



SEX-SPECIFIC PATTERNS OF MORPHOLOGICAL DIVERSIFICATION: EVOLUTION OF REACTION NORMS AND STATIC ALLOMETRIES IN NERIID FLIES

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The consequences of sex-specific selection for patterns of diversification remain poorly known. Because male secondary sexual traits are typically costly to express, and both costs and benefits are likely to depend on ambient environment and individual condition, such traits may be expected to diversify via changes in reaction norms as well as the scaling of trait size with body size (static allometry). We investigated morphological diversification within two species of Australian neriid flies (*Telostylinus angusticollis*, *Telostylinus lineolatus*) by rearing larvae from several populations on larval diets varying sixfold in nutrient concentration. Mean body size varied among populations of *T. angusticollis*, but body size reaction norms did not vary within either species. However, we detected diversification of reaction norms for body shape in males and females within both species. Moreover, unlike females, males also diversified in static allometry slope and reaction norms for static allometry slope of sexual and nonsexual traits. Our findings reveal qualitative sex differences in patterns of morphological diversification, whereby shape–size relationships diversify extensively in males, but remain conserved in females despite extensive evolution of trait means. Our results highlight the importance of incorporating plasticity and allometry in studies of adaptation and diversification.

KEY WORDS: Adaptation, allometry, diet, diversification, evolution, plasticity, reaction norms, secondary sexual traits.

In sexual populations, males and females experience sex-specific regimes of selection reflecting their divergent reproductive strategies. In particular, sexual selection typically acts with greater strength upon males, and it is within this sex that the expression of secondary sexual traits is usually most pronounced (Darwin 1871; Andersson 1994). The sex-specific nature of sexual selection may lead to differences in patterns of diversification of sex-specific morphologies. As a result of the complex, chaos-like

dynamics of sexual coevolution (Iwasa and Pomiankowski 1995; Gavrillets 2000), male secondary sexual traits are expected to evolve and diversify very rapidly relative to nonsexual traits—a prediction supported empirically for genitalia (Arnqvist 1998), display traits (Seehausen et al. 1999; Kolm et al. 2012), and weapons (Emlen and Nijhout 2000; Emlen et al. 2005). Male secondary sexual traits may thus diversify more rapidly than their female homologues. However, there are several reasons sex

differences in qualitative patterns of diversification may also be expected.

Because sexual selection typically displaces traits from their viability optima, the expression of secondary sexual traits can impose substantial viability costs, as well as confer great potential benefits in terms of increased mating success. These costs and benefits are both likely to depend strongly on environmental parameters that influence the costs of developing a secondary sexual trait (Kotiaho 2001; Vergara et al. 2012), as well as the potential mating-success gains from sexual trait expression (Seehausen et al. 1997; Boughman 2001; Cornwallis and Birkhead 2008). Thus, although most traits exhibit developmental plasticity (DeWitt et al. 1998; Pigliucci 2001; West-Eberhard 2003), secondary sexual traits are often especially plastic and condition dependent (Rowe and Houle 1996; David et al. 2000; Cotton et al. 2004). In particular, many secondary sexual traits exhibit heightened sensitivity to the developmental environment (Kasumovic and Brooks 2011; Emlen et al. 2012). The reaction norm—a function that characterizes developmental or phenotypic responses to an environmental variable—represents the nature and degree of plasticity for a trait. If environmental parameters vary spatially, then reaction norms for secondary sexual traits may be expected to differ between populations occupying distinct environments. Indeed, as a result of cryptic genetic variation, novel environments may expose “hidden reaction norms” or extend the expressed range of reaction norms, and selection may then act on the shape and elevation of those functions (Schlichting 2008). The diversification of secondary sexual traits may therefore be expected to involve changes in reaction norms for condition-dependent traits. In contrast, such changes may play a less important role in the diversification of female traits, which tend to be closer to their viability-selected optima and to exhibit less pronounced plasticity and condition dependence (David et al. 2000; Bonduriansky 2007a).

Investigating diversification solely in terms of phenotypic means can lead to an underestimation of the extent of diversification because similar means can be associated with very different reaction norms or, more generally, different physiological response curves (Stinchcombe et al. 2012). Studies of the evolution of reaction norms can also provide important tests of hypotheses about functional or genomic constraints (Kingsolver et al. 2007), and shed light on microevolutionary patterns (Fairbairn 2005). The incorporation of plasticity into studies of diversification is particularly timely, given increasing interest in the role of phenotypic plasticity in adaptation and evolution. Despite the long held assumption that plasticity would dull the force of natural selection and slow genetic evolution, a plastic phenotype is now thought by many researchers to play a considerable role in promoting adaptive evolution and diversification (Pigliucci 2001; Price et al. 2003; West-Eberhard 2003; Ghalambor et al. 2007; Pfennig et al.

2010). To test such ideas, it will be necessary to understand how reaction norms themselves evolve, and how the nature and degree of plasticity is associated with rates of diversification and adaptive evolution.

Despite the potential importance of plasticity, studies of diversification of sexual traits have typically focused on evolution of the phenotypic mean (e.g., Cuervo and Moller 1999; Emlen et al. 2005; Tataric and Cassis 2010; Gonzalez-Voyer and Kolm 2011). Nonetheless, several studies have reported variation among populations in the reaction norms of sexual traits, as well as evidence of divergent reaction norms between sexes. For example, four populations of hoverflies (*Eristalis arbustorum*) exhibit divergent temperature reaction norms for color pattern, body size, and other traits (Ottenheim et al. 1998). Guppies (*Poecilia reticulata*) from neighboring populations display divergent temperature reaction norms for courtship behavior (Rodd et al. 1997). Two populations of *Drosophila melanogaster* showed variation for abdominal pigmentation and its sexual dimorphism in response to temperature (Gibert et al. 2009). Although these studies provide some indication that sex-specific selection regimes could impact the way sex-specific morphologies diversify, they do not explicitly compare the diversification of reaction norms between sexes or sexual and nonsexual traits. Fairbairn (2005) carried out a direct comparison of among-population diversification patterns for temperature reaction norms of male and female morphological traits in the water strider *Aquarius remigis*: although reaction norms differed between sexes for some traits, sex differences in diversification of reaction norms were not detected. However, the secondary sexual trait examined in Fairbairn’s study was male genital size, a trait that tends to exhibit low variation among individuals within species (Eberhard et al. 1998; Eberhard 2009) and weak sensitivity to environment (Fairbairn 2005; House and Simmons 2007) relative to other traits. Further research is needed to test for sex-specific patterns of diversification for nongenital traits.

The diversification of secondary sexual traits can also involve the evolution of static allometry—that is, the scaling of relative trait size with body size among adults (Gould 1966). The static allometry slope is thought to reflect the scaling of both costs and benefits of trait expression with body size. For example, larger males may have a greater pool of resources to allocate to sexual signals and weapons, or they may be better able to tolerate the viability costs (e.g., predator avoidance) of bearing large secondary sexual traits (Rowe and Houle 1996). Larger males may also benefit more from expressing relatively larger secondary sexual traits if they are better able to wield such traits as signals or weapons (Lailvaux et al. 2004). Conversely, low allometric coefficients may evolve if selection favors individuals bearing average-sized traits regardless of their body size, as is the case for many genitalic structures (Eberhard et al. 1998; Eberhard 2009). If the body size–dependent costs and benefits of secondary

sexual trait expression vary among habitats, then populations may be expected to evolve differences in static allometry slopes of such traits. For example, populations could experience different levels of predation risk as a function of body size and secondary sexual trait size (Burk 1982), and differences among populations in operational sex ratio could result in differential benefits of investing in enlarged secondary sexual traits (Jirotkul 1999). Both experimental (Cayetano et al. 2011) and comparative (Voje and Hansen 2012) evidence indicates that static allometry slopes can evolve when the selective regime is altered (although see Egset et al. 2011, 2012).

