Costs increase as ritualized fighting progresses within and between phases in the sierra dome spider, *Neriene litigiosa*

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Magnitudes and patterns of energy expenditure in animal contests are seldom measured, but can be critical for predicting contest dynamics and understanding the evolution of ritualized fighting behaviour. In the sierra dome spider, males compete for sexual access to females and their webs. They show three distinct phases of fighting behaviour, escalating from ritualized noncontact display (phase 1) to cooperative wrestling (phase 2), and finally to unritualized, potentially fatal fighting (phase 3). Using CO_2 respirometry, we estimated energetic costs of male–male combat in terms of mean and maximum metabolic rates and the rate of increase in energy expenditure. We also investigated the energetic consequences of age and body mass, and compared fighting metabolism to metabolism during courtship. All three phases involved mean energy expenditures well above resting metabolic rate ($3.5\times$, $7.4\times$ and $11.5\times$). Both mean and maximum energy expenditure became substantially greater as fights escalated through successive phases. The rates of increase in energy use during phases 2 and 3 were much higher than in phase 1. In addition, age and body mass affected contest energetics. These results are consistent with a basic prediction of evolutionarily stable strategy contest models, that sequences of agonistic behaviours should be organized into phases of escalating energetic costs. Finally, higher energetic costs of escalated fighting compared to courtship provide a rationale for first-male sperm precedence in this spider species.

In many species, male reproductive success depends on the ability to win access to receptive females through the use of physical aggression (Andersson 1994). During fights, there are advantages to a male in assessing both the female's reproductive value (e.g. Austad 1983) and the chances of beating his opponent (Parker 1974; Enquist & Leimar 1983). By repeatedly weighing the costs and benefits of escalating a fight, rival males may mutually benefit by having the option to retreat without risking injury or death in an escalated fight against a superior opponent (Maynard Smith & Price 1973; Parker 1974; Maynard Smith & Parker 1976). In this context, ritualized contest behaviours, defined here as organized, noninju-

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rious styles of mutual assessment that demand a substantial level of honest signalling, have a clear adaptive purpose. They serve to extract accurate information about the individuals' ability to win a fully escalated fight while managing energy expenditure and the risk of injury.

However, exactly how animals extract this information during the course of a fight remains an open question. The various game-theory models of contest behaviours postulate different possible rules that an animal could use to update its information state and thus determine its next move. One way of assessing the accuracy of these models is to look at how well they predict the pattern of energy expenditure over the course of a contest. For example, the 'sequential assessment game' (SAG) of Enquist & Leimar (1983) dictates that individuals should fight with constant intensity within a phase and that each successive phase should yield more accurate (and presumably more costly) information. Hence it predicts energy expenditure to remain constant within a phase, but to increase across phases as the contest progresses. In contrast, the 'energetic

war of attrition' (E-WOA, Mesterton-Gibbons et al. 1996; Payne & Pagel 1996) requires only that the intensity of contestants be equally matched at all times; the actual magnitude of the energy expenditure is free to vary either up or down during the contest. A more recent model, Payne's (1998) 'cumulative assessment model' (CAM) is more flexible and allows for contestants' energy expenditures to both differ from each other and to vary up or down as the fight proceeds. Although testing such models is crucial to advancing our understanding of animal costs, the technical challenge of measuring energetic or metabolic costs of fighting has meant that these tests have rarely been attempted (but see Reichert 1988; Hack 1997; Briffa & Elwood 2001).

Following from general contest theory, we hypothesized that the energetic costs of contest behaviours would increase from phases 1 to 3. We tested this hypothesis by characterizing energy consumption in staged fights using estimates based on precision flow-through CO_2 respirometry. We also measured copulatory courtship and resting energetics (Watson 1991a, b; Watson & Lighton 1994) and compared these data to fight energetics to ascertain relative energy expenditures. We used the results as a guide to interpreting ritualized fighting in sierra dome spiders in an evolutionary framework.

METHODS

Fighting Behaviour

Sierra dome spider fights consist of three distinct phases. Phase 1 displays consist of the contestants closely approaching one another with their anterior legs spread apart and their second and third legs plucking the web. Phase 1 clearly allows for mutual assessment of (1) size, based on the spread of the front legs, and (2) weight, based on vibrations caused by the plucking behaviour. Phase 2 consists of pedipalp wrestling. Males extend their pedipalps and lock them over one another's coxae (i.e. 'shoulders'). Entry into the phase 2 configuration can only be done with mutual cooperation of the contestants. Once the spiders are in the phase 2 position, the anterior surfaces of their chelicerae are pressed together tightly, making it impossible for them to bite one another. During the wrestling match, the spiders attempt to jam one another through the dome of the web by pushing forward and upward using muscle power from the legs, pedicle and palpi. Phase 3 is unritualized biting and grappling. The phase 2 grip is released and opponents attempt to get into a position to bite while avoiding being bitten.

Animal Care

We collected all data at the University of Montana's Flathead Lake Biological Station, Polson, Montana, U.S.A., during July and August 1999. We gathered male *N. litigiosa* from their webs soon after maturation so that they had no previous adult fighting experience. Day of maturation was recorded for each male so we could determine his exact age during each respirometric recording. We housed the males in individual 600-cc containers under ambient conditions in an unheated laboratory building. We fed them a standardized diet of natural prey and misted them regularly with water. We recorded male body mass before each fight trial and respirometric test. To assure proper identification during fights, we marked each male with a unique set of small colour marks on the tibia of their hindmost legs using thinned fast-drying Testors enamel paint (Testor Corp., Rockford, Illinois, U.S.A.). The size and placement of these marks did not interfere with fighting (there was no paint on leg joints, and no indication that the painted area became tangled in silk during fights).

