

# Hemolymph loss during nuptial feeding constrains male mating success in sagebrush crickets

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Although costs of mating have been widely documented in females, intrinsic costs of copulation have been poorly documented in males, and there is little evidence that such costs constrain male mating success under natural conditions. Male sagebrush crickets, *Cyphoderris strepitans*, offer females an unusual somatic food gift at copulation that may constitute a significant cost of copulation: females chew on the ends of the males' fleshy hind wings and ingest hemolymph seeping from the wounds they inflict. Previous studies have shown that once a male has mated, his probability of obtaining an additional copulation is reduced relative to that of a virgin male seeking to secure his first mating. If the future mating prospects of nonvirgin males are diminished because of the costs of copulation, this could stem either from the resources required to manufacture a new spermatophore or through the energy needed to replenish hemolymph lost through female wing-feeding. To distinguish between these two alternatives, we experimentally depleted virgin males of varying amounts hemolymph in a way that mimicked hemolymph loss of nonvirgin males, without the attendant costs of spermatophore production. After they had been treated, males were released in the field and recaptured over the course of the breeding season to monitor their mating success. Control males mated significantly sooner than did males depleted of hemolymph. We conclude, therefore, that the depletion of hemolymph that occurs through female wing feeding is sufficient by itself to diminish a nonvirgin male's ability to secure another mating. *Key words*: costs of mating, *Cyphoderris strepitans*, mating success, nuptial food gift, sagebrush crickets, sexual selection. [*Behav Ecol* 15:845–849 (2004)]

Costs of mating can be partitioned into those arising from the pursuit of mates (extrinsic costs) and those arising from the act of copulation itself (intrinsic costs; Martin and Hosken, 2004). Because there is often a conflict between the sexes over the occurrence of mating (Chapman et al., 2003), copulation can be especially harmful to females when males use physical or chemical means to deter females from remating (Johnstone and Keller, 2000), or transfer substances that induce females to prematurely invest in egg production or oviposition (Wolfner, 2002). In addition to these female-specific costs, the much greater investment by females in their gametes relative to males has tended to promote the view that intrinsic costs of mating to males are trivial relative to that of females (Dewsbury, 1982; Trivers, 1972).

Notwithstanding the empirical focus on females, there has been a growing appreciation that intrinsic costs of mating can, under certain circumstances, limit the ability of males to invest in future matings (Bonduriansky, 2001). Not only do males expend time and energy in consummating matings (Sparkes et al., 2002; Woods and Stevenson, 1996), they may also invest considerable energy in the production of costly ejaculates or nuptial food gifts that constrains future mating success (for review, see Vahed, 1998). For example, costs incurred in the manufacture of ejaculates may limit the number of sperm that males can produce, and a number of studies have shown that males often exhibit strategic

allocation of sperm among their various mating partners (for review, see Wedell et al., 2002). Although an understanding of the relative costs of mating to males and females is critical to our understanding of sexual selection, there is little direct experimental evidence of the cost of copulation to males and even less evidence that such costs constrain male mating success under natural conditions. Experimental studies on giant waterbugs (Gilg and Kruse, 2003) and dung flies (Martin and Hosken, 2003) have shown that costs of copulation alone can reduce male lifespan, which presumably constrains male mating success. Indirect evidence for costs of copulation in gift-giving insects comes from studies in which males have been subject either to food deprivation (Engqvist and Sauer, 2000; Gwynne and Simmons, 1990; Jia et al., 2000) or parasitism (Lehmann and Lehmann, 2000); under these circumstances, the ability of males to synthesize nuptial food gifts, and hence their ability to secure matings, is constrained. However, these studies, although suggestive, cannot be taken as unambiguous evidence that the provision of food gifts actually constrains male mating success in the wild.