Static allometry slopes may also vary as a function of environmental factors that influence endocrine signals involved in the regulation of body and organ growth (Shingleton et al. 2008; Teleman 2009; Emlen et al. 2012), and this function (i.e., the reaction norm for the allometric slope) may evolve and contribute to diversification (Shingleton et al. 2007). In *D. melanogaster*, the scaling of several morphological traits with body size is affected by larval density, nutrition, and ambient temperature (Shingleton et al. 2009), whereas, in the neriid fly *Telostylinus angusticollis*, the static allometry slopes of male head capsule and antenna length (which serve as weapons and possibly signals) are affected by larval diet quality (Bonduriansky 2007a). The reaction norm for the allometric slope may evolve and diversify if local environments select differently on the developmental-genetic program linking trait expression to nutritional environment (i.e., on the nature and degree of condition dependence; Shingleton et al. 2007; Emlen et al. 2012; Kijimoto et al. 2012). For example, very large relative trait size in the largest males (i.e., a steep static allometry) may be favored in an environment characterized by intense sexual competition (e.g., highly concentrated resource patches, or male-biased operational sex ratio) coupled with the availability of nutrient-rich larval substrates. No study, to our knowledge, has investigated the diversification of reaction norms for static allometry slope.

We used a combination of experimental and comparative approaches to investigate sex-specific patterns of morphological diversification in trait means, reaction norms, and static allometries (Fig. 1). We reared flies from each of five populations of two species of Australian Neriidae (*Telostylinus angusticollis* and *Telostylinus lineolatus*) on three larval diets varying sixfold in nutrient concentration, and examined effects of population and larval diet on head, leg, and wing dimensions of both sexes. The *Telostylinus* species are endemic to the east coast of Australia, spanning roughly 18 degrees of latitude (Fig. 2). *Telostylinus angusticollis* aggregates and breeds on beetle-damaged bark of *Acacia longifolia* and other trees in New South Wales and southern Queensland, whereas *T. lineolatus* aggregates and breeds on rotting fruit in tropical North Queensland. As in all holometabolous insects, adult body size and shape of neriid flies are determined during the larval feeding and development phase. Previous studies

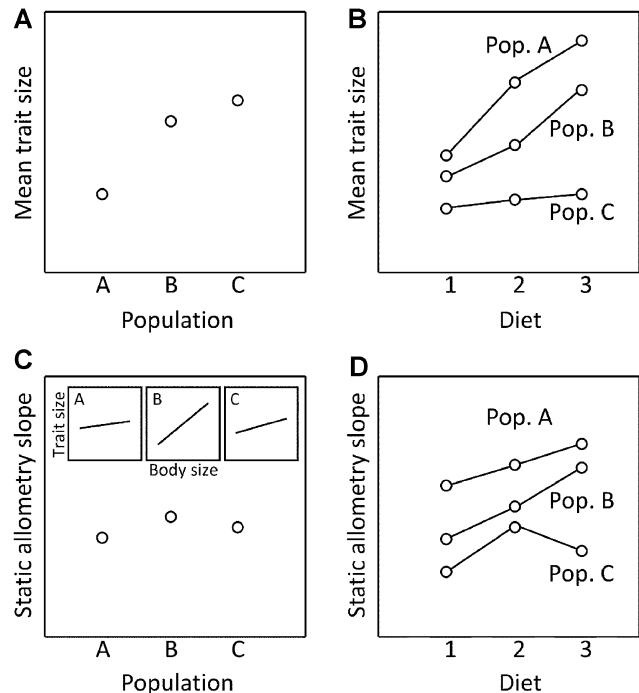


Figure 1. Schematic representation of potential forms of diversification: (A) variation among populations in mean trait size (population effect); (B) variation among populations in reaction norm for mean trait size (population \times environment effect); (C) variation among populations in static allometry slope (population \times body size effect), with inset showing slopes as regressions of trait size on body size for each population; (D) variation among populations in reaction norm for static allometry slope (population \times environment \times body size effect).

have shown that *T. angusticollis* exhibits a high level of phenotypic plasticity in response to larval nutrient intake (Bonduriansky and Head 2007; Bonduriansky 2007a, 2009). Males and females reared on a low-quality diet are small and very similar in body shape, whereas individuals reared on a high-quality diet are larger in size and exhibit sexual dimorphism, with males expressing elongated heads, antennae, and legs. Males use their heads and legs as weapons in combat, and also use their legs to encircle and guard ovipositing females (Bonduriansky 2006).

In *T. angusticollis*, male body size and shape are under sexual selection (Bonduriansky and Head 2007; C. Fricke, M. I. Adler, R. C. Brooks, and R. Bonduriansky, unpubl. ms.), whereas female body size and shape do not appear to be sexually selected. *Telostylinus lineolatus* displays similar morphology and sexual behavior to *T. angusticollis* (Bath et al. 2012), suggesting that male body size and shape are also sexually selected in this species. We therefore hypothesized that patterns of diversification of body shape components are sex specific in these species. In particular, we predicted that male but not female traits would exhibit diversification among populations in allometric slope and/or its reaction norm (Fig. 1, panels C and D). We also expected to see more

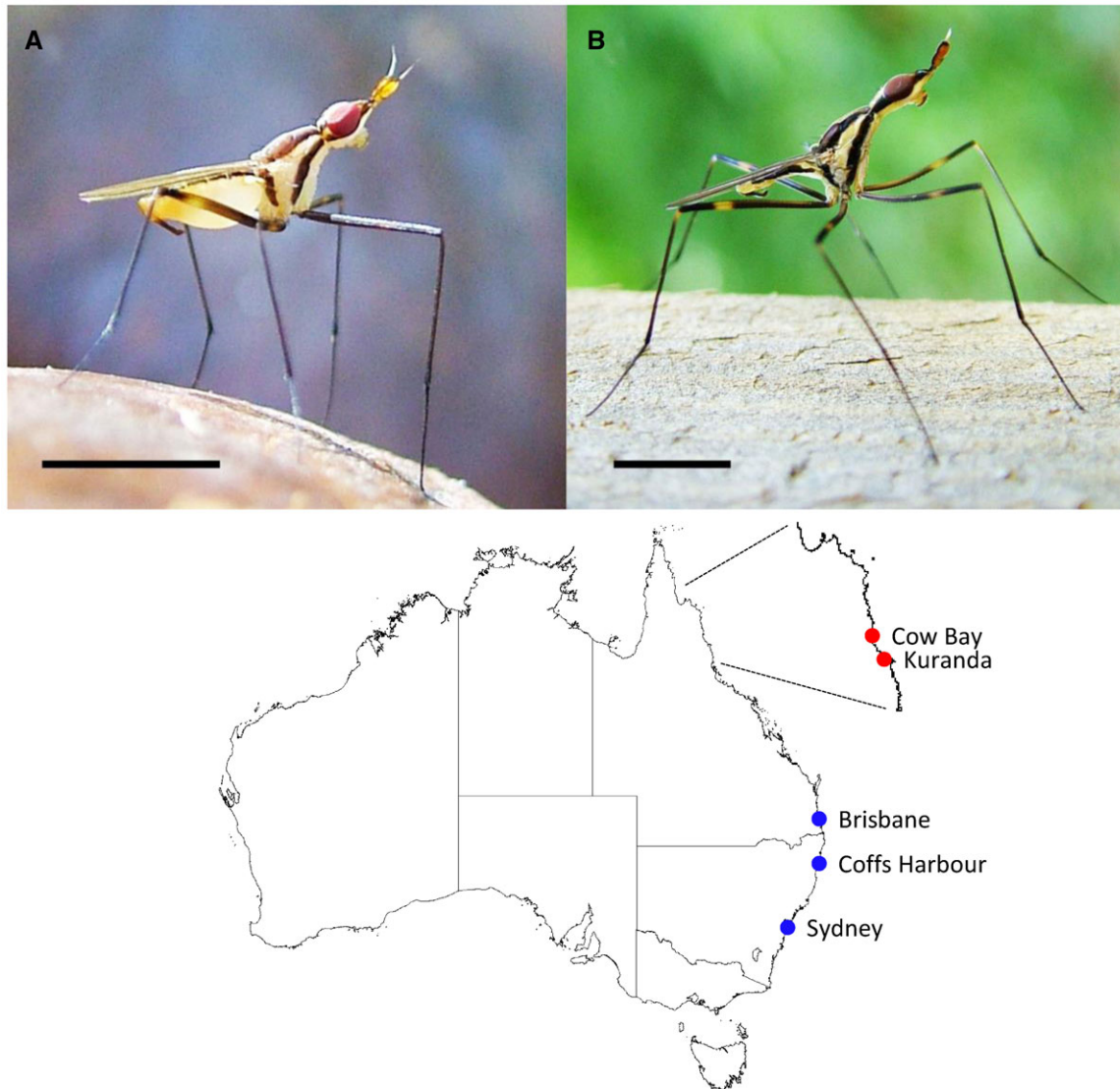


Figure 2. Male *Telostylinus lineolatus* (A) and *Telostylinus angusticollis* (B). Bars represent 1 cm.

evidence of diversification in traits that play direct roles in male sexual competition (i.e., the head and forelegs) than in other traits (mid- and hind-legs and wing). Given the apparent similarity in sexual morphology and behavior in the two *Telostylinus* species, we expected to see similar sex-specific patterns of diversification in both species. The two *Telostylinus* species were included in our study not for the purposes of conducting a detailed interspecific comparison, but as replicates that allowed us to generalize our approach and conclusions.