Measurement of Exercise Intensity during Fights

We used high-resolution flow-through CO_2 respirometry to estimate the energetics of fighting. We collected real-time gas exchange data using a Licor LI-6251 CO_2 analyser in a Sable Systems TR-3 flow-through respirometry system. Flask temperature data were incorporated into each respirometric record. During recordings, we entered behavioural codes into the respirometry record and took handwritten notes.

The respirometry flasks were custom-made, inverted Erlenmeyer-style 46-cc flasks. Because fights over mates occur on female webs in nature (Watson 1990), we allowed females to build webs in the respirometry flasks to serve as fighting arenas for males. During respirometric recordings, we suspended the flasks from the ceiling to buffer the spiders from room vibrations. Before each respirometric recording, we weighed contestants to the nearest 0.01 mg using a Sartorious M2P digital microbalance. For fights, we attempted to match males closely in weight to maximize the probability of prolonged and escalated fighting. The mean weight difference between contestants across all fights was 9.9% (range 0.01−34.8%).

Trials began and ended with a baseline analyser reading of a zero humidity, CO_2-free air stream. After the first baseline reading, we switched the air stream to a respirometry flask containing a single male, allowing ambient CO_2 and moisture to be flushed to the point of equilibration (i.e. when only CO_2 outputs of the male were being measured). This several-minute flushing period prepared the flask for the introduction of the second male. We introduced the second male quickly to the already equilibrated flask, in a manner that minimized infusion of outside CO_2 and moisture. We then rapidly re-equilibrated the flask at a high flow rate (500 ml/min). We were usually able to begin recording meaningful data on the combined CO_2 outputs of both males within 60 s, before fighting began. The flow-through rate was reduced to 150 ml/min during recordings of most fights.

We obtained measures of exercise intensity during the three fighting behaviours for 31 males. We calculated average overall metabolic rates during one to nine repeated fights ($\overline{X} \pm SD = 3.5 \pm 1.9$ fights) for each male in the study. Data on maximum (asymptotic) energy use

and rates of metabolic rate increase were drawn from the same pool of respirometric recordings, when high-quality data were available. Repeated observations on a given individual usually involved him fighting a new opponent. Repeated observations always were done on different days, usually 3 or more days apart and were counted as separate data points (see Fig. 3a, b for sample sizes). Because we controlled statistically for male body mass and age, we regard our estimates of male energy investment in each phase of fighting to be adequately independent between fights.

To compare the energy requirements of intense intersexual courtship versus male–male fighting, we measured maximum male energetic expenditures during preinsemination phase copulation (i.e. aspermic copulatory courtship; see Watson 1991a, b) using methods detailed in Watson & Lighton (1994). The 68 observations presented here were collected in the 1994 breeding season. The data were accumulated from 24 males copulating on different days (3–9 days between matings) with 16 randomly assigned virgin females. Each male contributed two to four matings to the data set.

We recorded temperature-standardized resting metabolic rates (SMR) for every individual at the time of first capture and throughout the fight trials. We used a 46-cc respirometry flask to measure SMR at a flow rate of 150 ml/min. We constantly observed the spiders during SMR recordings to ensure that they were motionless and that residual CO_2 from any prior activity had been respired and flushed from the flask. The 68 SMR records from 1994 were taken on the same day that copulatory courtship measurements were taken. The 65 SMR records from 1999 were from 31 males with no more than three repeated measures, all taken on different days.

Respirometric Conversions: Calculating $\dot{V}CO_2$ and Estimating Energetics

Along with the risk of injury and various opportunity costs, one of the most important costs of any intensive behaviour is the amount of energy consumed in performing it. Estimating behavioural costs in terms of energy consumption (e.g. microwatts: µW) is a meaningful way to address the overall fitness consequences of a behaviour. Our study was designed to estimate the energetic costs of three intrasexual fighting behaviours and to compare them to each other and, for perspective, to male energy use during complete rest and when maximally exerting themselves in intersexual courtship.

Like any measure of the fitness consequences of a behaviour or trait, measures of energy consumption are imperfect. An advantage of using small animals like the sierra dome spider is that they display complex behaviours normally in captivity and in respirometry chambers small enough that respired gasses flush out quickly. However, they also present certain technical problems when attempting to measure moment-to-moment variation in energy use during rapidly changing behavioural sequences. In such cases, one relies on instruments that measure the volume of respired CO_2

($\dot{V}CO_2$), rather than O_2 consumption, because only CO_2 analysers offer the requisite sensitivity. Unfortunately, estimates of energy use based on $\dot{V}CO_2$ are subject to inaccuracies whenever an activity intensifies or is sustained to a point where the organism must begin using a combination of aerobic and anaerobic metabolism to provide the required energy to its muscles. It is important to realize that the energetic data we derived, particularly those pertaining to phase 2 and 3 fighting, represent estimates of male metabolic rates based on the assumption that the spiders rely on aerobic energy production during their contests.

Physiologically, the problem is this: in anaerobic metabolism, two lactic acid molecules are produced for every hexose burned. The resulting decrease in the pH of the haemolymph results in release of CO_2 stored in the blood (e.g. as bicarbonate). To the extent that the animal's overall metabolism goes anaerobic, its total CO_2 production becomes the sum of CO_2 produced due to energy consumption plus that released from passive storage. Thus, energy consumption may be increasingly overestimated from CO_2 data as activity intensifies and anaerobic processes play an increasingly greater metabolic role. Ensuing estimation errors are difficult to correct because they are likely to be nonlinear in relation to the intensity and duration of the activity, due to, for example, the variable potency of pH buffering systems and the gradual exhaustion of stored CO_2 capable of being driven out of the haemolymph by physiologically plausible shifts in pH induced by lactic acid.

