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# Condition dependence of developmental stability in the sexually dimorphic fly *Telostylinus angusticollis* (Diptera: Neriidae)

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## Abstract

Developmental stability is widely regarded as a condition-dependent trait, but its relation to genotype and environment, and extent of developmental integration, remain contentious. In *Telostylinus angusticollis*, the dorsocentral bristles exhibit striking variation in developmental stability, manifested as fluctuating asymmetry (FA) in bristle position ('positional FA') and failure to develop some bristles ('bristle loss'), in natural and laboratory populations. To determine whether this variation reflects condition, I tested for effects of genotype and environment (larval diet quality), and examined covariation with condition-dependent traits. Positional FA was not affected by genotype or environment. However, positional FA covaried negatively with secondary sexual trait expression in males, and with sexual dimorphism in body shape, but covaried positively with body size in females. Bristle loss reflected both genotype and larval diet. Flies reared on poor-quality diet exhibited a similar rate of bristle loss as wild flies. Both positional FA and bristle loss were greater in males. These results suggest that the relation between developmental stability and condition is complex and sex dependent.

## Introduction

According to theory, selection generally favours a high degree of developmental stability, allowing a functional phenotype to develop despite genetic or environmental perturbations (Debat & David, 2001; de Visser *et al.*, 2003; Leamy & Klingenberg, 2005). Selection may thus drive the evolution of canalization (de Visser *et al.*, 2003), broadly defined as the propensity of a genotype to produce a stable phenotype (Debat & David, 2001), although some models suggest that strong canalization may be an inevitable property of complex developmental systems (Siegal & Bergman, 2002). For traits exhibiting adaptive phenotypic plasticity, where a given genotype can produce a range of phenotypes suited to a range of environmental conditions (Agrawal, 2001a; DeWitt & Scheiner, 2004), selection may still be expected to canalize the development of the optimal phenotype within each environment, producing a developmentally stable reaction norm. However, quantifying developmental stability is a challenge. The standard approach uses nondirectional differences between the left and right sides – fluctuating asymmetry (FA) – as an operational index of developmental stability (Palmer, 1994). Despite an enormous research effort, the causes and implications of developmental stability remain poorly understood (Leamy & Klingenberg, 2005).

Because developmental stability is assumed to affect fitness, variation among individuals in developmental stability is expected to reflect variation in condition (Møller & Pomiankowski, 1993; Møller & Thornhill, 1998; Blanckenhorn & Hosken, 2003). The condition dependence hypothesis predicts that, like other condition-dependent traits, developmental stability will exhibit both additive genetic variation and phenotypic plasticity in relation to environmental quality (Møller & Pomiankowski, 1993; Rowe & Houle, 1996; Møller & Thornhill, 1998; Hosken *et al.*, 2000; Cotton *et al.*, 2004a; see Bonduriansky, 2007). Although many studies have investigated the causes of developmental stability (operationalized as FA), the evidence remains equivocal. Some authors have argued that FA reflects genetic quality (Møller & Thornhill, 1997, 1998), but many studies have

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failed to detect significant levels of additive genetic variance or heritability for FA (Woods *et al.*, 1998, 1999; Polak & Starmer, 2001; Blanckenhorn & Hosken, 2003; Leamy & Klingenberg, 2005). Nonetheless, because FA offers a relatively crude index of developmental stability (Palmer, 1994; Palmer & Strobeck, 2003), much uncertainly remains about the genetic basis of this trait (Fuller & Houle, 2003). Effects of environment on developmental stability also remain controversial. Some studies have shown that FA is significantly greater in stressful environments, as expected for a condition-dependent trait (Clarke *et al.*, 2000; Leamy & Klingenberg, 2005), but other studies largely failed to detect such effects (Hurtado *et al.*, 1997; Blanckenhorn *et al.*, 1998; Hovorka & Robertson, 2000; Stige *et al.*, 2004), or detected a response in some traits only (Woods *et al.*, 1999; Hosken *et al.*, 2000). Moreover, Hosken *et al.* (2000) found that FA was increased by heat stress, but not by food limitation. However, most past studies have been correlational (but see Blanckenhorn *et al.*, 1998; Hosken *et al.*, 2000), and understanding of such effects may be enhanced by studies that manipulate environmental factors while controlling for genotype (Leamy & Klingenberg, 2005).

If developmental stability reflects condition, we should also observe phenotypic integration (i.e. developmental covariation: Pigliucci, 2003) between the degree of developmental stability and the expression of condition-dependent traits (Blanckenhorn *et al.*, 1998). Indeed, a key reason for continuing interest in developmental stability is the hypothesis that FA may serve as an honest indicator of genetic quality or phenotypic condition, leading to the evolution of female preferences for the most symmetrical males (Møller & Pomiankowski, 1993; Møller & Thornhill, 1998). This idea is supported by positive covariation between symmetry in secondary sexual traits and male mating success in some species (Møller, 1996; Hunt *et al.*, 2004). Secondary sexual traits also appear to exhibit higher variation in symmetry than other traits, thus potentially providing more reliable indicators of developmental stability (Møller & Höglund, 1991; Blanckenhorn *et al.*, 1998). Although previous studies have focused on developmental stability of secondary sexual traits themselves, the condition dependence hypothesis also predicts positive phenotypic covariation between developmental stability in nonsexual traits and the expression of male secondary sexual traits, as well as other traits related to fitness in both sexes, such as body size.

Although strong canalization is generally expected, considerable variation in developmental stability is observed among different traits within a species, and homologous traits in different species. For example, segment number is an invariant feature of most arthropod species at the adult stage, but segment number varied substantially within some species of Cambrian trilobites (Hughes, 1991; Hughes *et al.*, 1999). In wild-type *Drosophila melanogaster*, different bristle characters exhibit dramatically different levels of FA (Indrasamy *et al.*, 2000). The causes of such variation between and within species remain unknown. However, poorly canalized traits may be particularly useful as models for addressing basic questions about developmental stability. Because of the subtlety of FA in most traits, studies of FA often suffer from high measurement error and low repeatability (Palmer, 1994; Fuller & Houle, 2003; Palmer & Strobeck, 2003). Traits that exhibit large variation in developmental stability may thus yield a higher signal-to-noise ratio.