Materials and Methods

EXPERIMENTAL ANIMALS

Telostylinus lineolatus adults were collected on rotting fruit in Kuranda (16°83'S, 145°64'E), Cow Bay (16°23'S, 145°43'E), and Cape Tribulation (16°09'S, 145°46'E), Queensland; and *T. angus-*

ticollis adults were collected from the bark of *Acacia longifolia* trees in Brisbane (27°48'S, 153°03'E), Queensland, and Coffs Harbour (30°32'S, 153°09'E) and Sydney (33°91'S, 151°25'E), New South Wales (Fig. 2). These populations represent the extremes of the known range of each species along the east coast of Australia, as well as a population located roughly mid-way between the extremes for each species, and were selected for this study because we were interested in patterns of diversification across the geographic range of each species. One *T. lineolatus* population (Cape Tribulation) failed to survive in the laboratory and was not included in the experiment. About 10 individuals of each sex were used to found the lab colony for each population except Sydney, which was founded with about 30 individuals of each sex. The flies from each location were maintained as large, outbred populations, reared on rich larval diet, for two–four generations before the common-garden experiment.

DIET MANIPULATION

From each of the five populations, 30 male–female pairs were placed into a single cage containing oviposition medium and allowed to mate and lay eggs, and 20 randomly chosen eggs were transferred into each of 10 replicate containers of each of three larval diets. Thus, a total of 3000 eggs were individually transferred to controlled quantities of larval food, resulting in a total of 150 replicate larval containers (5 populations \times 3 larval diets \times 10 containers). The rich (R) diet was composed of 30 mL barley malt (Colonial Farms, Australia), 30 mL molasses (Conga Foods, Australia), and 32 g soy protein powder (Nature's Way, Australia) per liter of dry cocopeat (Galuku, Australia) hydrated with 800 mL of water. The poor (P) and very poor (VP) diets were three- and sixfold dilutions of the rich diet, respectively. Each replicate was provided with 200 mL of larval medium. Eggs were transferred to different larval diet treatment containers in alternating order to minimize possibility of bias, and larval containers were maintained at 27°C and watered periodically to keep the larval medium moist. Five to 10 days after adult emergence flies were frozen for measurement.

MORPHOMETRIC DATA

From each replicate jar, two randomly selected adults of each sex (where possible) were imaged, and eight measurements were taken on each individual: thorax length (TL), head capsule length (HL), head capsule width at the widest point across the eyes (HW), antenna length (AL), fore-tibia length (FL), mid-tibia length (ML), hind tibia length (RL), and the length of the R_{4+5} vein of the left wing from the r-m cross vein to the wing margin (WL). Measurements were made using ImageJ software (Rasband 1997–2009). For additional details, see Bonduriansky (2006, 2007a). Because sex ratio and emergence rate varied among replicate containers, not all replicates yielded two individuals of each sex. A total of 544 individuals were imaged and measured ($N = 90$ –120 per population).

ANALYSIS

All analyses were carried out on replicate means for log-transformed data, and a separate analysis was carried out for each species and sex. AL was removed from the analysis because it was not possible to obtain a measurement for this trait in a substantial number of individuals. HL, HW, and FL were classified as sexual traits, and the remaining traits were classified as nonsexual traits. TL was used as an index of body size because this trait loads very strongly on the first principal component in both sexes (Bonduriansky 2007a).

We evaluated among-population diversification in several ways. We asked whether populations differ in mean body size and mean trait size overall (i.e., across all larval diets). In the analysis, this is reflected in the main effect of population on body size (TL)

or relative trait size (with TL included as a covariate). We also asked whether populations differ in the response of body shape to larval diet quality (i.e., reaction norms for body shape). This is reflected in a population \times diet interaction for trait size, with thorax length included as a covariate. Finally, we asked whether populations differ in the response of the static allometry slope to larval diet quality (i.e., reaction norms for static allometry slope). This is reflected in a population \times diet \times TL interaction.

There is on-going debate over whether ordinary least-squares-based (OLS) analysis (i.e., linear regression and analysis of covariance, ANCOVA) or geometric mean regression (especially the reduced major axis, RMA) is best suited for investigation of static allometry (Seim and Saether 1983; Smith 2009; Hansen and Bartoszek 2012). Both approaches are based on restrictive assumptions about the relative measurement error variance in X and Y that are typically violated to some extent by biological data (McArdle 1988). We use OLS-based approaches in this study for two reasons. First, OLS facilitates statistical testing of the association between Y and X (Warton et al. 2006), and thus lends itself to hypothesis testing. Second, OLS is considered more appropriate when there is an inherent directionality in the relationship of Y and X (Smith 2009), and this is the case in our data: because overall body growth affects the growth of appendages such as legs and wings, it is appropriate to treat body size as the independent variable in regression analysis. Although OLS will generally yield lower slope estimates than RMA, our focus here is on variation in slopes among populations and larval diets rather than on slope magnitudes per se.

Population can be modeled as either a fixed or random factor depending on the nature and objectives of the analysis (Fairbairn 2005; Ovaskainen and Laine 2006; Sparkman et al. 2009). The populations included in our study were chosen to represent the latitudinal extremes of the known range of each species along the east coast of Australia, as well as one population located midway between the extremes. Thus, because our objective was to test for morphological differences between those geographically distant populations, rather than to estimate a variance component for a random sample of populations, we modeled population as a fixed factor.

For body size (TL), we constructed a model with population and diet as fixed, categorical predictors. For each of the other traits, we first used ANCOVA to test for differences among groups in allometric slope. We constructed a general linear model with population and diet as fixed, categorical predictors and TL as covariate:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}X + \alpha_i X + \beta_j X + (\alpha\beta)_{ij}X + \varepsilon_{ijk}, \quad (1)$$

where y_{ijk} is mean log trait size for replicate k within population i and diet j , μ is the overall mean trait size, α_i is the effect of

population i , β_j is the effect of diet j , $(\alpha\beta)_{ij}$ is the effect of the interaction of population and diet, X is the covariate (log TL), $\alpha_i X$, $\beta_j X$, and $(\alpha\beta)_{ij} X$ are the interactions of the fixed factors and their product with the covariate, and ε_{ijk} is unexplained error. Initially, all morphological traits were included in a single multivariate analysis of covariance (MANCOVA) for each sex within each species. Univariate analyses were then carried out for the separate traits. Where all interactions involving TL were nonsignificant, these interactions were removed, and simplified models (with TL as covariate) were used to test effects of population and diet. These analyses were performed using Statistica version 7 (©StatSoft Inc. 1984–2005).

When slopes were found to differ among groups, we tested population, diet, or population \times diet effects on mean trait size using the Wilcoxon test, which is a modification of the Johnson–Neyman procedure (Quinn and Keough 2002). This analysis allows for a pairwise comparison of groups that reveals covariate ranges (if any) where group means differ significantly, with P -values adjusted to account for the number of comparisons. A difference between populations in reaction norm for mean trait size was inferred when populations were found to differ significantly in mean trait size on any of the three larval diets. Wilcoxon tests were carried out using software written by Andrew Constable (<http://www.zoology.unimelb.edu.au/qkstats/software.html>).

In principle, the response in static allometry slope could be modeled as a single nonlinear reaction norm across the three larval diets within each population \times sex combination. However, we chose to fit a separate regression to each population \times sex \times diet combination for two reasons. First, it has been shown in a separate study that the relationship between trait size and body size varies among larval diets in *T. angusticollis* (Sentinella et al. 2013). Data from each diet treatment are therefore most appropriately modeled as separate reaction norms. Second, the scaling of trait size with body size for each population \times diet \times sex combination is adequately described by a linear function (see Results), allowing us to relate our findings directly to the static allometry literature, which focuses primarily on the slopes and intercepts of linear functions (e.g., Voje and Hansen 2012). Our approach should capture the same qualitative patterns that would be detected through analysis of nonlinear, continuous functions.