Nothing is known about the aerobic capacities of the sierra dome spider. Its aerobic capacities may be greater than those of some other spiders (see below). However, like other spiders, our study species surely uses 'book lung' and 'tracheal lung' systems, as well as diffusion of gasses across the thin nonwaxy abdominal cuticle, to supply oxygen to the haemolymph. Importantly, they lack the more efficient insectan system of tracheal oxygen delivery directly to muscle (Schmitz & Perry 2001). Another special problem for spiders in maintaining adequate oxygen levels in the prosoma during intense activity is probably hard to overcome in any species: the fact that some leg joints depend upon blood pressure to cause extension means that oxygenating blood flow in the prosoma during intense activity may be hampered.

To help compensate for their aerobic constraints, anaerobic metabolism is well developed in spiders that have been studied to date (Prestwich 1983). However, spider species do vary in the efficiency of oxygen transport to their leg musculature, possibly due in part to differential development in tracheal lung systems among species. For several reasons, one might suppose that, for spiders, male sierra dome spider aerobic capacities are relatively good. First, adult males have a nomadic, scramble-competition, polygynous lifestyle where sustained locomotion and brushes with predators are common. Second, males routinely fight one another for sexual access to females. The complexity of sierra dome spider fighting assures that this behaviour has a long evolutionary history that could have provided time for the evolution of respiratory enhancements that this mode of sexual

selection would certainly favour. Third, females demand energetic copulatory courtship, which is lengthy and therefore probably largely aerobic, as a prelude to insemination (Watson 1991a), another source of selection for respiratory enhancement.

Nevertheless, based on what is known about other spiders, we must assume that anaerobic metabolism plays a nontrivial role in sierra dome spider energy production, especially during phase 2 and 3 fighting. Although our results provide the best data on real-time fighting energetics available with current technology, we emphasize the limitations of our methods by expressing energy as 'estimated microwatts' (EμW). We also provide two Y axes on all graphical representations of behaviour versus exercise intensity, one showing EμW and the other $\dot{V}CO_2$. Note that for all our data, EμW can be converted to $\dot{V}CO_2$ simply by multiplying by the constant 0.1278. In spite of the possible inaccuracies in our estimates, our results provide a good representation of the relative intensity of male exercise during resting, copulatory courtship and the three phases of fighting.

We used Sable Systems Datacan V software to convert raw respirometric data to $\dot{V}CO_2$ (of CO_2 μl/h) and then to energetic data (EμW), as appropriate (see above), according to previously published methods (Watson & Lighton, 1994). Identical methods were used to convert fighting, courtship and resting data. Our calculations used standard equations that adjusted the data for recording-specific airflow rates. Data were standardized to 25°C using an empirically determined Q_{10} of 2.21 (P. J. Watson, unpublished data). We also adjusted for minor intra-recording baseline drifts (i.e. zero readings) and temperature drifts of the CO_2 analyser. We used an empirically determined respiratory exchange ratio of 0.713 (RQ) in the CO_2-to-energy conversion (Watson & Lighton 1994; P. J. Watson, unpublished data). This RQ estimate is an average based on the relative amount of oxygen consumed and CO_2 produced by intermittently locomoting spiders ($N = 16$ adult sierra dome males) over several hours in a constant volume of air at temperatures and nutritional states similar to those experienced by spiders used for energetic estimates. A standard Z transformation was used to sharpen the respirometric data to compensate for empirically determined flask-specific flushing rates (Bartholomew et al. 1981).

Statistics

We calculated the mean metabolic rates for each phase of fighting by averaging data values in the relevant section of the energy use curve of each fight. We determined maximum metabolic rates during each phase of fighting by averaging data points at the asymptote of energy use for each phase, only using records in which clear asymptotes were present. Rates of change of energy use for each phase of fighting were determined by calculating the slopes of relevant sections of each energy curve. We halved energetic measures from fights to obtain average energy consumption per individual, enabling direct comparison of resting and copulatory courtship energetics. For

energetic measures of copulatory courtship, female SMRs were subtracted from the data; this is justified because females are inactive during such courtship (Watson & Lighton 1994). For these measures, we used the highest metabolic rate attained by the male during the entire 1–3-h period of copulatory courtship. These values are known to represent energy use during normal copulatory courtship behaviour because we continuously observed all courtships.

When comparing the energetics of fighting phases, we statistically controlled for the effects of male weight and age by including the averaged body mass and age of the two males in each fight as covariates in our regression models. Previous studies have shown that both variables affect the resting and active metabolic rates of male sierra dome spiders (P. J. Watson, unpublished data). We used analyses of covariance and Tukey HSD multiple comparison regimes to estimate and compare three measures of least square mean (i.e. means adjusted for body mass and age) energy use during fighting phases 1–3: (1) average overall energy use, (2) asymptotic or maximal energy use (MR_{max}) and (3) rates of metabolic increase.

Least square (LS) means for MR_{max} and the rate of metabolic rate increase during copulatory courtship were estimated by adding a fourth category to the factor used above to code for fighting phase. Maximum MR fighting data were used in the analysis, along with maximum MR courtship data to produce the LS-mean for courtship shown in Fig. 3a. Age and body mass were controlled for statistically. Separate analyses using average costs of fighting and maximal costs of fighting in each phase were followed by Tukey HSD multiple comparisons to contrast courtship energetics to both average and maximal energy use during the three phases of fighting. Resting metabolic rates are also least square means for each year in which data were collected: 1994 for the courtship data and 1999 for the fighting data. These LS-mean SMRs were adjusted for body mass and age.

