

Male development tracks rapidly shifting sexual versus natural selection pressures

Michael M. Kasumovic* and
Maydianne C.B. Andrade

Theory predicts that interplay between sexual and natural selection shapes phenotypic distributions over evolutionary time [1–3]. We show that this also significantly affects individual development. Developmental plasticity, whereby individuals vary their ontogeny in response to variation in the selection to be encountered upon maturity [4–6], has previously been demonstrated in response to single selective pressures, such as predation and sperm competition [7,8]. We show that the relative magnitude of opposing natural and sexual selection can trigger developmental shifts and rapidly change the distribution of phenotypic traits critical to reproductive success.

Males of the sexually cannibalistic redback spider (*Latrodectus hasselti*) show a tactical, condition-dependent shift between conflicting developmental strategies favoured by scramble competition or surviving mate search. Male condition, developmental rate and body size changed with the relative importance of these selective pressures, which naturally fluctuate throughout a breeding season [9–11]. This has important implications for studies comparing fitness values of fixed traits without regard for plasticity.

We exposed penultimate-instar redback spider males to non-contact, pheromonal cues [12,13] simulating dense (females present) or sparse (females absent) populations. This treatment was combined with high, intermediate or low diet levels, respectively. When females are sparse, natural selection for provisioning to

survive mate searching, during which 80% of males perish [14], will be more intense than sexual selection for scramble competition for virgin females (90% median paternity for the first male to mate) [15]. As multiple males commonly settle on females' webs [16], male density will also affect competitive success. We, therefore, examined male growth, development time, and body condition as a function of diet, female treatment and male density. We also assessed male phenotypes as a function of female proximity in the field (Supplemental Data).

Our laboratory and field results collectively show that redback males adjust their development in response to pheromonal cues of female and male density. When females are absent, males trade-off rapid development for increased body size and condition (Table 1; Figure 1) as larger males in better condition are more likely to survive mate searching and direct competition when females are sparse. In contrast, males trade-off size and body condition for rapid development when females are present (Table 1; Figure 1), which would ensure that they can reach virgin females first [15]. Similarly, in the field, as female proximity increases, male size (Two-way ANCOVA, $F = 8.96$, $p = 0.004$) and body condition (Two-way ANCOVA, $F = 5.14$, $p = 0.028$) decrease. Sexual selection for scramble competition has been proposed to contribute to sexual size dimorphism as a side-effect of rapid development of males [17]. These results show that the degree of dimorphism could change within seasons as selection fluctuates.

Although increased female density resulted in smaller males of poorer condition that developed rapidly, increased male density led to the development of smaller males in better condition (Table 1). This independent effect of neighbouring males suggests a trade-off between size and body condition. Thus, the relative importance of these traits to male fitness apparently changes as a function of both female and male density.

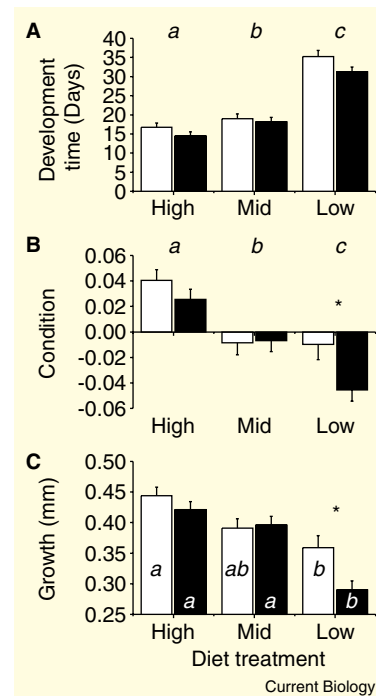


Figure 1. Developmental plasticity under varying selection.