Differences between sexes in patterns of diversification of static allometry slopes and reaction norms of static allometry slopes can be examined by testing population \times sex \times TL and population \times diet \times sex \times TL interactions in a model fitted to data for both sexes. However, such an analysis would have low power, given the large number of effects tested. Instead, we tested for sex differences in patterns of diversification by comparing effect sizes for males and females for population \times TL and population \times diet \times TL interactions, estimated from separate models fitted to data for each sex. Effect sizes were estimated as squared semipartial

correlations, which represent the contribution of an effect to the total variance explained by the model (Fritz et al. 2012). Effect sizes were calculated by excluding the focal interaction from the model and computing the resulting reduction in total adjusted R^2 . Effect size estimates for male and female traits were compared, separately for each interaction and in each species, using Wilcoxon tests. Because a directional prediction was available (i.e., greater diversification in static allometry slope or reaction norm for static allometry slope in males, relative to females), one-tailed tests were used. Similarly, effect sizes for population \times TL and population \times diet \times TL interactions for male sexual and nonsexual traits were compared by Mann–Whitney U -tests separately within each species, using one-tailed tests to assess the directional prediction of greater diversification in sexual traits.

In our analyses, we treat the six body shape components as independent units. Although these traits are correlated genetically to varying degrees, and probably experience correlated and correlational selection (see Discussion), it is also clear that morphological traits can have substantially different genetic architectures and functional roles (Bonduriansky and Rowe 2005), and analyses of variation among multiple morphological traits within species have provided important insights (e.g., Chenoweth and Blows 2005; Fairbairn 2005; Abbott et al. 2010; Emlen et al. 2012). We did not undertake correction for multiple testing (except in Wilcoxon tests, as described earlier) because we tested distinct hypotheses for different types of traits (e.g., sexual versus nonsexual, male vs. female) in this study (Perneger 1998; Bender and Lange 2001). Moreover, we do not draw conclusions on the basis of any single univariate result but, rather, examine the broad patterns that emerge from these analyses.

Results

BODY SIZE

Mean body size (thorax length) of both sexes differed significantly among populations of *T. angusticollis* (males: $F_{2,69} = 17.2$, $P < 0.0001$; females: $F_{2,70} = 17.2$, $P < 0.0001$), with an apparent decrease in mean body size with increasing latitude from Brisbane in the north to Sydney in the south (Fig. 3). There was no evidence of variation in mean body size between populations of *T. lineolatus* (males: $F_{1,54} = 0.1$, $P > 0.76$; females: $F_{2,70} = 2.6$, $P > 0.11$). Larval diet affected mean body size in both sexes of both species ($F_{2,54-70} > 69$, $P < 0.0001$), albeit with much more pronounced effects in *T. angusticollis* than in *T. lineolatus* (Fig. 3). However, there was no evidence of diversification of reaction norm for body size (population \times diet interaction) in either *T. angusticollis* (males: $F_{4,69} = 1.9$, $P > 0.11$; females: $F_{4,70} = 1.3$, $P > 0.26$) or *T. lineolatus* (males: $F_{2,54} = 1.1$, $P > 0.35$; females: $F_{2,54} = 1.3$, $P > 0.28$).

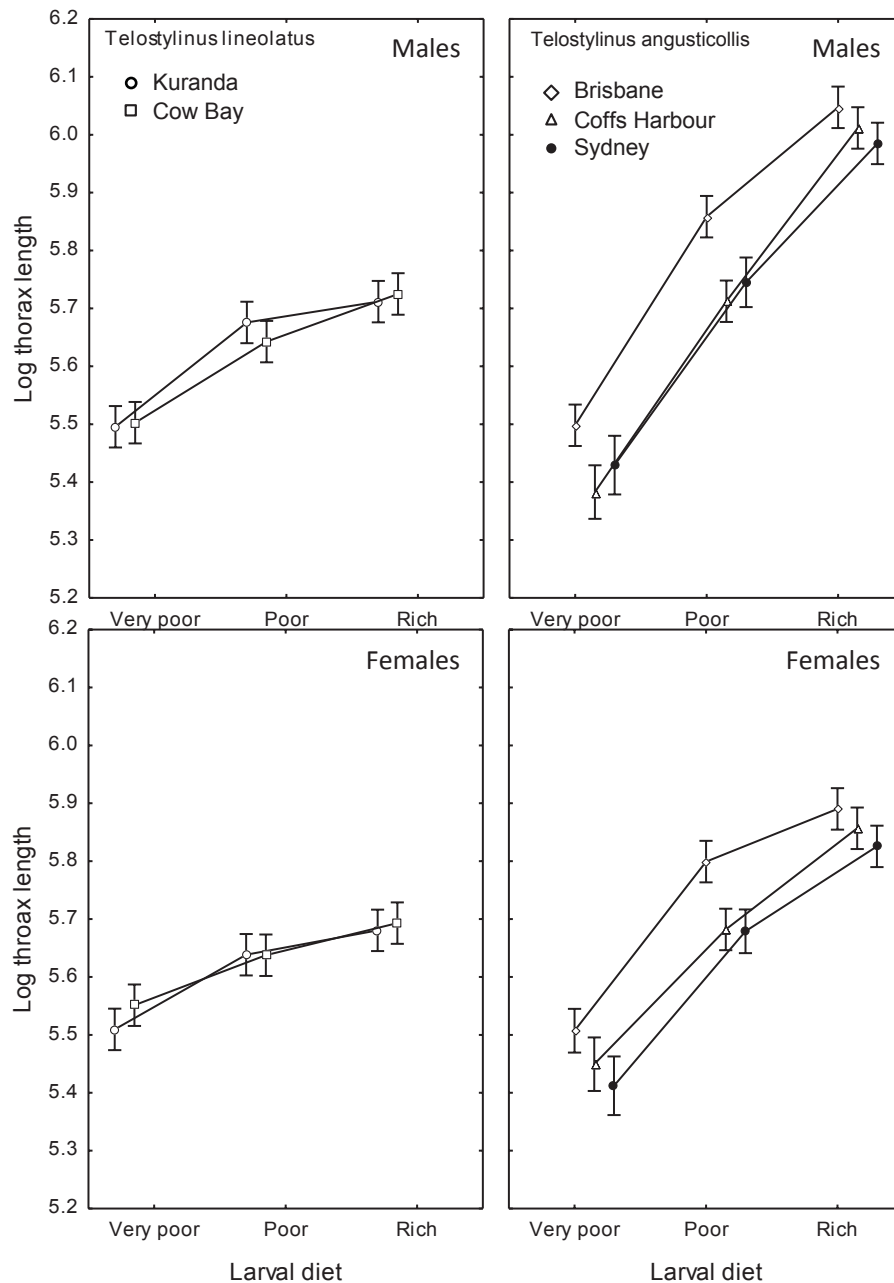


Figure 3. Reaction norms for body size (log thorax length) in populations of *Telostylinus lineolatus* (left panels) and *Telostylinus angusticollis* (right panels), with males shown in the top panels and females in the bottom panels. Bars indicate standard errors of the mean of replicates.

BODY SHAPE: STATIC ALLOMETRY

In *T. angusticollis* males, MANCOVA showed a near-significant population \times diet \times TL interaction, suggesting divergence among populations in reaction norm for allometric slope (Table 1). In *T. lineolatus* males, MANCOVA showed a trend toward a population \times TL interaction, suggesting divergence among populations in allometric slope. In contrast, MANCOVA provided no evidence of divergence in allometric slope in females of either species (all interactions with TL: $P > 0.2$). However, significant effects of

population, diet, and population \times diet on mean relative (body size corrected) trait size were observed in females of both species.

Univariate tests showed that, in *T. angusticollis* males, divergence in reaction norms of allometric slope, indicated by significant population \times diet \times TL interactions, occurred for all leg traits (FL, ML, RL; Table 2). This effect was driven by the non-linear effect of larval diet quality in the Coffs Harbour population, which exhibited marked changes in static allometry slope from < 1 on very poor diet to > 1 on poor diet to < 1 on rich diet

Table 1. Summary of multivariate analysis of covariance results for effects of population and diet, with thorax length (TL) as covariate, on head, leg, and wing dimensions in *Telostylinus angusticollis* and *Telostylinus lineolatus* (all data log-transformed). For females of both species, results for TL and its interactions with the categorical predictors are based on the full model, whereas results for population, diet, and population \times diet are based on simplified models refitted after removing the nonsignificant interactions with TL.