The neriid fly *Telostylinus angusticollis* offers a convenient model to investigate the condition dependence of developmental stability. The dorsocentral bristles (a group of four large bristles on the posterior thoracic notum) exhibit remarkable variation among individuals in their position on the thorax, and many *T. angusticollis* individuals also fail to develop one or more bristles (Fig. 1). Such variation is observed in both the *T. angusticollis* laboratory stock and in the wild source population. The average degree of FA exhibited by this trait (~12%: see Results) appears to be high in comparison with FA estimates for various traits in other species, which are typically between 0.1% and 5% in nonmutant samples (e.g. Møller & Höglund, 1991; Møller, 1996; Blanckenhorn *et al.*, 1998; Woods *et al.*, 1999). Although some bristle traits are quite variable, such variation may be unusual for bristles that are large, prominent and few in numbers. Indeed, the number and position of the dorsocentral bristles is highly conserved in many families of Diptera (McAlpine, 1987), and serves as a diagnostic feature within Neriidae (Enderlein, 1922; Hennig, 1937; Aczél, 1959).

I quantified FA in bristle position ('positional FA') as the difference between the left and right sides of the body in the distance between the anterior and posterior dorsocentral bristle (Fig. 1). Although much of the empirical research on developmental stability has focused on bristles (e.g. Woods *et al.*, 1998, 1999; Clarke *et al.*, 2000; Indrasamy *et al.*, 2000; Polak & Starmer, 2001; Polak *et al.*, 2002), evidence is almost entirely limited to *Drosophila* (but see Blanckenhorn *et al.*, 1998; Hunt *et al.*, 2004). Moreover, many studies have treated bristles as a meristic trait (i.e. quantifying bristle number) but, to my knowledge, asymmetry in bristle position has not been examined. I also investigated variation in the number of bristles that failed to develop ('bristle loss'). Bristle loss does not represent FA *per se* (for example, an individual missing both anterior dorsocentrals would be completely symmetrical) but, like FA, it describes how far a given phenotype deviates from the 'target' phenotype (Palmer, 1994; Palmer & Strobeck, 2003). Given the strong effect of larval diet on condition, secondary sexual trait expression and sexual dimorphism in *T. angusticollis* (Bonduriansky, 2007), this system offers a convenient opportunity to test experimentally for effects of environmental quality on developmental stability and its relation to other condition-dependent traits. I tested for environ-

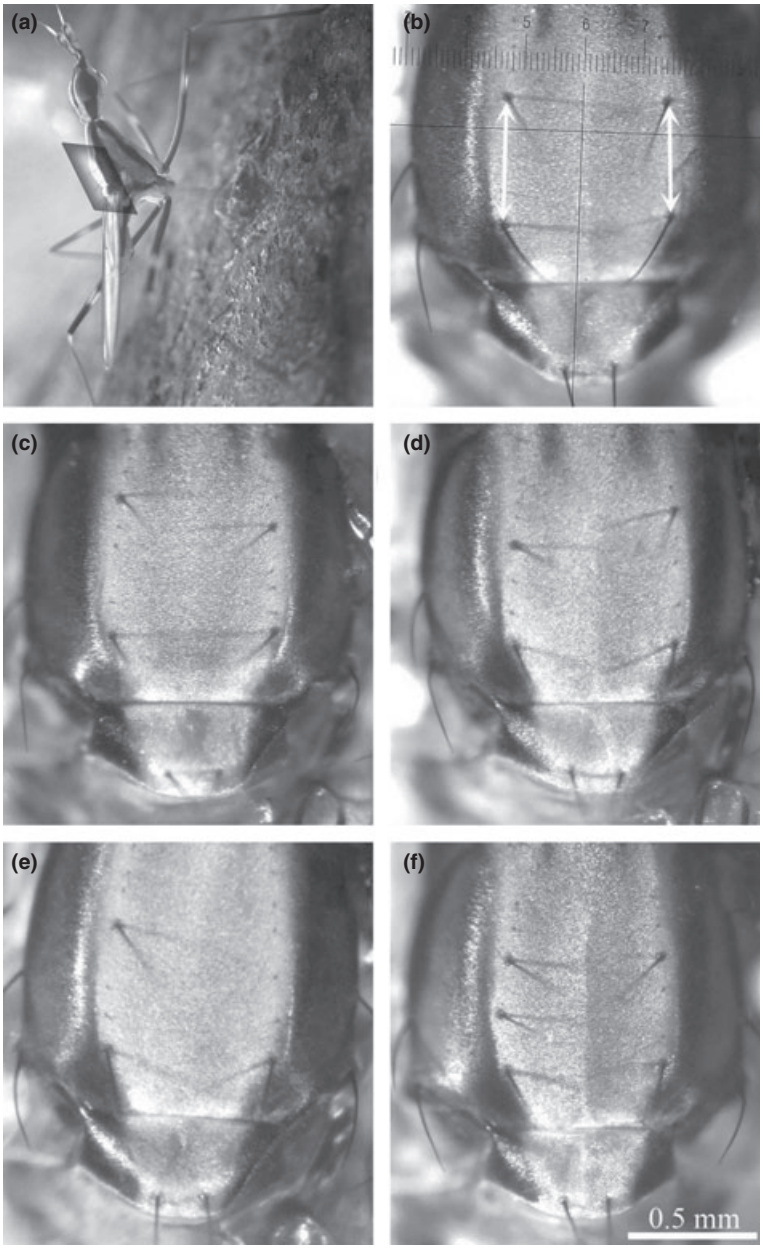


**Fig. 1** Developmental variation in the dorsocentral bristles of *Telostylinus angusticollis*: (a) a male with the location of the dorsocentral bristles (posterior end of the thoracic notum) indicated by the rectangular frame; (b) a normal phenotype, showing the distances between the anterior and posterior bristles on the left and right sides of the thoracic notum; (c) and (d) individuals exhibiting fluctuating asymmetry (FA) in bristle position ('positional FA'); (e) an individual exhibiting developmental loss of one dorsocentral bristle; (f) an individual with a supernumerary bristle.

mental effects by comparing FA in paired samples of full-sibs reared on either nutrient-rich or poor larval medium, and tested for effects of genotype by examining variation among families, and estimating heritability. I investigated the covariation of positional FA and bristle loss with body size, secondary sexual trait expression and sexual dimorphism. The hypothesis that developmental stability is a condition-dependent trait predicts that both indices of developmental stability (positional FA and bristle loss) would exhibit additive genetic variance, would be greater in flies reared on poor-quality larval diet, and would covary negatively with body size in both sexes, with the expression of secondary sexual traits in males, and with sexual dimorphism among families. I also compared positional FA and developmental bristle loss in laboratory and wild flies to determine how the environmental manipulation of condition relates to natural conditions. Few comparisons of FA in wild and lab populations have been carried out (but see Woods *et al.*, 1998).