The sagebrush cricket, *Cyphoderris strepitans* (Orthoptera: Haglidae), is an ideal candidate with which to assess the intrinsic costs of mating to males because males provide females with a somatic food gift at copulation (Morris, 1979), the magnitude of which is readily amenable to experimental manipulation (Eggert and Sakaluk, 1994; Johnson et al., 1999; Weddle and Sakaluk, 2003). Copulation is initiated when a receptive female climbs onto the dorsum of a male, at which time he attempts to transfer a spermatophore. During copulation, the female feeds on the male's fleshy hind wings and bodily fluids (hemolymph) leaking from the wounds she inflicts (Dodson et al., 1983; Eggert and Sakaluk, 1994; Sakaluk et al., 1995a). Field studies involving the mark-recapture of a large number of males have shown that once a male has

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mated, his probability of obtaining an additional copulation is significantly reduced relative to that of a virgin male securing his first mating (Morris et al., 1989; Snedden, 1995, 1996). Although the reduction in the future mating prospects of mated males is consistent with high intrinsic costs of copulation, we cannot rule out the possibility of an age effect in which males suffer a decline in sexual vigor as they get older.

If the future mating prospects of nonvirgin males are diminished because of the costs of copulation, this could stem either from the resources required to manufacture a new spermatophore or through the energy needed to replenish hemolymph lost through female wing-feeding. The relative costs of producing new spermatophores versus hemolymph replenishment are unknown; in mating trials staged in the laboratory, males are capable of producing two spermatophores within a night even when held without food (Eggert and Sakaluk, 1994; Sakaluk and Ivy, 1999), which suggests that spermatophore production does not constitute a major constraint. One difficulty in distinguishing between these alternatives is that nonvirgin males are invariably disadvantaged in both contexts. In the current study, we circumvented this problem by experimentally depleting virgin males of hemolymph in a way that mimics hemolymph loss of nonvirgin males, without the attendant costs of spermatophore production. If hemolymph lost through nuptial feeding constitutes a significant cost of copulation, we predicted that the experimental depletion of hemolymph in virgin males would lead to a reduction in their mating success relative to unmanipulated virgin males.

## METHODS

### Mass loss at mating

As part of a previous study designed to assess the importance of wing feeding to the subsequent mating behavior of females, we compared the remating propensity of females initially mated to virgin males with intact hind wings, and females initially mated to virgin males whose hind wings had been surgically removed, precluding wing feeding (Johnson et al., 1999). For a subset of these matings, we weighed the males before and after mating as a measure of the mass lost at mating. Although the weight-loss data were not reported in the original study, we include them here because it gives us a crude, but serviceable, means of calibrating the severity of the experimental depletion of hemolymph imposed on males in the current field study (see below). Because males whose wings are removed can only lose mass through transfer of the spermatophore, whereas those whose hind wings are left intact should lose mass both through hemolymph ingestion by females during wing feeding and the transfer of a spermatophore, the difference between treatments in mass lost represents the mass lost through hemolymph ingestion alone. This comparison assumes that mass lost through defecation and dehydration before mating was similar between the two treatments. Males of both treatments were weighed before mating trials and immediately after mating to the nearest 0.01 mg by using a Mettler-Toledo AG245 electronic balance. Many more males with intact wings mated ( $n = 40$ ) than did males lacking hind wings ( $n = 8$ ) because females frequently dismount males lacking hind wings before spermatophore transfer has occurred (Eggert and Sakaluk, 1994). A complete description of the protocol used in staging the matings is described in Johnson et al. (1999).

### Mark-recapture study

Sagebrush crickets occur exclusively in mountainous areas of the western United States, where they are often found in high-

altitude sagebrush meadow habitat (Morris and Gwynne, 1978). Adults become sexually active in late spring, shortly after the snow melts, and remain active for the next 4–6 weeks. The acoustic signals produced by males appear to be the primary means of pair formation (Sakaluk et al., 1995b; Snedden and Irazuzta, 1994; Snedden and Sakaluk, 1992). We conducted a mark-recapture study from 21 May–15 June 2003 in Grand Teton National Park, Wyoming. A rectangular study plot of approximately 3 ha was established in sagebrush meadow habitat adjacent to the Snake River at Deadman's Bar. During the early portion of the breeding season, we attempted to capture and mark all of the virgin males present in the study plot. Males were found at night by orienting to their calls and using head lamps to determine their exact location within a sagebrush bush. The mating status of males was determined by examining their hind wings for the wounds inflicted by females; only virgin males, as evidenced by intact wings, were used in experimental treatments. Each virgin male was placed in a collecting vial, numbered to correspond with a surveyor's flag placed at the capture location, and transported to the University of Wyoming–National Park Service Research Center, approximately 30 km away, for processing.

