

Natural and Sexual Selection in a Wild Insect Population

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The understanding of natural and sexual selection requires both field and laboratory studies to exploit the advantages and avoid the disadvantages of each approach. However, studies have tended to be polarized among the types of organisms studied, with vertebrates studied in the field and invertebrates in the lab. We used video monitoring combined with DNA profiling of all of the members of a wild population of field crickets across two generations to capture the factors predicting the reproductive success of males and females. The factors that predict a male's success in gaining mates differ from those that predict how many offspring he has. We confirm the fundamental prediction that males vary more in their reproductive success than females, and we find that females as well as males leave more offspring when they mate with more partners.

Insects are of fundamental importance to terrestrial ecosystems but are underrepresented in studies that aim to understand how natural and sexual selection drive evolution in wild populations. Although poorly understood in their natural habitats, crickets have become an important laboratory model system, revealing complex forms of sexual selection whereby females choose between males according to their songs (1), males fight (2), females manipulate sperm from several males to favor unrelated males (3, 4), and females lay eggs faster when mated to dominant males (2). However, although we now have many insights into the behavior and physiology of crickets in the laboratory, we have almost no idea how important these various aspects are in the insects' natural habitat. This discrepancy is a cause for concern: Laboratory situations remove some sources of selection that may be very important in wild populations and may create new pressures; for instance, it may be that males that sing more get more mates in the lab, but in the field such males may die younger.

Univoltine flightless field crickets, *Gryllus campestris*, hatch from eggs in early summer. Nymphs build burrows among the grass and spend the winter underground, emerging in spring to undergo one or two final molts to adulthood. Both sexes are highly territorial and spend the vast majority of their time in the immediate vicinity of a burrow entrance. A few days after becoming adults, males start to sing, and both males and females start moving frequently from one burrow to another in search of mates. To identify selective pressures affecting behavior and to observe how behavior is correlated with fitness, we built a network of 64 motion-sensitive, infrared-equipped

video cameras allowing us to monitor occupied burrows 24 hours a day throughout the breeding season. We tagged every newly emerged adult with a unique code to analyze their lives and behaviors, including mating partners, how long particular males and females spent together, the time that each male spent singing calling songs to attract females, and the fights that almost invariably occur when a male approaches a burrow occupied by another male. We used these fights to score males as either dominant or subordinate, reflecting the proportion of fights that he won (5). Although females never share burrows, they are only very rarely involved in aggressive interactions. Females visit or receive visits from neighboring males and frequently remain with a male for hours or days, sharing his burrow and mating repeatedly. From our videos, we inferred adult life span as the time from the observed emergence to the point when a cricket was either seen to be killed by a predator or was no longer found at any burrow.

We observed that females began mating a few days after becoming adults and laid eggs directly into the ground throughout the breeding season (burrows are narrow, so molting and mating take place just outside and are easily observed). The crickets in the field in the second year of our ob-

servations are therefore the offspring of the adults from the previous summer. Populations may experience some migration, but this is likely to be very limited in our study population. The meadow is relatively isolated, being surrounded by little suitable habitat, and the observed immigration rates of adults are low; therefore we had high success in assigning parentage within the population (5). All of these factors indicate that it is unlikely that substantial numbers of adult offspring were missed because of emigration. Lifetime reproductive success (LRS) was therefore inferred from the assignment of parentage from parents in 2006 to offspring in 2007 through the genotyping of all adults at 11 microsatellite loci.

A key prediction of the theory of sexual selection (6–8), assuming conventional sex roles and an even sex ratio, posits that males should have greater variance in LRS than females do. This prediction has been supported in a small number of studies of wild vertebrates [for example, (9)] and in laboratory experiments [although the lack of ecological context has led to debates over their relevance (10)]. Most studies of the cost and benefits of mates and matings in insects have been performed in the laboratory (11–13), and the only examination in the wild was of reproductive success estimated via the time female damselflies spent laying eggs after mating to a particular male (14). We directly examined both the number of mates that each individual had (controlling for differences in observational effort) and the number of descendants they left in the next generation. The sex ratio was very close to even, which constrained the means to be the same for both sexes. As expected, we found that mean numbers of mates per day [males = 0.27 (0.40 SD), females = 0.25 (0.32 SD)] and of offspring surviving to adulthood [males = 1.92 (3.66 SD), females = 1.79 (2.46 SD)] were, respectively, very similar. The small differences we observed were attributed to imperfections in observational data and in parentage assignment.

