



# Experimental evidence of condition-dependent sexual dimorphism in the weakly dimorphic antler fly *Protopiophila litigata* (Diptera: Piophilidae)

MATHIEU J. OUDIN<sup>1</sup>, RUSSELL BONDURIANSKY<sup>2</sup> and HOWARD D. RUNDLE<sup>1\*</sup>

<sup>1</sup>Department of Biology, University of Ottawa, Ottawa, Canada

<sup>2</sup>Evolution and Ecology Research Centre and School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, Australia

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Sexually dimorphic traits have a history of directional selection for exaggeration in at least one sex. Theory suggests that traits targeted by persistent selection should evolve heightened condition dependence whereby their expression reflects the availability and processing efficiency of metabolic resources. This joint dependence of sexual dimorphism and condition dependence on directional selection should result in a positive correlation between the extent of sexual dimorphism and the strength of condition dependence. However, because direct phenotypic evidence is predominately from species with highly exaggerated male traits, it remains unclear whether condition-dependent sexual dimorphism is characteristic of species with more typical levels of dimorphism. We manipulated condition via larval diet and quantified sex-specific responses in adult body size and shape in a moderately dimorphic dipteran species, the antler fly *Protopiophila litigata*. While dimorphism did not increase with diet quality within any trait, among traits the extent of dimorphism was positively associated with the strength of condition dependence in males but not females, as previously reported for highly dimorphic species. This finding suggests that a shared developmental basis to condition dependence and sexual dimorphism can arise via sexual selection on males even in lineages lacking highly exaggerated male traits. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **116**, 211–220.

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## INTRODUCTION

Sexual dimorphism is common in nature, with males and females often differing morphologically in body size, shape, and in the presence and exaggeration of various secondary sexual traits (Andersson, 1994). Sexual dimorphism arises ultimately from differences in selection between the sexes. While sexually antagonistic selection may arise from ecological interactions such as intersexual resource competition (Darwin, 1871), sexual selection is often thought to be involved because it is persistent and the divergent reproductive interests of males and females cause it to be sex-specific, often being present in one sex yet weak or absent in the other (Price, 1984; Fairbairn & Preziosi, 1996). The extent of dimorphism that evolves reflects an interaction between sexually

antagonistic selection and constraints that arise from a genome that is largely shared between the sexes (Lande, 1980).

Sexually dimorphic traits therefore have a history of directional selection for exaggeration in at least one sex. For traits subject to persistent directional selection, a component of fitness is an increasing function of investment in them, favouring increased allocation to their expression (Andersson, 1982; Nur & Hasson, 1984). The evolutionary exaggeration of such traits should eventually be halted when the benefit is offset by expression-dependent costs to other fitness components. Life history theory suggests that such costly traits should evolve heightened condition dependence, a form of plasticity that links trait expression to the availability and processing efficiency of metabolic resources, or more generally to an individual's ability to maintain optimal functionality (Rowe & Houle, 1996; Getty, 1998, 2006;

\*Corresponding author. E-mail: hrundle@uottawa.ca

Hill, 2011). The strength of condition dependence that evolves should reflect both the strength of selection and the costs of trait expression (Rowe & Houle, 1996).

For sexually dimorphic traits, the strength of condition dependence and the extent of sexual dimorphism are therefore both functions of persistent selection for exaggeration. This joint dependency should produce a positive association between condition dependence and sexual dimorphism such that the extent of sexual dimorphism should itself be condition-dependent (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). In particular, if condition dependence and sexual dimorphism coevolve then: (1) within a trait, sexual dimorphism should be greater among high- as opposed to low-condition individuals; and (2) among traits, there should be a positive association between the extent of dimorphism and the strength of condition dependence. In addition, if sexual dimorphism is the result of persistent directional selection on males, as might be expected if it involves sexual selection, then males should be more sensitive to changes in condition than females and the among-trait correlation of condition dependence and sexual dimorphism should be stronger in males than in females.

Empirically, while even non-dimorphic traits can be condition-dependent (Cotton, Fowler & Pomiankowski, 2004b), it is well established that dimorphic sexual displays and armaments are often highly so (Cotton, Fowler & Pomiankowski, 2004a). Consistent with the first expectation above, several studies have gone further to show that the extent of sexual dimorphism for a given trait is sensitive to environmental factors that probably alter condition, both for morphological traits (David *et al.*, 1994, 2000; Post *et al.*, 1999; Karan *et al.*, 2000; Bonduriansky & Rowe, 2005; Weladji *et al.*, 2005; Bonduriansky, 2007a; Boughman, 2007; Punzalan *et al.*, 2008) and, in a study in *Drosophila melanogaster*, for gene expression (Wyman, Agrawal & Rowe, 2010). However, the expectation of covariation among morphological traits has, to our knowledge, only been tested in two studies, both of which used species with pronounced sexual dimorphism in body size and shape (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). In many organisms, however, sexual dimorphism in morphology is more modest and obviously exaggerated traits are lacking. Traits in such species may still vary in both the extent of sexual dimorphism and the strength of condition dependence, but whether selection has been sufficiently strong to drive the coevolution of these is an open question. Hence, it remains unclear whether condition-dependent sexual dimorphism is a widespread feature of sexual taxa.

