Endurance in exercise is associated with courtship call rate in decorated crickets, *Gryllodes sigillatus*

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ABSTRACT

Question: Is sexual signalling (courtship call rate) determined by physiological fitness (energy metabolism at rest), endurance during physically demanding activity, an aspect of immune defence (lytic activity) or body mass?

Organism: Pedigree laboratory population of decorated crickets (*Gryllodes sigillatus*).

Methods: Behavioural trial of male courtship call rate and measurements of males' physiological performance. Covariance analysis exploring the determinants of courtship call rate.

Results: We found that endurance was strongly positively associated with the courtship call rate. However, neither the lytic activity nor the resting metabolic rate correlated with courtship call rate. Together with body mass and family effects, endurance explained over 77% of the variance in courtship call rate. Hence, only physically fit individuals can perform sexual displays well.

Keywords: condition, energy use, exercise, immunity, physiology, quality.

INTRODUCTION

Sexual signalling has traditionally been thought to convey information of male quality to females, benefiting females directly or indirectly (e.g. Kirkpatrick and Ryan, 1991; Kokko *et al.*, 2006). The theoretical approaches often address the link between sexual signalling and individual quality, viability or condition (e.g. Cotton *et al.*, 2004), but these vague concepts have rather little to offer the empiricist. Fortunately, recently there have been attempts to conceptualize 'condition' and 'quality' in a more empirically approachable way in terms of parasite resistance, energy allocation patterns, and whole-body performance (Siva-Jothy and Skarstein, 1998; Tomkins *et al.*, 2004; Hunt *et al.*, 2004; Irschick *et al.*, 2008; Ketola and Kotiaho, 2009).

Much of an individual's 'quality' is determined by that individual's relationship to its immediate biotic and abiotic environment. One of the most important biotic interactions is resistance against parasites and pathogenic diseases. This is also expected to be reflected in

sexual signals (Lawniczak *et al.*, 2006). Theoretically, resistance against the most prevalent parasites in the environment has been proposed to explain most of an individual's quality as well as sexual signals (Hamilton and Zuk, 1982; Taskinen and Kortet, 2002). In addition to this 'lock and key' relationship between the host and the parasite (e.g. Agraval and Lively, 2002), parasite resistance can also be viewed as the result of a more dynamic process, whereby allocating resources to resistance causes differences in quality, and thus variation also in sexual signals and courtship call rate. This might be mediated via pure resource allocation (Sheldon and Verhulst, 1996) or via sex hormones or other similar biochemicals affecting both sexual signal and immunological parasite resistance [i.e. the immunocompetence handicap hypothesis (Folstad and Karter, 1992; Wedekind and Folstad, 1994)].

Life-history theories emphasize the role of resource allocation in explaining the evolution of life histories (Roff, 2002). One of the most acknowledged resources is energy and its utilization capacity (e.g. Parsson, 2005; Ketola and Kotiaho, 2009). Therefore, all traits involved in mediating energy use should play an important role in natural selection and sexual selection. In particular, resting metabolic rate is expected to enable energetically costly activities and determine the maintenance costs of living (see Nilsson, 2002). Traditionally, a high resting metabolic rate has been thought to result from larger size of the intestines, and associated greater energy need. The larger intestines are thought to allow high energy release for various traits, despite higher maintenance metabolism (Konarzewski and Diamond, 1995). However, high-maintenance metabolism can also be considered to constrain energy use on activities such as sexual signalling (e.g. Nilsson, 2002). Even though sexual signals are often reported to consume energy (reviewed by Kotiaho, 2001), little is known about the detailed effects of energetic traits, such as maintenance metabolism, on sexual signals (Chappell *et al.*, 1997; Kotiaho *et al.*, 1998; Reinhold *et al.*, 1998; Radwan *et al.*, 2006).

