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Condition dependence of sexually dimorphic colouration and longevity in the ambush bug *Phymata americana*

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Abstract

Sexually selected traits that are costly are predicted to be more condition dependent than nonsexually selected traits. Assuming resource limitation, increased allocation to a sexually selected trait may also come at a cost to other fitness components. To test these predictions, we varied adult food ration to manipulate condition in the colour dimorphic bug, *Phymata americana*. We compared the degree of condition dependence in a sexually selected trait expressed in males to a nonsexually selected trait expressed in males and females. We also evaluated the effects of condition on longevity of both sexes. We found that the expression of these colour pattern traits was strongly influenced by both diet and age. As expected, the strength of condition dependence was much more pronounced in the sexually selected, male-limited trait but the nonsexual trait also exhibited significant condition dependence in both sexes. The sexually selected male trait also exhibited a higher coefficient of phenotypic variation than the nonsexually selected trait in males and females. Diet had contrasting effects on male and female longevity; increased food availability had positive effects on female lifespan but these effects were not detected in males, suggesting that males allocated limited resources preferentially to sexually selected traits. These results are consistent with the expectation that optimal allocation to various fitness components differs between the sexes.

Introduction

Sexually selected traits are expected to be exaggerated to the point that the benefits under sexual selection are balanced by the costs imposed by natural selection. Costs of bearing exaggerated traits may include increased predation risk or decreased foraging success, and there may be energetic and developmental costs associated with their production (Andersson, 1994; Johnstone, 1995). It is generally assumed that the optimal exaggeration of these traits varies with individual condition (i.e. the total pool of resources from which an individual can allocate), where the marginal benefit to fitness of further allocation to sexual traits is greater for individuals in high condition (Proulx *et al.*, 2002; Getty, 2006). Because condition reflects genetic or environmentally determined variance in the ability to acquire or assimilate resources, condition dependent traits may therefore honestly signal phenotypic and/or genetic quality (Zahavi, 1975; Andersson, 1986; Grafen, 1990; Iwasa & Pomiankowski, 1994; Rowe & Houle, 1996). In addition to its importance for models of sexual selection, condition dependence has broader implications for the maintenance of genetic variation in sexually selected and life history traits under persistent directional selection (Rowe & Houle, 1996; Houle, 1998; Tomkins *et al.*, 2004).

In contrast to these traits under directional selection, 'ordinary' metric traits (i.e. those not directly related to fitness) may be under weak stabilizing selection and therefore, lack dependence on condition. Consequently, a commonly held expectation is that sexually selected traits should exhibit a greater dependence on condition and be more variable than nonsexually selected metric traits (e.g. Alatalo *et al.*, 1988; Pomiankowski & Møller, 1995; Wilkinson & Taper, 1999; Cotton *et al.*, 2004b; Boughman, 2007). In spite of the prevalence of this view, a recent, critical evaluation by Cotton *et al.* (2004a)

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found only limited support for the prediction that sexually selected traits exhibit heightened condition dependence.

The concept of condition dependence is a key element in the integration of sexual selection and life history theory (Schluter *et al.*, 1991; Rowe & Houle, 1996; Hunt *et al.*, 2004b). In particular, most models of indirect sexual selection (e.g. 'good genes') have considered condition dependence as being indicative of genetic covariance between sexually selected traits and components of fitness (other than mating success). Seemingly in support of this view, many empirical studies have indeed reported a positive relationship between condition dependent traits and certain components of fitness (e.g. reviews in Møller & Alatalo, 1999; Jennions *et al.*, 2001). However, though condition dependent traits are expected to covary positively with *net* fitness, there is no basis to predict that such traits will necessarily exhibit positive correlations with *all* components of fitness. Limited resources will frequently lead to allocation trade-offs, making the relationship between a sexually selected trait and any given fitness component difficult to predict (Hansen & Price, 1995; Proulx *et al.*, 2002; Kokko *et al.*, 2003). Indeed, at least a few studies have reported negative relationships between the expression of a sexually selected trait and some life history trait (e.g. Brooks, 2000; Hunt *et al.*, 2004a).

Both the issues of condition dependence of sexually selected traits and their relationship to life history traits can be addressed with experimental manipulations of condition. Manipulation of food availability is a commonly employed method for simulating genetic or environmental variance in condition (e.g. Cotton *et al.*, 2004a,b; Hunt *et al.*, 2004a; Bonduriansky & Rowe, 2005b). In this paper, we tested the prediction that sexually selected traits exhibit stronger condition dependence than nonsexually selected traits by examining the effects of food ration on the expression of two sexually dimorphic traits in the ambush bug *Phymata americana*: dorsal colouration, which is expressed in both sexes but to a greater extent in males, and lateral colouration, which is apparently male-limited in expression (Punzalan, 2007). Previous studies have shown that lateral colouration is a target of consistently strong sexual selection whereas dorsal colour pattern is not (Punzalan *et al.*, 2008; Punzalan *et al.*, in press).

Unlike many previous studies of condition dependence of sexual traits, where either only male-limited traits (e.g. Hooper *et al.*, 1999; Grether, 2000) or sexually homologous traits were examined (e.g. Cotton *et al.*, 2004a; Bonduriansky & Rowe, 2005b), the current study provides a unique opportunity to investigate both simultaneously. Assuming that differences among traits in their degree of sex-limitation reflect differences in genetic architecture, the strength of condition dependence may vary considerably among traits (Bonduriansky & Rowe, 2005b). For example, sex-limited traits might be expected to exhibit a greater degree of condition dependence than sexually homologous traits as a result of the former being relatively free from the potential constraints imposed by intra-locus sexual conflict (Bonduriansky & Rowe, 2005a).

We also measured the effects of condition on longevity in both sexes to test for life history effects due to possible sex differences in the allocation of condition. There is some evidence that sex differences in the allocation of resources can result, not only in sexually divergent morphologies, but also in disparate life histories, such that condition affects the life history of the sexes differently (e.g. Hunt *et al.*, 2004a; but also see Judge *et al.*, 2008).

