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ENVIRONMENTAL ORIGINS OF SEXUALLY SELECTED VARIATION AND A CRITIQUE OF THE FLUCTUATING ASYMMETRY–SEXUAL SELECTION HYPOTHESIS

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Abstract.—Identifying sources of phenotypic variability in secondary sexual traits is critical for understanding their signaling properties, role in sexual selection, and for predicting their evolutionary dynamics. The present study tests for the effects of genotype, developmental temperature, and their interaction, on size and fluctuating asymmetry of the male sex comb, a secondary sexual character, in *Drosophila bipectinata* Duda. Both the size and symmetry of elements of the sex comb have been shown previously to be under sexual selection in a natural population in northeastern Australia. Two independent reciprocal crosses were conducted at 25° and 29°C between genetic lines extracted from this population that differed in the size of the first (TC1) and third (TC3) comb segments. These temperatures are within the documented range experienced by the species in nature. Additive and dominance genetic effects were detected for TC1, whereas additive genetic, and Y-chromosomal effects were detected for TC3. TC2 and TC3 decreased sharply with increasing temperature, by 10% and 22%, respectively. In contrast, positional fluctuating asymmetry (PFA) significantly increased with temperature, by up to 38%. The results (1) document an important source of environmental variance in a sexual ornament expected to reduce trait heritability in field populations, and thus act to attenuate response to sexual selection, (2) suggest that variation in ornament size reflects differences in male condition; and (3) support the general hypothesis that asymmetry in a sexual ornament is indicative of developmental instability arising from environmental stress. The ''environmental heterogeneity'' (EH) hypothesis is proposed, and supportive evidence for it presented, to explain negative size-FA correlations in natural populations. Data and theory challenge the use of negative size-FA correlations observed in nature to support the FA-sexual selection hypothesis, which posits that such correlations are driven by differences in genetic quality among individuals.

Key words.—*Drosophila bipectinata*, environmental variation, fluctuating asymmetry, genetic variation, genotype by environment interaction, sex comb, sexual selection, thermal stress, Y chromosome.

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Secondary sexual characters mediate competition over mates (Darwin 1871). This competition arises in the context of contest interactions for access to mates, and mate selection by individuals of one sex among members of the opposite sex (Darwin 1871; Andersson 1982). Sexual selection, which operates when either of these forms of competition generates variance in mating success, is a potent force in evolution: Secondary sexual traits are among the most rapidly evolving traits of animals (Andersson 1994; Panhuis et al. 2001).

As sexual ornaments become progressively larger, their evolution ultimately may be slowed or arrested by a variety of interacting processes. For example, when physiological costs associated with trait production suddenly rise, as might occur in a changing environment (Nur and Hasson 1984; Hoffmann and Parsons 1991), survival probability of males bearing the largest traits may decrease sharply. Such decrements in survival, arising when limited resources are channeled into a sexual trait(s) at the expense of somatic maintenance (Andersson 1994; Kotiaho 2001), can result in evolutionary reduction of ornament size (Fisher 1930; Lande 1981, 1982; Nur and Hasson 1984; Pomiankowski and Iwasa 1998).

The existence of resource-based trade-offs may also explain why costly sexual traits are often sensitive to environmental variation, more so than other classes of traits (Parsons 1995). Indeed, the environmental component of total phenotypic variance of secondary sexual traits is often higher than that in morphological traits not subject to sexual selection (Alatalo et al. 1988; Pomiankowski and Møller 1995). This increased variability arises from elevated sensitivity to environmental heterogeneity, and genotype $\times$ environment interaction, both of which are components of the environmental variance of phenotypic traits (Stearns 1989; Falconer and Mackay 1996; Qvarnström 1999).