Here, we investigate the relationship between sexual dimorphism and condition dependence in the antler fly *Protopiophila litigata* Bonduriansky (1995). Antler flies are small insects (1.5–3.2 mm body length) that breed on discarded cervid (i.e. moose and deer) antlers. Males aggressively defend mating territories on the surface of an antler and large males are both more successful at doing so and are more attractive to females (Bonduriansky & Brooks, 1998b, 1999). In addition to fighting over territories, males guard females during oviposition into cracks or pores in the antler (larvae feed and develop within the decaying bone matrix) and sometimes also attempt to dislodge other males that are mating, taking over the mating when successful (Bonduriansky & Brooks, 1998a). These aggressive intrasexual interactions, together with a highly male-biased operational sex ratio on antlers, suggest that sexual selection may be strong, although it is unclear to what extent it targets male morphology. Indeed, highly dimorphic secondary sexual traits are lacking in this species, and the sexes have similar body sizes. We investigated the condition-dependence of sexual dimorphism using a manipulation of larval diet quality to generate high- and low-condition individuals. While conventional studies of sexual dimorphism and trait allometry tend to focus on highly dimorphic secondary sexual traits (Bonduriansky, 2007c), here we analyse variation in a suite of morphological traits exhibiting subtle variation in dimorphism, including thorax, head, leg and wing dimensions. We also analyse variation in the male intromittent organ (the aedeagus) to gain insight into condition dependence and allometric scaling of this sex-specific trait (see online Supporting Information, Data S1).

## MATERIAL AND METHODS

### FLIES AND CULTURE TECHNIQUES

An outbred laboratory stock population of *P. litigata* (Fig. S1) was created from a large sample (> 500) of adult flies collected in the spring and early summer of 2012 at the Wildlife Research Station, Algonquin Park, Ontario, Canada. The population was maintained at the University of Ottawa via non-overlapping generations at 23 °C, 60% relative humidity and under a 17 : 7-h light–dark photoperiod. Each generation larvae developed in an oviposition dish that contained a basal layer of 2.5 g of ground beef covered by a foam sponge (to mimic the bone matrix) to which was added a variable amount of a 20% (w/v) ground beef solution up to three times per week to maintain moisture (Data S1).

Our experiment involved three larval diet treatments (high, mid and low quality) that differed in

the ratio of ground beef to fibre within the oviposition dishes, as well as the concentration and quantity of ground beef solution. Like the stock population, the high-quality diet used only regular ground beef while the intermediate (mid) diet consisted of a 9 : 1 mixture of homogenized ground beef/fibre ('Inulin', Exact). Preparations were stored at  $-20^{\circ}\text{C}$  prior to use. During larval development, oviposition dishes for the high- and mid-quality diets received 1.5 mL of a 10% (w/v) ground beef solution three times per week. A 10% solution was used to increase larval reliance on the ground beef/fibre. The low-quality diet had no ground beef, but received up to 3 mL (less if the oviposition foam pad became saturated) of a 20% ground beef solution added three times per week.

Our analyses were based on flies that had been reared for one generation on these three diets. To collect these flies, 50 adults of each sex were randomly chosen from the stock population (generation 6) and placed into each of five population cages for egg laying. Density was monitored daily and any dead individuals were replaced with new stock flies of the matching sex. Cages were housed under the same environmental conditions as the stock and received an abundant supply of granulated sugar and water. Eggs were collected by adding a single oviposition dish to each cage for 48 h, after which it was replaced with a new one. Six sequential rounds of egg collection were performed, creating six temporal blocks of offspring. Diet treatments were applied after removing an oviposition dish from a cage, preventing females from adjusting their egg laying in relation to diet quality. All three diet treatments were applied within each of the six temporal blocks and each cage contributed at least one dish to each diet treatment. After the diet treatment was applied, oviposition dishes were relocated to separate 250-mL mason jars with 10 g of dry coco peat layering the base and a mesh cap to allow air circulation. Ground beef solution was applied during subsequent larval development. Emerging adults were removed daily and held for 24 h to allow their cuticle to sclerotize, after which they were preserved in 99% ethanol and frozen at  $-20^{\circ}\text{C}$ .