Methods

Background on study organism

Phymata americana (Heteroptera: Phymatidae) is a predatory insect occurring on inflorescences and flowers in temperate North America (Balduf, 1939, 1941). The sexes exhibit striking differences in pigmentation on various parts of the body. Males generally have darker (see below for technical definition) pigmentation than females, and expression of some components of colouration (e.g. dark pigment on the head and lateral thoracic sclerites) are apparently absent in females from populations we examined in Southern Ontario (Fig. 1). Dark colouration is expressed only in adults. Phenotypic selection studies (conducted over multiple years) in wild populations of *P. americana* have consistently shown that lateral colouration is the target of strong, positive directional sexual selection (Punzalan, 2007; Punzalan *et al.*, in press). Laboratory studies have not found any evidence of colour pattern functioning as a visual signal in male–male or male–female interactions; instead, lateral colour pattern appears to mediate sexual selection via a thermoregulatory role; darker males achieve higher body temperatures and, therefore, can search for mates more actively in cool ambient temperatures (Punzalan *et al.* 2008). The evolutionary cause of dimorphism in dorsal colouration is not known but it is possible that dimorphism reflects divergent trait optima resulting from sex differences in ecology (Slatkin, 1984). For example, colour pattern in phymatids is typically attributed to selection for crypsis (Dodson & Marshall, 1984); if the optimal cryptic colouration of males and females differs, then dimorphism may be the result of divergent viability selection pressures on dorsal colouration.

Manipulation of condition and rearing

One hundred and forty-two live penultimate (fifth instar) nymphs were collected from a field site at the Koffler Scientific Reserve at Joker's Hill, King, Ontario, Canada (44°03′N, 79°29′W) between 12 July and

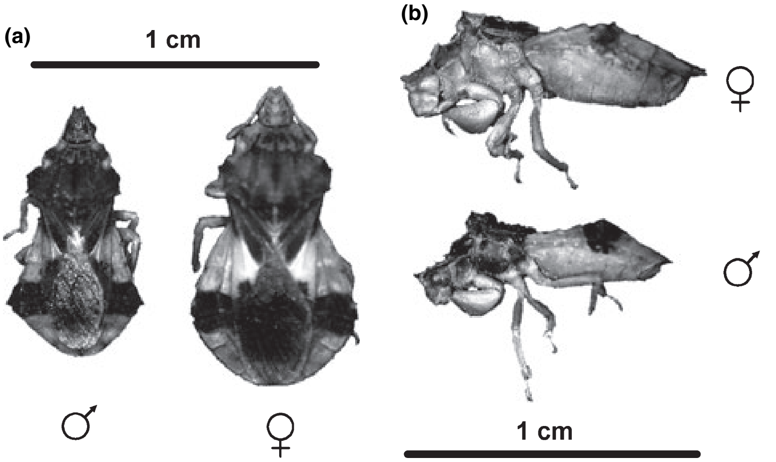

Fig. 1 Dorsal (a) and lateral (b) view of male and female *Phymata americana*. Dorsal colouration was defined as the total area of dark colouration visible from the dorsal aspect. Lateral colouration was defined as the total area of dark colour pattern on the head and thorax visible from the lateral aspect.

3 August, 2003 and maintained in a temperature-controlled room at 27 ± 2 °C, 40% RH under a 14L:10D fluorescent light cycle. Bugs were each individually housed in a separate cage consisting of a 1.5 mL polypropylene centrifuge tube pierced in several places for ventilation. Cages also contained a piece of moistened foam and a small piece of toothpick as a perch. Nymphs were provisioned with six live adult fruit flies (*Drosophila melanogaster*) daily until adulthood. Of the 142 nymphs collected, 134 successfully moulted to adult, of which 49 were male and 85 were female (Chi-squared test for deviation from 1 : 1, $\chi^2 = 9.67$, df = 1, $P = 0.002$). Whether this sex bias reflects differences in primary sex ratio is unclear. Relatively few adults were observed in the field during the collection period (at the same site, adults began to emerge approximately a week later, Punzalan, 2007), suggesting that we did not miss the peak period of emergence (i.e. sampling was not too late). It is possible that the over-representation of females in the sample reflects sex differences in development rates (e.g. if females develop more rapidly, and thus, reach the penultimate instar at the collection site before males).

Diet manipulation commenced upon emergence as adults (after which body size is fixed); individuals in their cages were randomly assigned to either a high- or low-food treatment. Individuals in the high-food treatment were provisioned with 10 fruit flies daily whereas those in the low-food treatment were provisioned with two fruit flies daily. Individuals of both diet treatments were also provisioned with one live adult blowfly (*Calliphora* sp.) weekly as a nutritional supplement. Upon random assignment to diet treatments, individual bug cages were randomly assigned to a position (by number) in one of two polystyrene trays that accommodate 125 cages in 15 × 15 square arrangement. Both trays (one full, the other partially filled) were maintained on the same shelf in the same walk-in environmental chamber. Cages were cleaned twice a week and were misted with water daily. Adult bugs were photographed at four different ages (as described below) as the colouration of *P. americana* may darken with age (Schuh, 1995). Cages were checked daily for mortality until bugs were 24 days old. Longevity for individuals surviving beyond this age was recorded as 24 days.