RESULTS

Relation of Mass and Age to Energetics

Figure 1 shows the univariate relationships between the average age and body mass of the two contestants of each fight versus the average costs of phase 1 intrasexual fighting (Fig. 1a, b, respectively), phase 2 intrasexual fighting (Fig. 1c, d), phase 3 fighting (Fig. 1e, f), the MR_{max} achieved by males during completed intersexual copulatory courtship (Fig. 1g, h), and average male SMRs (Fig. 1i, j). In most univariate analyses, age had significant negative relationships with estimates of energy use, whereas body mass had significant positive relationships with energy use.

The average number of days since sexual maturation ('age') and the average body mass of each pair of fighting males were used jointly as covariates in analyses comparing the estimated energy costs of phase 1–3 styles of combat. In these analyses, the average body mass of contestants had significant positive relationships with both average overall energy use and maximal energy use
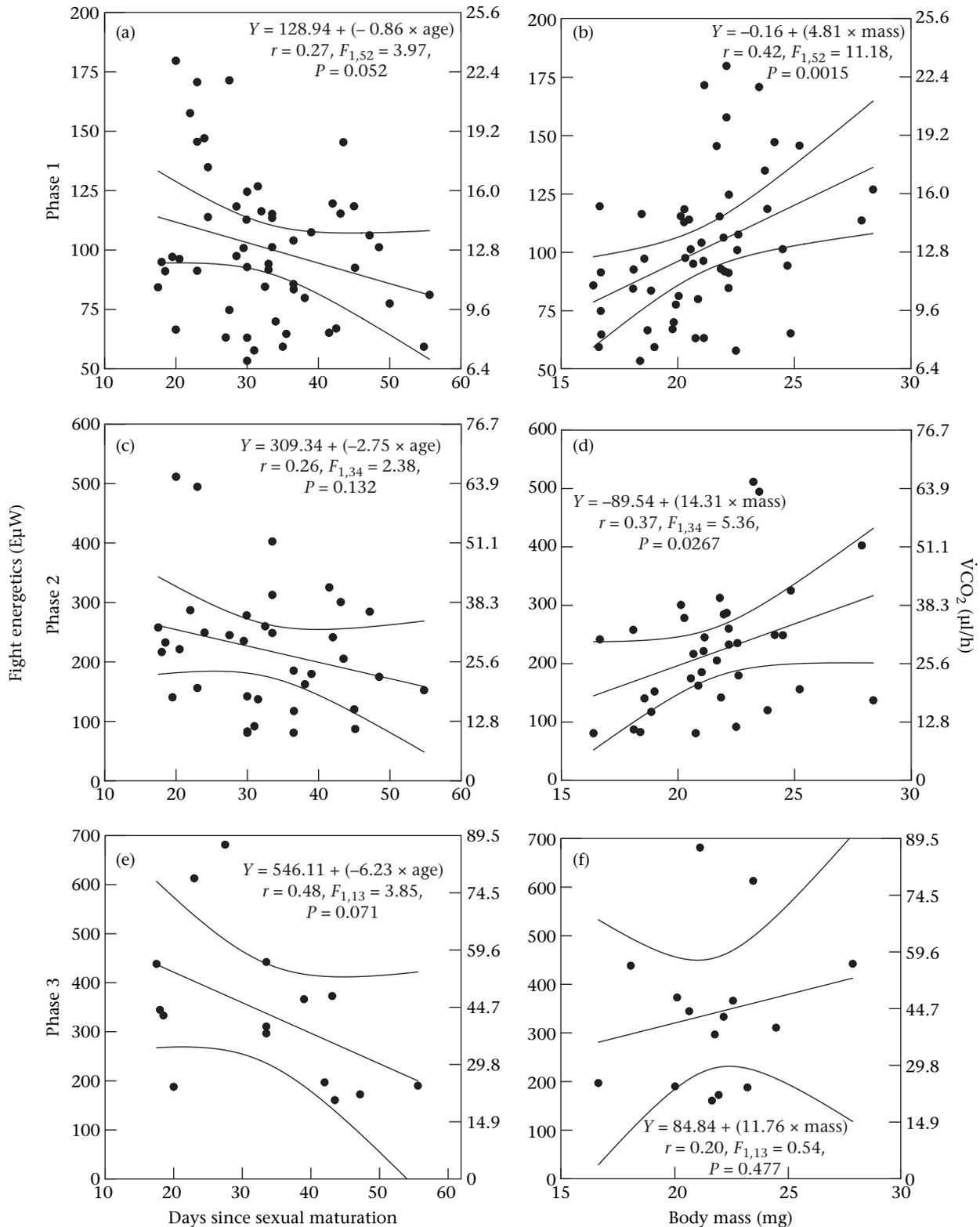