#### MORPHOMETRIC DATA

For each of the three diet treatments, 20 adults of each sex were measured. Developmental time differed among diets (see Results) and the measured individuals were chosen to have a developmental time that was approximately average for all individuals on that diet while still including individuals from all six temporal blocks. For each fly, ten linear measurements were made (Fig. S1): thorax length,

head width, head height, fore-tibia length, mid-tibia length, hind-tibia length, fore-tarsus length, mid-tarsus length, hind-tarsus length and wing length (from the inner r-m cross-vein to the distal end of the  $R_{4+5}$  vein). Prior to measurements, all appendages (legs, wings, head) were severed at the point of attachment to the body using McPherson-Vannas micro iris scissors (Miltex/Integra LifeSciences). Wings and legs were mounted on standard microscope slides using double-sided tape. The head and thorax were placed in a droplet of glycerol on a microscope slide to avoid desiccation. Samples were photographed using an A640 PowerShot Canon camera mounted on a Zeiss Discovery V.12 stereo microscope with an ocular micrometer. Images were captured using ZoomBrowser EX software (Canon) and measurements were performed using ImageJ software 1.47v (National Institute of Health). The measurement repeatability of each trait was estimated by re-mounting, re-imaging and re-measuring 20 randomly selected individuals. Re-mounting traits involved delicately removing them from the tape with insect pins and re-positioning them in a different orientation. Repeatabilities were estimated as the ANOVA-based intraclass correlation coefficient (Lessels & Boag, 1987) and were  $\geq 0.9$  for all traits (Table S1).

#### STATISTICAL ANALYSES

Traits covaried positively, as expected for morphological components that scale allometrically with body size. The relationship between the strength of condition dependence and the extent of sexual dimorphism can only be tested using a set of different traits, not multiple measures of the same trait (i.e. body size), and we therefore analysed variation in body size and the size-corrected traits (i.e. components of body shape). As an index of body size, we used the first principal component (PC1) of the correlation matrix of all traits across both sexes and all three diets. We used PC1 in place of other commonly used measures of body size such as thorax or wing length because these traits had the lowest loadings on PC1 (Table S2), suggesting each to be a poor index of body size on its own in this species. In addition, the allometric scaling of the majority of traits with thorax length and with wing length varied significantly between diet treatments. Such heterogeneity of slopes further indicates that thorax and wing length are biologically and statistically inappropriate indices of body size in this case. In contrast, the allometric scaling of the traits with PC1 showed no such heterogeneity of slopes (see below).

The effects of sex, diet and their interaction on body size were tested using a general linear mixed

model:

$$\text{PC1} = \text{Sex} + \text{Diet} + \text{Sex} \times \text{Diet} + \text{Block}, \quad (1)$$

where Block is the random effect of temporal block and all other terms are fixed. Our analysis demonstrated a significant overall effect of diet treatment on body size, but post-hoc comparisons revealed this to be due almost entirely to a significantly reduced size in the low-quality diet, with no difference between the mid and high treatments and no interaction with sex (see Results). Given little phenotypic effect of the mid- (relative to the high-) quality diet, our subsequent analyses of within- and among-trait variation in the various size-corrected shape traits used only the low- and high-quality diets. Doing this allowed us to reserve individuals from the mid-quality diet to provide an independent estimate of sexual dimorphism for our among-trait analyses, thereby avoiding any spurious association between the extent of sexual dimorphism and the strength of condition dependence that could result if some of the same data contributed to estimates of both (see below).

Effects on body shape were tested in separate analyses of the head, legs, and wing and body measurements by including body size (PC1) as a covariate in Eqn (1), thereby quantifying change in the relative size of these traits. For each trait, PC1 was re-calculated after excluding the focal trait (i.e. dependent variable) to avoid modelling a trait as a function of itself. While PC1 therefore differs for every trait (because a different focal trait is excluded in each case), the correlation among these different PC1s ranges from 0.9966 to 0.9993, indicating that they are all essentially the same trait. There was no evidence of heterogeneity of the allometric slopes between diets or the sexes for any of the traits (Table S3), so the interaction terms of these fixed effects with PC1 were excluded from the models. The only exception was a significant PC1  $\times$  Sex  $\times$  Diet effect for wing length. However, this effect was not strong (Fig. S2) and we therefore proceeded with the analysis using a single, common slope. All models were fit using restricted maximum likelihood.