Even if energetic traits and parasite resistance are potentially involved in sexual signalling, this should not be taken for granted. Performing sexual displays is most likely an interplay between several physiological traits, and depends on previous or immediate selection pressures (e.g. Hamilton and Zuk, 1982; Väänänen *et al.*, 2006). Thus, the effects of particular physiological mechanisms on sexual signals may vary considerably. However, recently there has been increased interest in whole-body performance (e.g. Husak and Fox, 2008; Irschick *et al.*, 2008). Whole-body performance is useful because it summarizes many physiological processes that enables demanding activities, such as sexual signalling, into the same measurement. Thus, even if exact physiological mechanisms according to current selection pressures might vary, whole-body performance should still be closely related to sexual signalling.

Our aim in this paper is to explore the relationships between sexual signalling (male courtship call rate), energy metabolism (resting metabolic rate), endurance during physically demanding activity, an aspect of immune defence (lytic activity), and body size.

METHODS

This study is a partial reanalysis of individuals included in a previous study (Ketola *et al.*, 2007) exploring the genetic basis of courtship call rate. In that study, we found that courtship call rate was an important determinant of female choice, whereas the other possible cues (coefficient of co-ancestry, body mass, morphological size, fluctuating asymmetry) had no effect. A sample of individuals from that previous study (Ketola *et al.*, 2007) was subjected to several physiological measurements: resting metabolic rate, endurance during forced exercise, and lytic activity.

Study population

Gyllodes sigillatus individuals for this study were obtained from a population that was kept in our laboratory at the University of Jyväskylä, Finland, for six generations prior to the experiment. The first generation was initiated from the eggs of 48 females that were wild-caught from a large population at the campus of the University of Western Australia.

A pedigree of individuals was maintained from the initiation of the laboratory population. Families were kept at a 12 h/12 h photoperiod at 30°C and relative humidity of 58%. They were provided with food (commercial dried cat and rabbit food) and water *ad libitum*. Individuals for the study were collected from the F_6-generation and they were reared individually from very early developmental stages in one-litre plastic containers with food and water *ad libitum*. This was done to exclude as far as possible any common environmental effects and to control for experience as well as virginity of the individuals. The individuals belonged to nine families with one to four individuals in each family. Average family size in our analysis was 2.33.

Courtship call rate

Courtship call rate of the mature crickets was measured at the age of 7 days (±1 day) after the final moult, at a mean temperature of 30°C (±2°C) under dim red lighting. The trial arena was a $25 \times 25 \times 20$ cm plastic container with 5 cm of vermiculite on the bottom. Trials were initiated by placing a weighed male and female in their own small containers inside the arena. After 5 min of acclimation, the small containers were carefully removed. For each trial we monitored courtship call rate for the first 2 min or until mating occurred if it was before the 2 min had elapsed. Courtship call rate was estimated by observing the male calling (call, not call) every 10 s. In this study, we did not consider males' long-distance calling but only included the courtship song that in this species precedes and determines the female's decision to mate with a male (Ketola *et al.*, 2007).

Resting metabolic rate and endurance

On the day following the behaviour trial, we measured male resting metabolic rate using an open-flow respirometer. Access to food was restricted 1 h before the metabolic measurements. In the open-flow respirometer, the incoming air was drawn by a pump through the respirometric system. The air first passed through moisture-absorbent Drierite (Hammond Drierite, Xenia, Ohio, USA) and then through soda lime to remove CO_2 from the incoming air. Next the dry, CO_2-free air was led to the measurement chamber (~9 cm^3). Before entering the CO_2-analyser (LI-6252, LI-COR, Lincoln, NB, USA), the moisture was once again removed from the air. A steady flow of air through the respirometric system was controlled by a mass flow controller (Sierra Instruments, Monterrey, CA, USA). The respirometric system was connected to a PC and the data acquired and further analysed by Datacan V software (Sable Systems, Henderson, NV, USA). The data are reported as voltages, thus offering only relative values of resting metabolic rate.