## Methods

### Flies and rearing conditions

The analysis reported here is based on two laboratory assays using *T. angusticollis* (Enderlein), and a sample

collected from the wild source population from which the laboratory stocks were derived. The wild population breeds on trunks of beetle-damaged *Acacia longifolia* trees in the Fred Hollows Reserve in Sydney, Australia. From this population, 110 individuals (56 males, 54 females) were collected from mating aggregations on tree trunks, and from other nearby trees and a fence. These individuals were used to found the laboratory stock, and then frozen. Laboratory assays were carried out using outbred, laboratory-reared F3–F5 descendants of these wild-caught flies. General culturing conditions are described in Bonduriansky (2007).

### Assays

In assay 1, outbred pedigrees were created under standardized conditions for analysis of genotypic effects. Adult females and males were randomly selected from the F3 lab-reared generation and crossed with randomly selected nonsiblings. Each male–female pair was housed in a 250-mL plastic container containing a 1-cm layer of moist cocopeat (Galuku Pty. Ltd., Sydney, NSW, Australia), 1 cm diameter dishes of molasses and soy protein for food, and a 3.5-cm Petri dish containing rich larval medium (see below) for oviposition. Petri dishes were checked daily for eggs. From each male–female pair, each of 20 eggs was transferred individually to a 20-mL glass vial (with mesh cap) containing 5 mL of 'rich' larval medium (see below). Vials were watered periodically. From among the F4 adults, some individuals were paired (as above) to produce the F5 generation, and others were allowed to sclerotize for at least 24 h and then frozen at −20 °C.

In assay 2, larval diet was manipulated to produce paired sets of full siblings in high and low condition. Genetic variation between condition treatments within families was minimized by random selection of eggs for transfer to rich and poor larval media. This design controls for genotype. Although average differences in genotype between treatments could arise through differential mortality in the two treatments, there was little evidence of treatment effects on mortality (Bonduriansky, 2007). Thus, treatment effects can be attributed to environmental rather than genetic differences. Details of the experimental protocol are given in Bonduriansky (2007). Briefly, 'rich' food was made up of 30 mL 'black strap' sugar cane molasses (Conga Foods Pty. Ltd., Preston, Victoria, Australia), 30 mL liquid barley malt (Colonial Farms brand, Select Foods Pty. Ltd., Smithfield, NSW, Australia), and 32 g of soy protein powder (Nature's Way brand; Pharm-a-care Pty. Ltd., Warriewood, NSW, Australia) per litre of dry cocopeat hydrated with 800 mL of purified water. 'Poor' food was made from 10 mL molasses, 10 mL malt and 10 g soy protein per litre of dry cocopeat and 800 mL water. From each of 16 male–female pairs, 20 eggs were transferred to each of the two larval diet treatments. Each egg was transferred individually to a 20-mL glass vial containing 5 mL of either rich or poor larval medium. Adults emerging inside the vials were left to sclerotize for 24–48 h, then frozen at −20 °C.

### Morphometric data

Flies were thawed and glued to entomological pins by the right mesopleuron. To quantify asymmetry, each fly's dorsal surface was photographed through the eye-piece of a Leica MS5 stereoscope at 16× magnification using a Fuji Finepix S7000 digital camera. From images, distances between the bases of the dorsocentral bristles on the left and right sides of the thoracic notum (denoted L and R, respectively; Fig. 1) were measured using image analysis software (ImageJ 1.34s; National Institutes of Health, Bethesda, MD, USA). In addition, bristle loss was recorded. Bristles that fail to develop are easily distinguished from bristles dislodged mechanically by the absence of the bristle base in the former (Fig. 1). Presence of supernumerary bristles (Fig. 1), observed in very few flies, was excluded from analyses.

Body size and shape were quantified from nine linear measurements on individuals from assay 1: thorax length (TL), head capsule length (HL), head width at the widest point across the eyes (HW), antenna length (AL), fore-tibia length (FL), mid-tibia length (ML), hind-tibia length (RL), wing-vein length ($R_{4+5}$ vein length from the r-m cross-vein to the wing margin; WL), and inter-setal width (distance between the bases of the posterior dorsocentral setae; IS) (see Bonduriansky, 2006). TL, HL, HW, AL and IS were measured using an ocular micrometer on a Leica MS 5 stereoscope. Wings and legs were mounted on stickers affixed to glass slides, and scanned on an HP Scanjet 4890. FL, ML, RL and WL measurements were made from the scans using image analysis software (see above). Repeatability of measurements is high for these traits in both sexes (see Bonduriansky, 2006).

Positional FA and bristle loss were estimated for all 110 wild-collected flies (56 males, 54 females). From the laboratory assays, estimates were obtained (where possible) for five randomly selected individuals of each sex from each family in assay 1, and from each larval diet treatment × sex combination in assay 2. For assay 1, the sample consisted of 62 families: 124 F4 parents (62 males, 62 females) and 591 F5 offspring (294 males, 298 females). For assay 2, the sample consisted of 16 families comprising 270 F4 individuals (141 males, 129 females), of which 144 flies were reared on rich diet and 126 flies were reared on poor diet. However, some families lacked individuals of one sex or from one diet treatment, and some traits could not be measured on some individuals.

### Analysis

There was no evidence of directional asymmetry in bristle position. Mean ($L-R$) did not differ from zero

(*t*-test: $t_{925} = -0.69$, $P = 0.49$). Likewise, in ANOVA testing for FA relative to measurement error (Palmer, 1994; Palmer & Strobeck, 2003), the side effect was not significant for either sex ($F_{1,46} < 2.4$, $P > 0.13$) or with sexes pooled (Table 1). After correction for body size (see below), there was no evidence of significant variation in $(L-R)$ among sexes (assay 1: $F_{1,398} = 0.12$, $P > 0.7$), families (assay 1: $F_{60,398} = 1.06$, $P > 0.4$) or diet treatments (assay 2: $F_{1,117} = 2.35$, $P > 0.15$), nor of any interaction effects (not shown). To estimate the contribution of measurement error to $(L-R)$, two replicate measurements were made on each side of the body for each of 46 F5 individuals from assay 1 (23 males, 23 females). Measurement error as a proportion of the actual between-sides variance was calculated as $100 \times MS_m/MS_{interaction}$, where $MS_m$ is the error mean square and $MS_{interaction}$ is the mean square for the side × individual interaction from ANOVA (Palmer, 1994; Palmer & Strobeck, 2003). This analysis showed highly significant variation among individuals, and highly significant FA (Table 1). Measurement error accounted for just 0.1% of total between-sides variance with sexes pooled, and similarly low proportions of variance for each sex (0.08% for males, 0.2% for females). Repeatability was > 0.99 for both sexes. $|L-R|$ covaried significantly with TL (linear regression: $\beta = 0.12$, $F_{1,520} = 7.75$, $P = 0.0056$). Positional FA was therefore quantified as $|L-R|/([L+R]/2)$, which yields a size-independent index of FA (Palmer, 1994; Palmer & Strobeck, 2003). Positional FA could not be calculated for individuals which lacked one or more bristles, and was not calculated for individuals whose thorax was damaged or distorted in shape. Bristle loss was quantified for each fly as 4 – bristle number. There was no evidence of directional asymmetry in bristle loss (not shown), and error in quantification of bristle number was negligible (there were no differences between the first and second estimates for the 46 flies used to calculate measurement error, above).