To explore the pattern of condition-dependent sexual dimorphism across traits, condition dependence was estimated as the difference in mean trait value between individuals raised on the high- vs. low-quality diets, separately in males and females. A spurious correlation of these values with the degree of sexual dimorphism could result if dimorphism was estimated using some of the same data (e.g. condition dependence of trait  $z$  in males =  $\bar{z}_{\text{male, high}} - \bar{z}_{\text{male, low}}$ ; sexual dimorphism =  $\bar{z}_{\text{male, high}} - \bar{z}_{\text{female, high}}$ ). To avoid this, dimorphism was calculated for each trait as the difference in mean size between males and females using individuals from the mid-quality diet. Body-size variation was

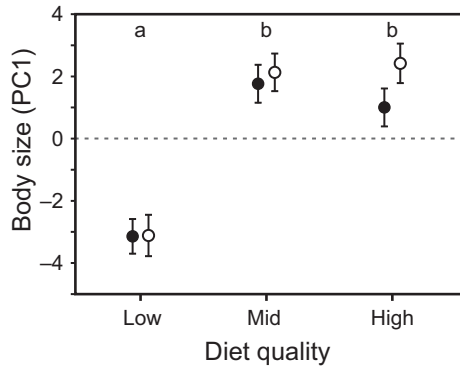
removed from each trait prior to analysis by taking the residuals from a regression of each trait against PC1 of all other traits (using a single, common slope and excluding the dependent trait in the calculation of PC1 in each case). This was done separately for the condition dependence and sexual dimorphism data sets, combining the sexes in each case. The resulting values were standardized [ $\sim N(0,1)$ ] across sexes prior to calculating sexual dimorphism (mid-quality diet only), and across diets (low and high quality) prior to calculating condition dependence scores separately for males and for females. The association across traits between the degree of sexual dimorphism and the extent of condition dependence was quantified within in each sex as the Pearson product-moment correlation and was visualized using reduced major axis regression, assuming equal error variances in both variables. Significance was determined via standard correlation analysis and the correlations were compared between the sexes using Fisher's  $r$ -to- $z$  transformation (Zar, 1984). Statistical analyses were performed using JMP version 10.0.0 (SAS Institute, Cary, NC, USA).

## RESULTS

Developmental time (from oviposition to adult emergence) increased significantly with decreasing diet quality (mean number of days  $\pm$  SE; high quality:  $32.6 \pm 0.3$ ; mid quality:  $39.5 \pm 0.5$ ; low quality:  $59.9 \pm 2.6$ ; ANOVA:  $F_{2,9.97} = 19.5$ ,  $P = 0.0004$ ). The average number of adults that emerged from each oviposition dish also decreased significantly with declining larval diet quality (mean  $\pm$  SE; high quality:  $65.3 \pm 16.1$ ; mid quality:  $40.2 \pm 6.6$ ; low quality:  $9.8 \pm 3.6$ ; ANOVA:  $F_{2,10} = 7.3$ ;  $P = 0.0111$ ), indicating differences in hatching success and/or larval survival across diets. Adult body size (PC1) varied significantly among diets ( $F_{2,93.34} = 51.5$ ,  $P < 0.0001$ ) but did not differ between the sexes ( $F_{1,96.94} = 2.2$ ,  $P = 0.144$ ), nor was there evidence of a sex  $\times$  diet interaction ( $F_{2,96.25} = 1.1$ ,  $P = 0.345$ ). The diet effect was caused by a reduced average size of the flies reared on the low-quality diet as compared with those reared on the mid- and high-quality diets, with these latter two groups not differing from each other (Fig. 1; Table S4). Given the non-significant difference in body size between the mid- and high-quality diet-reared individuals, our subsequent analyses of size-corrected shape traits focused on the low- and high-quality diets, reserving individuals from the mid-quality diet for use in the among-trait analysis of condition-dependent sexual dimorphism (see Methods).

Significant sexual dimorphism was detected for several shape traits (Table 1), with males having





**Figure 1.** Body size comparisons across diets for males (closed circles) and females (open circles). Letters above diet treatments represent post-hoc comparisons using Tukey’s HSD test. Points are least squares means from Eqn (1) ± 1 SE.

relatively longer fore-tibia, mid-tibia and hind-tibia, and relatively shorter fore-tarsi, wings and thoraxes, along with narrower heads. Diet quality had a significant effect on three traits, with a high-quality larval diet increasing relative hind-tibia length and relative head width and decreasing relative hind-tarsus length (Table 1). The sex × diet interaction was non-significant in all cases and, although it approached significance for relative mid-tarsus length ( $P = 0.0605$ ), the pattern was opposite to that expected under condition-dependent sexual dimorphism with males and females differing more when raised on the low- compared with the high-quality diet. Across these shape traits, there was a

positive correlation between the strength of condition dependence in males and females that approached significance (Fig. 2; Pearson correlation:  $N = 10$ ,  $r = 0.604$ ,  $P = 0.064$ ).