To obtain the resting metabolic rate, we measured and observed every individual for up to 30 min. The first 5 min, which we considered to be the period of acclimation, were excluded from the analysis. Moreover, if individuals were active, the time was extended until individuals became non-active. Resting metabolic rate was calculated as mean CO_2

production during the least active 8.5-s period. Respirometry is based on the fact that in aerobic conditions, energy use produces heat and carbon dioxide and depletes oxygen. Thus, the energy use of an individual can be estimated by measuring one of these variables (Hill and Wyse, 1989).

Many animal species are reluctant to spend time on their back, and forcing an individual into this position results in an immediate return to an upright position. Repeated turning is energetically demanding and exhausts individuals relatively quickly (e.g. Kotiaho *et al.*, 1998). We exploited this phenomenon to measure the endurance of males. All individuals were repeatedly turned on their back until they became exhausted. Based on our preliminary observations, we defined exhaustion as the point at which an individual could not return, within 3 s, to an upright position. Endurance was defined as the time elapsing from the beginning of exercise to exhaustion.

Lytic activity of haemolymph

The main elements of insect immunity are the inducible expression of a large array of antimicrobial peptides, and the constitutive melanization–encapsulation response (Hetru *et al.*, 1998; Hancock *et al.*, 2006). An enzyme thought to be important in the non-specific immune response of insects against bacterial infection is lysozyme, which hydrolyses β-1,4 linkages in the peptidoglycan of bacterial cell walls (Götz and Trenczek, 1991). Thus, lytic activity of insect haemolymph can be assayed by the clearance rate of bacterial suspension by an individual's haemolymph (Rantala and Kortet, 2003; Kortet *et al.*, 2007).

Within 2 h of respirometry, we anaesthetized males in a cold room for ease of handling. Then, using a micropipette, 2 μl haemolymph was taken from the abdomen and stored in 30 μl phosphate-buffered saline (PBS, pH 7.2). Lytic activity was measured turbidometrically against 200 μl *Micrococcus lysodeicticus* (Strain: M-3770; Sigma-Aldrich Corp., St Louis, MO, USA) in PBS (0.3 mg·ml^{-1}). Measurements were made in a 96-well assay plate (Corning Inc., Corning, NY, USA), absorbance of 492 nm, at 22°C at 1-min intervals for 30 min with a plate reader (Multiscan Plus, Labsystems, Finland). Since the reaction was fast, we used values over the first 5 min for lytic activity. Lytic activity (i.e. clearance rate) was calculated as the total change in absorbance. To help interpretation, the values were transformed by multiplying by −1. For practical reasons, we did not use standard curves (serial dilution of hen egg white lysozyme). Thus, we used relative rather than absolute values. A similar method has previously been used to measure lytic activity of the haemolymph in field crickets (e.g. Rantala and Kortet, 2003; Kortet *et al.*, 2007).

Data analysis

Sample sizes vary from trait to trait because we were unable to record all of the traits for all of the males. We first performed a simple correlation analysis followed by an analysis of covariance. We initiated our analysis of covariance from a full model including family as a factor and all measured continuous variables as covariates. This was done to resolve the effects of resting metabolic rate, endurance, lytic activity, and body mass on courtship call rate. The analysis with family as a factor was used to control for the non-independence of observations. If important, the family effect can cause resemblance between individuals (due to maternal effects, common environmental effects or shared alleles), thus precluding

the use of ordinary regression analysis. All statistical tests were conducted with SPSS (v. 14, SPSS Inc., Chicago, IL, USA).

RESULTS

Of the physiological traits measured, only resting metabolic rate and body size were significantly correlated ($r = 0.50$, $P = 0.028$, $n = 19$). The relationships between the remaining physiological traits were weak ($r < 0.14$) and non-significant (Table 1).