Captured males were randomly assigned to one of three treatments in which males were experimentally depleted of hemolymph to varying degrees: (1) males from which 5  $\mu$ l of hemolymph were drawn, (2) males from which 10  $\mu$ l of hemolymph were drawn, and (3) sham-operated control males. Hemolymph was extracted from males by making a small incision in the outside margin of one of the hind wings (usually the right), and drawing hemolymph from the wound by using a microhematocrit capillary tube (Weddle and Sakaluk, 2003). In sham-operated control males, a small incision was made in the hind wing of the male, resulting in minimal or no hemolymph loss. This treatment was established as a control for any detrimental effects of handling/surgery experienced by males in the other two treatments. Each male was marked individually with a numbered plastic tag secured to the pronotum with cyanoacrylic glue, and his femora were painted with fluorescent model paint (Testors) of a unique color that designated the treatment to which he had been assigned. Portable ultraviolet lanterns, the illumination of which caused the paint to fluoresce in the dark, were used to facilitate the capture of experimental individuals at night. The following evening at sunset, marked males were returned to their respective points of capture. We marked and released a total of 121 males (40 sham-control, 40 five- $\mu$ l hemolymph-depleted males, 41 ten- $\mu$ l hemolymph-depleted males) over the course of six nights (21–26 May).

After experimental males had been released, males were recaptured and examined for evidence of mating activity regularly over the course of the breeding season, usually every second night, weather permitting. We recaptured, on the average,  $30.0 \pm 5.6$  males ( $\pm$ SE) on any given night (range = 8–61,  $N = 12$  nights). Mating activity was inferred by loss of hind wing material in all treatments. Wing wounds were classified as "fresh" (visibly wet wounds with no discoloration, indicating that the male had mated on the night of capture) or "old" (dry darkened wounds, indicating that the male had mated at least one night previous to the night of capture).

Data were analyzed by using SAS (SAS Institute, 2000).

## RESULTS

### Mass loss at mating

Males with intact wings lost significantly more mass at mating than did males whose hind wings had been surgically removed before copulation (Mann-Whitney test,  $Z = -2.78$ ,  $p = .0054$ ).

The difference between the two treatments in the median mass lost by males was 24.5 mg (median mass loss [ $Q_1$ ,  $Q_2$ ] of intact males = 60.5 mg [46.5, 80.0]; wingless males = 36.0 mg [16.5, 48.0]), which can be taken as a crude estimate of mass lost through female hemolymph ingestion alone. This difference is within the range of the mass loss occurring as a consequence of the experimental depletion of hemolymph imposed on males in the mark-recapture study (see below).