Measuring colour pattern

Bugs were photographed live at four different ages past emergence: 3, 10, 17 and 24 days. Body size and colour pattern measures were obtained following the methods described in Punzalan (2007); dorsal photographs were obtained by placing an individual bug in a plastic Petri dish stuffed with a cut out layer of compressible foam to restrain the bug during photography. A standard ruler and Liquitex® Acrylic (Liquitex Artist Materials, Piscataway, NJ, USA) paint chips were permanently mounted on the transparent lid of the Petri dish, for scale and colour reference. To obtain lateral photographs, a paper clip was fashioned into a clamp (using pieces of cardboard and compressible foam glued to the arms of the clip) to restrain the bug. Permanently mounted on the visible surface of the clamp was a standard ruler for scale and paint chips of known colour values; in subsequent image analyses. Colour pattern data were collected after recalibrating each image according to the colour pattern standards (i.e. paint chips) to correct for possible differences in lighting during photography. Digital photographs were taken using a Nikon CoolPix 995 (Nikon Corporation, Tokyo, Japan) under standardized illumination (fibre optic light source). Images were analysed using Scion® Image software (http://www.scioncorp.com) to measure body size (measured as the width across the pronotum, between anterior and posterior tubercules when viewed from the lateral aspect; Mason, 1973) and area of dark colouration. The area of dark pigmentation (hereafter, simply referred to as *colouration*) was measured using the threshold function where 'darkly pigmented' was defined as a pixel with a value of 200 (on a scale of 0–255), based on measurements from pilot studies demonstrating this method as a reliable representation of the total amount of visible colouration that is darker than the colour primarily yellow/green integument (Punzalan, 2007).

For both males and females, we measured the total area of colouration visible from the dorsal aspect (hereafter referred to as *dorsal colouration*) (Fig. 1). For males only, we measured the total visible area of colouration on the lateral surface of the thorax (hereafter referred to as *lateral colouration*) (Fig. 1). Both sexes also express a patch of colouration on the latero-ventral surface of the abdomen (i.e. on the abdominal sternites and connexiva). Unlike the thorax, the abdomen is soft and became noticeably compressed by the restraining device during photography. This made it difficult to get a precise measure of colour pattern area of this patch and, therefore, this was not included in the analyses. Because colouration in *P. americana* is somewhat complex and colour patches are not easily defined with morphological landmarks, total area provided an objective measure. Although darkening in *P. americana* may also occur in terms of dark regions getting progressively darker (e.g. from dark brown to black), our analyses are restricted to measures of area.

Statistical analyses

To obtain measures of colour pattern corrected for body size and, hence, relative measures of colouration, area of dorsal and lateral colour patches were square root transformed (because area is expected to increase exponentially with a linear measure of body size) and divided by pronotum width (a good surrogate measure of overall body size; Mason, 1973). Subsequently, we analysed the effects of diet manipulation on these ratio-adjusted colour pattern scores. To estimate the condition dependence of male lateral colouration, we used a single-factor, repeated measures ANOVA, with diet treatment as a fixed factor and lateral colouration (at all four measured adult ages) as the dependent variable. Repeated measures (within group) comparisons were used to assess the effect of age on colour pattern development ('darkening'). The effect of diet treatment was used to estimate condition dependence and the age * diet interaction was interpreted as an estimate of the effect of condition on the rate of darkening. A similar procedure was used to estimate condition dependence of dorsal colouration, except in this case, sex was added as a second factor in a two-factor, repeated measures ANOVA. Here, we interpreted the diet * sex interaction as an estimate of sex differences in condition dependence. In addition, we interpreted the age * diet and age * sex interactions as estimates of the effects of condition and sex, respectively, on rate of darkening. The age * sex * diet interaction was interpreted as a measure of sex differences in the effects of condition on the rate of colour pattern development. Due to mortality across age classes, a number of individuals had to be excluded in the repeated measures analyses. However, separate tests comparing colour pattern development between sequential age classes (i.e. dorsal: two-factor repeated measures ANOVA, lateral: one-factor repeated measures ANOVA) did not reveal any departure from the trends observed in the full (across all four age classes) analyses.

Although the use of ratios to remove the effects of body size can be problematic, and the use of analysis of covariance (ANCOVA) using body size as a covariate is often a preferable method over the use of ANOVA on ratio-adjusted values (recently reviewed in Packard & Boardman, 1999) or residuals (Garcia-Berthou, 2001), we opted not to use this method because our study employed repeated measures of colour pattern from each individual. Though ANCOVA can also accommodate repeated measures, it introduces additional problems when the number of repeated samplings is not equal among individuals (i.e. in our study, sample sizes were reduced with each subsequent sampling period due to mortality). Because ANCOVA relies on estimating the slope of regression at each sampling period, loss of individuals between sampling periods can affect the slope of regression (between the trait and body size). This would lead to apparent changes in individual colour pattern scores (i.e. residual variation in colour pattern) solely as a result of removal of individuals at each subsequent sampling period. As a safeguard, we did perform the analyses using a repeated measures ANCOVA (with pronotum width as a covariate) and compared them to the analyses performed using the repeated measures ANOVA on the ratio-adjusted values. Qualitatively, the results did not differ from the original analysis and, therefore, we present only the latter here.

We also measured condition dependence by estimating the effect size of the diet manipulation for each colour pattern trait. This was calculated, for each trait at 17 days, as the difference in mean colour pattern score between the high-food and low-food treatments, standardized by subsequently dividing by the mean trait size in the low-food treatment (modified from Bonduriansky & Rowe, 2005b). Thus, effect size represented a standardized percent difference in trait size due to the diet treatments. We used age at 17 days (rather than age 24 days) because darkening appeared to reach an asymptotic value by age 17 days (Figs 2 and 3) and there was considerable mortality by age 24 days. Subsequent analyses of effect size using traits at age 24 days did not differ qualitatively from those using traits at 17 days and only the latter are reported here.

