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The physiological cost of courtship: field cricket song results in anaerobic metabolism

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During courtship interactions, males typically perform displays that are assumed to demonstrate some aspect of their quality. While some displays are elaborate and spectacular in appearance, others involve comparatively simple repetitive actions. The functions of these dynamic repeated courtship displays are hypothesized to fall into two broad categories. Either the signal is repeated as a process of validation to counter errors in transmission, or the process of repetition itself advertises the ability to bear signalling costs. The function of any repetitive courtship display can thus be identified by investigating the nature of production costs as well as the pattern of repetition. In the present study, I investigated the function of signal repetition using a commonly used organism, the Mediterranean field cricket, *Gryllus bimaculatus*. Male *G. bimaculatus* court females by producing acoustic signals using rapid, repeated movements of their wings. I tested for the presence of energetic costs by analysing haemolymph lactate concentrations after time-controlled courtship interactions. Males that had performed 5 min of courtship were found to have higher levels of haemolymph lactate relative to controls. Furthermore, those individuals producing more rapid song had higher levels of haemolymph lactate. The song produced by courting crickets therefore incurs significant energetic costs, and probably serves to advertise the energetic cost capacity of the calling male. Thus, I confirm that stridulation is an energetically costly signal and demonstrate that anaerobic metabolism appears to be a significant cost of repeated courtship displays.

During courtship interactions, the courting individual usually signals its quality as a potential mate, in its capacity to provide resources (Howard, 1978; Thornhill, 1976), parental care (Knapp & Kovach, 1991) or simply 'good genes' with which the courted individual should want to provision its young (Hamilton & Zuk, 1982). Such communication may involve elaborate multimodal displays involving colour, sound and motion cues to convey information about male quality to the female; these displays occur in a variety of animals from salticid spiders (Girard, Kasumovic, & Elias, 2011) to peacocks (Dakin & Montgomerie, 2009). The study of such systems has received enormous interest historically, but it has only recently been hypothesized that simple repetitive displays may be of comparable importance in mate choice (Mowles & Ord, 2012). The emphasis here is on identifying why courtship displays are performed repeatedly.

The functions of repetitive displays are hypothesized to fall into two broad categories. Repeated displays may be advantageous to animals by ensuring that information is accurately transferred (i.e. any error in transmission is reduced by repeating the signal; see Enquist & Leimar, 1983; Enquist, Leimar, Ljungberg, Mallner, & Segerdahl, 1990), or the repetitive nature of the display may itself be informative of the signaller's ability to withstand signalling costs. The costs of signal production may be extrinsic (i.e. circumstantial costs), occurring as a result of producing the display, such as reduced time for performing other activities or the risk of attracting predators (Tuttle & Ryan, 1981; Zuk, Rotenberry, & Tinghitella, 2006), or may be intrinsic, relating directly to the individual's ability to produce the display. The display may thus be limited by the physiological capacity of the individual to produce the repeated elements of the dynamic display (Kotiaho et al., 1998; Mappes, Alatalo, Kotiaho, & Parri, 1996). Endurance capacities, or 'stamina', may be informative in mate choice as they communicate the quality of the signaller by demonstrating (1) its energy reserves and thus its capacity to gather resources (which are then used in the production of display), or (2) its ability to perform well in other ecologically relevant activities (Irschick & Garland, 2001). A high degree of stamina may confer a greater ability to disperse, search for resources, search for mates, beat rivals and evade predators (Leal, 1999). These are qualities that are likely to confer survivability to potential offspring (see Mowles & Ord, 2012), and therefore repetitive displays relying on signaller stamina should be attended to by females.

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Courtship displays are thus produced repetitively because the signal either needs to be repeated to reduce error in transmission, in which case the signal is produced at a constant rate (Enquist et al., 1990; Enquist & Leimar, 1983; Mowles & Ord, 2012), or because some aspect of repetition itself contains the information being communicated, in which case signal escalation is predicted (Mowles & Ord, 2012; Payne & Pagel, 1997). In the present study I aimed to highlight the possible function of courtship signal repetition in a commonly used model organism, the Mediterranean field cricket, *Gryllus bimaculatus*. In this species, males court females by producing repetitive acoustic signals using movements of their wings, termed stridulations. This courtship song is composed of a sequence of soft, discrete 'ticks' created by bringing the wings together in individual movements (Rantala & Kortet, 2003), interspersed with occasional trills created by vibrating the wings rapidly. It has been shown that females prefer males that produce more vigorous courtship songs (Rantala & Kortet, 2003; Rebar, Bailey, & Zuk, 2009; Wagner & Reiser, 2000) and that these songs are energetically costly to produce as respiration increases during calling (Hack, 1998). What remains to be seen is the extent to which these energetic costs drive the signalling males to their aerobic limits and whether the accumulation of costs is related to the vigour of the display, consistent with a signal of stamina (Mowles & Ord, 2012; Payne & Pagel, 1997). We can investigate this by looking at whether signalling males have exceeded their aerobic capacities, indicated by the accumulation of lactic acid, the metabolic by-product of anaerobic respiration.