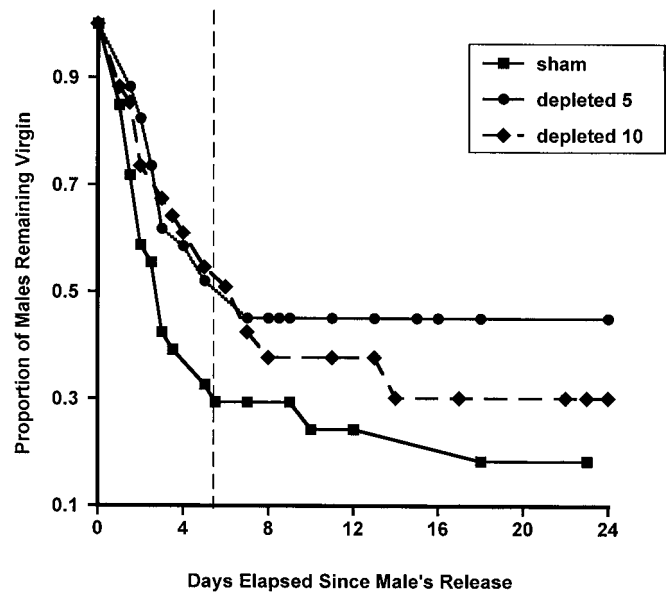
### Mark-recapture study

There was no difference between treatments in the initial mass of males after their capture (ANOVA:  $F_{2,118} = 0.97$ ,  $p = .38$ ); the mean mass of males ( $\pm$ SE) pooled across all three treatments was  $839.3 \pm 6.5$  mg. To compare the mass loss of males experiencing different levels of hemolymph depletion, we used a Kruskal-Wallis nonparametric ANOVA because mass loss was nonnormally distributed within treatments owing to regurgitation of gut contents or defecation of some males during handling (Shapiro-Wilk test for normality,  $p < .05$  for all treatments). Hemolymph depletion resulted in a significant weight loss of experimental males before their release in the field (Kruskal-Wallis  $\chi^2 = 75.1$ ,  $df = 2$ ,  $p < .0001$ ). The median mass loss ( $Q_1$ ,  $Q_3$ ) of males in each of the three treatments was 1.7 mg (1.4, 2.2) for sham-control males, 10.6 mg (8.0, 22.8) for 5- $\mu$ l hemolymph-depleted males, and 14.0 mg (11.9, 29.9) for 10- $\mu$ l hemolymph-depleted males. Post hoc pairwise comparisons using Mann-Whitney  $U$  tests revealed that both 10- $\mu$ l ( $Z = -7.48$ ,  $p < .0001$ ) and 5- $\mu$ l hemolymph-depleted males ( $Z = -7.03$ ,  $p < .0001$ ) lost more mass after treatment than did sham-control males, and that 10- $\mu$ l hemolymph-depleted males lost more mass than did 5- $\mu$ l hemolymph-depleted males ( $Z = -3.02$ ,  $p = .0034$ ).

Eighty-four percent of marked males were recaptured at least once (102/121), and there was no significant difference between treatments in the proportion of males recaptured (sham-control males: 82.5% [33/40]; 5- $\mu$ l hemolymph-depleted males: 87.5% [35/40]; 10- $\mu$ l hemolymph-depleted males: 82.9% [34/41]; likelihood ratio  $\chi^2 = 0.48$ ,  $p = .17$ ). Likewise, the number of times that males were recaptured (excluding those that were never recovered) was similarly homogeneous across treatments (median recapture frequency [range]; control = 3 [1–8]; 5  $\mu$ l = 3 [1–8]; 10  $\mu$ l = 3 [1–9]; Kruskal-Wallis  $\chi^2 = 2.13$ ,  $df = 2$ ,  $p = .34$ ).

Survival of experimental males was determined as the number of nights from the time a male was first captured to the night on which a male was last recaptured. We excluded from this calculation males that were never recovered after their initial release (see above) because these males may have lost their tags or immediately left the study area owing to the trauma of release. We used failure time analysis to compare survival across treatments, using time to last recapture as our measure of “failure time,” and classifying observations of males that were still alive on the last night of the study as “right censored” (i.e., observations for which we can only be certain that a male’s actual survival was greater than his last recorded recapture time). Failure time analysis compares groups’ survival trajectories over all failure times, taking into account not only censored data, but the shapes of the failure-time distributions (Fox, 1993). Omission of such data, as is frequently done in behavioral studies, may lead to a serious bias in comparisons across treatments (Fox, 1993). There was no difference in male survival across treatments (Wilcoxon  $\chi^2 = 0.16$ ,  $p = .92$ ).

Time to mating was determined as the number of nights from the time a male was first released until he was captured as a nonvirgin. Nonvirgin males bearing fresh wing wounds were assumed to have mated on the night they were captured.