Body size was quantified using PC1 scores from principal components analysis on the correlation matrix for the nine morphological traits in F5 individuals from assay 1, performed separately for each sex (Fig. 2). Body shape was quantified using PC2 scores: PC2 reflects relative (body size independent) elongation of the secondary sexual traits in males (antenna, head capsule, legs), and homologous traits in females (Fig. 2). As the head capsule is directly involved in male–male combat in *T. angusticollis* (Bonduriansky, 2006), and head capsule elongation is a highly condition-dependent secondary sexual trait in this species (Bonduriansky, 2007), I also constructed an index of relative head capsule elongation (henceforth 'head shape'). For each individual, I calculated residual HL and width from regressions of these traits on TL, and subtracted the width residual from the length residual to obtain the head shape score. Thus, individuals with relatively long, narrow heads have larger values than individuals with short, wide heads.

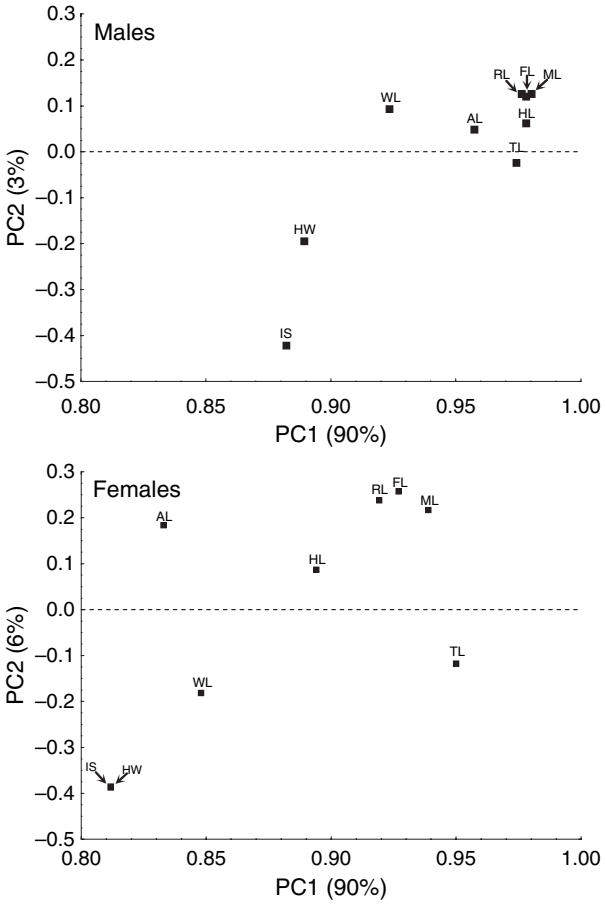


**Fig. 2** Ordinations for nine morphological traits (see Methods for trait labels).

**Table 1** Analysis of variance for between-sides variation, based on two replicate measurements on each side of each of 46 F5 individuals (23 males, 23 females) from assay 1. Separate ANOVAS for each sex yielded qualitatively identical results (not shown).

| | d.f. | MS | *F* | *P* |
|---|---|---|---|---|
| Individual | 45 | 58 501.91 | 8.80 | < 0.0001 |
| Side | 1 | 12 661.29 | 1.90 | 0.1744 |
| Individual × side | 45 | 6647.75 | 852.79 | < 0.0001 |
| Error | 92 | 7.80 | | |

I used factorial ANOVA to test for effects of genotype (family), sex and their interaction on positional FA, using F5 individuals from assay 1. To estimate heritability, I calculated offspring (F5)-parent (F4) regressions on family means for the 62 families. Because bristle loss data were highly non-normal in distribution, I used nonparametric tests (Kruskal–Wallis ANOVA, Spearman rank correlation, Wilcoxon test) to investigate family and sex effects on bristle loss, and to test for covariation between offspring and parent means. I used ordinary

least-square regression to test for covariation between positional FA and body size (PC1) and shape (PC2 and head shape score) in F5 individuals, and used F5 family means to test for covariation between positional FA and sexual dimorphism in body size and shape. Sexual dimorphism was calculated as the family mean for males minus the family mean for females, such that larger (more positive) values indicate greater trait expression in males, relative to their sisters. For bristle loss data, tests were carried out using Spearman rank correlations. I tested for covariation between positional FA and rate of bristle loss by calculating Pearson correlations based on means for F5 families (family means for bristle loss were continuously and normally distributed).

I used factorial ANOVA to test for effects of family, sex and larval diet on positional FA in assay 2. Nonparametric tests were used to test for sex effects on bristle loss, and to compare bristle loss in wild and captive flies. Statistical analysis was carried out using STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA).

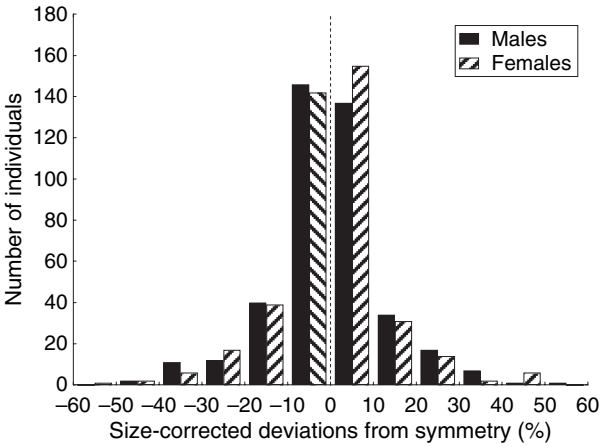


**Fig. 3** Distribution of positional fluctuating asymmetry values (grouped by intervals of 10%) for dorsocentral bristles in males and females.

