurs because, although female maximum fecundity increases with additional matings by an amount equal to nuptial gift value, male maximum fecundity increases by this amount plus any female pre-mating fecundity (equal to her fecundity if she were to receive only sperm and no gift; Lorch, 2005). Any matings resulting in nuptial gift transfer but no sperm transfer, as occurs in our experiment, will reduce the average maximum fecundity of single mated individuals in proportion to the number of failures (relative to the maximum when there are no failures, since a failure will produce no offspring). It is also clear that the maximum fecundity of males and females who mate once must be equal.

The maximum fecundity of males and females who mate twice is not equal when some matings result in a failure to transfer sperm. This is because twice mated males fall into three categories of maximum fecundity (males with two failures, with one success and one failure, and with two successes) while females fall into only two categories (two failures versus at least one success). Whether a female has one or two successful sperm transfers is irrelevant to her fecundity (assuming no additional effect of ovipositional stimulants from the second ejaculate) because one successful mating can transfer enough sperm to fertilize all her eggs (katydids ejaculate a lot of sperm; e.g., 6.3 million in *Poecilimon veluchianus*, Reinhold & von Helverson, 1997), while both matings transfer fecundity benefits from nuptial gifts. Thus, with failed insemination the average maximum fecundity of twice mated males and females will decrease at different rates (Figure 2A), leading to different effects on the upper limit of sexual selection (the rate of increase in maximum fecundity with additional mates; Figure 2B). If we assume that failures to transfer sperm are relatively rare (presumably they are costly and natural selection acts to reduce them), failures can actually increase the female upper limit on sexual selection because a second male provides two direct benefits to females: nutrition and insurance against a failure of sperm transfer in the first mating. By contrast, failures to transfer sperm always decrease male upper limits because a male must successfully inseminate each of his mates to gain from his investment in each mating (Figure 2B). This can lead to a situation where estimates of the female upper limit can actually exceed those of the male upper limit (Figure 2B) as was seen in our estimates. It can

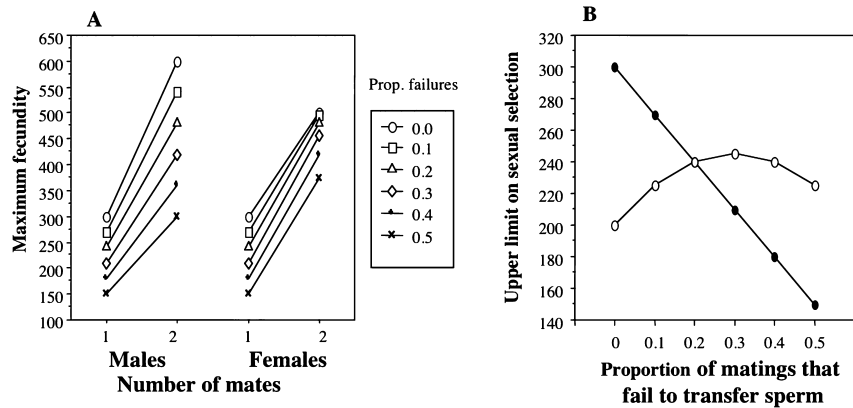


Figure 2. Effects of the proportion of matings that result in successful nuptial gift transfer but a failure to transfer sperm on the average maximum fecundity for a group of mating individuals (A) and how this affects the estimates of upper limits on sexual selection (B). Females are arbitrarily assumed to have a pre-mating fecundity (before they receive a nuptial gift) of 100 eggs, and nuptial gifts allow females to produce 200 extra eggs. Failures reduce estimates of maximum fecundity linearly for single mated individuals (of both sexes; numbers of mates = 1 in A) and for double mated males (male number of mates = 2 in A). However, failures do not reduce the fecundity of double mated females as fast as they do single mated females (females with 1 and 2 mates in A). Estimates of the upper limit on sexual selection (slopes in A plotted on the y-axis in B) are decreased by failures for males (closed circles), but failures can increase estimates for females (open circles; when failures are rare, < 0.5 of all matings).

also explain why the maximum fecundity of females who mate two times can exceed twice the maximum for females who have mated once (compare Figures 1 and 2).

Discussion

In our data the regression slopes expressing the relationship between maximum fecundity and numbers of mates were significantly positive for both sexes, indicating they both have the potential to gain fecundity by copulating with more than one mate. In other words there is the potential for sexual selection on both males and females in *C. nigropleurum* (Lorch, 2005). A positive relationship between fecundity and mating success has generally been assumed to be the case for males, but it may also often be true for females in many species (and not only where there is a clear reversal in the mating roles; Arnqvist & Nilsson, 2000). The cause of sexual selection is

different in the two sexes. This is reflected in what sets the upper limit on sexual selection for each sex. In *C. nigropleurum* and in other systems with nuptial gifts or paternal care, female fecundity increases primarily because males give females goods and/or services that allow them to produce more eggs, while male fecundity increase primarily when they are successful at fertilizing more eggs (Gwynne, 1993). Females may also gain fecundity by remating if they are sperm limited (Lorch et al., 1993), if some males in the population are genetically incompatible (Lorch & Chao, 2003) or through sperm selection (Simmons et al., 1996). Whatever the cause of increases in female fecundity with additional mates, it can generate the potential for sexual selection. Individuals of both sexes who are better able to take advantage of the potential represented by the upper limits on sexual selection leave more descendants.

