TESTING PARENTAL INVESTMENT AND THE CONTROL
OF SEXUAL SELECTION IN KATYDIDS:
THE OPERATIONAL SEX RATIO

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The study of sexual selection, which has a substantial theoretical base, has
begun to include more research that is empirical. Much of this research has
described the nature of sexual selection, particularly the measurement of sexual-
selection intensities and the adaptive significance of sexual differences, especially
regarding mate choice (review in Bradbury and Andersson 1987). There has been
far less empirical testing of the factors that ultimately control, or determine
the extent of, sexual selection and sexual differences. A major factor, outlined
in the theory of Williams (1966) and Trivers (1972) (review in Thornhill 1986),
is that relative investment by males and females in offspring controls the degree
to which each sex competes for or chooses mates. This control is achieved
through the relative numbers of sexually receptive females and males. The
ratio of fertilizable females to sexually active males is referred to as the operational sex ratio (OSR; Emlen and Oring 1977). The greater the degree of imbalance
in the OSR, the greater the expected variation in the reproductive success,
and thus sexual selection, among members of the limited sex (see also Emlen
1976). If the OSR is less than one, there should be more sexual competition
among males than females. The converse is expected if the OSR is greater than
one.

The initial OSR, resulting from parental-investment (PI) patterns, is expected to
be adjusted by ecological factors that affect the extent to which mates can be
monopolized (see Emlen and Oring 1977; Ims 1988). This secondary ratio would
reflect the intensity of sexual selection on the sexes. Therefore, measures of the
initial OSR are often difficult to carry out in nature because, in censuses of
breeding animals, intrasexual competition is likely to cause an underrepresentation
of the numbers of the limited sex (Emlen and Oring 1977). A test of the
influence of PI on the ratio of the numbers of females and males available for
mating should thus control intrasexual selection while varying investment by the
sexes.
Katydid, a family of orthopteran insects (Tettigoniidae), are appropriate subjects for examining this theory, since both sexes invest parentally by providing materials to eggs, and male investment appears to be associated with sexual selection acting on females. The male contribution is courtship feeding of the female: the greater portion of the spermatophore passed to the female is a spermfree mass, the spermatophylax, which she eats; in *Requena verticalis*, spermatophylax nutrients are translocated to eggs (Bowen et al. 1984) and increase both fecundity and the fitness of progeny fathered by the mating male (Gwynne 1984a, 1988a, 1988b).

The size of male investment in the spermatophylax of some katydids is substantial (Gwynne 1983), suggesting that the material contribution may, in these species, cause female reproduction to be limited by male offerings, resulting in female-female competition for receptive males. Such a courtship role reversal has been observed in two species with large spermatophores. In both *Anabrus simplex* (the Mormon cricket) and *Metallus litus*, females fight for access to sexually signaling males, and males discriminate among females (Gwynne 1981, 1984b, 1985). However, such a polyandrous mating system is not always the case. In some populations of these species, the mating system is polygynous, and the sexes show the more typical courtship roles. Experiments transferring *M. litus* individuals between populations revealed that the mating system is plastic, being determined by some aspect of the habitat (Gwynne 1985). I hypothesized that the courtship role reversal in polyandrous populations occurs only in habitats in which food limitation decreases the availability of spermatophores sufficiently to reduce female reproduction, thus eliciting sexual competition for the male nuptial gifts (Gwynne 1984b). Observations leading to this hypothesis were that individuals (of both sexes) compete for proteinaceous food items, such as dead arthropods, only at sites where polyandry is noted and that most males sampled from these sites do not have sufficient material in reproductive accessory glands to produce a spermatophore.

A test of the hypothesis that food availability in the habitat can ultimately determine sexual differences for katydids would represent an experimental test of the sexual-difference theory of Williams (1966) and Trivers (1972) (Thornhill 1986). The hypothesis predicts that male PI is more important in food-stressed habitats. The relative importance of the male contribution to offspring production (relative male PI) compared with that of the female is expected to increase with increasing food limitation (Gwynne 1985; Thornhill 1986). This would be due not only to a decrease in the availability of spermatophores to females, as already mentioned, but also to any tendency for females to increase mating frequency as a way of “foraging” for additional matings with nurturant males.