Figure 1. Univariate relationships between male age and body mass versus the estimated energetic costs (EμW; left Y axis) and the corresponding volume of respired CO_2 ($\dot{V}CO_2$) (right Y axis) of four behaviours. Mean energy use during phase 1 (a, b), phase 2 (c, d) and phase 3 (e, f) fighting versus age and mass, respectively. Maximal energy use during copulatory courtship versus (g) age and (h) mass. Temperature-standardized resting metabolic rate (SMR) for years 1994 ($-\bullet-$) and 1999 ($- -\blacktriangle- -$) versus (i) age ($41.0 + (-0.53 \times age)$); $r = 0.50$, $F_{1,66} = 22.38$, $P = 0.00001$ and $45.13 + (-0.47 \times age)$; $r = 0.50$, $F_{1,63} = 20.96$, $P = 0.00002$, respectively) and (j) mass ($2.52 + (1.47 \times mass)$; $r = 0.45$, $F_{1,66} = 17.17$, $P = 0.0001$ and $16.61 + (0.71 \times mass)$; $r = 0.23$, $F_{1,79} = 4.40$, $P = 0.039$, respectively). The slopes did not differ for SMR versus age or body mass between 1994 and 1999 ($F_{1,129} = 0.132$, $P = 0.72$ and $F_{1,146} = 0.19$, $P = 0.66$, respectively). Best fit regression lines are shown with 95% confidence bands.
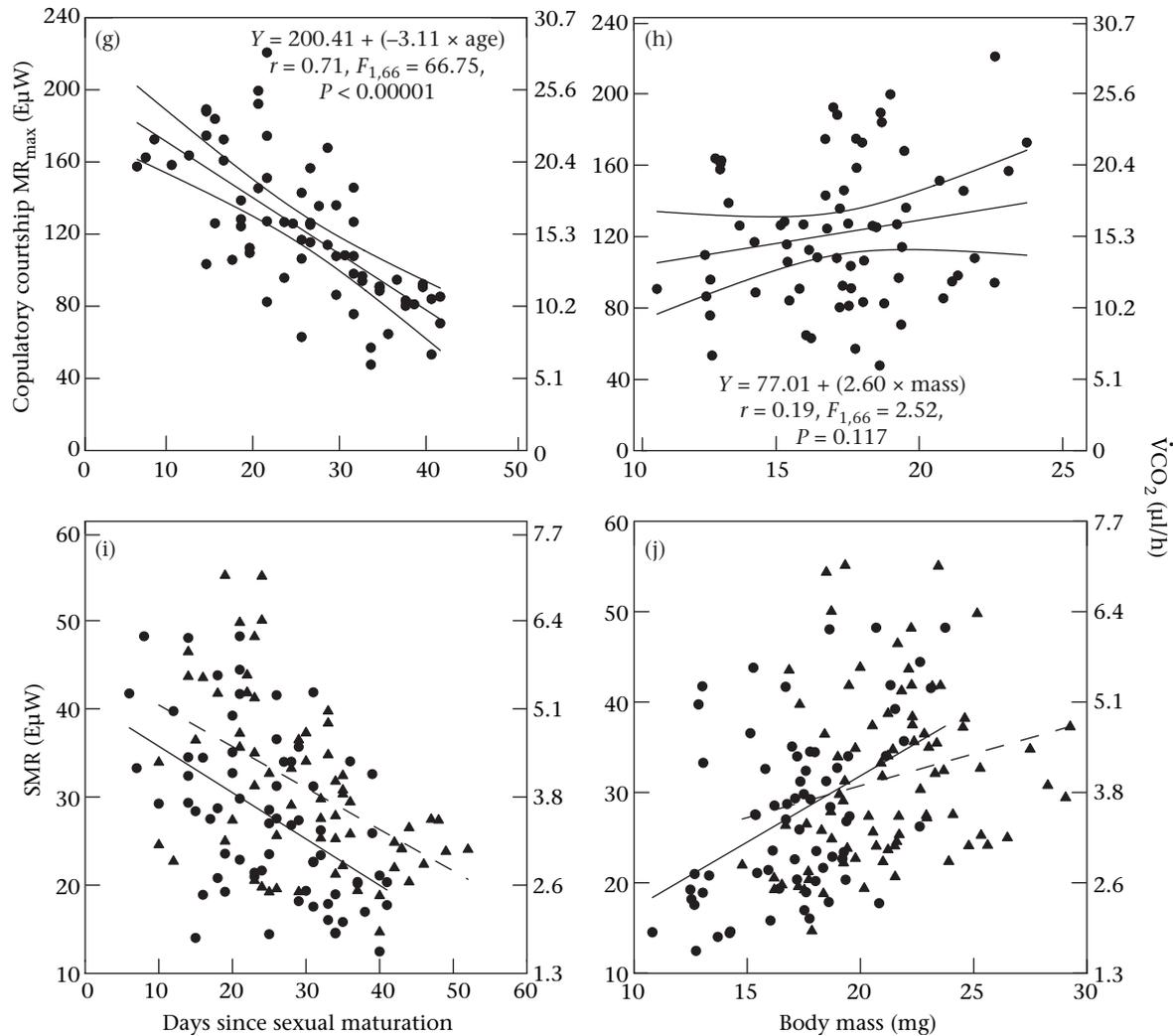
Figure 1. (*continued*)