Development time (A), body condition (B) and growth (C) of males reared in the absence (white bars) or presence (black bars) of virgin females across high, mid and low diet treatments (X-axis). Error bars represent one standard error. Asterisks and letters within bars represent significance in Tukey-Kramer HSD post-hoc tests. In (A and B), data for post-hoc tests were pooled across female presence treatments. Post-hoc tests in (C) were completed separately for the different female treatments due to a significant interaction between female presence and diet when controlled for male density. In (B and C), asterisks represent significant differences between female presence treatments within the low-diet.

Although conflicting selection usually results in non-optimal phenotypes with moderate fitness [18,19], in redback spiders, conflicting pressures acting on phenotypic plasticity yield phenotypes optimized for specific competitive challenges [6]. This is critical because redback males are under strong selection to succeed in their single mating opportunity [16,20]. Similar plasticity may be important in cases where males have few mating opportunities that occur over small spatial or temporal scales, where juveniles can detect cues reliably predicting adult challenges and/or where allocation of limited resources

Table 1. Female presence and diet affect growth, body condition and development time of males.

MANOVA				
	Pillai's Trace	F	d.f.	p
Female	0.086	6.01	3, 198	0.0006
Diet	0.584	27.27	6, 398	<0.0001
Female x Diet	0.059	1.83	6, 398	0.09
Males	0.084	5.73	3, 198	0.0009
Female x Males	0.009	0.48	3, 198	0.70
Diet x Males	0.016	0.97	6, 398	0.44
Female x Diet x Males	0.073	2.32	6, 398	0.032
Univariate Analyses				
	Source	F	p	
Development time	Female	4.80	0.03	
	Diet	80.70	< 0.0001	
	Female x Diet	0.25	0.78	
	Males	0.34	0.56	
	Female x Males	0.01	0.90	
	Diet x Males	0.058	0.95	
	Female x Diet x Males	0.08	0.92	
Body condition	Female	4.62	0.033	
	Diet	23.15	< 0.0001	
	Female x Diet	1.84	0.16	
	Males	15.22	0.0001	
	Female x Males	1.25	0.26	
	Diet x Males	2.68	0.07	
	Female x Diet x Males	2.67	0.07	
Experimental growth	Female	5.23	0.023	
	Diet	23.73	< 0.0001	
	Female x Diet	2.58	0.078	
	Males	4.41	0.037	
	Female x Males	0.06	0.81	
	Diet x Males	0.07	0.94	
	Female x Diet x Males	3.21	0.042	

Values in bold are significant (table-wise $p < 0.05$).

yields disproportionate increases in selected fitness components. Such conditions may occur in other mate-searching species with variable population density and relatively short male lifespan [17].

We demonstrate that short-term shifts in conflicting sexual and natural selection can be a major source of variation in male phenotypic traits important to sexual competitiveness [1,17]. Although decreases in resource availability can decrease male size and body condition (Table 1; Figure 1) [8], small size may also arise because the net fitness benefit of decreased development time outweighs the potential costs of small size in the local environment. Thus the maintenance of classes of individuals thought to be competitively inferior [1] could be explained by plasticity and fine-scale environmental heterogeneity, rather than variation in condition [7]. This highlights the critical need to

expand definitions of male quality beyond fixed adult traits to include an individual's ability to respond to changes in varying selection pressures [9]. Most importantly, for studies of sexual selection on male phenotypes, assessments of fitness effects measured without consideration of local conditions and development could seriously overstate the importance of fixed, heritable traits to lifetime reproductive success.

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Supplemental data

Supplemental data are available at <http://www.current-biology.com/cgi/content/full/16/7/R242/DC1/>

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Integrative Behaviour and Neuroscience Group, University of Toronto at Scarborough, Toronto, Ontario, M1C 1A4, Canada.

*E-mail: m.kasumovic@utoronto.ca

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

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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 ($[\text{adult size} - \text{penultimate size}] / \text{penultimate size}$). 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 $\log \sqrt{\text{weight}}$ on $\log \sqrt{\text{size}}$. 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 \pm SD; this study: $2.993 \pm 0.250\text{mm}$, data from [S4]: $2.913 \pm 0.399\text{mm}$) 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).

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