I present an initial test of this hypothesis by determining whether experimental variation in diet changes the mating frequency of males and females in the directions predicted. Since the objective of the study was to determine the influence of diet (and thus the relative importance of male and female PI) on the maximum number of matings achievable by the sexes, the experimental protocol paired single males and females so as to exclude intrasexual competition.
METHODS

The Species: Requena verticalis

Requena verticalis, a listroscelidine katydid endemic to Western Australia, was the subject of the experiment. In contrast to certain other species (including Metaballus litus and Anabrus simplex), the size and other aspects of the biology of this species make it an appropriate subject because it is easily raised and mated in the laboratory. Furthermore, previous work provides background information on the costs and consequences of spermatophore production in this species (Bowen et al. 1984; Gwynne 1984a, 1986, 1988a, 1988b; Gwynne et al. 1984; Davies and Dadour 1989).

Mating in R. verticalis starts when a sexually receptive female moves up to a male and raises her hind leg. If the mating is successful, a spermatophore is produced, consisting of a bi-lobed sperm ampula—which is inserted partly into the female gonopore—and a bi-lobed spermatophylax attached to the ampulla. A few minutes after the end of copulation, the female grasps the spermatophylax with her mandibles and removes and eats it. She takes about 5 h, on the average, to consume the spermatophylax. This is followed by consumption of the empty sperm ampulla (the ejaculate is transferred into the female during the first 2.5 h after mating; Gwynne et al. 1984; Gwynne 1986). Male investment in a spermatophore appears to be costly. Since mated males require more than 5 d to fully replenish the spermatophore-producing glands, with an unrestricted diet, the average 4-d remating interval of females (noted in Gwynne 1986) may not be as long as the remating interval of males (Davies and Dadour 1989).

Experimental Protocols

The experiment was conducted in a laboratory (at a photoperiod of 12L: 12D, at 25°C) and focused on a primary group of male and initially virgin females. For each of 17 consecutive days, individual members of the primary group were placed in a mating cage (12 cm in diameter and 7.5 cm in height) with a receptive member of the opposite sex taken from a secondary group. After each mating trial, both primary and secondary individuals were returned to their individual housing jars (8 cm in diameter and 6.5 cm in depth), which contained food. Primary individuals received either a low- or a high-quality diet. All secondary individuals were maintained on a high-quality diet.

Virgin females were obtained for the experiment by raising field-collected larval females in the laboratory on a high-quality diet (for details, see Gwynne 1984a, 1986). All males were collected as adults in the field; any variation in the ages and mating histories of these males was assumed to be consistent across treatments.

Most individuals in the secondary group were used to supply or receive a spermatophore only once. (An experimental animal never mated with the same member of the secondary group more than once.) Receptivity of secondary females was ensured by using un inseminated individuals (virgins and some individuals from which I had removed the spermatophore immediately after copulation in a previous mating). Primary animals were paired with secondary individ-
uals for about 5 h per day, either in the afternoon or immediately after dark, the two periods during which males appear to call most frequently. If the secondary animal was not observed responding sexually to the primary animal during the first hour, it was replaced with another secondary animal.

Experimental Diets

Primary *R. verticalis* males and females were fed either a low- or a high-quality diet. The low-quality diet was of plant origin; oatmeal was fed every third or fourth day, and the insects were allowed continuous access to apple and water. The high-quality-diet animals were allowed continuous access to water, apple, oatmeal, pollen, seeds, and Tetra-min fish food. The fish food included arthropod and other animal and plant products (shrimp, fish, cod liver, and algal meals). Field observations (unpublished data) indicate that arthropods form part of the diet of *R. verticalis*. Primary females were given a low-quality diet immediately after their adult molt. Primary males began the low-quality diet on the first day of placement with a female.

The composition of the diets was intended to simulate the natural variation observed among katydids that showed a variation in mating system. Observations of *Anabrus simplex* in nature (Gwynne 1984b) indicate great variation in the food eaten, ranging from habitats in which the katydids fed on abundant seeds, flowers, and insect prey (especially grasshopper nymphs) to habitats in which these food items appeared scarce and katydids competed aggressively for such food items, especially dead arthropods.