among fights ($F_{1,100} = 7.18$, $P = 0.009$ and $F_{1,38} = 4.53$, $P = 0.04$, respectively). When we controlled statistically for the phase of fighting and body mass, age had a significant negative relationship with average overall, but not maximal, energy use ($F_{1,100} = 8.03$, $P = 0.006$ and $F_{1,38} = 0.73$, $P = 0.40$, respectively). In similar analyses, body mass and age both were unrelated to the rate of metabolic increase during fights ($F_{1,101} = 0.18$, $P = 0.68$ and $F_{1,101} = 1.67$, $P = 0.20$, respectively). The relationship between age and body mass was not statistically significant using our SMR data set ($F_{1,130} = 0.47$, $P = 0.49$), courtship data set ($F_{1,66} = 0.82$, $P = 0.37$), or fighting data set ($F_{1,103} = 1.23$, $P = 0.27$).

Average Fighting Energetics

Compared to the average male resting metabolic rate of 32.09 μW, we estimated that average energy use was raised by factors of 3.5, 7.4 and 11.5 during fighting phases 1, 2 and 3, respectively. However, energy use during sierra dome spider fights is highly dynamic, especially when fights escalate beyond the noncontact display of phase 1.

A respirometric record from a representative fight including all three phases is shown in Fig. 2.

Average energy consumption differed significantly between the three phases of fighting ($F_{2,100} = 55.0$, $P < 0.0001$; Fig. 3a). Phase 2 assessments were estimated to be, on average, 2.1 times more costly than phase 1 assessments. Bouts of phase 3 combat were estimated to be an average of 3.3 times more costly than phase 1 and 1.6 times more costly than phase 2 assessments. All three phases differed significantly from each other in estimated mean energy cost (Tukey HSD: all Ps < 0.00003).

In a similar analysis, copulatory courtship MR_{max} tended to be higher than average energy use in phase 1 combat (124.6 EμW ± 9.5 versus 103.1 EμW ± 11.1, respectively), but the difference was not statistically significant (Tukey HSD: $P = 0.34$). Copulatory courtship MR_{max} was significantly lower than average estimated energy use during phase 2 and 3 fighting (Tukey HSD: $P < 0.0001$).

Maximum Fighting Energetics

We found significantly different estimates of MR_{max} between phases ($F_{2,38} = 18.9$, $P < 0.0001$; Fig. 3a), with the
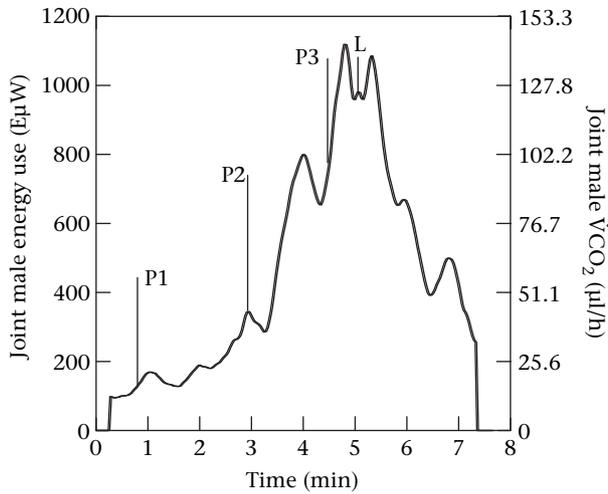
Figure 2. Joint male estimated energy use (left Y axis) and measured volume of expired CO_2 ($\dot{V}CO_2$; right Y axis) during a fight between a 19.3-mg male and 16.8-mg male in which a mild phase 1 (P1) display escalates quickly to an intense P1 display, followed by phase 2 (P2) and phase 3 (P3) combat. Markers show where fighting behaviours (P1, P2, P3) and postfight locomotion (L) began. The figure includes data collected immediately after the first baseline during which both males were stationary. It also includes postfight data: a combat-free period (beginning at 'L') during which energy use gradually dropped to a level representative of moderate, normal locomotion.

higher phases of escalation all tending to be more costly. We estimated that MR_{max} during phase 2 was, on average, 2.5 times greater than that during phase 1, and MR_{max} during phase 3 averaged 2.8 times greater than that during phase 1. However, MR_{max} during phase 3 was only 1.13 times greater than that during phase 2 fighting. Only phases 1 versus 2 and 1 versus 3 differed significantly in MR_{max} (Tukey HSD: $P < 0.0001$ and $P = 0.0007$, respectively). Our MR_{max} estimates for phases 2 versus 3 were statistically indistinguishable (Tukey HSD: $P = 0.77$).

When estimated in conjunction with maximal energy use during fights, MR_{max} attained during copulatory courtship was indistinguishable from that of phase 1 fighting (132.9 EμW $\pm$ 11.5 versus 138.1 EμW $\pm$ 19.0, respectively; Tukey HSD: $P = 0.99$; Fig. 3a). Maximum energy use during copulatory courtship again was significantly lower than that estimated for phase 2 or 3 fighting (Tukey HSD: $P < 0.001$; Fig. 3a).