## Results

### Variation in positional FA and developmental bristle loss

Mean positional FA (the difference between the left and right sides of the body in the distance between the anterior and posterior dorsocentral bristles) was ~12% (Table 2; Figs 1 and 3). The mean rate of bristle loss was ~0.16 (i.e. on average, 0.16 bristles of four were missing) (Table 2; Figs 1 and 4). About 13% of flies failed to develop the full complement of four dorsocentral bristles. Among families, there was no association between positional FA and bristle loss for either sex or with sexes pooled ($N$ = 61–62 families, Pearson $|r| < 0.08$, $|t| < 0.6$, $P > 0.5$).

### Sex effects

There was no significant effect of sex on positional FA within assay 1 (Table 3a), assay 2 (Table 3b) or the wild-collected sample (ANOVA: $F_{1,83} = 2.75$, $P > 0.1$). There was no significant difference in positional FA among assays or lab/wild samples (Table 3c). Pooling the data, there was a significant effect of sex, with males being ~14% more asymmetrical than females on average (Table 3c; Fig. 5). For bristle loss, there was a nearly-significant trend within families towards greater bristle loss in males than in their female siblings in assay 1 (Wilcoxon test: $N$ = 284 male–female sib comparisons, $T$ = 438.5, $Z$ = 1.92, $P$ = 0.0547). In assay 2, the sex difference was not significant within families (Wilcoxon test: $N$ = 11 families, $T$ = 12.0, $Z$ = 1.58, $P$ = 0.11) but bristle loss was greater in males than in females overall (Mann–Whitney $U$-test: $N_{male}$ = 141, $N_{female}$ = 129, $Z$ = 2.14, $P$ = 0.0323). Bristle loss did not differ between assay 1 and assay 2 flies reared on similar (rich) larval diet (Mann–Whitney $U$-test: $N_1$ = 591, $N_2$ = 144, $Z$ = 0.64, $P > 0.5$). Pooling lab assays, rate of bristle loss was greater in males than in females (Mann–Whitney $U$-test: $N_{males}$ = 434, $N_{females}$ = 427, $Z$ = 2.65, $P$ = 0.0080; Fig. 4).

**Table 2** Means, sample sizes ($N$) and standard deviations (SD) for the number of bristles of four that failed to develop (bristle loss), and positional FA for each sample by sex, and by larval diet in assay 2.

| | | | Bristle loss | | | Positional FA | | |
|---|---|---|---|---|---|---|---|---|
| Sample | Sex | Larval diet | Mean | *N* | SD | Mean | *N* | SD |
| Assay 1 (F4) | Male | Rich | 0.197 | 61 | 0.440 | 0.131 | 50 | 0.107 |
| | Female | Rich | 0.117 | 60 | 0.324 | 0.105 | 53 | 0.093 |
| Assay 1 (F5) | Male | Rich | 0.146 | 293 | 0.408 | 0.121 | 255 | 0.103 |
| | Female | Rich | 0.097 | 298 | 0.329 | 0.116 | 267 | 0.102 |
| Assay 2 | Male | Rich | 0.116 | 69 | 0.322 | 0.126 | 59 | 0.118 |
| | Female | Rich | 0.080 | 75 | 0.319 | 0.112 | 58 | 0.108 |
| | Male | Poor | 0.333 | 72 | 0.628 | 0.132 | 54 | 0.098 |
| | Female | Poor | 0.167 | 54 | 0.466 | 0.083 | 45 | 0.074 |
| Wild | Male | | 0.428 | 56 | 0.735 | 0.121 | 40 | 0.098 |
| | Female | | 0.185 | 54 | 0.438 | 0.090 | 45 | 0.072 |
| Grand mean | | | 0.158 | 1092 | 0.429 | 0.116 | 926 | 0.101 |

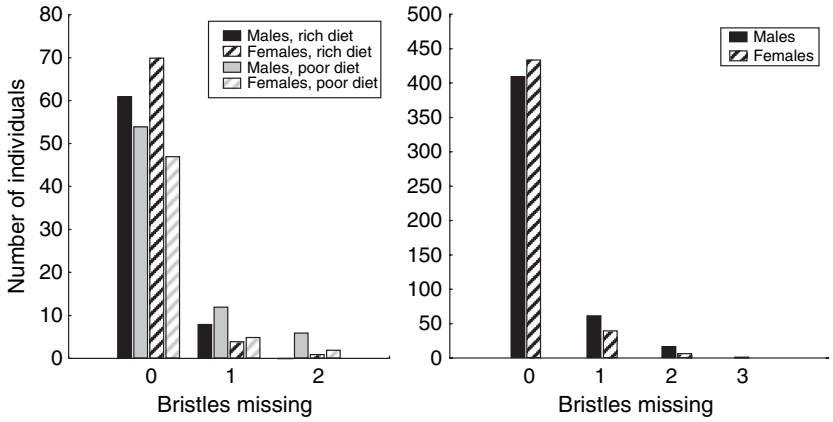


**Fig. 4** Developmental loss of dorsocentral bristles: number of bristles missing for males and females reared on rich and poor larval diets in assay 2 (left panel), and number of bristles missing by sex for the entire data set.

**Table 3** Analyses of variance for positional fluctuating asymmetry: (a) effects of family, sex and their interactions in F5 individuals from assay 1; (b) effects of family, diet, sex and their interactions in assay 2; (c) effects of assay (lab assays 1 and 2, and wild-caught) and sex.

| | d.f. | MS | *F* | *P* |
|---|---|---|---|---|
| (a) | | | | |
| Family | 60 | 112.13 | 1.04 | 0.4029 |
| Sex | 1 | 11.25 | 0.104 | 0.7467 |
| Family × sex | 60 | 85.27 | 0.790 | 0.8679 |
| Error | 398 | 107.89 | | |
| (b) | | | | |
| Family | 10 | 90.02 | 0.834 | 0.5972 |
| Diet | 1 | 1.87 | 0.017 | 0.8956 |
| Sex | 1 | 194.11 | 1.798 | 0.1826 |
| Family × diet | 10 | 64.84 | 0.600 | 0.8108 |
| Family × sex | 10 | 100.23 | 0.928 | 0.5101 |
| Diet × sex | 1 | 26.61 | 0.246 | 0.6205 |
| Family × diet × sex | 10 | 71.55 | 0.663 | 0.7569 |
| Error | 117 | 107.99 | | |
| (c) | | | | |
| Assay | 2 | 63.31 | 0.622 | 0.5374 |
| Sex | 1 | 574.19 | 5.637 | 0.0178 |
| Assay × sex | 2 | 142.37 | 1.398 | 0.2477 |
| Error | 817 | 101.85 | | |