There was no evidence that experimental diets affected the survival of males or females during the experiment or for the month that animals were monitored after its completion. Only one individual (a low-quality-diet male) died during the experiment (data for this male were not included in the analysis). The death was caused by a predatory attack by a female. This male appeared to have been healthy, since he was observed producing a calling (sexual) signal on the day before the attack.

Statistical Analysis

The variances of mating frequencies among the four treatments (low- and high-quality diets and two sexes) showed significant heterogeneity (Cochran’s test, *P* < .05; fig. 1). After log transformation, the variances remained heterogeneous. Mann-Whitney tests (one-tailed) were therefore used to test for the effects of diet on mating frequency for each sex.

The effects of diet and the number of previous matings on the mass of the spermatophore were analyzed by a repeated-measures ANOVA (Wilkinson 1988). Since this analysis depends on an equal number of repeated measures for each individual and since males from different treatments differed with respect to mating frequency, two analyses were conducted. In the first, the sample size of males was reduced in order to analyze the first five spermatophores (i.e., individuals with a mating frequency lower than five were deleted). In the second, only the first three spermatophores were analyzed (thus increasing the sample size of males analyzed).
RESULTS

Mating Frequency

Males on a high-quality diet mated more frequently (median = 5, n = 8) than males on a low-quality diet (median = 4, n = 7; Mann-Whitney, P = .05). The converse was true for females: low-quality-diet females mated more frequently (median = 4, n = 9) than females on a high-quality diet (median = 2, n = 8; Mann-Whitney, P < .05; fig. 1).

The data (fig. 1) were used to estimate the ratio of sexually receptive Requena verticalis females and males for each diet. Data for mating females were divided by those for mating males. Because sample sizes for each treatment were not equal, means instead of totals were used to calculate these ratios for each diet. These estimates indicate that, for a high-quality diet, there would be more than twice as many sexually active males as receptive females (means, female, 2.25; male, 5.0; female : male ratio = 0.45). For a low-quality diet, the converse, more receptive females than sexually active males, was predicted (means, female, 4.33; male, 4.14; ratio = 1.05).

Spermatophore Mass and Remating Interval

Figure 2 shows the effect of diet and the number of previous matings on the mass of the spermatophore produced by a male (estimated as male weight loss
following mating). Analysis of the first five spermatophores produced by males revealed no significant effect of diet ($F = 1.38; \text{df} = 1, 8; P = .27$) but a significant effect of the number of previous matings (mating history; analysis conducted on males that mated at least five times: 7 of 8 high-quality-diet males and the 3 of 8 low-quality-diet males ($F = 3.61; \text{df} = 4, 32; P = .02$). The interaction of diet and mating history was also significant ($F = 3.26; \text{df} = 4, 32; P = .02$). Simple-effects testing of mating frequency within each diet revealed the source of the interaction: there were significant differences among the masses of spermatophores produced by males on a low-quality diet ($F = 10.70; \text{df} = 4, 8; P = .003$) but not those produced by males on a high-quality diet ($F = 1.03; \text{df} = 4, 24; P = .41$). The differences within the low-quality diet were caused by a reduction in the size of the last spermatophore produced by each of the five low-quality-diet males that mated four or five times: analysis of the masses of just the first three spermatophores revealed no significant effect of diet ($F = 0.80; \text{df} = 1, 13; P = .38$) or the number of previous matings ($F = 1.70; \text{df} = 2, 26; P = .19$). There was no significant interaction ($F = 0.25; \text{df} = 2, 26; P = .80$).