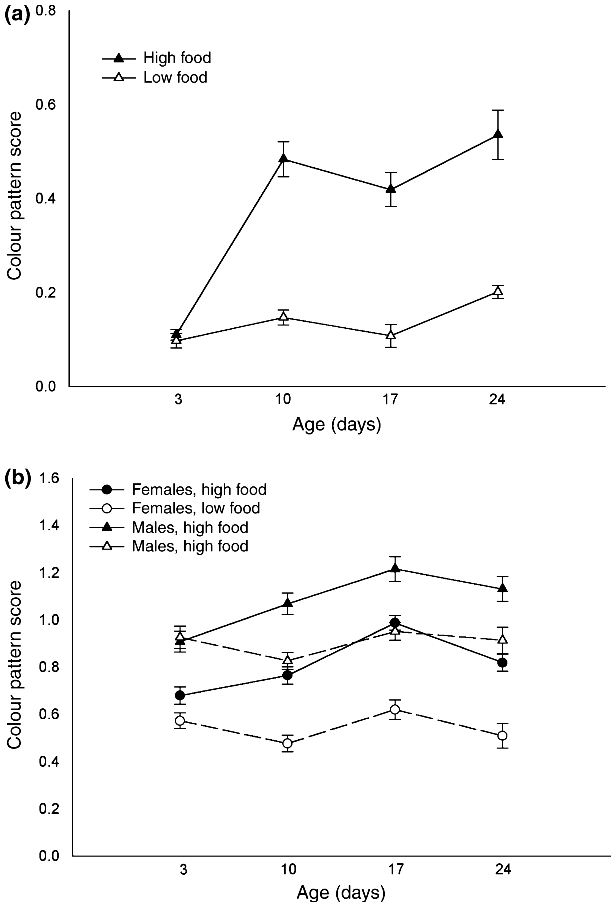

Fig. 2 Effects of diet on the expression of: (a) sexually selected lateral colouration (males) and (b) nonsexually selected colouration (males and females) in *Phymata americana* over the first 24 days of adulthood. Depicted are mean (±SE) colour pattern scores (see Methods) for males (triangles) and females (circles) maintained on high food (closed symbols) and low food (open symbols) treatments at four adult ages.

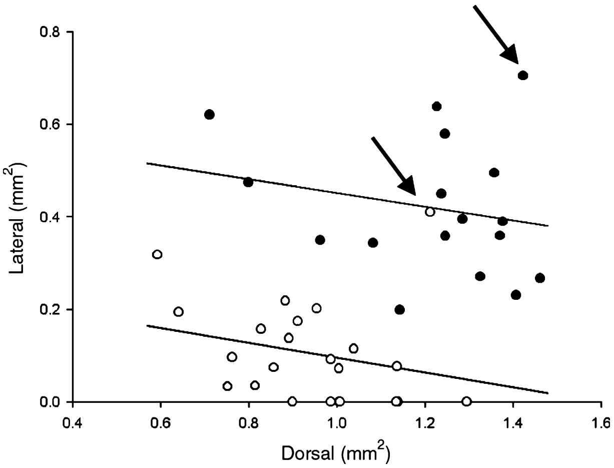

Fig. 3 Phenotypic correlations between male lateral and dorsal colour pattern scores at age 17 days (see Methods). Filled circles represent males from the high food treatment, open circles represent males from the low food treatment. The relationship between dorsal and lateral colouration exhibits a negative trend when examined within treatments but an overall positive trend when examined across treatments. Separate regression lines for the high and low food treatment include outliers (indicted by arrows).

To estimate the effects of diet manipulation on trait variance, for each trait, we calculated the coefficient of variation

$$\mathrm{CV} = 100\ {}^{*}(\text{trait variance})^{1/2}/\text{trait mean}$$

This standardized estimate is particularly useful for comparing phenotypic variance when trait means differ. We report CVs separately for each diet treatment as well as after pooling treatments. Comparisons of trait variability (pooled across diet treatments) were performed in a pair-wise manner; following the method outlined by Lewontin (1966), the F-statistic was calculated for the ratio of the variances (S) for each trait (x and y) according to:

$$F = (S_{\ln x})^2/(S_{\ln y})^2,$$

where the numerator and denominator degrees of freedom equal N_x-1 and N_y-1, respectively. Statistically significant (at a Bonferroni-corrected $\alpha = 0.0167$) F-ratios were taken to indicate significant differences in trait variability.

To evaluate the relationship between expression of dorsal and lateral colouration within and across food treatments, we first performed an ANCOVA with lateral colour pattern at 17 days as the response variable, food treatment as a fixed effect and with dorsal colour pattern at 17 days as a covariate. We examined the diet * dorsal interaction term to determine whether the relationship between male traits differed between diet treatments. Subsequently, we plotted the relationship between dorsal and lateral colour pattern at age 17 days separately for each treatment and calculated the Pearson's product moment correlation for each. We also evaluated the relationship between the two male traits after pooling data across diet treatments. Two data points were detected as outliers in the ANCOVA (Studentized residuals = 3.164 and 3.207; shown in Fig. 3); to be cautious, we present the results for analyses with outliers included and with the outliers removed.

We conducted a right-censored survival analysis to estimate the effects of diet on longevity. We employed a (Cox-regression) proportional hazards model that included sex, diet treatment and the sex * diet interaction as predictor variables and longevity (until 24 days) as the response variable. A similar analysis was performed on each sex separately to identify the sex-specific effects of diet treatment.