Among traits, there was a significant positive association between the degree of sexual dimorphism and the strength of condition dependence in males (Fig. 3A; Pearson correlation:  $N = 10$ ,  $r = 0.650$ ,  $P = 0.042$ ). In females (Fig. 3B), variation in the strength of condition dependence was less and the association was not significant (Pearson correlation:  $N = 10$ ,  $r = 0.486$ ,  $P = 0.154$ ), although the pattern was similar and the correlations did not differ significantly between the sexes ( $z = 0.457$ ,  $P = 0.648$ ). Results were qualitatively unchanged if sexual dimorphism was estimated using individuals from the high-quality diet instead.

DISCUSSION

A positive association between the extent of sexual dimorphism and the strength of condition dependence should arise from their joint dependence on directional selection (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a), but phenotypic tests of this have been limited to species with exaggerated secondary sexual traits. To assess the generality of this pattern, we tested this prediction in the weakly dimorphic antler fly *P. litigata*. While dimorphism was not greater in high- as compared with low-condition flies, there was a correlation among traits between the extent of dimorphism and the strength

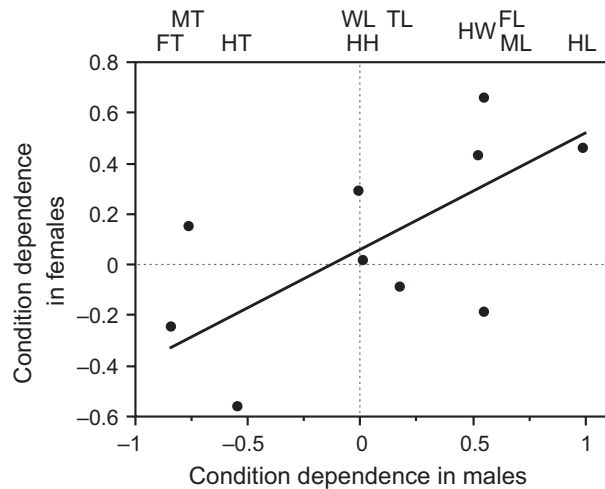
**Table 1.** Least-squares means\* of various shape traits for males and females when reared on low- and high-quality larval diets; model coefficients and their significance are also shown for the fixed effects of sex, diet and their interaction (sex × diet) from the separate analysis of each trait

Shape trait†	Low-quality diet		High-quality diet		Model coefficient ( $P$ -value)‡		
	Males	Females	Males	Females	Sex	Diet	Sex × Diet
Fore-tibia (FL)	0.467	0.437	0.473	0.448	<b>-12.7 (&lt; 0.001)</b>	3.0 (0.152)	1.3 (0.400)
Fore-tarsus (FT)	0.479	0.496	0.469	0.488	<b>9.0 (&lt; 0.001)</b>	-4.5 (0.130)	0.6 (0.738)
Mid-tibia (ML)	0.547	0.534	0.553	0.529	<b>-0.9 (&lt; 0.001)</b>	0.2 (0.922)	-2.6 (0.156)
Mid-tarsus (MT)	0.553	0.540	0.533	0.536	-2.4 (0.269)	-6.1 (0.118)	4.2 (0.061)
Hind-tibia (HL)	0.536	0.517	0.557	0.532	<b>-10.7 (&lt; 0.001)</b>	<b>7.6 (0.003)</b>	-1.5 (0.422)
Hind-tarsus (HT)	0.597	0.604	0.583	0.587	2.8 (0.124)	<b>-7.6 (0.014)</b>	-0.6 (0.730)
Wing (WL)	1.030	1.058	1.043	1.079	<b>15.9 (0.001)</b>	8.3 (0.237)	2.2 (0.619)
Head width (HW)	0.699	0.722	0.733	0.743	<b>8.2 (0.004)</b>	<b>13.9 (0.001)</b>	-3.3 (0.223)
Head height (HH)	0.513	0.516	0.514	0.525	2.4 (0.316)	1.2 (0.715)	0.1 (0.953)
Thorax (TL)	0.782	0.814	0.794	0.821	<b>14.8 (0.045)</b>	4.5 (0.716)	-1.2 (0.867)

\*Least squares means for the sex × diet interaction from Eqn (1) with body size (PC1) also included.