Courtship call rate was positively related to endurance and body mass but did not correlate with lytic activity or resting metabolic rate (Table 1). Despite the fact that resting metabolic rate is commonly corrected by mass prior to analysis, this practice is not recommended (Packard and Boardman, 1999). However, as body mass has an influence on metabolic rate (Table 1), we included both body mass and resting metabolic rate together with the remaining independent variables and family in an analysis of covariance on courtship call rate. We continued our analysis from the full model to determine the most redundant subset of traits explaining courtship call rate. This was done by stepwise removal of the least significant traits. The best model was chosen based on adjusted R^2, which is R^2 penalized by the number of parameters fitted (Table 2). Finally, the best model, which explained 77.9% of the variation in courtship call rate, included endurance, body mass, and family (Table 2).

Table 1. Descriptive statistics and correlations between measured traits

		Courtship call rate	Resting metabolic rate	Lytic activity	Endurance (s)	Body mass (mg)
	M	0.268	0.209	0.027	94.78	252.49
	S.D.	0.281	0.060	0.013	29.97	43.01
	N	121	19	100	21	119
Resting metabolic rate	r	0.174				
	P	0.476				
	n	19				
Lytic activity	r	0.026	−0.134			
	P	0.796	0.585			
	n	100	19			
Endurance (s)	r	0.629	0.052	0.080		
	P	0.002	0.832	0.732		
	n	21	19	21		
Body mass (mg)	r	0.248	0.503	0.130	−0.010	
	P	0.007	0.028	0.202	0.965	
	n	119	19	98	21	

Note: Mean (M), standard deviation of the mean (S.D.), total number of individuals measured (N), Pearson correlation coefficients between the traits (r), their significance (P), and associated sample size (n). Courtship call rate is expressed as the frequency of courtship call rate, resting metabolic rate is expressed as volts, and lytic activity as the change in absorbance over 5 min multiplied by −1. For more details, see 'Materials and methods'.

Table 2. Results of stepwise analysis of covariance

Model	R^2/R^2adj.	Traits	d.f.	B	S.E.	F	P
1	0.795/0.384	Endurance	1	0.006	0.002	11.694	0.014
		Body mass	1	−0.001	0.002	0.690	0.438
		Lytic activity	1	0.844	5.099	0.027	0.874
		Resting metabolic rate	1	−0.112	0.915	0.015	0.906
		Family	8			1.185	0.430
2	0.801/0.557	Endurance	1	0.006	0.001	18.559	0.002
		Body mass	1	−0.002	0.001	1.818	0.210
		Lytic activity	1	0.947	3.831	0.061	0.810
		Family	8			2.167	0.135
3	**0.779/0.598**	**Endurance**	**1**	**0.006**	**0.001**	**21.529**	**<0.001**
		Body mass	**1**	**−0.002**	**0.001**	**1.986**	**0.189**
		Family	**8**			**2.386**	**0.099**
4	0.759/0.563	Endurance	1	0.006	0.002	17.998	<0.001
		Family	8			2.081	0.129

Note: The best model, highlighted in bold, was chosen according to the adjusted R^2. Continuous traits were fitted as covariates and family as a fixed factor. The associated degrees of freedom are denoted as d.f.; F = F-test statistic, P = statistical significance, B = regression slope of continuous trait and S.E. its standard error. $N = 19$.

From the analyses above, it is evident that lytic activity and resting metabolic rate were not related to courtship call rate. However, our main statistical test did not utilize all lytic activity or body mass data, only those for 19 crickets. However, a similar result was obtained if the effects of lytic activity and body mass on courtship call rate were examined without endurance and resting metabolic rate ($n = 97$, results not shown).

DISCUSSION

Sexually selected traits are thought to be dependent on male quality or condition, and thus indicative of potential benefits to choosy females (Andersson, 1994; Kotiaho, 2001; Kotiaho *et al.*, 2001; Tomkins *et al.*, 2004; Kokko *et al.*, 2006). Here we explored selected potential physiological traits that could be linked to male performance and furthermore to male courtship call rate. However, neither lytic activity nor resting metabolic rate was related to courtship call rate. Nevertheless, we found a strong and significant positive relationship between endurance and courtship call rate. This result is important since the courtship call rate is a clear determinant of mate choice in this species (Ketola *et al.*, 2007).

