Coping with the cold: temperature and mating activity of male sagebrush crickets *Cyphoderris strepitans* (Orthoptera: Haglidae)

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Abstract. The effect of temperature on male sexual displays is well documented but its role in mediating the differential mating success of males is less well established. Male sagebrush crickets *Cyphoderris strepitans* Morris and Gwynne occur in high-elevation sagebrush meadows in mountainous areas of the western U.S.A., and often are observed singing at temperatures as low −8 to −4 °C to attract sexually receptive females. Males exhibit differential mating success based on their previous mating experience: virgin males have a higher probability of obtaining a mating than do non-virgin males of securing an additional mating. This differential mating success is mediated in part by an unusual form of nuptial feeding behaviour. Females feed on males' hind wings at mating and the costs imposed on males as a consequence of this behaviour constrain male mating success. In the present study, to determine whether sagebrush crickets are capable of some degree of thermoregulation, and also to investigate whether this ability is influenced by male mating experience, thoracic temperatures of males of varying mating status were measured in relation to ambient temperature throughout the breeding season. A full factorial model examining the effect of site of capture, mating status, body mass and ambient temperature reveals that only ambient temperature has a significant effect on thoracic temperature after controlling for all other factors. It is concluded that differences in the calling times of virgin and non-virgin males and their ability to secure mates does not result from differences in their ability to cope physiologically with low temperatures.

Key words. Calling behaviour, *Cyphoderris strepitans*, Haglidae, mating success, nuptial food gift, sagebrush crickets, thermoregulation.

Introduction

Temperature has a pervasive effect on the physiology and behaviour of ectotherms, including many insects (Angilletta *et al.*, 2002). The primary means of ectothermic regulation in insects involves the exploitation of sunlight (May, 1979). This means of thermoregulation is largely unavailable to nocturnally active insects such as crickets. However, even nocturnal insects may thermoregulate to some degree by seeking out warmer parts of their environment (e.g. rocks that retain

heat longer; Hedrick *et al.*, 2002). In addition, some insects, including several orthopteran species, are capable of endothermic regulation and, in these species, the primary source of metabolic heat is the flight musculature (May, 1979, 1985; Heinrich, 1993). Although the effect of temperature on male sexual displays and mating behaviour is particularly well documented (May, 1979, 1985; Heinrich, 1993), its role in mediating the differential mating success of males is less well established (Willmer, 1991).

The sagebrush cricket *Cyphoderris strepitans* Gwynne and Morris (Orthoptera: Haglidae) is one of only three extant species of hump-winged grigs in North America, which comprise relatively obscure ensiferans that are restricted to mountainous areas of western North America (Morris & Gwynne, 1978; Kumala *et al.*, 2005). *Cyphoderris strepitans* occurs in

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high-elevation sagebrush meadows nestled within coniferous forests in Wyoming and Colorado (Morris & Gwynne, 1978). In Grand Teton National Park, where the majority of field studies on *C. strepitans* are conducted (Morris *et al.*, 1989; Sakaluk *et al.*, 1995; Johnson *et al.*, 1999; Weddle & Sakaluk, 2003), sexual activity commences in mid-May, which is an arduous time of year at the higher elevations when patches of snow remain scattered on the ground and night-time temperatures frequently fall below freezing. On each night of the breeding season, males emerge from the ground cover to secure a calling perch in sagebrush or lodgepole pine, where they sing to attract sexually receptive females (Snedden & Sakaluk, 1992; Snedden & Irazuzta, 1994). Males are observed to sing at temperatures as low as −8 to −4 °C, which is an unprecedented feat for an acoustic insect (Morris & Gwynne, 1978; Dodson *et al.*, 1983).

Once a calling male has attracted a female, the female mounts the male dorsally to initiate an approximately 3–5 min mating that ends with the transfer of a spermatophore to the female (Dodson *et al.*, 1983). During the time that females remain mounted on males, they feed on the males' fleshy hind wings and ingest haemolymph seeping from the wounds that they inflict. Males exhibit differential mating success based on their previous mating experience: virgin males have a higher probability of obtaining a mating than do non-virgin males of securing an additional mating (Morris *et al.*, 1989; Snedden, 1996). The decreased likelihood of non-virgin mating apparently arises from the loss of haemolymph and costly immune responses that occur as a result of wing wounding during copulation (Sakaluk *et al.*, 2004; Leman *et al.*, 2009). However, it also is possible that the thermoregulatory properties of males are altered by the loss of haemolymph.