Environmental stress, such as exposure to unfavorable weather conditions, toxins, or parasites, may often reduce sexual trait expression because stress triggers the adaptive diversion of limited resources to somatic maintenance away from traits not critical for immediate survival (Hoffmann and Parsons 1991). It therefore follows that variation in the expression of sexual traits within a given environment should reflect differences in the internal state, or condition, among males, a prediction for which there is growing support (Andersson 1982; Griffith et al. 1999; Qvarnström 1999; Kotiaho 2001; Kotiaho et al. 2001; Badyaev and Duckworth 2003). Indeed, evolutionary models predict that environmentally labile traits will more reliably indicate male quality, because traits with high environmental variance (reduced canalization) are expected to be more condition-dependent compared to traits that are strongly canalized (e.g., Dominey 1983; Andersson 1986).

But increasing the environmental variance underlying trait expression may also lower heritability (assuming that genetic variance remains constant) (Price and Schluter 1991; Qvarnström 1999; Endler 2000), and erode the potency of sexual selection acting directly on the trait for effecting a microevolutionary response (Hoffmann 2000). Therefore, a detailed knowledge of both genetic and environmental effects on trait expression is required for understanding the signaling properties of sexual traits and for predicting their evolutionary responses, at least in the short term.

The present study tests for the effects of developmental temperature, genotype, and their interaction, on the size and asymmetry of the male sex comb in *Drosophila bipectinata* Duda. Two independent reciprocal crosses between high and low sex comb genetic lines are performed at two temperatures within the natural range documented for the population. Comparisons of progeny means to parental values are made to gain preliminary insight into the mode of inheritance of elements of the sex comb, and the degree to which genotype-specific ornament values are temperature stress labile.

The effect of temperature on both trait size and fluctuating asymmetry (FA), as well as the relationship between size and FA, are evaluated, because the study of patterns of FA in sexual ornaments, especially under variable environmental conditions, may offer insight into the signaling properties of sexual traits (Parsons 1990, 1995; Møller and Swaddle 1997; Uetz and Taylor 2003). Fluctuating asymmetry refers to subtle deviations from perfect bilateral symmetry in morphological structures, and results from underlying developmental instability (DI, the outcome of processes that disturb development along a particular trajectory under a defined set of environmental conditions) (Ludwig 1932; Waddington 1957; Van Valen 1962; Nijhout and Davidowitz 2003). The role of positional fluctuating asymmetry (PFA), which reflects asymmetry in the placement (as opposed to mere number) of components of meristic traits, is of particular interest because both a developmental model (Starmer et al. 2002) and empirical data (e.g., Polak 1997; Polak et al. 2004) suggest it may be a sensitive indicator of developmental instability.

We specifically relate our results to a key component of the FA-sexual selection hypothesis (Møller and Höglund 1991; Møller and Pomiankowski 1993; Møller and Swaddle 1997; Tomkins and Simmons 2003). This hypothesis proposes that FA in secondary sexual traits reveals individual quality to competitors and mates because FA reflects ability to cope with stress (Møller 1992a; Møller and Pomiankowski 1993; Watson and Thornhill 1994; Tomkins and Simmons 2003). Because only high quality males are expected to be able to produce sexual traits that are both large and symmetrical, the FA-sexual selection hypothesis predicts that trait size and FA will be negatively correlated. We refer to this model as the ''quality heterogeneity'' hypothesis, where differences in male genetic quality simultaneously drive variation in trait size and symmetry (Møller 1993a, b; Møller and Pomiankowski 1993; Møller and Swaddle 1997; Tomkins and Simmons 2003). However, this ''quality heterogeneity'' hypothesis implicitly assumes that males were exposed to similar kind and magnitude of stress during development, an assumption that often may be false in natural populations. The data presented herein provide supportive evidence for an alternative explanation for negative size-FA covariation in condition-dependent traits in natural populations: heterogeneous exposure to environmental stress (Polak et al. 2004).