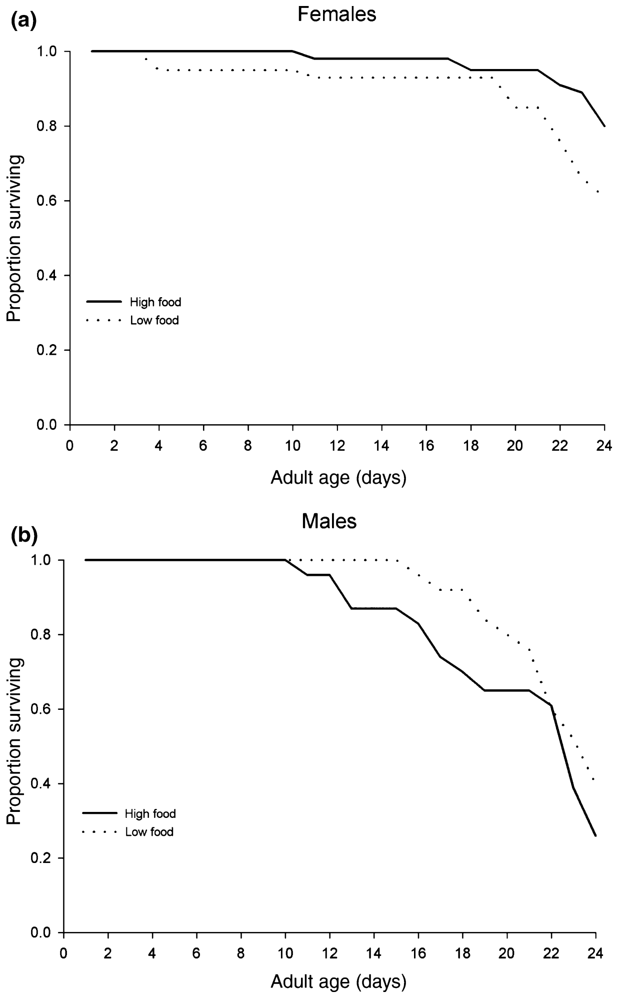

Fig. 4 Survivorship for (a) female and (b) male *Phymata americana* maintained on high food (solid line) and low food (dashed line) treatments over the first 24 days of adulthood.

Analyses were performed using SYSTAT® (Systat Software Inc., San Jose, CA, USA) software (version 10.0) except for the survival analysis, where parameters were estimated using the Cox proportional hazards function in the base package in 'R': A Language and Environment for Statistical Computing, version 2.4.1 (available at: http://www.R-project.org).

Results

Age and condition dependence of a sexually selected male trait

High-food males were darker in lateral colouration than low-food males ($F_{1,14} = 11.28$, $P = 0.005$) by day 24. Lateral colouration darkened significantly with age in both treatments ($F_{3,42} = 10.60$, $P < 0.001$) (Fig. 2) but high-food males darkened faster ($F_{3,42} = 4.31$, $P = 0.010$) than low-food males.

Age and condition dependence of a nonsexually selected, homologous trait

Males were darker in dorsal colouration than females ($F_{1,67} = 47.83$, $P < 0.001$) (Fig. 2). Individuals of both sexes in the high-food treatment were darker in dorsal colouration than in the low-food treatment ($F_{1,67} = 36.20$, $P < 0.001$). The sexes did not differ in their response to the diet treatments ($F_{1,67} = 0.69$, $P = 0.410$). Individuals darkened significantly with age ($F_{3,201} = 9.07$, $P < 0.001$) and those in high-food treatments darkened at a faster rate ($F_{3,201} = 6.55$, $P < 0.001$) (Fig. 2) but the sexes did not differ in this rate ($F_{3,201} = 0.850$, $P = 0.468$) nor was there a sex difference in the effects of condition on the rate of darkening (beyond age 3d) ($F_{3,201} = 0.507$, $P = 0.678$).

Comparing condition dependence among sexual and nonsexual traits

The effect size of the diet manipulation was considerable for all measured traits (Table 1). Effect size was greatest for male lateral colouration and weakest for male dorsal colouration. Effect size of diet treatment for male lateral coloration was ten times larger than that for male dorsal colouration. Male lateral colouration exhibited a higher coefficient of phenotypic variation compared to male dorsal colouration ($F_{32,39} = 10.54$, $P < 0.0001$) and female dorsal colouration ($F_{32,79} = 3.03$, $P < 0.0001$). Trait variability of female and male dorsal colouration was not significantly different ($F_{39,79} = 0.288$, $P = 0.999$). Coefficients of variation were consistently larger in the low-food treatments (summarized in Table 1).

Table 1 Colour pattern scores (mean ± SD) and their coefficient of phenotypic variation (CV) for bugs (age 17 days) reared on two food regimes.

Sex	Diet treatment	Dorsal colouration (√area/pronotum width)	CV lateral colouration (√area/pronotum width)	CV
Female				
Low (37)	0.620 ± 0.247	39.9	–	–
High (43)	0.988 ± 0.240	20.8	–	–
Pooled (80)	0.818 ± 0.290	35.5	–	–
Effect size	59%		–	–
Male				
Low (23)	0.945 ± 0.174	18.4	0.105 ± 0.110	105.5
High (17)	1.215 ± 0.216	17.8	0.419 ± 0.149	35.5
Pooled (40)	1.060 ± 0.290	22.0	0.239 ± 0.202	84.8
Effect size	29%		299%	

Sample sizes in parentheses.

Trade-offs between male sexually and nonsexually selected traits

The relationship between dorsal and lateral colouration did not differ significantly between the diet treatments (diet * dorsal interaction with outliers included: $F_{1,36}$ = 0.001, $P = 0.991$; with outliers removed: $F_{1,34}$ = 0.015, $P = 0.903$). Within diet treatments, there was some evidence suggesting a trade-off between expression of male dorsal and lateral colouration in both the high food treatment (with outliers included: $r = -0.182$, $P = 0.485$; with outliers removed: $r = -0.406$, $P = 0.118$) and low food treatment (with outliers included: $r = -0.211$, $P = 0.334$; with outliers removed: $r = -0.530$, $P = 0.011$; Fig. 3).

When pooled across treatments, however, the dorsal and lateral colouration exhibited an overall positive phenotypic correlation (outliers included: $r = 0.336$, $P = 0.034$; with outliers removed: $r = 0.249$, $P = 0.132$).