The opportunity for selection can be estimated by comparing variances or coefficients of variation (15). We examined with a randomization-

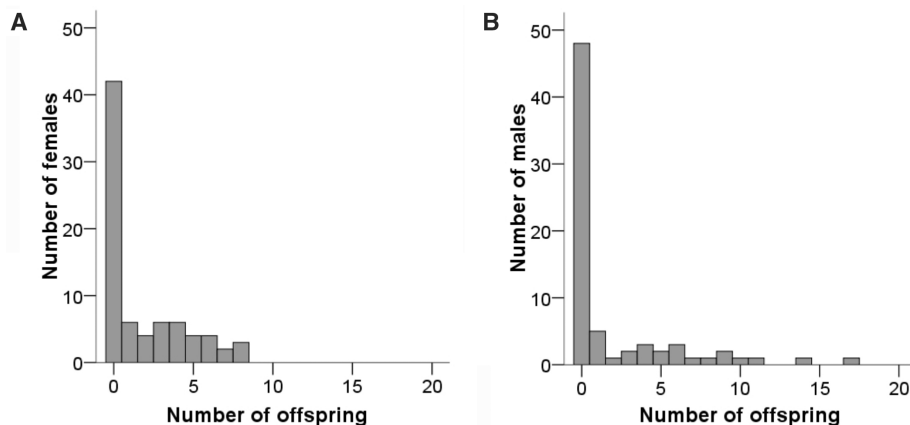


Fig. 1. Number of adult offspring per individual. Frequencies for (A) females and (B) males. Males have significantly greater variance in offspring number relative to females.

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based test (5) the prediction that males should show more variation than females, and we found no difference between the sexes in variance in the number of mates ($P = 0.39$) but significantly more variance in the number of offspring produced by males ($P = 0.033$) (Fig. 1) (5). Similar results were found when controlling for the small differences in means between males and females (number of mates, $P = 0.39$; number of offspring, $P = 0.028$). It is, however, striking that although males varied more than females, the overall patterns were similar, with many females failing to leave any descendants.

We found that both sexes benefit from multiple mates. Females may use a single ejaculate over their reproductive lifetime, raising questions about why they mate with more than one male (16). Examination of traits expressed by both sexes indicated that individuals with a higher number of mates (standardized for monitoring effort) had a higher number of offspring for both male parents (Spearman's $\rho = 0.53$, $n = 47$ individuals, $P < 0.0001$) and female parents (Spearman's $\rho = 0.37$, $n = 55$, $P = 0.005$). This suggests that the factors affecting the number of offspring produced by males are the same as those affecting the number of offspring produced by females. Because there are numerous other correlations between traits, we also examined whether the number of mates was correlated with other behavioral and life-history traits by using a generalized linear model (5). This approach indicated that there were no interactions between sex and number of mates affecting LRS, nor any other significant interactions. Individuals of both sexes that were either larger, longer-lived, or had more mates had significantly higher LRS (analysis of deviance: size deviance = 4.58, $F_{1, 99} = 4.58$, $P = 0.035$; longevity deviance = 117.14, $F_{1, 99} = 38.74$, $P < 0.0001$; number of mates deviance = 44.47, $F_{1, 99} = 14.71$, $P = 0.0002$) (Fig. 2). This demonstrates that not only do males increase their reproductive success

through an increased number of mates, but females do better by mating with multiple partners, too. Although there is greater variance in male reproductive success than in female reproductive success, this increased variance is not due to different effects of mate number between the sexes.