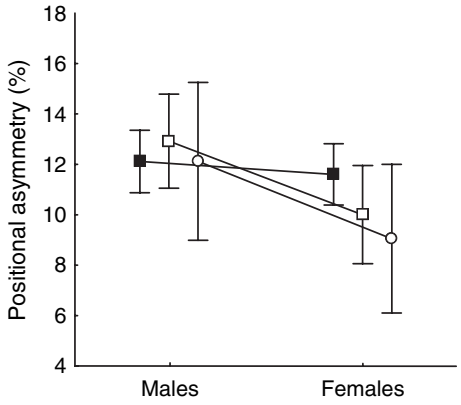


**Fig. 5** Mean positional fluctuating asymmetry by sex and sample: closed squares: assay 1; open squares: assay 2; open circles: wild flies (bars represent standard errors).

## Genotype effects

There was no evidence of family effects on positional FA in assays 1 (Table 3a) or 2 (Table 3b). Likewise, there was no evidence of significant heritability for positional FA. Father–son, mother–daughter and midparent–midoffspring regressions on family means for positional FA were all nonsignificant ($F_{1,43–52} < 1.6$, $P > 0.3$), except for a marginally significant negative father–son regression ($\beta = -0.28$, $r^2 = 0.08$, $F_{1,49} = 4.06$, $P = 0.0494$). In contrast, there was some evidence of genotype effects for bristle loss. I detected significant variation among families (assay 1) in females (Kruskal–Wallis ANOVA: $N = 61$ families, $H = 83.3$, $P = 0.0303$), but not males (Kruskal–Wallis ANOVA: $N = 61$ families, $H = 71.7$, $P = 0.14$). Moreover, there was a near-significant correlation for mean bristle loss between male and female siblings (Spearman rank correlation: $N = 61$ families, Spearman $R = 0.24$, $P = 0.0642$). However, Spearman rank correlations for bristle loss in offspring and their parents were all nonsignificant ($N = 60–62$ families, Spearman $|R| < 0.08$, $P > 0.5$).

## Larval diet effects

There was no effect of larval diet treatment on positional FA (Table 3b). However, mean bristle loss was lower in flies reared on rich larval diet than in their siblings reared on poor larval diet (Wilcoxon test: $N = 11$ families, $T = 3.0$, $Z = 2.50$, $P = 0.0125$; Fig. 4). Although rate of bristle loss did not differ between assay 1 flies and assay 2 flies reared on rich larval diet (see above), there was a significant difference between assay 1 flies (rich diet) and assay 2 flies reared on poor larval diet (Mann–Whitney *U*-test: $N_{rich} = 591$, $N_{poor} = 126$, $Z = 2.92$, $P = 0.0035$). Pooling assays, rate of bristle loss was greater in flies reared on poor diet than on rich diet (Mann–Whitney *U*-test: $N_{rich} = 735$, $N_{poor} = 126$, $Z = 3.14$, $P = 0.0017$).

### Comparison of captive and wild flies

There was no effect of assay or sample (lab/wild) on positional FA (Table 3c). Likewise, positional FA did not differ between wild flies and flies reared on rich or poor larval diets in the laboratory (Mann–Whitney *U*-tests: $N_{wild} = 110$, $N_{lab} = 99–117$, $Z < 0.4$, $P > 0.7$). However, wild flies exhibited greater bristle loss than flies reared on a rich larval diet (assay 2: Mann–Whitney *U*-test: $N_{wild} = 110$, $N_{lab} = 144$, $Z = 3.15$, $P = 0.0016$; assays 1 and 2 pooled: Mann–Whitney *U*-test: $N_{lab} = 735$, $N_{wild} = 110$, $Z = 3.85$, $P = 0.0001$). In contrast, wild flies did not differ in rate of bristle loss from flies reared on a poor larval diet (Mann–Whitney *U*-test: $N_{wild} = 110$, $N_{lab} = 126$, $Z = 0.57$, $P > 0.5$).

### Covariation with size and shape

Positional FA did not covary with body size (PC1) in males ($F_{1,248} = 0.03$, $P > 0.8$), but covaried negatively with male secondary sexual trait expression, quantified as PC2 ($F_{1,248} = 5.01$, $P = 0.0261$) or head shape ($F_{1,251} = 4.69$, $P = 0.0313$; Fig. 6). In females, positional FA increased with body size ($F_{1,254} = 4.40$, $P = 0.0370$), but did not covary with PC2 ($F_{1,254} = 0.18$, $P > 0.6$) or head shape ($F_{1,262} = 0.23$, $P > 0.6$). Among families, mean positional FA did not covary with sexual dimorphism in PC1 ($F_{1,59} = 0.02$, $P > 0.8$), but covaried negatively with sexual dimorphism in PC2 ($F_{1,59} = 4.85$, $P = 0.0317$) and (near significantly) with sexual dimorphism in head shape ($F_{1,59} = 3.54$, $P = 0.0647$; Fig. 7). In contrast, bristle loss did not covary with PC1, PC2 or head shape in either sex (Spearman rank correlations: $N = 61–62$ families, Spearman $|R| < 0.18$, $P > 0.15$; Fig. 6). There was no evidence of covariation between mean bristle loss and any index of sexual dimorphism ($N = 61$ families, Spearman $|R| < 0.19$, $P > 0.15$).

## Discussion

Positional FA did not vary significantly among families, and did not exhibit significant heritability, suggesting that there is little or no additive genetic variance for developmental stability in this trait. Nor did positional FA reflect larval diet quality, or differ between laboratory and wild flies. However, mean positional FA was greater in males than in females. Positional FA covaried positively with female body size, but did not covary with female body shape. In contrast, positional FA did not covary with body size in males, but more asymmetrical males had less developed secondary sexual traits (i.e. less elongated appendages and heads). Among families, positional FA covaried negatively with sexual dimorphism in body shape. Bristle loss exhibited substantially different patterns. I found evidence of variation among families, suggesting the presence of genetic variation, but found no evidence of heritability. Flies reared on poor larval diet exhibited greater bristle loss than their siblings reared on rich larval diet. Bristle loss was lower in flies reared on rich larval medium than in wild flies, but did not differ between poor-diet flies and wild flies.