The methodology adopted here allowed us to confirm that the expectation of sexual selection theory – that is, that only males in good condition can perform sexual signals well – holds (Grafen, 1990; Andersson, 1994; Kotiaho, 2000; Cotton *et al.*, 2004). This is because there was a positive relationship between endurance and courtship call rate. However, neither resting metabolic rate nor lytic activity contributed significantly to courtship call rate or endurance. Endurance in physical activity also appears to explain courtship call rate in a few other species (e.g. Kotiaho *et al.*, 1998; Lailvaux and Irschick, 2006). The observed association between

endurance and courtship is important because it highlights the potential role of endurance in studies of sexual selection. Moreover, there is a vast amount of information about traits enabling high endurance (e.g. Joyner and Coyle, 2008), opening up promising new avenues for more direct mapping of determinants of sexual signalling, especially in species where sexual signalling is a physically demanding activity. There are numerous mechanisms of endurance, many of which are associated with maximal aerobic capacity, the lactate threshold, and muscle fatigability (e.g. Joyner and Coyle, 2008). Interestingly, these traits are closely related to muscle performance *per se*, whereas traditional conceptualizations of 'quality' or 'condition' in the life-history and sexual selection literature focus on the mechanisms that indirectly affect muscle performance, such as via resource allocation (e.g. Sheldon and Verhulst, 1996; Tomkins *et al.*, 2004). However, endurance, in principle, summarizes these all under the same measure.

One possible drawback of using endurance on its own as an 'index of condition' is that the importance of the different physiological mechanisms of courtship call rate is not clear. However, many potential traits enabling endurance in activity are directly or indirectly related to energy metabolism (e.g. Nilsson, 2002; Joyner and Coyle, 2008). Previous studies have found that resting metabolic rate is positively linked to sexual attractiveness in some species (Reinhold *et al.*, 1998), but not in others (Kotiaho *et al.*, 1998; Radwan *et al.*, 2006). Despite the suggestion that resting metabolic rate mediates energy-demanding activities, this relationship has seldom been observed (e.g. Johnston *et al.*, 2007). It could be argued that because our sample size was small, concluding that there is no effect of resting metabolic rate on courtship call rate is premature. However, with equal sample size, the endurance indicated a strong and significant result. Therefore, the difference in the effect sizes is clear.

Endurance and courtship call rate should, in principle, be influenced by the effect of energy or resource use on maintenance (resting metabolic rate), in addition to allocation to other key traits, such as parasite resistance (Sheldon and Verhulst, 1996). The sexual selection theories (and resource partitioning theories) suggest that good-quality males can afford both energetically costly parasite resistance and energetically costly sexual signalling, suggesting that male signalling could indicate male quality (van Noordwijk and de Jong, 1986; Rowe and Houle, 1996). This would be indicated by a positive relationship between sexual signals and immune defence at the population level, even if the life-history theories predict the opposite (i.e. trade-off) at the individual level (Stearns, 1992). In the present study, we did not find strong relationships between lytic activity, endurance or sexual signalling. However, it should be noted that lytic activity alone does not equal immune defence. Moreover, no clear pattern is yet to emerge of the relationship of lytic activity and other aspects of immunity on sexual signalling, as previously reported results vary depending on the immunity trait, population, and species (Rolff and Siva-Jothy, 2003; Ryder and Siva-Jothy, 2003; Lawniczak *et al.*, 2006; Väänänen *et al.*, 2006).

In conclusion, our results show that endurance could be a useful trait when studying behaviour and mate choice in an attempt to understand the complex relationship between condition and sexual signalling. We propose that the next step should be to explore the physiological knowledge we have on determinants of endurance, and to exploit this knowledge in detecting and understanding the underlying physiological determinants of condition, quality, and sexual signalling.

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