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# The active metabolic rate predicts a male spider's proximity to females and expected fitness

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Conspicuous traits, such as weaponry and body size, are often correlated with fitness. By contrast, we understand less about how inconspicuous physiological traits affect fitness. Not only is linking physiology directly to fitness a challenge, but in addition, behavioural studies most often focus on resting or basal metabolic rates, resulting in a poor understanding of how active metabolic rates affect fitness. Here we use the golden orb-web spider (*Nephila plumipes*), a species for which proximity to a female on the web predicts a male's paternity share, to examine the role of resting and active metabolic rates in fitness. Using a semi-natural experimental set-up, we show that males closer to a female have higher active metabolic rates than males further from females. This higher metabolic activity is paralleled by increased citrate synthase activity, suggesting greater mitochondrial densities. Our results link both higher active metabolic rates and increased citrate synthase activity with fitness. Coupled with the behaviour and life history of *N. plumipes*, these results provide insight into the evolution of physiological systems.

## 1. Introduction

Decades of research on male competition have resulted in a solid understanding between the correlation of various morphological traits and reproductive success. In most cases, larger animals or those with larger weapons are more likely to win agonistic encounters and have higher fitness in competitive contexts [1]. Our understanding of the relative importance of inconspicuous traits such as resting and active metabolic rates for fitness is less clear, despite metabolic rates often being invoked as a physiological trait that influences fitness [2,3].

In endotherms, resting metabolic rates are linked to fitness by conferring thermoregulatory and survival benefits [4]. In ectotherms, which do not thermoregulate by metabolic heat production, the role of resting metabolic rates may be viewed as an energetic cost of maintaining cellular integrity [5]. Activity and behaviour in ectotherms, on the other hand, are supported by active/maximal metabolic rates [6], which may therefore be more closely linked to fitness than resting rates. Additionally, in many ectotherms, anaerobic metabolism can contribute substantially to short-term activity [7,8] so that measures of oxygen consumption by themselves may not capture the interactions between metabolism, behaviour and fitness.

Our aims were to examine the relative importance of resting and active metabolic rates in modulating reproductive fitness, and to determine the association of three important metabolic pathways in the production of energy required for metabolic demands. We used the golden orb-web spider (*Nephila plumipes*), a species for which the male's proximity to a female at the

hub of the web predicts his paternity share. Males closest to females can expect on average twice the paternity (45%) of males ranked third (23%) [9]. Although larger males often secure positions closer to the female [10], size is not an absolute predictor of position [10,11].

To examine whether metabolic rate can explain male settlement patterns associated with fitness, we released size-matched males into an artificial enclosure, and examined differences in resting and active oxygen consumption between the males closest and furthest to a female's hub. We predicted that males closer to the hub would have higher active metabolic rates. We also examined the activities of different enzymes to determine which metabolic pathways played a role in the energy required for the increase in active rates. These results combined with information on the life history and behaviour of *N. plumipes* provide important new insights into the evolution of physiological mechanisms.

## 2. Material and methods

We collected 210 male and 12 female *N. plumipes* from around Sydney, Australia (151.183 E, 33.891° S). After collection, we measured the cephalothorax width (size) using an ocular micrometer and weighed males to the nearest 0.1 mg using an Ohaus Pioneer balance. We marked spiders using water-based paint (Gouache paint: Reeves; Harrows, England) for individual identification, which did not detectably change their mass.

To create a naturalistic mate choice situation, we used a 2.5 m<sup>3</sup> wooden frame covered with shade cloth within a glass house. The day before a trial, we allowed three similarly sized and aged females to build a web overnight. The next morning, we released 12 mass-matched males (see the electronic supplementary material, table S2), three at the base of each wall of the enclosure. As males select a female's web within 15–30 min in our enclosure (M. M. Kasumovic 2012, personal observation), we returned after 1 h and noted their distance to the female. We collected all the males and prepared the enclosure for the next release by cleaning the walls of remaining silk strands. We completed a total of nine replicate trials using six females and 108 males. Thirty-one males (28.7%) did not settle on a female's web, resulting in a mean of  $3.86 \pm 1.15$  (mean  $\pm$  s.d.; range 2–6) males per web. Although there were a total of 36 males in the experiment, two high-ranked males were cannibalized by the female during collection, and a third male was misplaced after the metabolic analysis. This resulted in a total of 34 males for the metabolic analyses and 33 males for the enzymatic analyses.