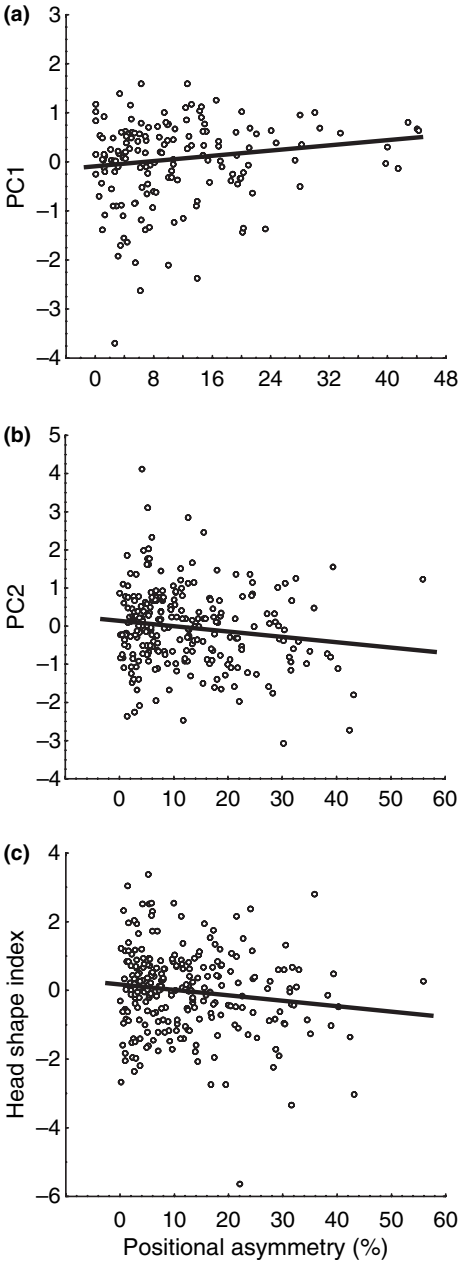


**Fig. 6** Positional fluctuating asymmetry and body size and shape: (a) positive covariation with PC1 in females; (b) negative covariation with PC2 in males; (c) negative covariation with head shape in males (lines are least-square regressions).

Because positional FA could not be determined for individuals lacking one or more bristles, analyses of positional FA could lack power if bristle loss is developmentally related to positional FA. In particular, if bristle

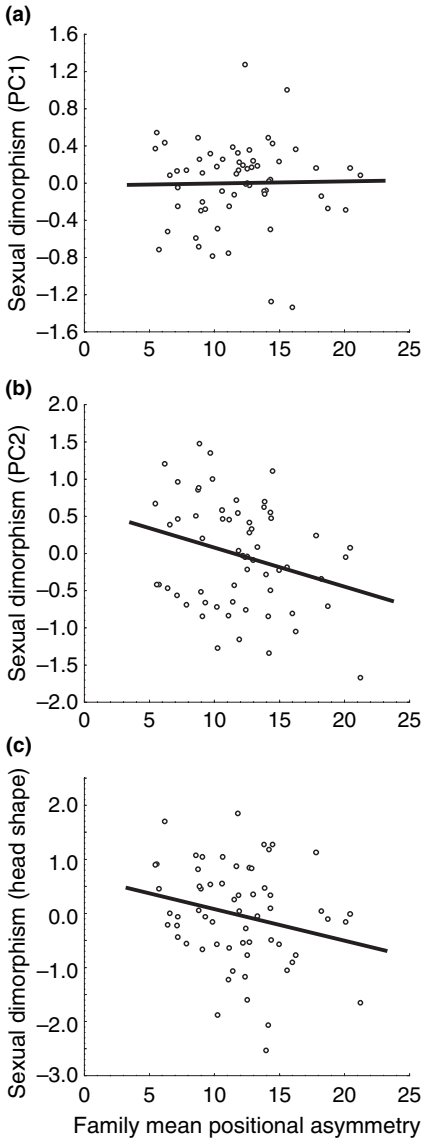


**Fig. 7** Positional fluctuating asymmetry and sexual dimorphism: (a) lack of covariation with sexual dimorphism in body size (PC1); (b) negative covariation with sexual dimorphism in PC2; (c) negative covariation with sexual dimorphism in head shape (lines are least-square regression).

loss is associated with extreme positional FA (whereby a particular bristle is displaced to such an extent from its 'normal' location that it completely fails to develop), then data on positional FA are, in effect, lacking the most extreme cases of asymmetry, and loss of power could potentially account for failure to detect genetic or environmental effects on positional FA. However, this scenario is not consistent with the data. If bristle loss represented an extreme case of positional FA, then families exhibiting the highest rates of positional FA would also be expected to show high rates of bristle loss, but I found no evidence of such covariation. This suggests that any association between these indices of developmental stability is very weak, and unlikely to affect results. However, the discontinuous nature of variation in bristle loss is likely to result in low statistical power in some analyses. In particular, in tests for offspring–parent correlations for this trait, the parental phenotype (effectively, the loss of 0, 1 or 2 bristles) probably estimates the parental breeding value very imprecisely. This may account for failure to detect an offspring–parent resemblance for bristle loss, despite the presence of family effects.

## Developmental stability and condition

If developmental stability is a condition-dependent trait, then it should exhibit both additive genetic variance (Rowe & Houle, 1996) and sensitivity to environmental determinants of condition, such as diet quality (Emlen, 1994, 1997; David *et al.*, 2000; Cotton *et al.*, 2004a,b; Bonduriansky & Rowe, 2005; Bonduriansky, 2007). It should also exhibit phenotypic integration (i.e. positive covariation) with the expression of other condition-dependent traits, such as body size, male secondary sexual traits, and sexual dimorphism.