Davies and Dadour (1989) predicted that the remating time of *R. verticalis* males would be at least 4–5 d. They based this prediction on data showing that the masses of spermatophore glands from males 5 d after mating were significantly smaller than those from unmated (control) males. Data from the present study indicate that a 4–5-d remating is an overestimate; males on a high-quality diet (similar to the diet used in Davies and Dadour 1989) mated for a second time after 2.6 d ± 0.2 ($\bar{X} \pm \text{SE}$). This second mating resulted in a mean loss of 12.5% of premating body mass. A comparison of this with data reported by Davies and
Dadour indicates that almost all of the spermatophore-gland material would have been used for the second mating. Males appear to be able to transfer nutrients quickly to the spermatophore glands, since the third mating (for high-quality-diet males) took place 3.8 d ± 0.6 after the second and the fourth mating occurred 5.1 d ± 0.4 after the third (the differences between these three remating intervals are significant: $F = 11.8$; df = 2, 14; $P < .001$). The time costs of remating for male katydids are clearly much greater than those for a related group, the crickets (Gryllidae): for *Gryllus bimaculatus*, which does not produce a spermatophylax, remating occurs in about an hour (Simmons 1988); and for the spermatophylax-producing *Gryllodes supplicans*, remating occurs in about 3 h (Sakaluk 1985).

**DISCUSSION**

**Food Availability and the Control of Sexual Selection**

Theories about the control of sexual selection and sexual differences predict that relative investment of the sexes in offspring strongly affects the numbers of males and females available for mating (the operational sex ratio, OSR) and thus the extent to which one sex or the other engages in sexual competition. Field studies with katydids indicate that there can be significant spatial variation in the intensity of sexual selection acting on females, ranging from a low variation in mating success with no observable female-female competition to high variation in mating success and extreme sexual competition (Gwynne 1984b).

The present results explain how changes in the relative parental investment (PI) attributable to diet may determine much of this variation by controlling the OSR (Gwynne 1985; Thornhill 1986). First, the number of available spermatophylaxes decreases with decreasing food quality. Thus, the relative amount of male PI increases, since male courtship gifts become more costly to make (with the increased effort needed to acquire food items). Second, as food quality declines, this male PI becomes more important to female fitness, not only because there are fewer nuptial meals available but also because females appear to need more of these meals (they increase mating frequency).

The adaptive significance of this increase in "foraging" for matings by most females on a low-quality diet is probably related to fitness increases from the additional male-derived nutrition. Previous work indicated that *Requena verticalis* females feeding on a low-quality diet obtain two sorts of nutritional benefits from male spermatophores: increased fecundity from eating several spermatophores (Gwynne 1984a, 1988a) and apparently specialized nutrients that increase offspring fitness (Gwynne 1988a).

The different estimates of the average number of sexually receptive females and males on the two diets predict contrasting OSRs and thus contrasting mating systems in *R. verticalis* populations eating these sorts of diets in nature. For a diet similar to the high-quality one, polygyny and sexual competition among males would be expected since there would be more than twice as many sexually active males as fertilizable females. However, under diets similar to the low-quality one, a slight excess in the number of fertilizable females is expected. This would
probably result in sexual competition among females, which may exceed the
degree of sexual competition among males, thus producing a polyandrous mating
system. Detailed information on either the variation in mating behavior or the
range of diets eaten by *R. verticalis* in nature is not available. Although prelimi-
nary observations indicate that males show mate rejection (W. Bailey, personal
communication), this species does not appear to show the extreme reversals in the
courtship roles observed for *Anabrus simplex* and *Metaballus litus* (Gwynne 1981,
1985). A possible explanation of the difference between *R. verticalis* and these
two species concerns the large relative size of the spermatophore in *A. simplex*
and *M. litus*. If large meal size has evolved because of the higher relative value of
nutrients in the male contribution, the effects of food limitation on spermatophore
production are likely to be more pronounced in species that produce large sper-
matophores.

*Multiple Mating by Females: A Conflict with Male Interests?*

In *R. verticalis*, multiple mating by females before the first eggs are laid appears
to counter the reproductive interests of certain males. In laboratory experiments,
the first of two males to mate fathers virtually all the subsequent offspring. The
second male provides a large nutrient investment for another male’s offspring
(Gwynne 1988b). Such a high level of cuckoldry is probably somewhat lower in
nature because recently mated females seeking further copulations lose in competi-
tion with other females for access to sexually active males (A. J. Lynam, S.
Morris, and D. T. Gwynne, unpublished manuscript).