†PC1 was included as a covariate for all traits and was highly significant in all cases ( $P < 0.0001$ ).

‡For display purposes? coefficients have been multiplied by 1000 in all cases. Bold font denotes probabilities < 0.05.

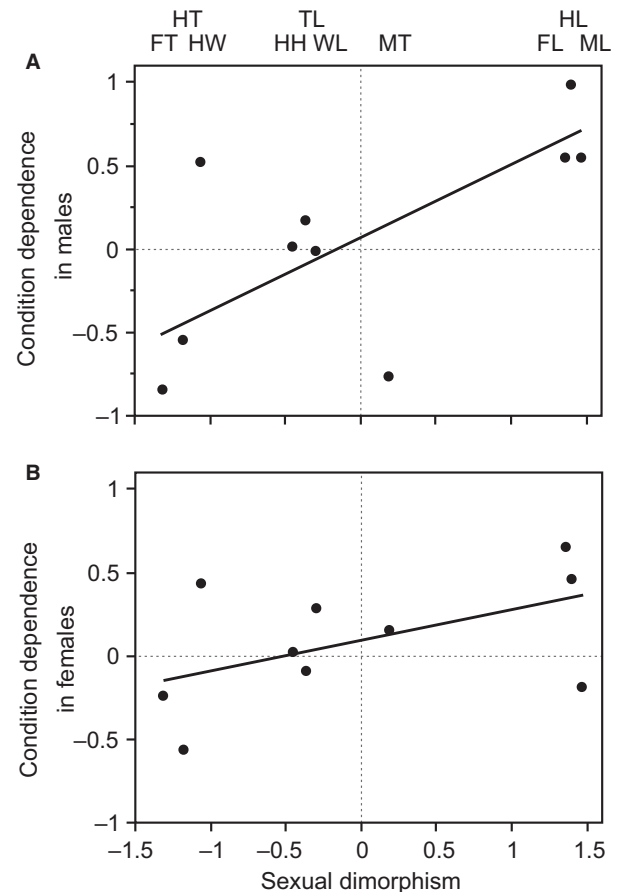


**Figure 2.** Covariation of degree of condition dependence (mean difference between diets: high – low quality) in males and females. Fitted line is from a reduced major axis regression ( $r = 0.604$ ,  $P = 0.064$ ). Trait labels (see Table 1) align vertically with their respective points.

of condition dependence that was present in males but not in females. Our findings suggest that the coevolution of condition dependence and sexual dimorphism is a widespread feature of sexual species.

#### WITHIN-TRAIT VARIATION

Body size was condition-dependent in that individuals raised on the mid- and high-quality diets were significantly larger than those raised on the low-quality diet, but sexual dimorphism in body size was not significant overall. While dimorphism did tend to increase across the three diets, from near monomorphism on the low-quality diet to noticeable dimorphism on the high-quality diet (Fig. 1), this was not sufficient to generate a diet  $\times$  sex interaction and was driven largely by a slight reduction in male size on the high-quality diet. Whether significant sexual size dimorphism may emerge among even higher quality individuals is an open question, but our results reveal a similar pattern of condition dependence in both sexes, suggesting that body size has been the target of directional selection for exaggeration in both males and females. Sexual selection for larger males is consistent with an apparent female preference for larger-bodied males (Bonduriansky & Brooks, 1998b) and increased size probably also benefits males during intrasexual competition (Bonduriansky & Brooks, 1999). In females, fecundity selection may favour increased body size (Bonduriansky & Brooks, 1998b) as may sexual selection given that



**Figure 3.** Covariation among traits in the strength of condition dependence (mean difference between high- and low-quality diet) and the extent of sexual dimorphism (mean difference between males and females) for (A) males and (B) females. Fitted lines are from a reduced major axis regression in each sex. Trait labels (see Table 1) align vertically with their respective points.

males have been suggested to be choosy (due to the high apparent costs of courtship, mating and mate guarding) (Bonduriansky & Brooks, 1998a) and may prefer larger females due to their increased fecundity.

With respect to body shape, male antler flies had relatively longer tibial segments than females on each of the three pairs of legs (i.e. fore, mid and hind), suggesting these traits, or overall relative leg length, as potential targets of sexual selection. Males attempt to dislodge other mounted males and take over mating with the female, sometimes resulting in prolonged wrestling bouts (Bonduriansky & Brooks, 1998a). Longer legs may therefore function in resisting such attempts and/or preventing females from escaping. Mid and hind legs are also involved in mating, probably serving to aid in both mounting and in actual copulation (Bonduriansky & Brooks, 1998a),

and after mounting, males use their mid and hind legs to tap the females' abdomen, possibly as a means to assess gravidity given that female abdomen width is a better predictor of egg load than is female body size (Bonduriansky & Brooks, 1998a, b). Hind-tibia length was the only one of these traits to exhibit significant condition dependence, with relatively greater values in males reared on high- as compared with low-quality food. However, there was no indication of stronger condition dependence of any of these traits in males compared with females (i.e. a sex  $\times$  diet interaction).