Rates of Increase in Metabolic Rates

Higher phases of escalation raised the contestants' metabolic rates more rapidly ($F_{2,101} = 33.7$, $P < 0.0001$; Fig. 3b); this analysis excluded courtship data and used male age ($F_{1,101} = 3.24$, $P = 0.075$) and body mass ($F_{1,101} = 0.23$, $P = 0.63$) as covariates. Phase 2 assessments, on average, raised male MR 3.7 times faster than phase 1
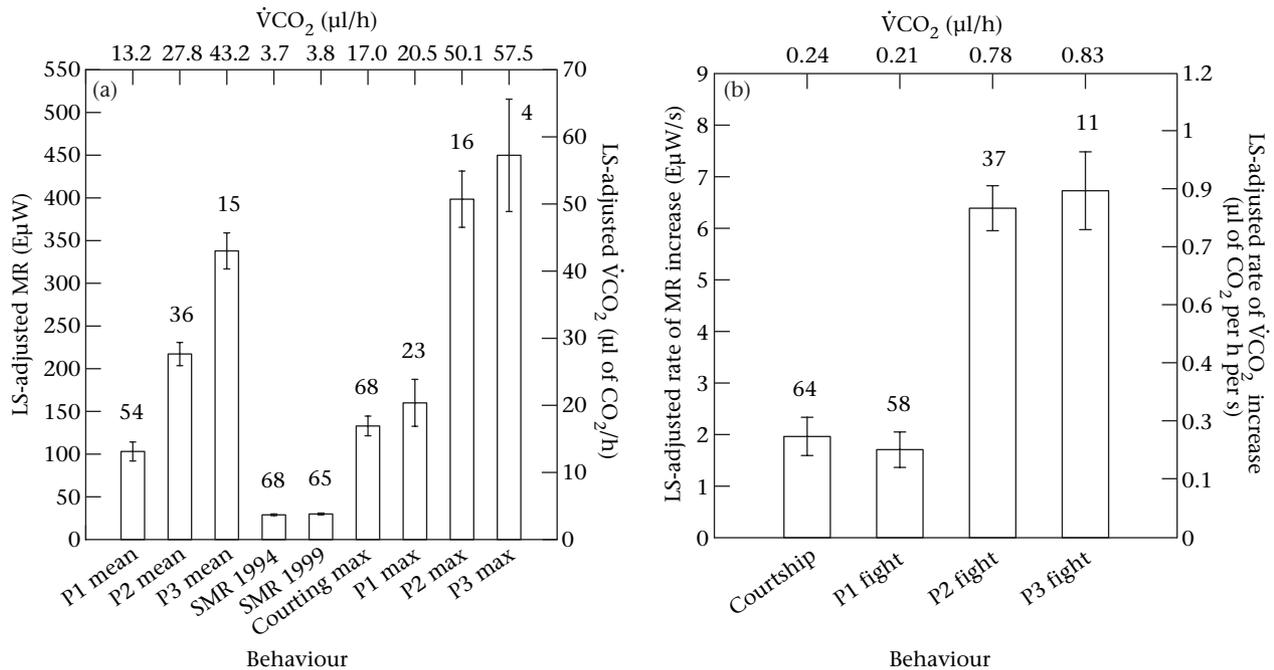
Figure 3. Comparative energetics of male–male fighting, copulatory courtship and resting metabolism. (a) The means of individual male mean and maximum energetic expenditures (MR) and volume of CO_2 production ($\dot{V}CO_2$) during phases 1–3 of fighting. For perspective, means are also graphed showing courtship at the maximum intensity achieved during each courtship, and energy use during sustained rest (SMR). (b) Rates of increase in estimated energy use and $\dot{V}CO_2$ during phases 1–3 (P1–P3) of fighting and copulatory courtship. Data represent estimated microwatts and actual CO_2 production per male. The heights of all bars represent least square means adjusted via multiple regression for the average age and body mass of the contestants, or the actual age and mass of the courting or resting male. Error bars span ± 1 SE. Numbers above bars, below the top scale, give the number of observations (N) used to calculate each mean. Numbers above bars, above the top scale, give the exact $\dot{V}CO_2$ value for each bar.

assessments. Phase 3 combat caused male MR to rise 3.9 times more quickly than in phase 1. Again, only phases 1 versus 2 and 1 versus 3 differed significantly in their associated rates of MR increase (Tukey HSD: $P<0.0001$). Accelerations of the metabolic rate during phase 2 versus 3 fighting were statistically indistinguishable (Tukey HSD: $P = 0.92$).

Males commencing steady preinsemination phase copulatory courtship from a state of rest or mild locomotion attained rates of increase in their metabolic rates similar to those seen during phase 1 fighting (Tukey HSD: copulatory courtship versus phase 1: $P = 0.88$; versus phase 2: $P = 0.006$; versus phase 3: $P<0.0001$); this analysis included courtship data as a fourth behavioural category and again controlled for age ($F_{1,164} = 0.91$, $P = 0.34$) and body mass ($F_{1,164} = 0.02$, $P = 0.89$).

DISCUSSION

Sierra dome spider males incur substantial energetic costs during intrasexual contests as they engage one another in phase 1 noncontact displays, phase 2 pedipalp wrestling, and phase 3 unritualized biting and grappling. Phase 1 behaviour raised the mean metabolic rate more than three times above the resting rate. The more energetically costly behaviours in phases 2 and 3 were estimated to raise average energy consumption more than seven and nine times above resting levels, respectively. Some portion of the estimated energy increases, especially during phase 2 and 3 fighting, probably were due to the release of CO_2 dissolved in the blood prior to fighting (see Methods). Although metabolic rates for phases 2 and 3 may reflect both aerobic and anaerobic respiration, they fall within a range of values obtained from other studies of arthropod energetics. Forced locomotion in various spiders has yielded metabolic rates that range from 2 to 22 times that of resting metabolic rates (Humphreys 1977; McQueen 1980; Prestwich 1983; Kotiaho et al. 1998; Schmitz & Perry 2001). Our estimates of metabolic rates in fighting sierra dome spiders also are in the vicinity of those recorded in the few studies of fight energetics in non-arachnid arthropods (e.g. Hack 1997). Our results provide strong support for a basic prediction of the evolutionary theory of animal contests, namely, that the more advanced stages of contests should represent the exchange of increasingly costly information concerning fighting ability (Enquist & Leimar 1983).

For distinctly different ritualized phases of fighting to evolve within a species, such as the phase 1 and 2 assessment behaviours of the sierra dome spider, they must provide qualitatively or quantitatively different information to the combatants (Enquist & Leimar 1983). For a less accurate assessment phase to persist, the phase that provides more accurate information on the probability of winning an unritualized fight must not always be necessary. That is, there must be conditions under which the expected cost of a more accurate but more expensive and risky method of assessment, on average, outweighs the expected benefits. Such conditions may include a large mismatch in body mass or vigour between contestants.