Traits that confer success in gaining mates may differ from those that predict reproductive success because of differences among males in post-mating fertilization success and the viability of offspring (17). We counted the number of offspring an individual had in the following generation to measure directly the reproductive success of our field crickets. We found differences between predictors of mating success and predictors of reproductive success. By comparing generalized linear models predicting mating success (measured as the number of females with which each male was observed to mate) with lifetime reproductive success (number of offspring surviving to adulthood), we identified factors that predicted the reproductive success of an individual male. Mating success was predicted by dominance and an interaction between size and singing activity. Offspring number was also predicted by interactions between size and singing, but there was an additional interaction between longevity and singing and no significant effect of dominance. Contrary to expectations from lab studies that show that dominant males can monopolize mating access to females (18), who also prefer the odor of dominant males (19), we found that dominant males had fewer mates than did subordinate males (analysis of deviance: dominance deviance = 2.22, $F_{1, 24} = 9.58$, $P = 0.004$) (Fig. 3A). This result is unexpected but reflects an ambiguous role for dominance in predicting mating success across species (20). For smaller males, the amount of singing was strongly correlated with the number of mates they obtained, whereas for larger males, singing activity was not associated with gaining more mates (size \times singing deviance = 0.89, $F_{1, 23} =$

4.53, $P = 0.044$) (Fig. 3B). Similarly, for small males, singing effort affected the number of offspring (analysis of deviance: size \times singing deviance = 25.93, $F_{1, 41} = 8.12$, $P = 0.007$) (Fig. 3C). In addition, short-lived males had more offspring when they sang more, whereas reproductive success in long-lived males was not dependent on a high rate of singing (longevity \times singing deviance = 13.91, $F_{1, 41} = 4.35$, $P = 0.043$) (Fig. 3D).

These interactions between naturally and sexually selected traits affecting different measures of reproductive success indicate that the benefits of sexually selected traits may vary according to other aspects of an individual's phenotype. The costs of sexually selected traits are expected to be lower in individuals of higher phenotypic condition, which in turn reflects the overall genetic quality (21). If males with high genetic quality are able to achieve a large size at adult emergence, they would be predicted to sing more. However, we observed no correlation between size and singing activity within males (Spearman's $\rho = 0.185$, $P = 0.2$, $n = 47$). Our results suggest that in this population, either size is not a reliable indicator of condition or male singing activity is not condition-dependent, despite its metabolic costs (22) and association with increased rates of parasitism (23) and predation (24). Size and singing activity are individually correlated with reproductive success, but the fact that smaller males benefit more from singing is the opposite of what we would expect if larger males are deemed to be those with the higher condition. This suggests that adult size is not an appropriate proxy for phenotypic condition in these animals and possibly in other insects.

Longevity is a fundamentally different trait from size, because it is not fixed at emergence to adulthood and hence can continue to be affected by other traits. The interaction between singing and longevity affecting reproductive success occurs because daily singing effort has a major effect on offspring number in shorter-lived males