Females had larger values than males for several shape traits including relative head width and thorax, fore-tarsus and wing length, with only relative head width increasingly significantly on the high-quality diet. There was no indication that the strength of condition dependence varied between the sexes for any of these traits. Whether this female-biased dimorphism is the result of selection on males for smaller relative sizes of these traits, and/or selection on females for larger values, is unknown. Males use their wings to fend off take-over attempts by other males during mating (Bonduriansky & Brooks, 1998a), so selection for smaller wings seems unlikely (unless smaller wings are more damage-resistant).

Condition-dependent sexual dimorphism within a trait requires males and females to differ more when raised on a high- as opposed to low-quality diet. Such differences constitute a particular form of sex-specific (i.e. non-parallel) reaction norms to the diet treatments whereby sexual dimorphism is greater in high- compared with low-condition individuals. Neither body size nor any of our measured shape traits demonstrated such a pattern. This contrasts with past results in the neriid fly *Telostylinus angusticollis* and the piophilid fly *Prochyliza xanthostoma* in which dimorphism in body size and some (but not all) shape traits increased on a high-condition diet (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). Insufficient power, resulting from the comparatively low sexual dimorphism that exists in antler flies compared with the species used in past studies, may have contributed to our inability to detect such an effect. Among-individual variation within a given trait may also be less than the among-trait variation, making the covariance of sexual dimorphism and condition dependence harder to detect in analyses conducted within as compared with among traits.

Alternatively, dimorphism may not increase with condition if the genetic basis of condition dependence is shared between the sexes. Such a shared genetic basis may indicate unresolved intralocus sexual conflict and would unconstrain the developmental plasticity of the sexes to respond similarly to increased resources, reducing or even preventing an

increase in dimorphism (Bonduriansky & Rowe, 2005; Bonduriansky, 2007b; Wyman *et al.*, 2010). Covariation of condition dependence among homologous traits in males and females has been previously observed (Bonduriansky & Rowe, 2005) and was substantial and near-significant in our experiment ( $r = 0.604$ ,  $P = 0.064$ ; Fig. 2). Sexually antagonistic selection is likely to favour sex-specific patterns in developmental plasticity that may lead to a breakdown of the intersex genetic correlation for condition dependence (Bonduriansky, 2007b). In antler flies, sex-specific selection may not have been sufficiently strong to drive this for our measured shape traits.

#### AMONG-TRAIT VARIATION

In males there was a significant association among traits between the strength of condition dependence and degree of sexual dimorphism, consistent with the evolution of condition-dependent sexual dimorphism. Such an association among morphological traits has been previously demonstrated in two other insect species with pronounced dimorphism (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a), and in a transcriptome analysis in *D. melanogaster* (Wyman *et al.*, 2010). Our results reveal the coevolution of sexual dimorphism and condition dependence in a species lacking highly exaggerated male sexual displays and armaments, suggesting a common genetic basis to sex-dependent and condition-dependent trait expression. There was some evidence of a similar pattern in females, although traits were less sensitive to the diet manipulation than they were in males and the association was non-significant (Fig. 3). This weaker and non-significant association in females suggests that condition-dependent sexual dimorphism has evolved primarily in response to persistent selection on males strengthening the condition dependence of the target traits in this sex, suggesting sexual selection as a possible cause. However, this difference between the sexes in the strength of the association is not itself significant and it is therefore conceivable that some of these traits may have been targets of persistent selection in females or in both sexes.

The pattern in male antler flies differs somewhat from that previously observed in the neriid fly *Telostylinus angusticollis*, and in the piophilid carrion fly *Prochyliza xanthostoma*. In these species, traits were always relatively larger in high- as opposed to low-condition males, with the magnitude of this effect being greater for traits that were more male biased (i.e. more sexually dimorphic, with males as the larger sex) (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). In antler flies, the strength of condition dependence tended to be greater for traits that were more sexually dimorphic,

but this was not only because traits that were normally larger in males became even more so in high-condition flies, but also because traits that were relatively smaller in males tended to become even more so in high condition flies. In other words, although traits responded in different directions to the diet manipulation, those that were more dimorphic responded to a greater degree and the response tended to be in the direction of increasing dimorphism, causing high-condition males to become even more 'male-like'.