The present study addresses two main questions with respect to the thermal biology of *C. strepitans* by measuring thoracic temperatures of males throughout the breeding season and relating these to male mating status: (i) is there any evidence that sagebrush crickets are capable of some degree of thermoregulation, as documented in other orthopteran species (May, 1979, 1985; Heinrich, 1993) and (ii) do virgin and non-virgin males differ in the manner in which their thoracic temperature varies with ambient temperature, which might contribute to the mating advantage of virgin males?

Materials and methods

The study was conducted from 21 May to 23 June 1991 in Grand Teton National Park, Wyoming. Sagebrush crickets were captured at two sites within the park: Dead Man's Bar and Pacific Creek. Males were found at night by orienting to their calls and using head lamps to determine their exact location within a sagebrush bush. Females were randomly encountered, usually in bushes already occupied by calling males.

Temperature measurements were made using an Omega® temperature microprocessor (model no. HH23; Omega Engineering, Inc., Stamford, Connecticut) equipped with two probes: a mini-hypodermic probe for determining the tho-racic temperature of crickets (model no. HYP-30-1/2-T-G-60-SMP-M, type 'T' calibration (copper-constantan), needle outer diameter = 0.3 mm); and a fine wire thermocouple (model no. 5SC-TT-T-36-72, type 'T' calibration, outer diameter = 0.13 mm) for determining the ambient temperature at the precise location that the subject was found calling (or perched in the case of females). Upon locating a cricket, it was induced to fall or jump into a plastic cup lined with Styrofoam (Dow Chemical Company, Midland, Michigan) by gently touching the branch or twig upon which it was perched. A blunt Styrofoam probe was used to hold the cricket in place in a depression carved into the Styrofoam-lined lining of the cup when its thoracic temperature was measured. The mini-hypodermic probe was inserted into the thorax approximately half-way down the length of the probe (i.e. 7.5 mm), but no further, because a Styrofoam 'spacer' inserted around the probe's needle maintained a constant insertion depth. It took typically approximately 5 s to measure a cricket's thoracic temperature once it had been coaxed into the Styrofoam-lined cup. Immediately after the thoracic temperature had been measured, ambient temperature was measured by positioning the end of the thermocouple directly above the cricket's original perch. After the ambient and thoracic temperatures had been recorded, the height of the male's calling perch above the ground was measured to the nearest centimetre using a metre stick.

The mating status of males was determined by examining their hind wings for the wounds inflicted by females. Virgin males were identified by their intact wings, whereas non-virgin males were identified by wounds or scars visible on their hind wings. Wing wounds were classified as 'fresh' (i.e. visibly wet wounds with no discoloration, indicating that the male had mated on the night of capture) or 'old' (i.e. dry, melanized wounds, indicating that the male had mated at least one night previous to the night of capture). The mating status of females could not be determined.

Crickets whose thoracic temperature had been measured were placed in uniquely numbered collecting vials and transported to the University of Wyoming–National Park Service Research Center, approximately 30 km away, where they were weighed to the nearest 0.2 mg using a Roller-Smith torsion balance (Roller-Smith, Inc., Bethlehem, Pensylvania). The next evening, crickets were released at the site at which they had been captured but were first marked with a spot of red nail polish so that, if these individuals were encountered again, repeated measurements of the same individuals could be avoided.

In total, 207 different crickets were measured: 77 at Dead Man's Bar (three females, 47 virgin males, 23 old-wound males, four fresh-wound males) and 130 at Pacific Creek (four females, 43 virgin males, 82 old-wound males, one fresh-wound male). All statistical tests were performed using SAS, version 9.1 (SAS Institute, 2004).

Results

A two-way analysis of variance examining the effect of site (Dead Man's Bar or Pacific Creek) and mating status (virgin

or old-wound males) on male body mass is shown Table 1. Freshly-wounded non-virgin males were excluded from this analysis because of the small sample size for this group. There was a significant effect of mating status on male body mass ($F_1 = 12.69$, $P = 0.0005$) but not of locality ($F_1 = 1.08$, $P = 0.30$); there was no significant interaction between the two main effects ($F_1 = 1.08$, $P = 0.30$). The mean mass $\pm$ SE of virgin males (681.0 $\pm$ 6.8 mg, $n = 90$, range = 549.8–851.6) was significantly greater than that of old-wound (non-virgin) males (651.5 $\pm$ 6.1 mg, $n = 105$, range = 420.4–810.4). The average mass of females (919.0 $\pm$ 42.8 mg, $n = 7$, range = 733.6–1,087.8) was significantly greater than that of males pooled across all mating categories (665.5 $\pm$ 4.7 mg, $n = 200$, range = 420.4–851.6; $F_{1,205} = 94.17$, $P < 0.0001$).