**Figure 1**

The proportion of male sagebrush crickets remaining unmated as a function of the time elapsed since their initial release. Sham-control males mated significantly sooner than hemolymph-depleted males (pooled; Wilcoxon  $\chi^2 = 5.06$ ,  $p = .024$ ). The dotted line marks the 5-day interval after the males’ release, the period during which the mating trajectories showed the most rapid divergence in the mating success.

Nonvirgin males bearing old wing wounds were assumed to have mated at least one night previous to their capture or, if they had not been captured in the previous census, we recorded the night of mating as the midpoint of the earliest time they could have mated and the latest time they could have mated. Males that had still not mated by the time of their last capture were treated as “censored” observations. We used failure time analysis to (1) compare time to mating across all three treatments and (2) compare time to mating of control males with that of all hemolymph-depleted males combined. The first analysis showed no significant difference across treatments in the time taken by males to obtain their initial copulations (Wilcoxon  $\chi^2 = 5.18$ ,  $p = 0.075$ ), but inspection of the data in Figure 1 reveals that the lack of a difference can be attributed more to the similarity in the trajectories of the 5- $\mu$ l and 10- $\mu$ l hemolymph-treatments than to the absence of an effect of hemolymph depletion per se. This was confirmed by the second analysis, which showed that sham-control males mated significantly sooner than did hemolymph-depleted males (Wilcoxon  $\chi^2 = 5.06$ ,  $p = .024$ ). We might expect that as the study progressed, hemolymph-depleted males would have been able to require the resources required to replenish the hemolymph lost upon their initial treatment. In support of this possibility, the results shown in Figure 1 shows the greatest divergence in mating success of control males and hemolymph-depleted males in the first 5 days after males’ release (Wilcoxon  $\chi^2 = 6.08$ ,  $p = .013$ ) (dotted line in Figure 1).

### DISCUSSION

Experimental depletion of hemolymph had a significant effect on the subsequent mating success of virgin male sagebrush crickets: control males mated significantly sooner than did males depleted of 5 or 10  $\mu$ l of hemolymph. Although the precise amount of hemolymph ingested by females at mating is unknown, the experimental volumes were

probably less than the amount typically ingested based on a comparison of the mass lost at mating by males whose hind wings had been experimentally removed and mass lost by males whose hind wings had been left intact. We conclude, therefore, that the depletion of hemolymph that occurs through female wing feeding is sufficient by itself to diminish a nonvirgin male's ability to secure another mating. The most plausible explanation for this effect is that nonvirgin males, having lost a substantial portion of their energy reserves through sexual cannibalism by females and the transfer of a spermatophore, are unable to sustain the costly acoustical signaling activity required for the passive attraction of additional females. In support of this hypothesis, electronic assays of male signaling behavior have shown that virgin male *C. strepitans* call for significantly longer durations than do recently mated males, at least in the short term (Sakaluk and Snedden, 1990; Sakaluk et al., 1987); however, the extent to which differences in calling account for differential mating success remains unknown.

Although control males enjoyed a significant mating advantage, there was no discernible difference in the mating trajectories of the 5- $\mu$ l and 10- $\mu$ l hemolymph-depleted males. It may be that there is a threshold for hemolymph depletion beyond which male investment in sexual advertisement is compromised irrespective of the level of depletion. Even in that case, however, we might expect that those males that had been more severely depleted would recover more slowly than would those males experiencing a lower degree of depletion, but this was not evidenced by the mating trajectories of the 5- $\mu$ l and 10- $\mu$ l hemolymph-depleted males. In any event, males do appear to recover from hemolymph depletion as there was no differences in survival across treatments. Moreover, the greatest difference in the decline of the proportion of males remaining virgin occurred in the first 5 days after treatment, whereas the slopes of the trajectories for all three treatments were fairly similar after this interval.