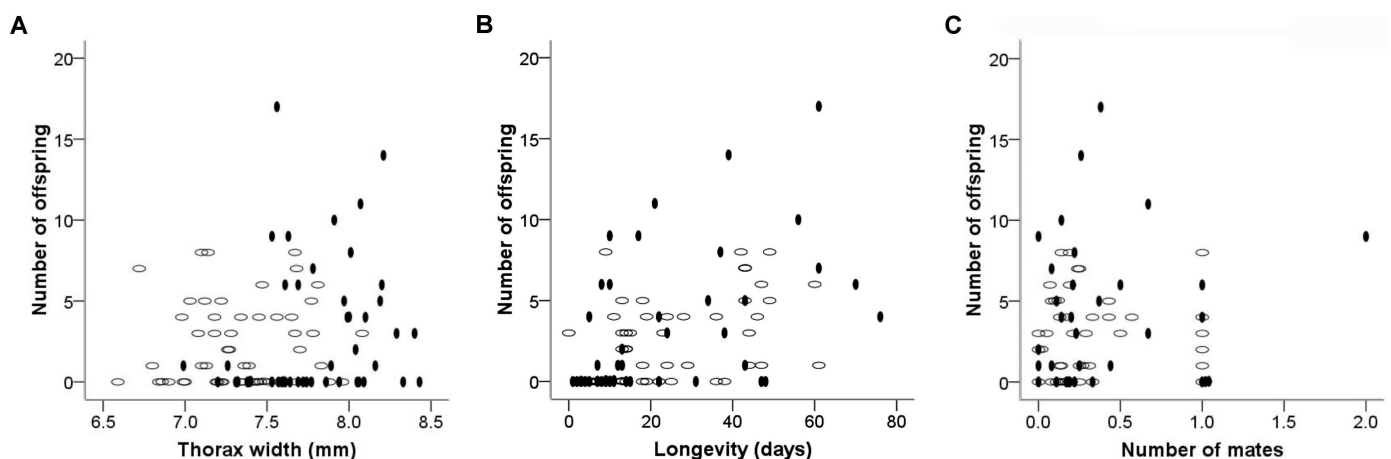


Fig. 2. Determinants of male and female reproductive success measured as number of adult offspring in the following generation. Females are indicated by open symbols. (A) Body size, (B) longevity (days), and (C) number of mates per day. To aid in distinguishing data, where data points overlap, a small increment (0.02 to x and y coordinates) has been added.

Also, in (C), a number of points overlap at 0, 0 (16 males and 12 females) and would obscure further data, so have not received an increment. Larger body size, greater longevity, and a higher number of mates are independently associated with increased reproductive success in both males and females.

but has no discernible effect in longer-lived males (Fig. 3D). This may be because if males live a long time, singing effort can be reduced once they have attracted a female to their burrow, whereas the population of short-lived males includes more individuals that are in poor condition and die young without mating or singing very much. In the laboratory, male crickets kept on a high-protein diet sang more, but lived for a shorter time (25). In the wild population, there was a strong positive correlation between daily singing effort and life span (Spearman's $\rho = 0.54$, $P < 0.0001$, $n = 47$), most likely indicating that those that sang more were of higher quality.

Our findings confirm the basic prediction that male reproductive success, while being constrained to be equal to that of females, is likely to vary more. Bateman's prediction (7) that this variation is due to the potentially higher mating rate of males does not appear to be borne out; variance in the number of mates a male had was no greater

than that of females. Rather, it appears that in these insects, some males gain a disproportionately large share of the offspring in the following generation through either greater success in postcopulatory sexual selection or greater viability and survival of their offspring.

Both sexes have higher LRSs when they have more mates, and there is no interaction between sex and number of mates that affects LRS. This demonstrates that polyandrous females have more offspring in the next generation, supporting previous laboratory experiments (26). Because polyandrous females tend to have more matings as well as more mates, it is still unknown whether these benefits accrue from some direct source such as ejaculate components or a need to replenish sperm stores, or whether they are the result of genetic benefits to the offspring of polyandrous females. It may be that polyandrous females can increase offspring fitness by preferentially fertilizing their eggs with sperm from unrelated males

(3) and that, by mating with multiple males, females increase their chances of producing offspring with unrelated males.

The number of mates was a strong predictor of the number of descendants that both males and females left in the next generation. Similarly, traits associated with having several mates, such as male size and singing activity, were also associated with LRS, indicating that, in general, mating success is likely to perform quite well as a surrogate for overall reproductive success. However, it is clear that if we wish to understand selection on individual traits in natural populations, careful consideration must be given to how we measure reproductive success: Some traits that affected an individual's number of mates failed to predict LRS, and other traits associated with high LRS were not good predictors of mating success. Furthermore, interactions between naturally and sexually selected traits affecting both mating and reproductive success indicate that studying a single trait in isolation may be misleading.