Anaerobic respiration is an extremely costly process owing to the production of lactic acid (and the lactate ions readily formed from this), which is damaging to tissues and causes muscle fatigue. Furthermore, anaerobic respiration is less efficient than aerobic respiration as it liberates significantly less energy as ATP from each molecule of glucose (Sadava, Heller, Orians, Purves, & Hillis, 2008). Animals should thus try to avoid anaerobic respiration as much as possible as, even though it may confer an additional opportunity to continue displaying, the resulting fatigue will limit the scope for performing other activities postdisplay. Lactic acid has successfully been used as an index of anaerobic respiration in other signalling systems (e.g. the aggressive repeated display of shell rapping in shell-fighting hermit crabs, *Pagurus bernhardus*, Briffa & Elwood, 2001; and the threat posturing of side blotched lizards, *Uta stansburiana*, Brandt, 2003). My aim in this study was thus to determine the function of the courtship song of *G. bimaculatus*, whether it constitutes an energetically costly display and what qualities this might communicate about the male.

METHODS

Study Organisms

Mediterranean field crickets were maintained in a laboratory colony established at the University of Nottingham, U.K., using crickets sourced from the University of Derby, U.K. The crickets were kept at 28 °C in a temperature-controlled environment under a 12:12 h light:dark regime and were fed ad libitum a diet of dried rabbit food, with supplementary fresh vegetables (see Wynn & Vahed, 2004). Crickets were kept in continuous culture, and were separated into single-sex tanks once it was possible to identify males and females based on the appearance of visible ovipositors, which are identifiable on females at approximately 4 weeks post-hatching. This single-sex storage method ensured that only virgin crickets were used in the experiments. The crickets used in the experimental trials were all aged between 4 and 12 days after eclosion. Interactions were all staged at a constant temperature of 28 °C and involved only crickets free from obvious physical damage.

Behavioural Trials

Trials were conducted using three treatment groups designed to ascertain the costliness of male song and the natural level of courtship effort required to stimulate mating. These were (1) courtship encounters involving a tethered stimulus female resulting in prolonged song, (2) mating trials involving free female choice and (3) control trials that did not include a female.

Courtship encounters ($N = 34$) were staged in clear acrylic tanks (18×10 cm and 13 cm high) with a sand substrate, divided in half by an opaque partition. A male cricket was placed in one half of the tank, and allowed to acclimate overnight (for at least 16 h). Twenty minutes before the observation period, a tethered female cricket was introduced into the other half of the arena. The female had a 5 cm long cotton tether attached to her pronotum by superglue, the other end of which was attached to a cardboard anchor placed beneath the substrate. When the dividing partition was removed, the male attempted to court the female, but the tether prevented the female from mating with the male. Each male was allowed to court for 5 min before the encounter was terminated. Mating trials ($N = 42$) were staged as above, except that a male and female cricket were placed in each tank, one on each side of the partition, and each was allowed to acclimate overnight (for at least 16 h). After the acclimation period, the opaque divider was removed and the crickets were allowed to interact. Each interaction was terminated once the female had decided to mate with the male (but before mating occurred). This ensured that any physiological differences between individuals were a result of courtship performance, and were not due to engaging in mating. Control trials ($N = 31$) were also staged, in which a female was not included in the arena. As with courtship encounters, the partition was removed, and the male allowed to explore the whole arena for a 5 min period, but in the absence of a female with which to interact. Each interaction was recorded from above using a Sony HDRXR160EB Handycam Camcorder.

Immediately after each encounter, each male was placed in a 2.0 ml plastic tube and humanely killed by rapidly freezing in liquid nitrogen. Each cricket was then stored at −80 °C until it was analysed for physiological costs. The widths of male head capsules were subsequently obtained by first photographing each cricket alongside a scale bar using a Fujifilm Finepix S7000 camera on the macro setting, then measuring the head capsule width via image analysis using ImageJ software (http://rsbweb.nih.gov/ij). This process provided a measure of the size of each animal, which may influence haemolymph lactate as a relationship between size and lactate has been observed in other organisms (e.g. Doake, Scantlebury, & Elwood, 2010). After the courtship encounters, the tethers were gently removed from each female without damaging them, after which each female was added to the breeding colony.

Physiological Analysis

Individual crickets were removed from the freezer and allowed to defrost gradually at room temperature. A single front leg was removed from each cricket at the trochanter and the cricket held over a 0.5 ml Eppendorf tube such that the resulting bead of haemolymph (ca. 10 µl) could be captured. The haemolymph was then analysed for lactate using a standard laboratory kit (Trinity Biotech PLC, Bray, Co Wicklow, Ireland).

Quantifying Signal Escalation

To quantify the vigour of courtship accurately, the digital recordings of each encounter were played back at half-speed and scored using JWatcher version 1.0 event recording software