If hemolymph depletion compromises the future mating success of males, why have the hind wings of males apparently been modified so as to promote wing feeding, and why do males permit females to wreak such damage on them during mating? The primary benefit to males appears to be that wing feeding keeps the female preoccupied during the time it takes the male to transfer the spermatophore, a benefit that has been attributed to nuptial food gifts in other insect species (see Sakaluk, 1984; Thornhill, 1976). Eggert and Sakaluk (1994) showed that females were more likely to dismount males before spermatophore transfer had occurred when a male's hind wings had been surgically removed than when they had been left intact. An alternative explanation for wing-feeding is that it represents a form of male parental investment (Morris, 1979). However, if this were true, we might expect males to tolerate wing feeding even after the spermatophore was transferred, but this is normally not the case: immediately after the male transfers the spermatophore, he actively pulls away from the female, terminating hemolymph ingestion even while she persists in her attempt to feed (data not shown).

Partly as a consequence of the hemolymph loss during nuptial feeding, the opportunity for sexual selection in males appears to be reduced relative to species in which males make no such mating investments (Snedden, 1996). Given the constraints placed on a male's future mating potential by the loss of hemolymph and passage of the spermatophore in an initial mating, we might expect that males would be selective of prospective mating partners as has been documented in certain orthopteran species that exhibit a sex-role reversal (see Gwynne, 1981; Gwynne and Simmons, 1990). However, unlike these other species, male *C. strepitans* invariably court

any female with which they have been placed (data not shown), and we have never witnessed males rejecting females that have mounted them, either in the field or the laboratory. This suggests that notwithstanding the limited number of matings that males can expect to secure over their lifetime (Snedden, 1996), the intensity of sexual selection appears to be higher in males than in females.

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## REFERENCES

- Bonduriansky R, 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol Rev* 76:305–339.
- Chapman T, Arnqvist G, Bangham J, Rowe L, 2003. Sexual conflict. *Trends Ecol Evol* 18:41–47.
- Dewsbury DA, 1982. Ejaculate cost and male choice. *Am Nat* 119:601–610.
- Dodson G, Morris GK, Gwynne DT, 1983. Mating behavior in the primitive orthopteran genus *Cyphoderris* (Haglidae). In: *Orthopteran mating systems: sexual competition in a diverse group of insects*, (Gwynne DT, Morris GK, eds). Boulder, Colorado: Westview Press; 305–318.
- Eggert A-K, Sakaluk SK, 1994. Sexual cannibalism and its relation to male mating success in sagebrush crickets, *Cyphoderris strepitans* (Orthoptera: Haglidae). *Anim Behav* 47:1171–1177.
- Engqvist L, Sauer KP, 2001. Strategic male mating effort and cryptic male choice in a scorpionfly. *Proc R Soc Lond B* 268:729–735.
- Fox GA, 1993. Failure-time analysis: emergence, flowering, survivorship and other waiting times. In: *Design and analysis of ecological experiments*, (Scheiner SM, Gurevitch J, eds). New York: Chapman & Hall; 253–289.
- Gilg MR, Kruse KC, 2003. Reproduction decreases lifespan in the giant waterbug (*Belostoma flumineum*). *Am Midl Nat* 149:306–319.
- Gwynne DT, 1981. Sexual difference theory: mormon crickets show role reversal in mate choice. *Science* 213:779–780.
- Gwynne DT, Simmons LW, 1990. Experimental reversal of courtship roles in an insect. *Nature* 346:172–174.
- Jia Z, Jiang Z, Sakaluk SK, 2000. Nutritional condition influences investment by male katydids in nuptial food gifts. *Ecol Entomol* 25: 115–118.
- Johnson JC, Ivy TM, Sakaluk SK, 1999. Female remating propensity contingent on sexual cannibalism in sagebrush crickets, *Cyphoderris strepitans*: a mechanism of cryptic female choice. *Behav Ecol* 10:227–233.
- Johnstone RA, Keller L, 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am Nat* 156:368–377.
- Lehmann GUC, Lehmann AW, 2000. Spermatophore characteristics in bushcrickets vary with parasitism and remating interval. *Behav Ecol Sociobiol* 47:393–399.
- Martin OY, Hosken DJ, 2004. Copulation reduces male but not female longevity in *Saltella sphondylii* (Diptera: Sepsidae). *J Evol Biol* 17: 357–362.
- Morris GK, 1979. Mating systems. paternal investment and aggressive behavior of acoustic Orthoptera. *Fla Entomol* 62:9–17.
- Morris GK, Gwynne DT, 1978. Geographical distribution and biological observations of *Cyphoderris* (Orthoptera: Haglidae) with a description of a new species. *Psyche* 85:147–167.