An analysis of covariance examining the effect of site (Dead Man's Bar or Pacific Creek) and ambient temperature on the height at which males called is shown in Table 2. There was a significant interaction between locality and ambient temperature in their effect on male calling height ($F_1 = 7.39$, $P = 0.0072$). Therefore, the effect of temperature on male calling height was assessed separately for each locality using linear regression. At Dead Man's Bar, the height at which males established their calling perches increased with increasing ambient temperature ($r^2 = 0.33$, $F_{1,68} = 34.08$, $P < 0.0001$), whereas, at Pacific Creek, temperature had no significant effect on the height at which males called ($r^2 = 0.02$, $F_{1,123} = 2.83$, $P = 0.095$). The average height at which males established calling perches at Dead Man's Bar was 25.1 $\pm$ 1.8 cm ($n = 74$, range = 0–66), whereas, at Pacific Creek, it was 32.0 $\pm$ 1.8 cm ($n = 126$, range = 0–129).

Males consistently maintained a higher thoracic temperature than the ambient temperature (Fig. 1). A full factorial model examining the effect of site of capture, mating status, body mass and ambient temperature explained a significant proportion of the variation in male thoracic temperature ($r^2 = 0.91$, $F_{15,179} = 120.3$, $P < 0.0001$) but only ambient temperature had a significant effect on thoracic temperature after controlling for all other factors ($F_1 = 7.86$, $P = 0.0056$). Thoracic temperature increased linearly with ambient temperature (linear regression: $F_{1,193} = 1,785.6$, $P < 0.0001$; Fig. 1) but the slope of the regression was significantly greater than unity ($b = 1.136 \pm 0.027$, $t_{193} = 5.064$, $P < 0.001$).

Table 1. Two-factor analysis of variance showing the effect of collecting locality (Dead Man's Bar or Pacific Creek) and mating status (virgin or non-virgin male) on male body mass.

Source	d.f.	SS	MS	F	P
Model	3	51 424	17 141	4.24	0.0063
Error	191	771 464	4039		
Total	194	822 889			
		Type III SS			
Locality	1	4375	4375	1.08	0.2993
Mating status	1	51 248	51 248	12.69	0.0005
Locality × Status	1	5609	5609	1.39	0.2401

d.f., degrees of freedom.

Table 2. Analysis of covariance showing the effect of collecting locality (Dead Man's Bar or Pacific Creek) and ambient temperature (covariate) on the height at which males called.

Source	d.f.	SS	MS	F	P
Model	3	9078	3026	9.08	< 0.0001
Error	191	63 631	333		
Total	194	72 709			
		Type III SS			
Locality	1	2612	2612	7.84	0.0056
$T_{ambient}$	1	6784	6784	20.36	< 0.0001
Locality × $T_{ambient}$	1	2462	2462	7.39	0.0072

d.f., degrees of freedom.

Females were excluded from this analysis because too few females were captured.

Discussion

Male sagebrush crickets consistently maintain a higher thoracic temperature than the ambient temperature and appear to be unusually tolerant of the cold. The slope of the regression of male thoracic temperature against ambient temperature is significantly greater than one indicating that the difference between the thoracic temperature of male sagebrush crickets and ambient temperature increases with increasing temperature. Normally, a slope of 1.0 is taken as evidence of a lack of thermoregulation, whereas a slope less than one may indicate some degree of thermoregulation (May, 1985). It is doubtful, however, that the observed relationship in *C. strepitans* in the present study reflects any thermoregulatory ability on the part of male*s*. Instead, it is more likely that, with increasing temperature, the muscles that power stridulation in males operate more efficiently, permitting greater calling effort. In some
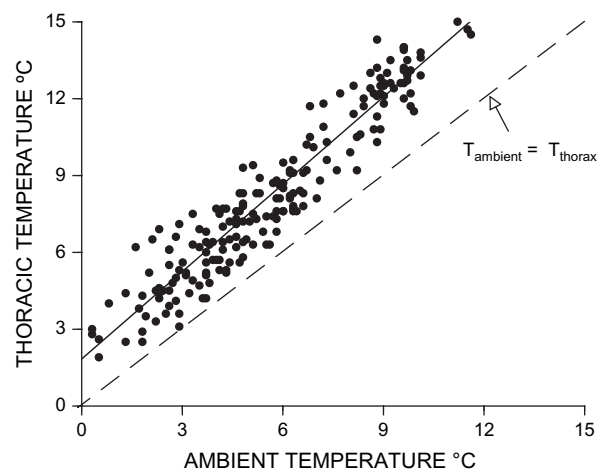

Fig. 1. Regression of thoracic temperature of male sagebrush crickets *Cyphoderris strepitans* on ambient temperature ($r^2 = 0.90$, $F_{1,193} = 1,785.6$, $P < 0.0001$).

katydids (Orthoptera: Tettigoniidae), for example, temperature has a marked influence on the contraction kinetics of the singing muscles, and muscle contractions become more rapid with increasing temperature (Josephson, 1973, 1981; Heinrich, 1993). If this is also true for male *C. strepitans* over the range of temperatures measured in the field, then the increased deviation of thoracic temperature from ambient temperature at higher temperatures may simply reflect the additional heat generated by the higher rate of muscle contraction.