The male sex comb in *D. bipectinata* is comprised of stout black bristles, or ''teeth,'' arranged either singly or in oblique rows on the first and second tarsal segments of the front legs of males (Kopp and True 2002; also see fig. 1 in Polak et al. 2004). During courtship, males contact either side of the female's abdomen with their combs, possibly delivering tactile stimuli to her body, although whether females detect and respond to variation in sex comb size is yet unknown. The sex comb differs significantly across populations throughout the geographic range of the species, indicating incipient diversification of this sexual ornament (Polak et al. 2004). Field work in northeastern Queensland, Australia, has demonstrated significant sexual selection operating in natural populations for increasing comb size, suggesting that sexual selection may be contributing to this observed evolutionary diversification (Polak et al. 2004). These findings have motivated the present research into the environmental and genetic determinants of phenotypic variation in the structural elements of the comb to understand more fully the signaling properties of the sex comb and its potential for microevolution by sexual selection.

Materials and Methods

Isofemale Lines

Copulating pairs of *D. bipectinata* were captured by aspirating them directly from the surface of exposed flesh of jackfruit between 0515 and 0700 h, October 10–14, 2001. Each pair was placed in an 8-fluid-dram shell vial containing 1.7 g Instant *Drosophila* Medium (Carolina Supply Co., Burlington, NC), 6 ml water, and 2 ml of a banana-live yeast slurry. A single virgin pair of offspring resulting from this original field-caught copulating pair was used to initiate an isofemale line. In the next generation, groups of eight males and eight females from each line were placed into food vials in which oviposition was allowed to take place for 36 h. Flies were transferred to a fresh food vial and a second 36 h round of oviposition was allowed to take place. Cultures were maintained at 25°C and a 12:12 L:D photoperiod. Upon emergence, males were harvested from vials, and thorax length and sex comb size determined (see below). The use of two replicate vials per line permitted assessment of the environmental component of variation in this sexual trait (Polak and Starmer 2001). A total of 28 isofemale lines were established, and 3–22 males per vial were processed (mean = 10.9, n = 56 vials).

Processing of Males and Genotypic Differences

Each male was killed with ether fumes and under an Olympus SZX12 stereomicroscope, thorax length (distance from the anterior edge of the thorax to the distal end of the scutellum) was measured, and sex comb teeth counted. Male foretarsi were removed and placed on double-sided adhesive tape on a microscope slide, and counts of the teeth were made against a white background. The sex comb teeth in *D. bipectinata* occur in three rows, designated C1, C2, and C3 (fig. 1 in Polak et al. 2004). The first tarsal segment bears C1 and C2, two oblique rows of 2–8 and 4–11 teeth, respectively. C3, the third segment of the comb, occurs on the second tarsal segment and consists of 0–4 teeth per tarsus. TC1, TC2, and TC3 refer to the total number of teeth in each comb segment per fly, calculated as the sum across tarsi.

TC1,2 is the sum of TC1 and TC2, and is the measure of trait size associated with positional fluctuating asymmetry.

Positional fluctuating asymmetry (PFA) was calculated as |(right C1/right C2)–(left C1/left C2)| (Polak 1997); C1 and C2 were used to calculate comb PFA because these components appear to be developmentally integrated (Polak and Starmer 2001, p. 510). Measurement error in tooth counts has been assessed previously (Polak et al. 2004): the foretarsi of 25 males were placed on double-sided adhesive tape on a microscope slide. Two replicate counts of the teeth in each comb segment were made 14 days apart by one observer (MP), and in different order. No counting errors were made between replicate counts.

To test for interline (genotypic) differences, and ascertain which comb attribute(s) most strongly differentiated the 28 lines, MANOVA was first used to examine the effects of line and vial (random factor nested within line) on TC1, TC2, TC3, and PFA. Roy's greatest root *F*-statistic was used to derive statistical conclusions regarding the overall effect of line. In subsequent ''protected'' univariate ANCOVAs in which each response variable was analyzed separately, either thorax length (in the case of TC1, TC2, and TC3) or TC1,2 (in the case of PFA) was the covariate. Counts of TC1, TC2, TC3, and TC1,2 each approached a normal distribution (Shapiro-Wilk statistic, $W = 0.94, 0.95, 0.83, 0.91$, respectively).