The time interval between matings by *R. verticalis* females has been interpreted
as an outcome of sexual conflict (see Parker 1979; Gwynne 1986); the positive
relationship between the length of the sexual refractory period and the time of
attachment of the sperm ampulla (for the first half of the attachment period)
suggested that a factor in the ejaculate induced the refractory period (Gwynne
1986). However, from the male’s standpoint, such a factor would appear redu-
dant because the first male to mate has almost 100% confidence of paternity when
the female mates again at the end of the refractory period. An alternative hy-
pothesis is that the sexual refractory period is less the outcome of male-female
conflict over the remating interval and more a result of female interests alone. The
sexual refractory period may be controlled almost completely by the female’s
level of hunger. This is supported by the present results: the refractory period of
low-quality-diet females after the first mating (median, 3 d) was significantly lower
than the refractory period of high-quality-diet females (median, 7.5 d; Mann-
Whitney, $P < .05$).

*Spermatophore Mass and Reproductive Tactics*

The main reason for the large effect of diet on the OSR of *R. verticalis* is that,
except for the final matings of some low-quality-diet males, males delay remating
until they can produce a large spermatophore. This result contrasts with our
knowledge of butterflies, a taxon in which important spermatophore nutrients are
also transferred to females (and absorbed in the genital tract; Boggs and Gilbert
1979; Marshall 1982; Rutowski et al. 1987). In all butterfly species studied, males
with access to receptive females remate before they can produce large spermatophores; the relative mass of a spermatophore in the second mating can be as low as 10%–15% of the first mating (Rutowski et al. 1987; Oberhauser 1988 and references therein).

For the katydid *R. verticalis*, males appear to have the opposite reproductive tactic since virtually all males do not reduce spermatophore size in favor of maximizing mating frequency. Other katydid species may act similarly: Simmons (in press) showed that males of an Australian zaporchiline (an undescribed genus) do not produce a sexual signal (the calling song) for about 5 d after mating; data on the weights of spermatophore glands of signaling *A. simplex* males indicate that they do not call until much of the gland material has been replenished (Gwynne 1984b).

The maintenance of a large spermatophore size, and thus the size of the spermatophylax gift, by *R. verticalis* males with constant access to receptive females supports the argument that nutrient investment in this species is maintained by the selective advantage of producing high-quality offspring (Gwynne 1986, 1988b; Davies and Dadour 1989; but see Wedell and Arak 1989). Males mating with virgin females (and probably females that have recently laid eggs) donate nutrients to their own progeny (Gwynne 1988b); any decrease in the size of the courtship gift could well compromise the fitness of these offspring (Gwynne 1988a).

SUMMARY

Theory predicts that relative parental investment by the sexes controls the ratio of the numbers of fertilizable females and sexually active males (the operational sex ratio, OSR) and thus sexual selection and sexual differences. This paper tests the hypothesis that variation in parental investment controls the numbers of males and females available for mating. In katydids, males and females invest parentally by providing material investment to eggs, with the male donation derived from spermatophore materials eaten by the female. Previous work with katydids in nature has shown intraspecific variation in sexual selection acting on females; only certain populations show a courtship role reversal in which females compete for access to males. These observations led to the hypothesis that the availability of food in nature determines the frequency of spermatophore production and thus the number of males available for mating. In the present study, male and female katydids were maintained on diets that differed in food quality. Both sexes were given daily access to sexually receptive members of the opposite sex. As predicted, males on a low-quality diet mated less frequently than males on a high-quality diet. The opposite trend was observed for females: females on a low-quality diet increased mating frequency, apparently to obtain additional spermatophore nutrients. These data suggest that male parental investment in the spermatophore increases in its relative importance when diet is low in quality. The effects of diet on mating frequency result in different estimates of the OSR, which accord with observations in nature: for the high-quality diet, there are fewer fertilizable females than sexually active males; similar conditions in nature would
be predicted to result in a male-to-male competitive (polygynous) mating system. For the low-quality diet, there were slightly fewer fertilizable females, indicating that such a diet may result in competition among females.

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LITERATURE CITED