### (a) Metabolic analyses

We measured the active and resting metabolic rates of the closest and furthest male from each web 30–60 min after collection. To examine metabolic rates, we placed males in 9.6 cm<sup>3</sup> sealed respirometers with a 3  $\times$  8 mm magnetic stir bar. We used a Fibox3 oxygen system (see the electronic supplementary material) to examine oxygen consumption. To measure the active metabolic rate for all males, we placed the respirometer over a magnetic stirrer at the lowest setting. The spinning stir bar elicited prey capture behaviour, including high levels of locomotion and continuous silk deposition. We visually monitored males during the trial to ensure continuous activity, and measured oxygen consumption (for 5–10 min) as ecologically relevant measures of active metabolic rates [12]. As soon as the stirbar was stopped, males settled in a web, and we monitored oxygen consumption until a steady state was established (approx. 30 min) to determine a resting metabolic rate. We visually ensured that males were stationary during this time.

### (b) Enzyme assays

Activities ( $K_{\max}$ ) of regulatory enzymes in metabolic pathways indicate the maximal capacities that may potentially be limiting activity. Hence, enzyme activities are a useful measure to assess whether metabolic capacity is limiting. We measured the activity of creatine kinase and lactate dehydrogenase to assess the capacity of rapid, anaerobic ATP production and the activity of citrate synthase to determine mitochondrial capacity [13]. After determining metabolic rates, we froze males in liquid nitrogen and transferred them to a  $-80^{\circ}\text{C}$  freezer. We homogenized whole spiders in a Bullet Blender (Next Advance, New York, USA) and used a briefly centrifuged homogenate in the assays. Enzyme activities were determined according to published protocols [14]. Saturating substrate concentrations were ascertained in pilot assays and enzyme activity was expressed as  $\mu\text{mol}$  of substrate converted  $\text{min}^{-1} \text{g}^{-1}$  wet tissue mass in duplicate at  $25^{\circ}\text{C}$  (the average air temperature during the behavioural trials).

### (c) Statistical analyses

To examine whether metabolic rate explained variation in settlement position, we only used males that settled closest to (high rank) and furthest (low rank) from the hub in webs containing a minimum of three males (only one web had two males). Settlement patterns are a result of multiple dyadic contests between players, such that rankings of the highest and lowest individuals are not directly dependent upon one another. As a result, we used a GLM with a binomial distribution (logit link) to examine whether rank was explained by mass, size and resting and active metabolic rates. We used a separate GLM to examine whether rank was predicted by citrate synthase, lactate dehydrogenase and creatine kinase activity. We also provide a correlation matrix of all the variables in the electronic supplementary material, table S2. All the data are submitted to Dryad (<http://dx.doi.org/10.5061/dryad.jv25p>).

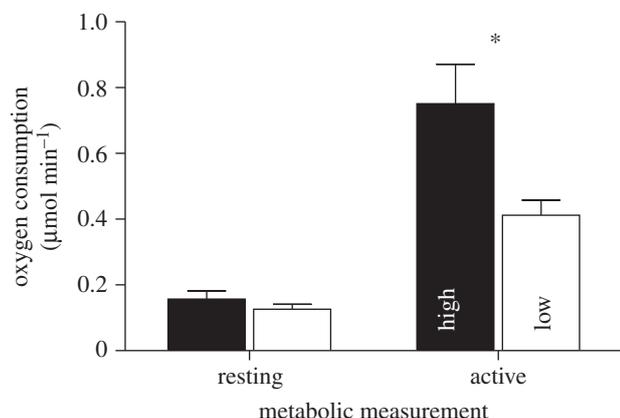
## 3. Results

A higher active metabolic rate significantly predicted male rank ( $\chi^2 = 8.68$ , d.f. = 1, 29,  $p = 0.003$ ; figure 1) in a model which controls for the effect of the other variables. In this model, resting metabolic rate ( $\chi^2 = 1.09$ , d.f. = 1, 29,  $p = 0.30$ ), mass ( $\chi^2 = 0.30$ , d.f. = 1, 29,  $p = 0.59$ ) and size ( $\chi^2 = 0.45$ , d.f. = 1, 29,  $p = 0.50$ ) had no effect. This is despite the fact that the active and resting metabolic rate was correlated (see the electronic supplementary material, table S2). Interestingly, there was also no correlation between mass and either metabolic rate (see the electronic supplementary material, table S2) or differences in mass between the high-ranked males ( $16.74 \pm 1.02$  mg) and low-ranked males ( $16.63 \pm 1.10$  mg).