Field Temperatures of Larval Substrates

On January 14 and 15, 2004, temperature recordings of necrotic tissue of jackfruit (*Artocarpus heterophyllus* Lam., Moraceae) inhabited by fly larvae were made with a thermocouple on the grounds of the Cape Tribulation Farmstay, northeastern Queensland, Australia. The thermocouple probe was inserted 2.5 cm into tissue teaming with larvae. A total of 15 fruits (either hanging from a tree or on the ground) were sampled, and 1–3 readings were taken per fruit, each from a different pocket of necrotic tissue. ''Nighttime'' measurements were taken at 2300 h and 0530 h, and daytime temperatures at 1130 h and 1300 h. Daytime temperatures were taken from fruits either entirely in the shade under the parent tree or partially exposed to the sun.

Interline Crosses and Temperature Treatments

Because MANOVA revealed that TC3 explained more variation between lines than either of the other three variables (see Results), lines for the cross were chosen on the basis of this comb segment. Two independent pairs of lines were chosen that differed significantly in mean TC3; each pair contained a ''high'' (H) and a ''low'' (L) parental isofemale line. Adults from the parental lines were collected as virgins, allowed to mature at 25°C, and H and L lines were selfed and crossed reciprocally to form four lines: H, F1, F1r, and L. The two replicate crosses, designated A and B, were each conducted at 25° and 29°C. Lines within either temperature treatment were reared from two vials, each seeded with five virgin females and five males.

Thorax length and sex comb tooth number of 11–58 males (mean = 33.8, $n = 32$ vials) emerging from each vial were determined as above. ANCOVA was used to test for the effects of replicate cross (A, B), line (H, F1, F1r, L), temperature treatment (25°, 29°C), vial (1, 2), and all interactions. Cross and vial were treated as random factors; *F*-statistics were calculated using appropriate mean squares. For TC1, TC2, and TC3, the covariate was thorax length (tl), whereas the covariate for PFA was trait size (TC1,2). The homogeneity of slopes assumption across replicate cross, line, and temperature was verified in separate analyses by testing for the interaction between the relevant covariate and each factor. In no case was the interaction significant. All analyses were conducted using SAS (SAS Institute 1990).

TABLE 1. Results of ANCOVA, with thorax length (tl) as the covariate, showing effects of cross, line, and temperature on TC3 (number of teeth in the third comb segment). Nonsignificant interactions are not shown.

Source	df	MS	*F*	*P*
tl	1	3.81	6.06	0.01
Cross	1	20.43	15.27	0.001
Line	3	77.49	57.83	<0.0001
Temp	1	35.16	28.36	<0.0001
Cross × line	3	9.53	7.03	0.003
Vial	16	1.39	2.22	0.004
Error	1050	0.63		

RESULTS

Genotypic Variability

A total of 28 isofemale lines derived from nature were screened for variation in ornament size. Nested MANOVA revealed strong overall effects of line on the structural elements of *D. bipectinata*'s sex comb ($F_{27,\,552} = 11.26$, $P < 0.0001$). Standardized canonical coefficients were greatest for TC3 (1.02), followed by TC1 (0.67), TC2 (0.34), and PFA (0.023), identifying TC3 as most important in explaining variation between lines. In subsequent ANCOVAs, with thorax length as the covariate, significant line effects were detected for each of these segments (all Ps ≤ 0.003). Vial effects were nonsignificant in each case.

These results indicate that significant genotypic variability underlies expression of the sex comb elements, whereas the environmental component of variation under the standard conditions at which isofemale lines were reared was negligible. In the case of PFA, neither line nor vial effects were significant.