Our system bridges the divide between laboratory and field studies in evolutionary biology and indicates that, with the above caveats, conclusions drawn from laboratory studies on crickets and, most likely, other insects as well are generally consistent with studies in the wild. We demonstrate here that the combination of video technology and genetic parentage assignment means that tracing reproductive success in wild invertebrates is no longer impractical and that we can now conduct quantitative and functional genetic studies in natural populations.

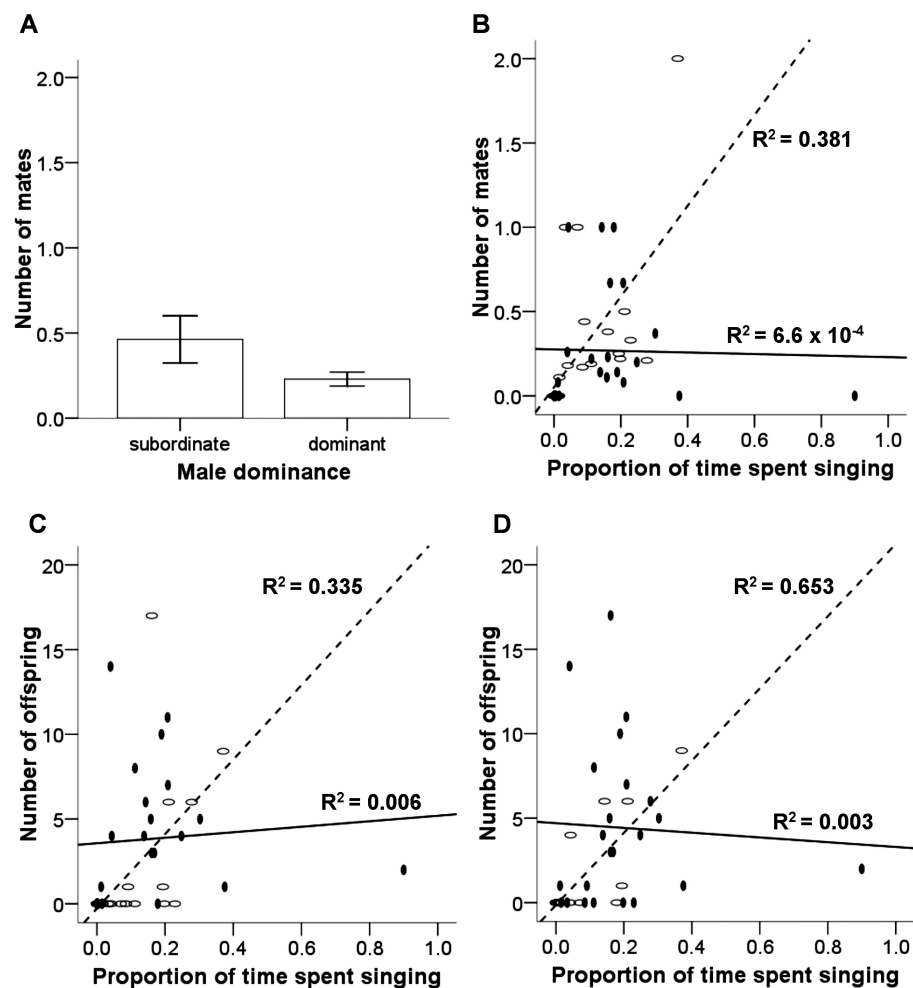


Fig. 3. Male sexual traits and mating and reproductive success. The effect on number of mates of (A) dominance and (B) the interaction between size and amount of singing, and the effect on number of adult offspring of (C) the interaction between size and the amount of singing and (D) the interaction between longevity and the amount of singing. For illustrative purposes only, the 47 males were split into two groups by size (24 smaller and 23 larger males) or by longevity (24 shorter-lived and 23 longer-lived males). Open symbols and hatched lines indicate smaller [(B) and (C)] or shorter-lived (D) males. A number of data points overlap at 0, 0: nine smaller males and five larger in (B) and (C), and 14 short-lived males in (D).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/328/5983/1269/DC1

Materials and Methods

Figs. S1 and S2

Table S1

References

Movies S1 to S8

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