Effects of condition on longevity

Diet had a significant effect on longevity (diet: $P = 0.023$) but these effects differed between the sexes (sex * diet interaction: $P = 0.016$; Table 2). When analysed separately, females in the high-food treatment experienced significantly lower mortality than those in the low-food treatment (diet effect: $P = 0.022$, df = 1, $n = 85$; Fig. 4a). In contrast, males in the high-food treatment had higher mortality than did males in the low-food treatment, though this difference was not statistically significant (diet effect: $P = 0.28$, df = 1, $n = 48$; Fig. 4b).

Discussion

Our data show that food limitation strongly influenced the expression of colour pattern in *P. americana*, consistent with previous studies reporting condition dependence of sexually selected colouration in a variety of taxa (e.g. Kodric-Brown, 1989; Hill, 1990; Candolin, 1999; Hooper *et al.*, 1999; Fitzstephens & Getty, 2000; Grether, 2000; McGraw *et al.*, 2002). In support of our prediction, we found that sexually selected colouration exhibited a greater degree of condition dependence than nonsexual colouration in this species. This result is consistent with the few existing previous studies showing heightened condition dependence of sexually selected traits compared with nonsexually selected traits (Cotton *et al.*, 2004a,b; Bonduriansky & Rowe, 2005b; Boughman, 2007).

Table 2 Effects of sex and diet treatment on survival of *Phymata americana* during the first 24 days of adulthood.

	Variable	Coefficient	Std error	*z*	*P*-value
a) Full model†	Sex	0.422	0.346	1.22	0.220
	Diet	−0.890	0.391	−2.28	0.023
	Sex * diet	1.276	0.528	2.42	0.016
b) Females‡	Diet	−0.895	0.391	−2.29	0.022
c) Males§	Diet	0.384	0.355	1.08	0.280

Hazard coefficients (i.e. the direction and effect of a particular term on survival probability), standard errors, the test statistic *z* and statistical significance were estimated using a Cox proportional hazards model for the (a) full model, (b) females separately and (c) males separately.

†Likelihood ratio test statistic = 21.6, df = 3, $P < 0.001$, $n = 133$.

‡Likelihood ratio test statistic = 5.57, df = 1, $P = 0.0183$, $n = 85$.

§Likelihood ratio test statistic = 1.17, df = 1, $P = 0.279$, $n = 48$.

Although lateral colouration exhibited the strongest condition dependence, the effect of condition was not restricted to the male sexually selected trait. The nonsexually selected, dorsal colouration in males and females also exhibited significant condition dependence, albeit to a lesser degree. The weaker condition dependence of this trait is in accord with many reports of nonsexual traits being relatively insensitive to variance in condition (examples in Cotton *et al.*, 2004a,b; but see Bonduriansky & Rowe, 2005b). The conventional view is that nonsexual traits subject to stabilizing selection should be only weakly related to condition (e.g. Wilkinson & Taper, 1999). Furthermore, strong stabilizing selection on a trait predicts canalization (de Visser *et al.*, 2003). However, this does not account for the possibility that genetic covariance among traits may result in a correlated response in the condition dependence of nonsexually selected traits. The condition dependence of male dorsal colouration could be the result of common developmental or physiological pathways underlying the expression of both components of male colour pattern. For example, selection may favour a general increase in the production of pigment (or its precursors) due to direct selection on lateral colouration but because of a common physiological mechanism, also results in similar effects of resource availability on dorsal colouration. On the other hand, a common resource pool can result in resource allocation trade-offs among traits, particularly when traits share similar developmental origins (Nijhout & Emlen, 1998; Moczek & Nijhout, 2004). Such an allocation trade-off may be reflected in the negative relationship observed between male dorsal and lateral colouration within treatments, particularly under low food conditions, despite a weak positive phenotypic correlation observed between dorsal and lateral colouration when pooled across food treatments. This pattern of positive correlations between traits, in spite of a trade-off between them, is consistent with the standard acquisition/allocation model proposed by van Noordwijk & de Jong (1986); also see Houle (1991) – allocation trade-offs among traits are often masked by the large degree of variance among individuals in available resources (i.e. acquisition).

We also observed condition dependence of female dorsal colouration; the strength of condition dependence was similar to that observed for the homologous trait in males. Intersexual genetic correlations are one possible explanation for the similar pattern of condition dependence observed in both sexes (Lande, 1980; Rice & Chippindale, 2001). Bonduriansky & Rowe (2005a,b) observed both that sexually homologous traits showed condition dependence and significant intersexual genetic correlations among these traits in the sexually dimorphic fly *Prochyliza xanthostoma*; this suggested similar genetic architecture underlying condition dependence in males and females. Interestingly, Bonduriansky & Rowe (2005b) found that the degree of condition dependence (in males) was inversely related to the magnitude of the intersexual genetic correlation, possibly indicating that intralocus sexual conflict constrains the degree to which males can optimally allocate condition to sexually selected traits. Whether the heightened condition dependence of sex-limited (lateral) colouration observed in our study represents the resolution of this conflict via adaptive changes in genetic architecture is unclear but offers an intriguing avenue for future research.

In accord with our expectation, male lateral colouration was more phenotypically variable than dorsal colouration, regardless of treatment. This result is consistent with the pattern of phenotypic variation observed for colour pattern traits ($CV_{lateral} > CV_{dorsal}$) in natural populations of *P. americana* (Punzalan, 2007). These data are also consistent with condition dependence resulting in relatively high levels of standing trait variance for sexually selected traits. Although it was not possible to estimate genetic variances in the present study, previous studies have found that phenotypic and genetic variances are often highly correlated and that sexually selected traits exhibit greater variability than nonsexual traits (Alatalo *et al.*, 1988; Pomiankowski & Møller, 1995; Cotton *et al.*, 2004b).