In contrast to our original expectation, male mating status has no significant effect on thoracic temperature, nor is there any significant interaction between mating status and ambient temperature in their influence on thoracic temperature. Hence, any differences in the calling times of virgin and non-virgin males (Sakaluk *et al.*, 1987; Sakaluk & Snedden, 1990), as well as their ability to secure mates (Morris *et al.*, 1989; Snedden, 1996), apparently do not result from differences in their ability to cope physiologically with low temperatures. Non-virgin males do, however, have significantly lower body mass, which presumably reflects the loss of haemolymph and the transfer of the spermatophore at mating (Dodson *et al.*, 1983; Sakaluk *et al.*, 2004). The loss of mass at mating could in theory influence heat retention because, all else being equal, insects of smaller mass cool more rapidly than those of larger mass (Bartholomew, 1981). However, the difference in body mass between virgin and non-virgin males evidently is insufficient to affect their thoracic temperatures and, even within males of the same mating status, there is no discernible effect of body mass on thoracic temperature.

One aspect of male singing behaviour that is influenced by ambient temperature is the height at which males call, although this is true only at Dead Man's Bar. Here, males are found to sing higher in the foliage with increasing ambient temperature, whereas no such effect is observed at Pacific Creek. In theory, an interaction such as this could occur if there were significant differences in the height of vegetation at the two sites; for example, if sagebrush was significantly shorter at Pacific Creek, the absence of an effect of temperature on male calling height could be the result of a constraint on the maximum height at which a male could establish a calling perch. However, the results of the present study show that no such constraint exists and, indeed, the mean calling height is greater at Pacific Creek than at Dead Man's Bar. Instead, it is suggested that this interaction occurs as an incidental consequence of the times at which males at the two sites were sampled. Because the population at Dead Man's Bar typically becomes active earlier in the spring than the population at Pacific Creek (S. K. Sakaluk, unpublished data), males at the Dead Man's Bar population are sampled earlier, on average, than those at Pacific Creek [mean Julian sampling date (range): Dead Man's Bar = 152 (141–161), Pacific Creek = 167 (159–174)]. This means that males at Dead Man's Bar first begin calling when night-time temperatures are lower relative to when males begin calling at Pacific Creek. If there is a threshold temperature above which males readily climb up into the foliage to sing, then the absence of an effect of temperature at Pacific Creek could be the result of nightly temperature usually being above the threshold

required to elicit climbing behaviour (i.e. males at Pacific Creek readily attain their maximum calling height); by contrast, when nightly temperatures are close to freezing (i.e. below the hypothetical threshold), as they frequently are early in the season at Dead Man's Bar, then an effect of temperature on calling height would be expected to emerge.

Why is the breeding season of *C. strepitans* restricted to the spring, a time of year that, in the mountainous regions in which it occurs, can pose a real challenge to any true poikilotherm, and also represents a time when many other insects have not yet become active? One possibility is that the life history of *C. strepitans* has evolved, in part, as a mechanism to avoid temporal overlap with potential predators. For example, population densities of montane voles in Grand Teton National Park (and, presumably, insectivorous rodents as well) do not peak until mid-summer (Pinter, 1986, 1988), long after the mating activity of sagebrush crickets has ceased. That predation has been important in shaping the behaviour of *C. strepitans* is demonstrated by a suite of conspicuous anti-predatory adaptations. When handled, male sagebrush crickets frequently bite or flip over on their backs and produce loud disturbance sounds and simultaneously wave their legs. Alternatively, they feign death if they succeed in eluding capture by falling from their calling perch (Sakaluk, 1991). Owls are also reported to feed exclusively on *Cyphoderris* at certain localities in the Cascade Mountains of Oregon (Marshall, 1942); indeed, at the Dead Man's Bar site, one member of a pair of nearby breeding owls was observed to fly conspicuously between bushes containing singing males, suggesting that the owls are attracted to their sounds and capture them on the wing (W. A. Snedden, personal communication). Based on these observations, it is plausible that the unusual life history of *C. strepitans*, at least as reflected in the timing of its reproductive activity, has been shaped, in part, by predation pressure.

Acknowledgements

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