Effect	<i>T. angusticollis</i>				<i>T. lineolatus</i>			
	Males		Females		Males		Females	
	Wilk's λ	<i>P</i>	Wilk's λ	<i>P</i>	Wilk's λ	<i>P</i>	Wilk's λ	<i>P</i>
Population	0.832 ¹	0.5650	0.440 ¹	<0.0001	0.785 ⁴	0.0919	0.420 ⁴	<0.0001
Diet	0.675 ¹	0.0312	0.705 ¹	0.0323	0.672 ⁵	0.1132	0.609 ⁵	0.0149
P \times D	0.539 ²	0.0537	0.451 ¹	0.0007	0.751 ⁵	0.3691	0.503 ⁵	0.0005
TL	0.092 ³	<0.0001	0.151 ³	<0.0001	0.220 ⁴	<0.0001	0.398 ⁴	<0.0001
P \times TL	0.827 ¹	0.5376	0.822 ¹	0.5222	0.786 ⁴	0.0937	0.896 ⁴	0.5495
D \times TL	0.675 ¹	0.0314	0.799 ¹	0.3938	0.673 ⁵	0.1153	0.738 ⁵	0.3133
P \times D \times TL	0.536 ²	0.0501	0.649 ²	0.4137	0.753 ⁵	0.3756	0.728 ⁵	0.2760

¹Effect df = 12, error df = 108–124.

²Effect df = 24, error df = 189.6–217.5.

³Effect df = 6, error df = 54–62.

⁴Effect df = 6, error df = 43–48.

⁵Effect df = 12, error df = 86–96.

(Table S1 and Figs. 4, 5). In *T. lineolatus* males, a marginally significant divergence in reaction norm of allometric slope for ML was observed, whereas all leg traits (FL, ML, RL) exhibited evidence of divergence in population-mean allometric slope (population \times TL interaction). These effects were driven by the

substantial difference in static allometry slope between the two populations on the rich diet (Figs. 4, 5). Only one female trait, HL in *T. lineolatus*, showed a marginally significant divergence in reaction norm of static allometry slope, reflecting a difference in slopes between the two populations on rich larval diet (Table 2

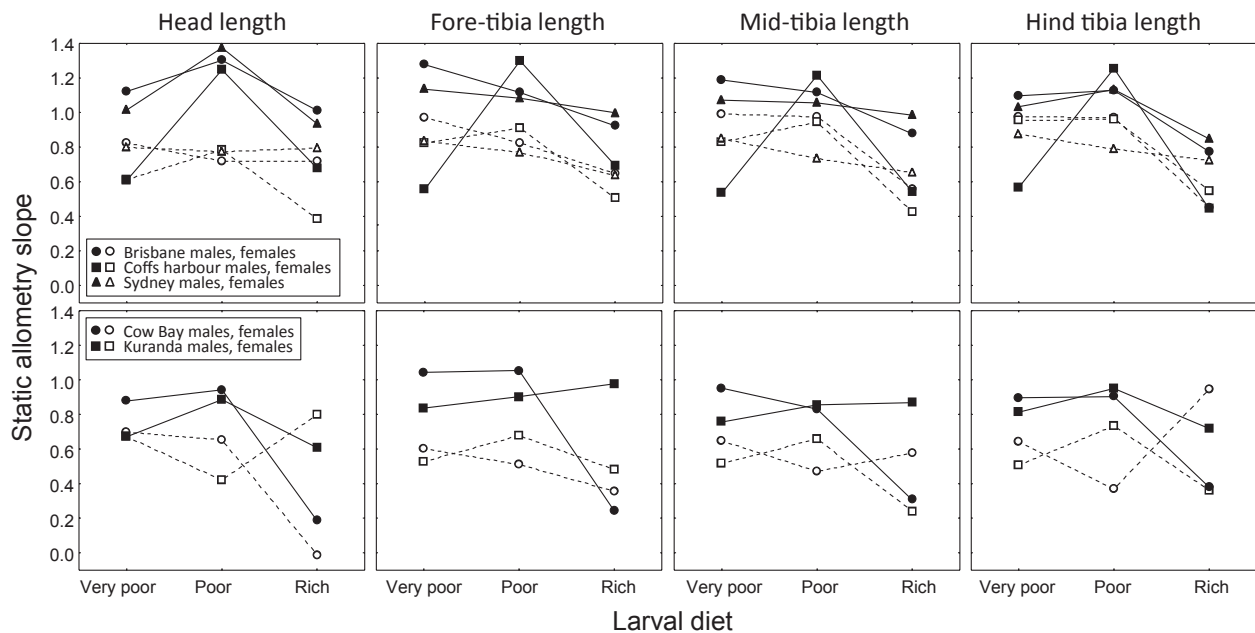


Figure 4. Reaction norms for static allometry slope of head capsule length, fore-tibia length, mid-tibia length, and hind tibia length in populations of *Telostylinus angusticollis* (top panels) and *Telostylinus lineolatus* (bottom panels). Male reaction norms are denoted by closed points and solid lines, and female reaction norms are denoted by open points and dashed lines. Standard error bars are omitted for clarity.

Table 2. Results of univariate analyses of covariance and Johnson–Neyman (Wilcox) tests (underlined) for effects of population (P), diet (D), and their interaction on morphological trait sizes in *Telostylinus angusticollis* and *Telostylinus lineolatus*. The covariate (TL) was significant ($P < 0.0001$) in each case. Effect df is shown for each species below the relevant effect, and error df and whole-model R^2 are shown below the table. When interactions with TL were nonsignificant, results for simplified models are shown. Probabilities are shown for significant and near-significant effects.

Trait	Population	Diet	P × D	P × TL	D × TL	P × D × TL
<i>T. angusticollis</i> ¹	2	2	4	2	2	4
Males						
Head length	ns	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	ns	$P = 0.0012$	ns
Head width	ns	$P = 0.02$	ns	ns	ns	ns
Foretibia length	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	ns	ns	$P < 0.0001$
Mid- tibia length	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	ns	ns	$P = 0.0012$
Hind tibia length	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	ns	$P = 0.0258$	$P = 0.0114$
Wing length	ns	ns	ns	ns	ns	ns
Females						
Head length	$P < 0.0001$	ns	$P = 0.0133$	ns	ns	ns
Head width	ns	$P = 0.0048$	$P = 0.0444$	ns	ns	ns
Foretibia length	$P < 0.0001$	$P = 0.0527$	$P = 0.0365$	ns	ns	ns
Mid- tibia length	$P < 0.0001$	ns	ns	ns	ns	ns
Hind tibia length	$P < 0.0001$	$P = 0.0341$	$P = 0.0059$	ns	ns	ns
Wing length	ns	ns	$P = 0.0022$	ns	ns	ns
<i>T. lineolatus</i> ²	1	2	2	1	2	2
Males						
Head length	$P = 0.0018$	ns	ns	ns	ns	ns
Head width	<u>$P < 0.05$</u>	<u>$P < 0.05$</u>	ns	ns	$P = 0.008$	ns
Foretibia length	ns	ns	ns	$P = 0.0548$	ns	ns
Mid- tibia length	ns	ns	<u>$P < 0.05$</u>	$P = 0.0153$	ns	$P = 0.0590$
Hind tibia length	ns	ns	ns	$P = 0.0379$	ns	ns
Wing length	ns	ns	ns	ns	ns	ns
Females						
Head length	ns	ns	ns	ns	ns	$P = 0.0499$
Head width	$P = 0.0005$	$P = 0.0001$	$P = 0.0021$	ns	ns	ns
Foretibia length	$P < 0.0001$	ns	$P = 0.0666$	ns	ns	ns
Mid-tibia length	$P < 0.0001$	ns	$P = 0.0180$	ns	ns	ns
Hind tibia length	$P = 0.0012$	ns	ns	ns	ns	ns
Wing length	$P = 0.0009$	ns	$P < 0.0001$	ns	ns	ns

¹Error df = 60–61, adjusted $R^2 = 0.97–0.99$.

²Error df = 48, adjusted $R^2 = 0.87–0.97$.

and Figs. 4, 5). Allometric slopes and their standard errors for all treatment combinations are shown in Tables S1 and S2.

BODY SHAPE: MEAN TRAIT SIZE

Univariate models refit with nonsignificant interactions with TL removed showed that, in both species, females exhibited significant population and population × diet effects for most head, leg, and wing dimensions, indicating divergence in mean relative trait size or reaction norm for mean relative trait size (Table 2 and Fig. 6). Significant population and population × diet effects were also observed for mean relative head capsule length in *T. lineolatus* and *T. angusticollis* males, respectively. For traits that exhibited significant interactions with TL, Wilcox tests showed

that all male leg dimensions in *T. angusticollis*, and ML in *T. lineolatus*, also exhibited diversification of reaction norms for mean relative trait sizes (population × diet interaction).