We also found that the expression of colour pattern generally increased with adult age in *P. americana*, though expression was asymptotic. A similar, age-related change was observed for sexually selected wing colouration in the damselfly *Mnais costalis* (Hooper *et al.*, 1999). In insects, cuticular darkening is common during the teneral period following emergence into adulthood (Andersen, 1974); however, darkening is typically a more rapid physiological process, being completed within several days (e.g. Thompson *et al.*, 2002). The prolonged development of colouration observed in our study may indicate that resource limitation (i.e. in the low-food treatment) resulted in reduced rates of colour pattern development in males and, to a lesser extent, in females. The failure of low-food individuals to achieve high asymptotic colour pattern scores suggests that darkening in *P. americana* is severely limited by resource availability.

Traits that are correlated with age and/or condition are thought to be likely targets of mate preferences because they are honest indicators of quality (Andersson, 1982). In *P. americana*, however, lateral colouration does not seem to function as a sexual ornament; previous studies in the wild have shown sexual selection on this trait but lab studies suggest this trait plays no role in male–male or male–female sexual signalling (Punzalan, 2007; Punzalan *et al.* in press). It is useful to point out that, despite the fact that most studies emphasize the importance of condition dependent traits for the evolution of sexual signals, condition dependence is not exclusive to sexual ornaments; condition dependence is characteristic of life history traits (Houle, 1991, 1998) and, generally, is expected for any costly trait closely related to fitness (Rowe & Houle, 1996; Houle, 1998).

Condition and lifespan

In many systems, male total fitness is primarily determined by mating success, whereas female total fitness is primarily determined by lifetime egg production (i.e. fecundity and longevity) (Bateman, 1948). As a result, the sexes may exhibit markedly different optimal strategies for allocation to various fitness components. Here, we found that manipulating diet (and thus the availability of resources) had different effects on lifespan of the two sexes; increased food availability had positive effects on female lifespan but these positive effects were absent in males. A very similar sex difference in condition dependent life history trade-offs was observed in the cricket *Teleogryllus commodus* (Hunt *et al.*, 2004a), where high resource availability resulted in longer lifespan in females but shorter lifespan (and increased calling effort – a sexually selected trait) in males. These studies suggest that high investment in sexually selected traits may be accomplished at the cost of resources that might otherwise be allocated to longevity/survival. Although the negative relationship between investment in a sexual trait and longevity has seldom been reported (but see Brooks, 2000), it probably represents an example of the common, general phenomenon of evolutionary trade-offs (Roff, 2002). Despite the ubiquity of trade-offs between components of fitness, many authors have interpreted age (an estimate of longevity), or traits correlated with age, to be honest indicators of genetic quality. Though, under some circumstances, age dependent traits may actually be reliable indicators (e.g. Kokko, 1997, 1998; Proulx et al., 2002), clearly, longevity need not covary positively with total fitness (e.g. Hansen & Price, 1995; Kokko, 1997, 1998).

Conclusions

In accord with theoretical predictions, we found that a sexually selected trait (lateral colouration in males) exhibited stronger condition dependence and greater levels of trait variance than nonsexually selected traits. However, even the nonsexually selected colouration exhibited some degree of condition dependence; the reason for this is unclear, though it may reflect genetic covariance between the nonsexually selected trait and the condition dependent sexually selected trait. Condition had contrasting effects on longevity in the two sexes; condition and longevity were positively associated in females but increased resource availability had no positive effects on male longevity. This is consistent with the expectation that males and females allocate limited resources differently among fitness traits, for example, with males allocating preferentially to mating success (via costly sexually selected traits).

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References

Alatalo, R.V., Höglund, J. & Lundberg, A. 1988. Patterns of variation in tail ornament size in birds. *Biol. J. Linn. Soc.* **34**: 363–374.

Andersen, S.O. 1974. Evidence for two mechanisms of sclerotisation in insect cuticle. *Nature* **251**: 507–508.

Andersson, M. 1982. Sexual selection, natural selection and quality advertisement. *Biol. J. Linn. Soc.* **17**: 375–393.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* **40**: 804–816.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ, USA.

Balduf, W.V. 1939. Food habits of *Phymata pennsylvanica americana* Melin (Hemip.). *Can. Ent.* **71**: 66–74.

Balduf, W.V. 1941. Life history of *Phymata pennsylvanica americana* Melin (Phymatidae, Hemiptera). *Ann. Entomol. Soc. Am.* **34**: 204–214.

Bateman, A.J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349–368.

Bonduriansky, R. & Rowe, L. 2005a. Intralocus sexual conflict and the genetic architecture of sexually dimorphic traits in *Prochyliza xanthostoma* (Diptera: Piophilidae). *Evolution* **59**: 1965–1975.

Bonduriansky, R. & Rowe, L. 2005b. Sexual selection, genetic architecture and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution* **59**: 138–151.

Boughman, J.W. 2007. Condition-dependent expression of red colour differs between stickleback species. *J. Evol. Biol.* **20**: 1577–1590.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature* **406**: 67–70.

Candolin, U. 1999. The relationship between signal quality and physical condition: is sexual signaling honest in the three-spined stickleback? *Anim. Behav.* **58**: 1261–1267.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004a. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. Lond. B* **271**: 771–783.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004b. Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanii* (Diptera: Diopsidae). *Evolution* **58**: 1038–1046.

Dodson, G. & Marshall, L. 1984. Mating patterns in an ambush bug *Phymata fasciata* (Phymatidae). *Am. Midl. Nat.* **112**: 50–57.

Fitzstephens, D.M. & Getty, T. 2000. Color, fat and social status in male damselflies, *Calopteryx maculata*. *Anim. Behav.* **60**: 851–855.

Garcia-Berthou, E. 2001. On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *J. Anim. Ecol.* **70**: 708–711.

Getty, T. 2006. Sexually selected signals are not similar to sports handicaps. *Trends Ecol. Evol.* **21**: 83–88.

Grafen, A. 1990. Sexual selection unhandicapped by the Fisher process. *J. Theor. Biol.* **144**: 473–516.

Grether, G.F. 2000. Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution* **54**: 1712–1724.