Such conditions often occur in meetings between male sierra dome spiders on female webs. Adult male sierra dome spiders vary in body mass from 6 to 28 mg. Contests involving a difference of more than 15–20% in body mass seldom escalate beyond phase 1 (P. Keil, P. J. Watson, S. Field & T. N. deCarvalho, unpublished data). Copulatory courtship performance, a measure of many components of male vigour such as metabolic competence, coordination and probably developmental competence, also varies from two to up to 30 intromissions per 3-min measurement period (Watson & Lighton 1994). Many male–male encounters occur in which the less rigorous assessment of phase 1 will suffice. However, despite the variation between males in size and vigour, many fights do occur, and those involving fairly well-matched contestants are common, providing selection pressure for the evolution of phase 2 fighting. The striking similarity of the MR_{max} and the rate of metabolic increase achieved by males during phases 2 and 3 suggest that phase 2 assessment provides contestants with high-quality information about their own and their rivals' metabolic capacities during ritualized fighting. Thus, phase 2 assessment provides an opportunity for each contestant to make a well-informed withdrawal before proceeding to dangerous unritualized phase 3 fighting.

Implications for Models of Contest Behaviour

In keeping with the predictions of the SAG, our results show that energy expenditure increases markedly when contestants switch from one behavioural phase to the next (Fig. 2). However, in contrast to SAG predictions, energy expenditure also increases within phases. This may be due to an increased reliance on anaerobic metabolism over time. Spiders could be maintaining a constant behavioural intensity, but rapidly depleting energy storage compounds, such as glucose, as they rely more on anaerobic metabolism. However, observations of the fighting style of sierra dome spiders suggest otherwise. Behavioural intensities of each individual undergo obvious shifts within phases. Sudden explosive efforts, as well as temporary withdrawals, are frequently evident. This is not surprising, given that it is in the interest of both contestants to attempt, at minimal cost, a determination of their relative resource holding power adequate to predict the outcome of a further escalated contest.

A more appropriate model would allow fight intensity to vary over the course of a fight (as do the CAM and the E-WOA) and between contestants (as does the CAM alone). Formally testing the predictions of either of these models would require energetic tracings of fighting individuals, which are not available at this point. However, the behavioural evidence strongly suggests that fight intensities of the contestants are not matched, as required by the E-WOA, and thus, the CAM is the only currently existing model that approximates the reality of sierra dome spider fighting. The difficulty of obtaining individual energetic traces means that testing of the CAM will rely on future work that seeks to establish relationships between specific behavioural acts and energetic costs.

Implications for Sperm Precedence

Electrophoretic paternity analyses on free-living sierra dome spider females, all their mates, and a random sample of their offspring, showed that first mates sire, on average, 60–70% of all offspring (Watson 1991a, b). Using various techniques, first-male sperm precedence has also been seen in other linyphiid species (Austad 1982; Martyniuk & Jaenike 1982), as well as in members of two other families of entelegyne spiders (see Kaster & Jakob 1997), in which females have conduit style, 'first in/first out' sperm duct systems. Based on gross spermathecal morphology, Austad (1984) predicted the above pattern of sperm use by female entelegyne spiders, in contrast to haplogynes, which have cul-de-sac, 'first in/last out' spermathecae and, consequently, probably a high likelihood of last-male sperm precedence (see also, West & Toft 1999).

Although spermathecal morphology is likely to play a role in determining patterns of sperm use, many subtle morphological or physiological features of the sperm duct and storage systems of any species could modify, and even reverse (as happens in some multiply mated sierra dome spider females), sperm usage patterns (see Watson 1991b). The question about sperm precedence at the evolutionary level of analysis, namely, why mechanisms promoting first-mate sperm precedence exist, remains open.

The results reported here shed light on the possible adaptive significance of first-male sperm precedence in sierra dome spiders and possibly other entelegyne species. Respirometric work on male courtship behaviour suggests that courting males spend much of their time at or near their maximum long-term sustainable aerobic capacity. Some males may 'go anaerobic' for the final 1–2 min of copulatory courtship, as indicated by a 5–10% increase in their CO_2 output without a corresponding increase in behavioural courtship performance (Watson & Lighton 1994; P. J. Watson, unpublished data). We found that the maximal intensity of exercise per unit time attained in the course of several hours of copulatory courtship was comparable to the average intensity maintained during several minutes of intensive phase 1 fighting display. Fights that have the greatest impact on male reproductive success, namely, those over nearly mature or mature virgin females, typically occur in an orderly multimale 'championship' series (Watson 1990). Such a series of fights, many of which escalate beyond phase 1, precede most females' first matings. In contrast, males seldom are required to fight even once to become a female's secondary mate. Thus, the male–male competition process that leads to the determination of all females' first mates should yield a more stringent test of male metabolic competence, an important aspect of viability, than intersexual copulatory courtship; this provides an adaptive rationale for the evolution of first-male sperm precedence in sierra dome spiders (Watson 1990, 1991a, 1998). It is especially likely that multimale fighting prior to the first mating provides the best test of explosive strength, which probably is an integrative measure of a male's ability to mobilize energy via a combination of aerobic and anaerobic metabolism. Interestingly, first mates also perform copulatory courtship, which provides additional information to females.

Copulatory courtship performance, however, has not been found to impact the fertilization success of first mates, but only that of secondary mates (Watson 1991a, b).

When attempting to obtain the most accurate and statistically powerful comparisons of the energetics of contest and courtship behaviours in short-lived species, our study illustrates that it is important to control for individuals' ages, in addition to their weights. Both of these factors may have significant effects on the costliness of fighting and assessment, individual capacities to sustain expensive behaviours, and thus, the evolution of contingent behavioural strategies that individuals use in male–male competition and intersexual courtship.

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