Crosses

Two independent crosses (A and B) were each conducted at 25° and 29°C. Results of the ANCOVA model for TC3 are presented in Table 1, which revealed strong effects of cross, line, and temperature. No interactions were significant, except for the cross-by-line effect (Table 1), which was caused by a different pattern of separation between F1 and F1r progeny in the two crosses (Fig. 1). In replicate cross A, F1 and F1r means were considerably closer together (at 25° these means were nearly identical) than in replicate B.

At the high temperature (29°C) in replicate A, and at both temperatures in replicate B, mean F1 and F1r values differed significantly from each other, and each tended toward the parental line from which it inherited its Y chromosome (Fig.

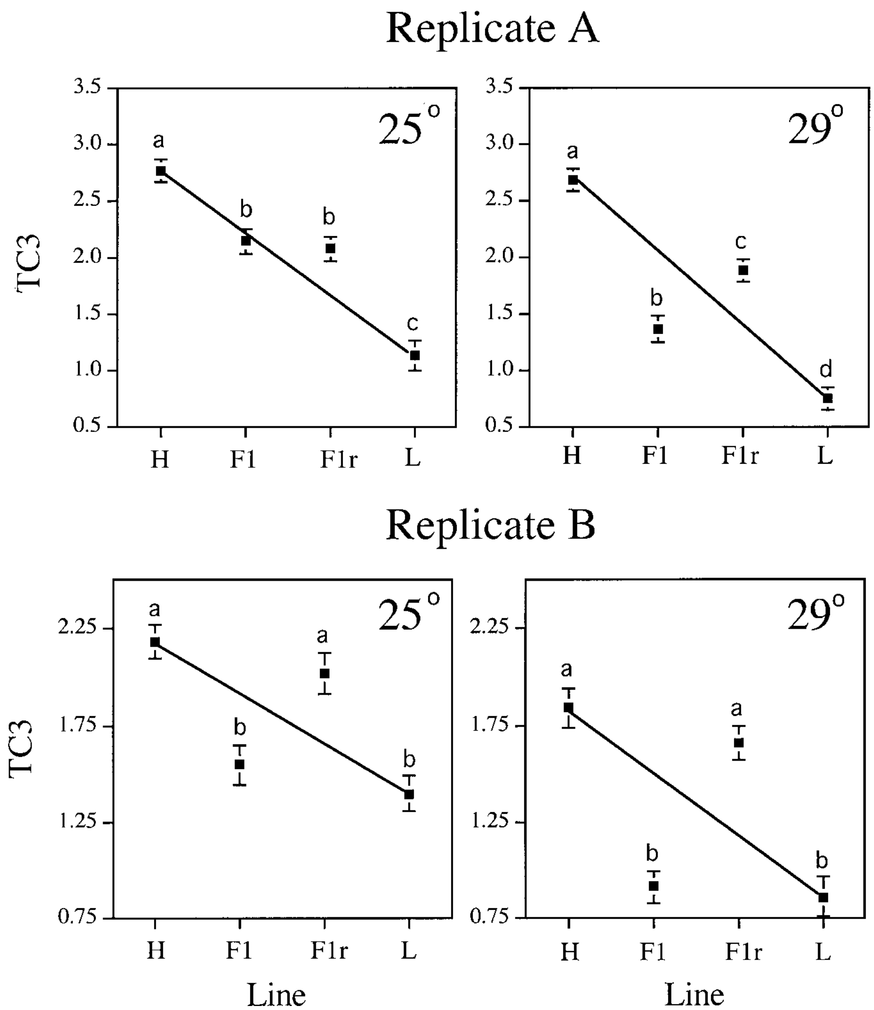

FIG. 1. Mean total tooth number in the third comb segment (TC3) in parental and hybrid lines of reciprocal crosses A and B performed at two temperatures. Error bars are ± 1 SE, and means sharing a letter are not significantly different by the Tukey-Kramer procedure (α = 0.05).