The strength of the association between condition dependence and sexual dimorphism we observed in antler flies was weaker than that previously seen in *T. angusticollis* (Bonduriansky, 2007a). This is perhaps not surprising as limited dimorphism suggests weaker selection for morphological trait exaggeration in *P. litigata* males, and hence a more moderate strengthening of condition dependence for these traits. In such a situation, other factors affecting sexual dimorphism and/or condition dependence may also be important. For example, ecological and social conditions may mediate sex-specific resource acquisition and plasticity in its subsequent allocation (De Lisle & Rowe, 2014), and failure to account for these may weaken the observation association between sexual dimorphism and condition dependence. Nevertheless, the association we detected was stronger than that observed in the highly dimorphic *P. xanthostoma* (Bonduriansky & Rowe, 2005), indicating that the extent of dimorphism itself is not a strong indicator of the pattern of among-trait variation. Theory predicts that the strength of condition dependence should increase with the strength of directional selection among traits and there is some empirical support for this (Delcourt & Rundle, 2011). For sexually dimorphic traits, among-trait variation in the strength of condition dependence may therefore associate more strongly with estimates of the strength of sexually antagonistic selection than the extent of dimorphism itself.

One caveat to the interpretation of our results stems from the significant decrease in the number of adults that emerged as diet quality declined. The diet treatments in our experiment were not applied until after eggs were laid, meaning that the observed differences among treatments were due to differences in hatching success and/or subsequent larval survival. In theory, effects on trait means could therefore have arisen not from treatment-specific developmental reaction norms in response to diet, but rather from sampling effects caused by selective deaths in each treatment. The extent to which this may have occurred is difficult to assess, although such a process would not be expected to produce the

observed association among traits between sexual dimorphism and condition dependence.

Our results suggest that condition dependence and sexual dimorphism coevolve in species lacking highly exaggerated secondary sexual traits. Condition-dependent sexual dimorphism may therefore be a general and widespread feature of sexual taxa, although data to date are restricted to only four species of insects. The condition dependence of sexual dimorphism has interesting implications for the developmental-genetic basis of trait expression in that it suggests that condition dependence and sexual dimorphism may have a common genetic basis. Among traits, a positive genetic correlation may therefore be expected between the strength of condition dependence and the extent of sexual dimorphism, although as far as we are aware this has never been tested. Condition-dependent sexual dimorphism also has potential implications for sexual selection and sexual coevolution, given that environmental variation that affects condition may simultaneously alter the degree of phenotypic differentiation between the sexes, and thereby affect the nature of inter- and intra-sexual interactions. Research on other species is required to verify these conjectures.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Measurement repeatabilities for all morphological traits in *Protopiophila litigata*, estimated as the ANOVA-based intraclass correlation coefficient ( $N = 20$  in all cases).

**Table S2.** Eigenvectors of the first principal component (PC1) for all measured traits across males and females from the low-, mid- and high-quality diets.

**Table S3.** Mixed model results testing for heterogeneity between the sexes and diet treatments in the allometric slopes representing the scaling of various traits with body size (PC1).

**Table S4.** Mean absolute measurement ( $\text{mm} \pm \text{SE}$ ) of various traits in males and females raised on low-, mid- and high-quality diets.

**Figure S1.** Morphological traits measured in the antler fly *Protopiophila litigata*. Shown are: wing length (WL), fore-tarsus length (FT), fore-tibia length (FL), head height (HH), head width (HW), thorax length (TL) and male aedeagus length (AD). Mid-tibia length (ML), mid-tarsus length (MT), hind-tibia length (HL) and hind-tarsus length (HT) are not shown, but were measured analogous to that shown for the fore-leg.

**Figure S2.** Allometric scaling of wing length with body size (PC1 of all other traits) for females (circles) and males (triangles) when raised on low-quality (open symbols) or high-quality (filled symbols) diet. Fitted lines are from ordinary least-squares regressions separately for each sex  $\times$  diet combination (solid lines) or across both sexes and diets (red dashed line).

**Figure S3.** Allometric scaling of the length of the aedeagus (the male intromittent organ) with body size (PC1 of all other morphological traits) for males reared on a low-quality (open circles) or high-quality (filled circles) diet. Solid line is from an ordinary least-squares regression across both diet treatments; dashed lines are from separate reduced major axis regressions for the low- and high-quality diets.

## SHARED DATA

Data deposited in the Dryad digital repository (Oudin MJ, Bonduriansky R, Rundle HD, 2015).