Supplemental Data: Male development tracks rapidly shifting sexual versus natural selection pressures

Michael M. Kasumovic and Maydianne C.B. Andrade

Supplemental Experimental Procedures
Laboratory experiment

Spiders were outbred offspring of individuals collected in Perth (2000) and Sydney, Australia (2001, rearing protocols in [S1]). Spiderlings were held in separate 2x2x3 cm plastic cages and fed *Drosophila sp.* twice weekly. For our development experiment, 212 penultimate (4th) instar males (identified by developing copulatory organs [S2]) were removed from the population and reared in the presence or absence of females and their webs, on one of three diets (high, mid, or low). High and mid-diet males were fed 3 times per week (6 and 3 *Drosophila*, respectively); low-diet males received 1 *Drosophila* each week. In the female-present treatment, each male's cage was surrounded by 4 web-building virgin females (each in their own cage). There was no direct or visual contact between males and females or their webs, but cages were porous to allow the passage of airborne pheromones. In the female-absent treatment, males were kept in a similar room, adjacent to cages of other males, but with no female spiders present. In both treatments, the number of neighbouring males varied throughout the experiment due to the constant addition of newly moulted penultimate males and the removal of mature males from treatments. Temperature and light cycle was the same for all males. Males were monitored daily and date of adult moult noted. We measured the length of the patella-tibia of each male's two front legs at the penultimate instar and the adult stage using digital images and measurement software (Simple PCI, Compix Inc. Imaging systems, 2002). Adult males were also weighed (Ohaus explorer balance accurate to 0.1 mg), then returned to their cages.

Male size was the average of the two leg measurements, and growth was the change in size after males were placed into a treatment, adjusted for variation in penultimate size ([adult size – penultimate size] / penultimate size). We ensured that variation in our measure of growth was not biased by pre-experimental differences in male size (i.e., size at the penultimate instar) ($F_{1, 210} = 2.44$, $P = 0.12$).

Male body condition was estimated using residuals of log (cubed root of male weight) regressed on log (size) (see below). We analyzed the data using a MANOVA and a three-way ANCOVA with (i) growth, (ii) adult body condition, and (iii) development time as the dependent variables, and (a) feeding treatment, (b) female presence/absence as the independent variables, and (c) average number of neighbouring males as a covariate for each male. We examined differences between diet treatments using a Tukey-Kramer HSD post-hoc test [S3].

Field data

We tested our prediction that size and body condition of adult males should decrease as the distance to the nearest potential mate decreased in the

field [field site described in S4]. We monitored solitary penultimate instar males (found on the webs on which they developed) daily in the field and weighed and measured them (as above) on the day they matured. We recorded distance to the nearest adult or penultimate female's web. We only used males observed during their penultimate instar or found during or immediately following moulting to insure accurate assessment of proximity to females during development and body condition at final moult.

Estimating male body condition

There are a variety of ways to estimate body condition, but there is considerable debate about which is the most statistically appropriate or biologically relevant [S5-S13]. Although there is no current consensus on any one method, it is desirable in many studies to estimate the relative size of energy stores available for use by individuals [S8, S12-S14]. Although body condition indices may not directly correlate to fat reserves as measured by lipid content, they nevertheless measure a biologically relevant trait related to fitness in many studies [S8, S13, S14]. One recommended method is to include body size as a covariate in a general linear model analysis of variables of interest [S5, S11]. While this method has some advantages [S5, S10, S11] it constrains the types of analyses available, and does not yield individual indices of body condition, as is required in many studies.

One commonly used body condition index, recently found to perform well statistically and to be biologically relevant [S13, S14], is the residual index, which estimates body condition as the residual from a regression of body weight on linear size [S8, S13]. Although the use of residuals has been questioned [S5, S10, S11], new research demonstrates that using Ordinary Least Squares (OLS) regression satisfies all critical statistical assumptions and performs better than Reduced Major Axis (RMA) regression [S13]. The relationship between weight and size has been argued to be log linear [S8], so residual indices are often based on a regression of $\log \sqrt[x]{weight}$ on $\log \sqrt{size}$. However, the exponent of the log relationship of weight may vary across species, and depends on how weight scales with size [S6].

To determine the relationship between log (weight) and log (size) for redback spiders, we used two independent groups of males. The first group was from a lab reared population (N = 60, Andrade, unpublished data), and the second group was from a field captured population (N = 400, see [S4]). For each group we performed a separate Model II regression (reduced major axis regression, RMA) because of the error associated with measuring both the dependant and independent variables [S3, S11]. We regressed male weight on size using RMA [S15] and found the average slope of the relationship across the two analyses was 3 (Group 1: 2.615 ± 0.1826, 95% CI: 2.249 – 2.989; Group 2: 3.667 ± 0.1141 ; 95% CI: 3.443 – 3.890). We ran our statistical analyses using each calculated slope value independently and using the average slope (3), but found no qualitative differences in our results. Thus we report our analyses using the average value (3) as our best estimate of the true exponent (Table 1).

Statistical Analyses

There was no difference in the initial size of penultimate-instar males placed in treatments (Two-way ANOVA, all P > 0.31). Variation in adult male size at the end of the experiment was within the range of variation seen in wild-caught males (mean ± SD; this study: 2.993 ± 0.250mm, data from [S4]: 2.913 ± 0.399mm) suggesting experimental diets were reasonable simulations of natural diets. Adult size and body condition were normally distributed.

We used a MANOVA to test for effects of diet treatment, female density, male density (and all possible interactions) on development time, experimental growth, and body condition. Because of the significant multivariate interaction between diet, female density, and male density (Table 1), we also report separate univariate ANOVA's for each treatment to determine how each factor influences the dependant variables.

We tested for post-hoc differences between and within diet treatments using a Tukey-Kramer HSD post-hoc test [S3]. Development time decreased and body condition at maturity increased significantly with each increase in food availability (low to mid to high-diet, Figure 1A, B). When data were examined within each diet treatment, only in the low-diet treatment did female presence lead to a significant decrease in male body condition and increase in growth (Figure 1B, C). Due to a significant interaction between diet and female presence (controlled for male density, Table 1), we examined effects of diet on growth separately within each female treatment [S3]. In the absence of females, high-diet males grew more than low-diet males (Figure 1C). In the presence of females, high and mid-diet males grew more than low-diet males (Figure 1C).

Supplemental References
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