Reaction norms for four representative traits (head capsule length and width, fore-tibia length, and wing length) are shown in Figure 6. In *T. angusticollis*, relative head capsule length and fore-tibia length (as well as mid- and hind tibia lengths, not shown) increased strongly with increasing larval diet quality in males, whereas the relative sizes of these traits decreased slightly with increasing larval diet quality in females in most populations. Relative wing length increased with increasing larval diet quality in both sexes, whereas relative head width increased with increasing larval diet quality in females only. In *T. lineolatus*, relative head

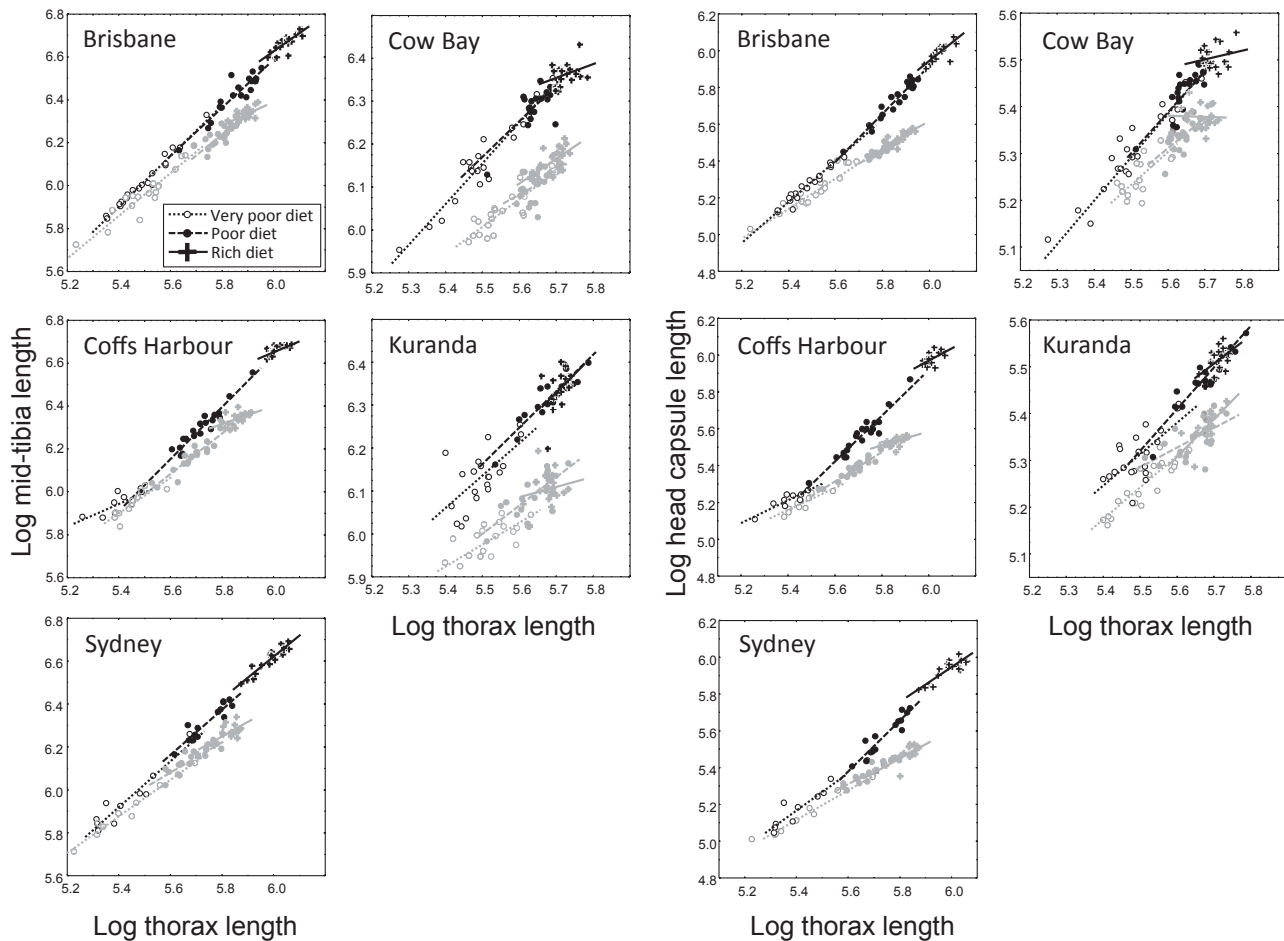


Figure 5. Static allometry slopes for mid-tibia length and head capsule length for male and female flies reared on different larval diets in *Telostylinus angusticollis* (Brisbane, Coffs Harbour, and Sydney populations) and *Telostylinus lineolatus* (Cow Bay and Kuranda populations). Black points and lines represent male data, and gray points and lines represent female data. Points represent individual flies.

capsule length and fore-tibia length (as well as mid- and hind tibia lengths, not shown) were unaffected by larval diet quality in males and decreased with increasing larval diet quality in females. Relative wing length did not show a consistent effect of larval diet quality in either sex, whereas relative head capsule width tended to increase with increasing larval diet quality in both sexes.

COMPARISON OF MALE AND FEMALE TRAITS

Multivariate analyses of variance and univariate tests suggested differences between sexes in patterns of diversification, with males but not females exhibiting interpopulation differences in static allometry slope (*T. lineolatus*) or reaction norm of static allometry slope (*T. angusticollis*). To test these sex-differences directly, we compared effect sizes for males and females. In *T. angusticollis*, effect sizes for diversification of static allometry slopes (population \times TL interaction) did not differ significantly between sexes (males: $\bar{x} = 0.000069$, $N = 6$ traits; females: $\bar{x} = 0.000094$, $N = 6$ traits; Wilcoxon test: $Z = 0.365$, one-tailed $P >$

0.3), but effect sizes for diversification of reaction norms for static allometry slope (population \times diet \times TL interaction) were significantly greater in males than in females (males: $\bar{x} = 0.001204$, $N = 6$ traits; females: $\bar{x} = 0.000156$, $N = 6$ traits; Wilcoxon test: $Z = 2.201$, one-tailed $P = 0.0139$). In *T. lineolatus*, effect sizes for diversification of static allometry slopes were significantly greater in males than in females (males: $\bar{x} = 0.002456$, $N = 6$ traits; females: $\bar{x} = 0.000758$, $N = 6$ traits; Wilcoxon test: $Z = 1.753$, one-tailed $P = 0.0398$), but effect sizes for diversification of reaction norms for static allometry slope did not differ significantly between sexes (males: $\bar{x} = 0.001497$, $N = 6$ traits; females: $\bar{x} = 0.002583$, $N = 6$ traits; Wilcoxon test: $Z = 0.105$, one-tailed $P > 0.4$).

COMPARISON OF SEXUAL AND NONSEXUAL TRAITS

We also tested for differences between male sexual and nonsexual traits by comparing effect sizes for static allometry slope and its reaction norm. We found no significant difference