Hansen, T.F. & Price, D.K. 1995. Good genes and old age: do old mates provide superior genes? *J. Evol. Biol.* **8**: 759–778.

Hill, G.E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Anim. Behav.* **40**: 563–572.

Hooper, R.E, Tsubaki, Y. & Siva-Jothy, M.T. 1999. Expression of a costly, plastic secondary sexual trait is correlated with age and condition in a damselfly with two male morphs. *Physiol. Entomol.* **24**: 364–369.

Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* **45**: 630–648.

Houle, D. 1998. How should we explain variation in the genetic variance of traits? *Genetica* **102/103**: 241–253.

Hunt, J., Brooks, R.C., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussiere, L.F. 2004a. High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**: 1024–1027.

Hunt, J., Bussiere, L., Jennions, M.D. & Brooks, R.C. 2004b. What is genetic quality? *Trends Ecol. Evol.* **19**: 329–333.

Iwasa, Y. & Pomiankowski, A. 1994. The evolution of mate preferences for multiple sexual ornaments. *Evolution* **48**: 853–867.

Jennions, M.D., Møller, A.P. & Petrie, M. 2001. Sexually selected traits and adult survival: a meta-analysis. *Q. Rev. Biol.* **76**: 3–36.

Johnstone, R.A. 1995. Sexual selection, honest advertising and the handicap principle: reviewing the evidence. *Biol. Rev.* **70**: 1–65.

Judge, K.A., Ting, J.J. & Gwynne, D.T. 2008. Condition dependence of male life span and calling effort in a field cricket. *Evolution* **62**: 868–878.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female mate choice. *Behav. Ecol. Sociobiol.* **225**: 393–401.

Kokko, H. 1997. Evolutionary stable strategies of age-dependent sexual adverstisement. *Behav. Ecol. Sociobiol.* **41**: 99–107.

Kokko, H. 1998. Good genes, old age and life-history trade-offs. *Evol. Ecol.* **12**: 739–750.

Kokko, H., Brooks, R., Jennions, M.D. & Morely, J. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B* **270**: 653–664.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**: 292–305.

Lewontin, R.C. 1966. On the measurement of relative variability. *Syst. Zool.* **15**: 141–142.

Mason, L.G. 1973. The habitat and phenetic variation in *Phymata americana* Melin. *Syst. Zool.* **22**: 271–279.

McGraw, K.J., McKillop, E.A., Dale, J. & Hauber, M.E. 2002. Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally-based ornamental plumage. *J. Exp. Biol.* **205**: 3747–3755.

Moczek, A.P. & Nijhout, F.H. 2004. Trade-offs during the development of primary and secondary sexual traits in a horned beetle. *Am. Nat.* **163**: 184–191.

Møller, A.P. & Alatalo, R.V. 1999. Good-genes effects in sexual selection. *Proc. R. Soc. Lond. B* **266**: 85–91.

Nijhout, H.F. & Emlen, D.J. 1998. Competition among body parts in the development and evolution of insect morphology. *Proc. Nat. Acad. Sci. USA.* **98**: 3685–3689.

van Noordwijk, A.J. & de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**: 137–142.

Packard, G.C. & Boardman, T.J. 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol.* **122**: 37–44.

Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B* **260**: 21–29.

Proulx, S., Day, T. & Rowe, L. 2002. Older males signal more reliably. *Proc. R. Soc. Lond. B* **269**: 2291–2299.

Punzalan, D. 2007. *Evolution of Sexual Colour Pattern Dimorphism in Phymata americana*. Ph.D. Thesis. University of Toronto, Toronto, Canada (pp. 188).

Punzalan, D., Rodd, F.H. & Rowe, L. 2008. Sexual selection mediated by the thermoregulatory effects of male colour pattern in the ambush bug *Phymata americana*. *Proc. R. Soc. Lond B* **275**: 483–492.

Punzalan, D., Rodd, F.H. & Rowe, L. 2008. Contemporary sexual selection on sexually dimorphic traits in the ambush bug *Phymata americana*. *Behav. Ecol.* doi: 10.1093/beheco/arn042.

Rice, W.R. & Chippindale, A.K. 2001. Intersexual ontogenetic conflict. *J. Evol. Biol.* **14**: 685–693.

Roff, D.A. 2002. *Life History Evolution*. Sinauer Associates Inc., USA.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**: 1415–1421.

Slatkin, M. 1984. Ecological causes of sexual dimorphism. *Evolution* **38**: 622–630.

Schluter, D., Price, T.D. & Rowe, L. 1991. Conflicting selection pressures and life history trade-offs. *Proc. R. Soc. Lond B.* **246**: 11–14.

Schuh, R.S. 1995. *True Bugs of the World*. Cornell University Press, USA.

Thompson, J.W., Armitage, S.A.O & Siva-Jothy, M.T. 2002. Cuticular colour change after imaginal eclosion is time-constrained: blacker beetles darken faster. *Physiol. Entomol.* **27**: 136–141.

Tomkins, J.L., Radwan, J., Kotiaho, J.S. & Tregenza, T. 2004. Genic capture and resolving the lek paradox. *Trends Ecol. Evol.* **19**: 323–328.

de Visser, J.A.G.M., Hermisson, J., Wagner, G.P., Ancel Meyers, L., Bagheri-Chaichian, H., Blanchard, J.L., Chao, L., Cheverud, J.M., Elena, S.F., Fontana, W., Gibson, G., Hansen, T.F., Krakauer, D., Lewontin, R.C., Ofria, C., Rice, S.H., von Dassow, G., Wagner, A. & Whitlock, M.C. 2003. Evolution and detection of genetic robustness. *Evolution* **57**: 1959–1972.

Wilkinson, G.S. & Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. *Proc. R. Soc. Lond. B* **266**: 1685–1690.

Zahavi, A. 1975. Mate selection – a selection for a handicap. *J. Theor. Biol.* **53**: 205–214.

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