The potential for sexual selection on female *C. nigropleurum* appears to be due to increased fecundity that comes from increased lifespan (for a review of similar results see Arnqvist & Nilsson, 2000; Wagner Jr. et al., 2001). Mating twice almost doubles post-mating lifespan of females without increasing their egg laying rate over this period. Our data do not demonstrate statistically significant levels of potential for sexual dimorphism. If anything, it appears that there are fewer fitness benefits to be gained by male *C. nigropleurum* than females, since the upper limit estimate for females was marginally larger (2 times) than that of males. However, this difference may have been the result of a short interval between matings causing a smaller spermatophore meal in male second matings. Such a short interval may not occur in nature if, for example, recently-mated males lose in competition for available females (Feaver, 1977).

Maximum male fecundity (from matings with virgin females) is expected to increase with increased numbers of mates at a rate equivalent to the fecundity of females who have mated once (Lorch, 2005). In our experiment there was a trend toward the male upper limit on sexual selection being less than the mean fecundity of once mated females. It is possible that some aspect of male quality or compatibility with particular females (Cunningham & Birkhead, 1998) is preventing some males from attaining their maximum rate of gain from remating. The failure of sperm transfer during mating attempts can also result in an increase in estimates of the female upper limit on sexual selection while reducing the male upper limit, leading to the seemingly paradoxical result where the male upper limit on sexual selection does not exceed the female upper limit.

Female *C. nigropleurum* have the potential to gain significant fecundity by remating. The potential for sexual selection on females that these gains create is in line with results from the majority of Bateman's (1948) replicates with *D. melanogaster*, with work on dark-eyed juncos and brown-headed cowbirds, with yellow-pine chipmunks and with two other *Drosophila* species. Similar gains in fecundity are known to accrue to females in other nuptial gift-giving systems (Arnqvist & Nilsson, 2000; Vahed, 1998), though these gains are generally not thought of as creating potential for sexual selection on females. In fact, the same potential is expected for all animals where males contribute significant levels of parental care (Arnold & Duvall, 1994) because female fecundity may increase with additional mates.

As mentioned in the introduction, there are important differences between estimates of the potential for sexual dimorphism (e.g., male/female upper limits on sexual selection) and measures of actual differences in Bateman gradients (e.g., male/female Bateman gradients). Studies that use genetic markers to estimate the average rate of gain in fecundity with increased numbers of mates (Bateman, 1948; Ketterson et al., 1998; Jones et al., 2000, 2002; Woolfenden et al., 2002) will arrive at estimates of sexual selection gradients for males and females that are lower than the upper limits estimated by using maximum fecundities. Even if there is a large difference between the male and female upper limits on sexual selection (i.e., there is high potential for sexual dimorphism), the difference between the actual sexual selection gradients may be small. In fact this discrepancy may tell us about the mating system or environmental constraints acting in a population at the time when the gradients were estimated. Such a discrepancy is likely to be the case in waterstrider species (Hemiptera: Gerridae). Repeated mating is costly to females that have to carry males during extended copulation bouts, making females more vulnerable to predators from below the water surface. There should, therefore, be a large potential for sexual dimorphism (i.e., a large difference in the upper limit on male and female Bateman gradients). We might not expect very high actual levels of sexual dimorphism in species like waterstriders, because females increase the cost of mating for males by struggling with them (see Arnqvist, 1997). Estimating both the upper limits and the actual Bateman gradients can, therefore, be informative.

Failure of sperm transfer

Our results highlight some of the pitfalls in estimating the potential for sexual dimorphism. As was the case with our data, whenever the estimate of

the male upper limit on sexual selection is less than the average fecundity of females who have mated once, there is reason for concern. In the case of *C. nigropleurum* we believe that this discrepancy can result partly from males giving smaller second nuptial gifts to females and partly from matings where sperm transfer was unsuccessful. The smaller second gift may have been due to unnaturally short remating intervals in this experiment. Little is known about natural remating intervals in insects and nothing is known for *C. nigropleurum*. What is known in other katydids suggests that natural intervals can be on the order of a week (Gwynne & Snedden, 1995). Failure to transfer sperm, on the other hand, affects maximum fecundity estimates for double-mated males and females in different ways with consequent divergent effects on estimates of the potential for sexual dimorphism. In Mormon crickets (*Anabrus simplex*), another katydid with a large spermatophore gift, of all females observed to mate one to four times (in field cages), 21% had at least one mating that transferred no sperm, while 33% of single matings in the lab failed to transfer sperm (Gwynne, 1993). We have argued that when failures to transfer sperm occur rarely and independently of nuptial gift transfer, they can increase estimates of female upper limits on sexual selection and decrease male estimates of the upper limit. This can lead to a situation where the female estimate exceeds the male estimate, especially when it is compounded (as it was in our data) with the fact that males were transferring smaller nuptial gifts during second matings. Such errors in estimating the upper limits on sexual selection could lead to a mistaken impression of the potential for sexual dimorphism. Estimating and using more natural remating intervals for males rather than pairing them with a new female every day until they mate, as was done here, might eliminate the reduction in nuptial gift size between matings which would reduce error in the estimates.

Alternatively, to estimate the male upper limit on sexual selection, one could simply use the estimate of the average fecundity of once mated females. The rate at which males maximum fecundity increases with additional ideal mates should be equal to the fecundity of females who mate once (Lorch, 2005). Since both reductions in secondary spermatophore size and failures to transfer sperm can affect female fecundity, this alternative is also of limited use here. Further empirical work comparing both approaches would be fruitful, particularly when something is known about natural mating intervals.

Acknowledgements

This research was funded by a National Science and Engineering Research Council operating grant to D.T. G. We thank Seaver Soon for helping collect eggs. Cynthia Thomas, Vijanti Ramlogan Murphy and Jenny McEwan helped with measuring and weighing. Steve Arnold, Nick Collins, Locke Rowe, Helen Rodd and several anonymous reviewers commented on earlier drafts of this manuscript. Experiments comply with the current laws of Canada.

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