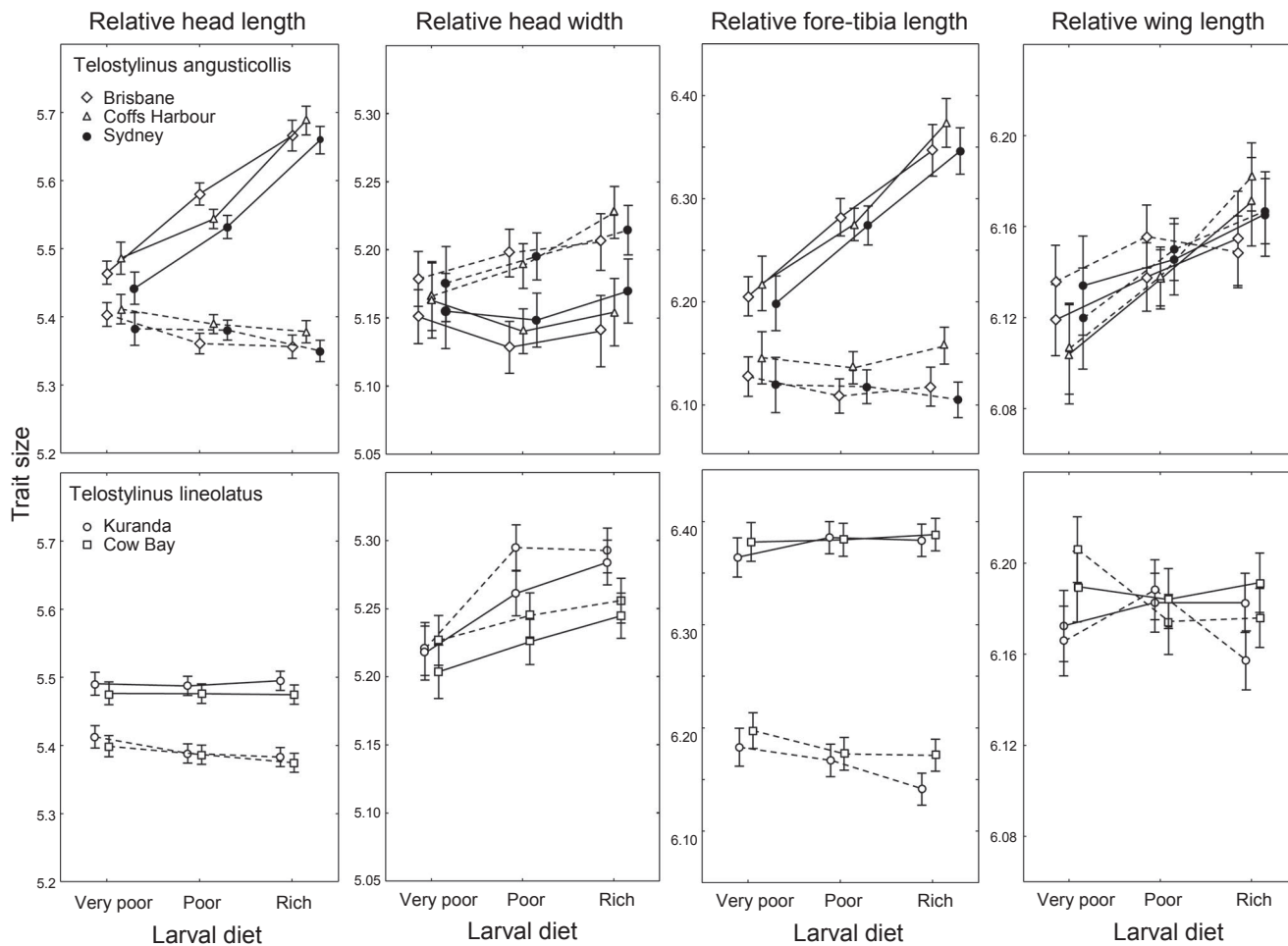


Figure 6. Reaction norms for relative log head capsule length and width, fore-tibia length, and wing length of *Telostylinus angusticollis* (top panels) and *Telostylinus lineolatus* (bottom panels). Solid lines denote male reaction norms, whereas dashed lines denote female reaction norms. Relative trait sizes represent least squares means from univariate analyses of covariance with TL as covariate. Bars indicate standard errors of the mean of replicates.

between sexual and nonsexual male traits in diversification of static allometry slopes (population \times TL interaction) or reaction norms for static allometry slope (population \times diet \times TL interaction) in *T. angusticollis* (population \times TL: sexual traits: $\bar{x} = 0.000123$, $N = 6$ traits; nonsexual traits: $\bar{x} = 0.000040$, $N = 6$ traits; Mann–Whitney U -test: $Z = 0.0$, one-tailed $P = 0.5$; population \times diet \times TL: sexual traits: $\bar{x} = 0.000672$, $N = 6$ traits; nonsexual traits: $\bar{x} = 0.000688$, $N = 6$ traits; Mann–Whitney U -test: $Z = 0.655$, one-tailed $P = 0.35$). Similarly, there were no significant differences in diversification of either static allometry slopes or their reaction norms in *T. lineolatus* (population \times TL: sexual traits: $\bar{x} = 0.000944$, $N = 6$ traits; nonsexual traits: $\bar{x} = 0.002270$, $N = 6$ traits; Mann–Whitney U -test: $Z = -0.655$, one-tailed $P = 0.35$; population \times diet \times TL: sexual traits: $\bar{x} = 0.002597$, $N = 6$ traits; nonsexual traits: $\bar{x} = 0.001484$, $N = 6$ traits; Mann–Whitney U -test: $Z = 0.655$, one-tailed $P = 0.35$).

Discussion

We investigated sex-specific patterns of morphological diversification in Australian neriid flies by rearing individuals from five populations belonging to two species on three larval diets varying sixfold in nutrient concentration. This enabled us to test for differences between populations in trait means and reaction norms for trait means, as well as static allometry slopes and reaction norms for static allometry slopes. Our study is the first, to our knowledge, to investigate the evolution of reaction norms for static allometry slopes, or to compare patterns of diversification in reaction norms and static allometries of sexually homologous traits in the sexes.

Our results reveal qualitative sex differences in patterns of diversification for components of body shape (i.e., head, leg, and wing dimensions in relation to body size). We found that female morphology of both species mainly diversified in mean trait size

or reaction norms for mean trait size. In contrast, significant population \times diet \times TL interactions (in *T. angusticollis*) or population \times TL interactions (in *T. lineolatus*) were detected for several male traits, but such interactions were nonsignificant in almost every case for female traits. Across the six head, leg, and wing traits, these interactions also explained significantly more variance in males than in females. Both sexes diversified in reaction norms for mean trait size. Body size also diversified in one species (*T. angusticollis*), although the pattern was similar in the sexes: *T. angusticollis* populations varied in mean body size of both sexes, but not in reaction norms for body size; whereas *T. lineolatus* populations did not diversify in mean body size. Overall, our results also show dramatically greater developmental plasticity in body size and shape in *T. angusticollis* than in *T. lineolatus*.

Diversification of allometric slope for male traits is consistent with the expectation that male body shape imposes viability costs and therefore develops in a condition-dependent manner. Given that body size reflects condition to a considerable extent (Blanckenhorn 2000), and that the costs and benefits of secondary sexual trait expression are likely to scale differently with body size in different ambient and social environments (Lailvaux et al. 2004; Bonduriansky 2007b), the optimum static allometry slope for such traits is likely to vary among populations and nutritional environments. Differing allometric responses to nutrient abundance suggest that optimal static allometry slope, or the investment in a given trait per unit of body size, varies depending on larval nutritional environment. Our results suggest that the costs and benefits of trait investment under the same nutritional conditions differ between populations. For example, in *T. angusticollis*, the static allometry slope of male fore-tibia, mid-tibia, and hind tibia length shows very little effect of larval diet in the Brisbane and Sydney populations, resulting in a near-linear scaling of tibia length with thorax length across the three diets within each population, whereas these traits exhibit marked changes in static allometry slope across the range of larval diets in the Coffs Harbour population, resulting in a markedly sigmoidal scaling of tibia length with thorax length across the range of larval diets (Fig. 5). As the forelegs (and to a lesser extent the mid- and hind legs) are involved in sexual competition, these populations may vary in the degree to which males benefit from leg elongation in relation to body size (see Fairbairn and Preziosi 1996).

Diversification among populations can, of course, result from genetic drift rather than selection (Coyne and Orr 2004). Although we cannot exclude the possibility that drift contributed to diversification of static allometries of male traits in these populations, we believe that local adaptation under sex-specific selection has played a substantial role for two reasons. First, the scaling of male secondary sexual traits is likely to be under strong selection through its effects on viability and mating success. Substantial effects of drift on the scaling of these traits thus seem unlikely.

Second, we observed diversification of static allometries almost exclusively for male traits, consistent with the effects of sex-specific selection. In contrast, under drift, a similar degree of diversification would be expected in both sexes. To verify the role of selection, sexual and viability selection on trait scaling must be quantified within each of our study populations.

Voje and Hansen (2012) compared diversification of static allometry slopes in male and female stalk-eyed flies (Diopsidae) and found more evidence of diversification in male slopes than in female slopes. Our findings for Australian neriid flies are consistent with Voje and Hansen's results. Our findings are also consistent with previous demonstrations that static allometry slope of some morphological traits is dependent on environmental factors such as larval nutrition (Bonduriansky 2007a; Shingleton et al. 2009). Here, we have gone a step further by showing that, in *T. angusticollis*, static allometry slope reaction norms diversify among populations and, furthermore, that such diversification has occurred only for male traits in this species.