Citrate synthase activity was significantly higher in the high-ranked males ( $\chi^2 = 4.76$ , d.f. = 1, 29,  $p = 0.029$ ; figure 2). There was no difference in either lactate dehydrogenase ( $\chi^2 = 0.10$ , d.f. = 1, 29,  $p = 0.76$ ) or creatine kinase ( $\chi^2 = 0.074$ , d.f. = 1, 29,  $p = 0.83$ ) between males of different rank.

## 4. Discussion

Morphological traits are often strong predictors of fitness [1]. Our study demonstrates that, when males are matched for morphological traits, the level of active metabolic rate, related to amount of citrate synthase, explains settlement patterns and hence the predicted proportion of offspring an

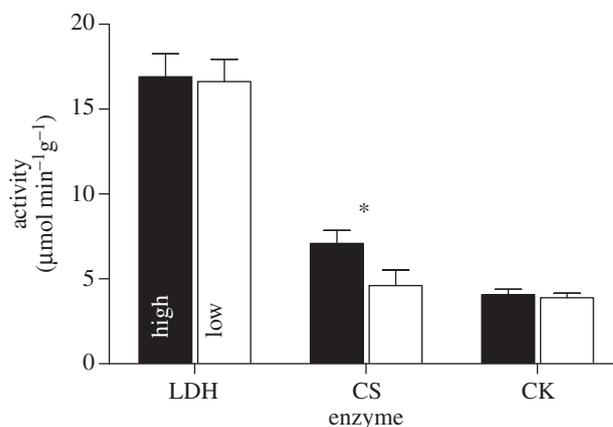


**Figure 1.** The mass specific oxygen consumption of high-ranking males (black bars) and low-ranking males (white bars) summarized as resting and active metabolic rate. The asterisk denotes a significant difference.

individual is likely to sire. There is the possibility that the interactions between males caused the difference in active metabolic rates. However, we do not believe this to be the case because resting metabolic rates did not differ between spiders of different rank and the short trial periods were not long enough to produce a training-effect to improve the active metabolic rate. We thus provide a strong link between a male's perceived fitness and his active metabolic rates, and also provide a mechanism by which these higher ranked males achieve closer proximity to females.

There are numerous examples where contests between similar-sized rivals lead to escalated contests in which morphological traits fail to predict contest outcomes (e.g. spiders [15] and fish [16]). We suggest that greater active metabolic rates may contribute to determining outcomes in these situations. In addition, differences in active metabolic rates may explain the 'Napoleon effect', whereby smaller males display increased aggression [17] without the need to invoke errors in animal judgement [18] or resource limitation (i.e. desperado effect; [19]). We thus caution against using morphological traits alone as estimates of competitive ability [20].

Increased citrate synthase activity generally denotes greater mitochondrial capacities, which are often selected in situations, where sustained energetic resources are required [13]. In addition to direct contests between rivals [10], *N. plumipes* males are required to sustain activity while mate



**Figure 2.** The mean lactate dehydrogenase (LDH), citrate synthase (CS) and creatine kinase (CK) activity of high-ranked males (black bars) and low-ranked males (white bars). The asterisk denotes a significant difference.

searching for several days [21], and while moving along a female's web and interacting with rivals [10]. Our enzymatic and metabolic results coupled with the behavioural and life-history understanding of *N. plumipes* indicate that citrate synthase (indicative of mitochondrial densities) and active metabolic rates are under selection for the benefit they confer on male mating success.

There may, however, be a trade-off with the greater resource allocation required for increased mitochondrial density, so that higher metabolic capacities and rates may be advantageous only under strong competition. Given that *N. plumipes* is developmentally plastic, altering size and mass in response to both rival density and female availability [22], coupled with results from the spider *Latrodectus hasselti* demonstrating similar plasticity [23], as well as shifts in the resting metabolic rate in response to male density during development [24], we suggest that investment in mitochondrial biogenesis may also be plastic in response to the social environment. This suggestion must be tested experimentally, but could provide novel insight into the factors affecting the evolution of metabolic rates.

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