- Morris GK, Gwynne DT, Klimas DE, Sakaluk SK, 1989. Virgin male mating advantage in a primitive acoustic insect (Orthoptera: Haglidae). *J Insect Behav* 2:173–185.
- Sakaluk SK, 1984. Male crickets feed females to ensure complete sperm transfer. *Science* 223:609–610.
- Sakaluk SK, Bangert PJ, Eggert A-K, Gack C, Swanson LV, 1995a. The gin trap as a device facilitating coercive mating in sagebrush crickets. *Proc R Soc Lond B* 261:65–71.
- Sakaluk SK, Ivy TM, 1999. Virgin-male mating advantage in sagebrush crickets: differential male competitiveness or non-independent female mate choice? *Behaviour* 136:1335–1346.
- Sakaluk SK, Morris GK, Snedden WA, 1987. Mating and its effect on acoustic signalling behavior in a primitive orthopteran, *Cyphoderris strepitans* (Haglidae): the cost of feeding females. *Behav Ecol Sociobiol* 21:173–178.
- Sakaluk SK, Snedden WA, 1990. Nightly calling durations of male sagebrush crickets, *Cyphoderris strepitans*: size, mating and seasonal effects. *Oikos* 57:153–160.
- Sakaluk SK, Snedden WA, Jacobson KA, Eggert A-K, 1995b. Sexual competition in sagebrush crickets: must males hear calling rivals? *Behav Ecol* 6:250–257.
- SAS Institute, 2000. SAS/STAT user's guide, version 8. Cary, North Carolina: SAS Institute.
- Snedden WA, 1995. Correlates of male mating success and their implications for sexual selection in a primitive orthopteran insect (PhD dissertation). Toronto: University of Toronto.
- Snedden WA, 1996. Lifetime mating success in male sagebrush crickets: sexual selection constrained by a virgin male mating advantage. *Anim Behav* 51:1119–1125.
- Snedden WA, Irazuzta S, 1994. Attraction of female sagebrush crickets to male song: the importance of field bioassays. *J Insect Behav* 7: 233–236.
- Snedden WA, Sakaluk SK, 1992. Acoustical signalling and its relation to male mating success in sagebrush crickets. *Anim Behav* 44:633–639.
- Sparkes TC, Keogh DP, Orsburn TH, 2002. Female resistance and mating outcomes in a stream-dwelling isopod: effects of male energy reserves and mating history. *Behaviour* 139:875–895.
- Thornhill R, 1976. Sexual selection and nuptial feeding behavior in *Bittacus apicalis* (Insecta: Mecoptera). *Am Nat* 110:529–548.
- Trivers RL, 1972. Parental investment and sexual selection. In: *Sexual selection and the descent of man, 1871–1971* (Campbell B, ed). Chicago: Aldine-Atherton; 136–179.
- Vahed K, 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biol Rev* 73:43–78.
- Weddle CB, Sakaluk SK, 2003. Ingestion of male hemolymph and mating propensity of female sagebrush crickets: no evidence of a male-derived anti-aphrodisiac. *Anim Behav* 65:83–88.
- Wedell N, Gage MJG, Parker GA, 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol Evol* 17:313–320.
- Wolfner MF, 2002. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* 88:85–93.
- Woods WA Jr, Stevenson RD, 1996. Time and energy costs of copulation for the sphinx moth, *Manduca sexta*. *Physiol Zool* 69:682–700.