Interpopulation diversification of reaction norms of allometric slope was seen in *T. angusticollis* but not *T. lineolatus* males. This difference between species may be related to the much greater degree of developmental plasticity and condition dependence in *T. angusticollis* males (Figs. 3, 6). These differences may stem from the variation in nutrient availability and environmental heterogeneity in the habitats of the two species. The natural larval environment of *T. angusticollis*, rotting tree bark, is long lasting and likely to vary considerably in nutrients across a small spatial gradient: some patches are moist and rich in tree sap and edible fungi, whereas others are dry and relatively devoid of resources (unpublished data). This may result in selection for a high degree of developmental plasticity in response to nutrient availability, allowing *T. angusticollis* to take advantage of abundant nutrients when available, but still develop normally when nutrients are scarce, by altering its development rate and duration. In contrast, *T. lineolatus* develops in rotting fruit—a rich but ephemeral nutrient source that may represent a less variable larval diet than that of *T. angusticollis*, selecting for a lower level of developmental plasticity. Plasticity is assumed to be costly, and should be maintained by selection only in environments where the advantages outweigh the costs (DeWitt et al. 1998; Auld et al. 2009). Given that plasticity and condition dependence of body shape are relatively weak in *T. lineolatus*, variation among populations in these parameters may also be difficult to detect. Nonetheless, the finding that diversification of static allometry slopes occurred almost exclusively in males within both *T. angusticollis* and *T. lineolatus*, despite markedly different degrees of plasticity in these species, supports the hypothesis that sex-specific selection drives diversification of static allometry slopes in male traits.

Contrary to our predictions, diversification of allometric slope was seen in both sexual and nonsexual traits. This

suggests that sexual selection is acting upon male body shape as a whole: even though some traits are more directly involved in male–male and male–female sexual interactions, and may therefore be more directly targeted by sexual selection, other traits may undergo correlated or correlational responses to selection as a result of genetic correlations or functional interactions between traits (Lande and Arnold 1983; Brodie 1992; Sinervo and Svensson 2002). Correlated suites of characters that maximize whole organism performance appear to be widespread (Irschick et al. 2008). For example, geometric morphometric analysis of stalk-eyed flies (*Teleopsis dalmanni*) illustrates complex patterns of shape variation with increase in size (Worthington et al. 2012), whereas a study on *Anolis carolinensis* lizards shows how sexual selection can favor suites of characters that enhance male–male combat performance (Lailvaux et al. 2004). Similarly, whole organism performance in *Telostylinus* males may reflect overall body shape.

Although *Telostylinus* females gain a viability and reproductive benefit from increased body size (Bonduriansky and Head 2007), female body shape is expected to approximate the viability optimum, and to undergo stabilizing selection (Darwin 1871; Andersson 1994). Female body shape is therefore not expected to exhibit strongly body size–dependent expression. Consistent with this expectation, we found very little evidence of diversification of allometric slope in females. Nonetheless, females of both species displayed extensive diversification of reaction norms for body shape. It is not clear how selection acts on female body shape in these species. However, one intriguing possibility is that the observed variation in reaction norms for female traits reflects varying degrees of resolution of intralocus sexual conflict in these populations. Selection on reaction norms and static allometries for male body shape components could displace homologous components of female body shape from their viability optima as a result of intersexual genetic correlation, and this will result in selection for modifications to the genetic architecture that reduce the genetic correlation, allowing sexual dimorphism to evolve in these traits (Lande 1980, 1987; Bonduriansky and Chenoweth 2009). However, different populations may exhibit varying stages in this process, resulting in variation among populations in the magnitudes of intersexual genetic correlations for developmental responses and growth patterns. A quantitative-genetic analysis of developmental plasticity of body shape in these populations is needed to test this possibility. Diversification of reaction norms for female trait means could also have resulted from genetic drift, particularly if female body shape is under weak stabilizing selection.

Although our study is based on just two to three populations from each of the two Australian *Telostylinus* species, it is unlikely that inclusion of additional populations in the study would have altered our major conclusion—that static allometries and/or their

reaction norms diversify primarily in males. Within each species, we deliberately sampled populations spanning a wide latitudinal range (Fig. 2), and therefore likely to exhibit substantive differentiation. Geographically less distant populations are likely to exhibit smaller interpopulation differences, so it is unlikely that diversification of static allometries in females would be detected among such populations. Our results also cannot be explained by a lack of power to detect diversification of static allometries in females. Sample sizes were similar for both sexes, measurement repeatabilities for the morphological traits examined do not differ between sexes (Bonduriansky 2006, 2007a), and static allometry slopes were estimated with similar confidence in both sexes (Fig. 5 and Tables S1, S2). Similarly, the absence of significant differences in diversification rates between male sexual and non-sexual traits does not appear to result from a lack of statistical power, because there is no trend toward a difference between these classes of traits in our data.

We have argued earlier that the observed differences between sexes in patterns of diversification are probably attributable to the presence of sexual selection on body shape in males only. The presence of sexual selection on male body shape is consistent with observations of male–male sexual competition in these species (Bonduriansky 2006; Bath et al. 2012), and with experimental findings (C. Fricke, M. I. Adler, R. C. Brooks, and R. Bonduriansky, unpubl. ms.). However, further work is required to gain a more complete understanding of selection on body shape in both sexes. Sexually dimorphic traits are not necessarily subject to strong sexual selection in males (Fairbairn and Preziosi 1996). Conversely, weakly dimorphic structures could be subject to correlated selection. It is also possible that female body shape is subject to selection through female–female competition (Clutton-Brock 2007, 2009), although lack of body elongation in *T. angusticollis* females reared on a rich larval diet suggests that, unlike males, females do not stand to benefit from exaggerated morphology.

Our findings show that diversification can proceed in several distinct ways (Fig. 1), and suggest a need to adopt an experimental approach to the study of this process. Incorporation of reaction norms and static allometry enabled us to identify vectors of differentiation among populations that we would not have been able to detect had we restricted this study to analysis of trait means. This suggests that comparative studies based on field-collected samples of individuals, representing a heterogeneous mix of environmental influences, may often underestimate the extent of diversification and, more importantly, may fail to identify some modes of adaptation. An experimental approach, whereby samples of genotypes from different populations or species are subjected to a range of relevant environmental conditions, is needed to detect divergence in reaction norms. This may be especially important in studies of the evolution of static (or ontogenetic) allometries. It

has been suggested that allometric slopes are evolutionarily conserved (Gould 1966), and some empirical studies have detected divergence in allometric intercept but not slope among natural or experimental populations (Egset et al. 2011, 2012), or concluded that the rate of slope evolution is limited (Voje and Hansen 2012). However, because allometric slopes can be plastic (Bonduriansky 2007a; this study; Shingleton et al. 2009; although see Okada and Miyatake 2010), studies of the diversification of this trait should incorporate experimental analysis of reaction norms.

Further research is needed to establish whether the sex-specific patterns of diversification observed in *Telostylinus* also occur in other taxa. Sexually homologous components of body shape, such as limb size (Zeh et al. 1992; Tseng and Rowe 1999), head width (Judge and Bonano 2008), relative lengths of the abdomen and genitalia (Fairbairn 2005), and flower size and shape (Delph et al. 1996; Barrett and Hough 2012), are particularly useful for such comparisons. Further work is also needed to verify the causes of sex differences in diversification patterns. We suggest that these differences reflect the presence (or greater intensity) of sexual selection in males, which results in the evolution of costly phenotypes and, in turn, favors environment- and condition-dependent expression of these male traits. Given the high and context-dependent costs of trait expression, diversification is largely driven by selection on the pattern of environment and condition dependence of such traits, resulting in the evolution of reaction norms for trait means and static allometry slopes. As a test of this hypothesis, it would be interesting to investigate patterns of diversification for costly female traits, such as secondary sexual traits in role-reversed species (Clutton-Brock 2007), or traits involved in maternal care. In such cases, the reverse pattern is expected, such that the evolution of (environment-dependent) static allometry slopes should be more prevalent in female traits than in their male homologues.

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Supporting Information

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

Table S1. Ordinary least squares static allometry slopes (with thorax length as the index of body size) for male head, leg, and wing dimensions in *Telostylinus angusticollis* and *Telostylinus lineolatus* individuals descended from several source populations and reared on three different larval diets (see Methods).

Table S2. Ordinary least squares static allometry slopes (with thorax length as the index of body size) for female head, leg, and wing dimensions in *Telostylinus angusticollis* and *Telostylinus lineolatus* individuals descended from several source populations and reared on three different larval diets (see Methods).