The two indices of developmental stability examined in this study yielded substantially different results and, in both cases, the evidence was equivocal with respect to the condition dependence hypothesis. Positional FA exhibited neither genetic variation nor sensitivity to larval diet quality. Moreover, whereas large body size generally reflects high condition in insects (Blanckenhorn, 2000), and body size increases with larval diet quality in *T. angusticollis* (Bonduriansky, 2007), larger females were more asymmetrical. These results suggest that positional FA does not reflect condition. However, positional FA covaried negatively with secondary sexual trait expression in males, and with sexual dimorphism in body shape (i.e. degree of secondary sexual trait exaggeration in males, relative to their sisters). Such covariation with highly condition dependent components of male body shape suggests that positional FA is associated in some way with condition in males. In addition, males exhibited greater mean positional FA than females, a pattern also observed for FA in some traits of *Drosophila* (Vishalakshi & Singh, 2006). The male phenotype may be affected more strongly by deleterious mutations (Agrawal, 2001b), environmental factors (Serrano *et al.*, 2008), or genotype × environment interactions (Gurganus *et al.*, 1998), although neither genetic nor environmental effects were detected for positional FA in this study. Positional FA thus conforms to some of the expectations for a condition-dependent trait in males, but not in females. It is possible that variation in positional FA reflects micro-environmental (inter-vial) variation that is unrelated to larval diet treatments, or variation among ovules within clutches in epigenetic factors, and that

such variation has sex-specific effects on development. In contrast to positional FA, developmental bristle loss was subject to genetic and environmental effects, as expected for a condition-dependent trait. However, there was no evidence of covariation with other phenotypic traits – a result that cannot be attributed to low statistical power because family means for bristle loss were continuously distributed. Thus, like positional FA, bristle loss appears to conform to some but not all predictions of the condition dependence hypothesis.

Two broad conclusions can be drawn from these results. First, two different indices of developmental stability of the same character, the dorsocentral bristles of *T. angusticollis*, yielded almost completely different results (the only common finding being greater mean instability in males). This finding suggests that bristle number and bristle position may have a substantially different genetic and developmental basis. It also shows how sensitively the results of studies on developmental stability may depend on the choice of trait, or even the type of measurement used to quantify variation in a given trait. Such sensitivity may explain why a consensus on the underlying causes of variation in developmental stability has remained elusive, despite much study. Second, results suggest that developmental stability of the dorsocentral bristles is not a condition-dependent trait in the conventional sense, as it conforms to some but not all expectations for a condition-dependent trait. A similar conclusion was reached by Blanckenhorn *et al.* (1998) in a study on the genetic and environmental determinants of asymmetry in the dung fly *Sepsis cynipsea*. Such a conclusion is also broadly consistent with the spectrum of results obtained by previous studies (Fuller & Houle, 2003; Palmer & Strobeck, 2003; Leamy & Klingenberg, 2005). These findings highlight the need to understand the genetic basis of condition dependence, and its relation to developmental stability. The apparent difference between sexes in the sign of the relation between positional FA and condition, and males' greater developmental instability, raise particularly interesting questions about the sex dependence of phenotypic integration.

## FA in the laboratory and the wild

Wild animals may be expected to exhibit greater mean developmental stability than captive-reared ones because of 'developmental selection' – the demise of poor-quality individuals at pre-adult stages (Polak *et al.*, 2002). The more intense viability selection operating in the wild should eliminate a larger proportion of low quality, developmentally unstable individuals, compared with the lab. However, the effects of selection may be countered by the effects of harsh and stressful conditions on trait expression in wild animals (Kawasaki *et al.*, 2008), which could amplify developmental instability. Perhaps owing to such conflicting effects, empirical results are mixed. Woods *et al.* (1998) established laboratory lines from three field-collected samples of *D. melanogaster* and found that, compared with the wild source population, FA in wing and bristle traits increased, remained unchanged, and decreased in the three samples, respectively. In gilthead sea bream, FA was higher in cultured stocks than in wild fish (Palma *et al.*, 2001). In *T. angusticollis*, I detected no difference between wild and captive flies in positional FA, consistent with the lack of effects of genotype and larval diet on this trait. However, I observed greater bristle loss in wild flies than in captive flies reared on rich larval medium. This difference between lab and wild samples is unlikely to reflect genetic differences because the laboratory stock was recently derived from the wild population. Rather, this result suggests that environmental factors (e.g. diet, temperature) experienced by wild flies amplify developmental instability, relative to flies reared in benign laboratory conditions. One implication of this finding is that relatively 'harsh' housing conditions or treatments created in the laboratory may approximate the conditions experienced by wild populations better than benign laboratory treatments.

## A poorly canalized trait?

Although a high degree of canalization and developmental stability is expected for most traits, there are several potential reasons why some traits may be less developmentally stable. First, in meristic traits, the magnitude of phenotypic variation may be related to the mean number of units. For example, arthropods with a larger number of body segments also tend to exhibit greater variation in segment number (Hughes *et al.*, 1999). In flies, bristle traits comprising a larger mean number of bristles may tend to exhibit greater absolute variation in bristle numbers between and within individuals (e.g. see McAlpine, 1987). Second, recently-evolved traits may exhibit lower developmental stability if canalization evolves less rapidly than the phenotypic mean (Clarke & McKenzie, 1987). Third, variation in developmental stability may be related to the strength of selection. In costly traits under directional selection, such as some secondary sexual traits, large variation in asymmetry among individuals may reflect strong condition dependence of trait expression (Møller & Höglund, 1991). Conversely, traits under very weak stabilizing selection may remain poorly canalized and exhibit large variation in developmental stability among individuals and genotypes simply because such variation is almost neutral with respect to fitness (assuming that canalization is trait-specific rather than general), and mutations that disrupt development of the trait may accumulate. Two of these explanations seem consistent with the considerable variation in developmental stability observed in the dorsocentral bristles of *T. angusticollis*. Given that other species in the genus

have only one pair of dorsocentrals (Aczél, 1959), the four-bristle phenotype may be recently evolved, and canalization for this trait may still be evolving. In addition, this trait may be under very weak selection. Bristle distribution is a complex trait (Held, 1991; MacKay, 1996; MacKay & Lyman, 2005), and there is some evidence of selection on bristle traits in flies (Nuzhdin *et al*., 1995; Markow *et al*., 1996; Acebes *et al*., 2003; Hunt *et al*., 2004; Polak & Stillabower, 2004). However, in *T. angusticollis*, there is no evidence of a direct role for the dorsocentral bristles in sexual interactions (Bonduriansky, 2006), and their number and position may have little functional significance.

## Conclusions

The hypothesis that developmental stability is a condition-dependent trait remains contentious. Traits that exhibit considerable variation in developmental stability, such as the dorsocentral bristles of *T. angusticollis*, provide useful models for investigation because they allow for the quantification of indices of developmental stability with little measurement error. Results of this study suggest that developmental stability of the dorsocentral bristles of *T. angusticollis* is not a typical condition-dependent trait. The two indices used to quantify variation in developmental stability in this morphological character yielded substantially different results, and each index conformed to some but not all expectations for a condition-dependent trait. These findings highlight a need to understand the genetic basis of condition dependence and developmental stability.

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