1). The Y-chromosome effect on TC3 was stronger in replicate cross B: at both temperatures, mean F1 and F1r values differed from each other, and each was closer to, and not significantly different from, the parental line from which it inherited its Y chromosome. In a follow-up analysis, in which crosses were treated separately, a contrast designed to partition the line sums of squares (cross A and B sums of squares = 215.49 and 77.43, respectively, both df = 3) according to the source of the Y chromosome (High + F1r versus Low + F1), showed significant effects ($P < 0.001$) that accounted for 63% (cross A) and 92% (cross B) of the line variation.

For TC1, both cross ($F_{1,17.3} = 10.62$, $P = 0.0045$) and line ($F_{1,17.3} = 6.12$, $P = 0.005$) effects were significant, whereas temperature effects and all interactions were not significant. In neither replicate cross did F1 and F1r values differ significantly from each other (Fig. 2). In replicate A, both values were similar to the low parental line (L), whereas in replicate B, F1 and F1r values fell approximately midway between H and L lines (Fig. 2). Because line effects were not detected for TC2 and PFA, these variables are treated in the next section only.

Temperature Effects

Field temperatures of jackfruit tissues containing developing larvae ranged from 24.8 to 33.0°C, and the grand mean was 27.8 ± 2.1°C (± SD.). Thus, the experimental temperatures employed in the present laboratory study were within the range encountered by wild individuals.

For TC2, the effect of temperature was strong and consistent across the replicates (Table 2, Fig. 3). Overall (i.e., replicates pooled), TC2 decreased by 10% with increasing temperature, or on average by 1.6 teeth per male.

The effect of temperature on TC3 was also strong (Table 1), and the direction of the shift was similar to TC2 (Fig. 3). Overall, TC3 decreased by 22% with increasing temperature.

The effect of temperature on PFA was also significant (Table 3), but in contrast to either size measure, PFA *increased* with temperature. There was a significant cross-by-temperature interaction affecting PFA (Table 3), resulting from a marked magnification of the temperature effect in replicate B (Fig. 3). In replicates A and B, PFA increased by 11 and 38%, respectively. Overall, PFA increased by 23% with increasing temperature.

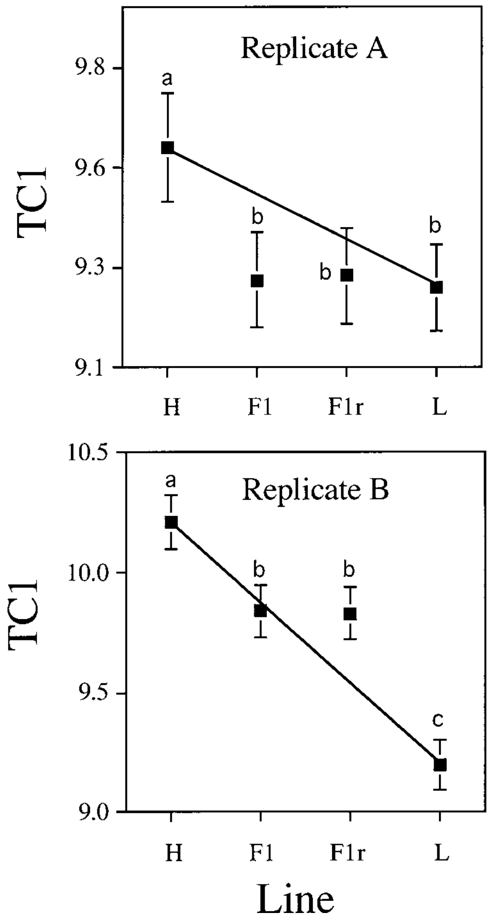

FIG. 2. Mean total tooth number in the first comb segment (TC1) in parental and hybrid lines of reciprocal crosses A and B. Error bars are ± 1 SE, and means sharing a letter are not significantly different by the Tukey-Kramer procedure (∝ = 0.05).

Figure 4 plots PFA against TC2 at both temperatures and demonstrates how a negative size-FA correlation would arise in a population composed of subsets of individuals experiencing different levels of environmental stress. These two traits are represented here because both have been found to be under sexual selection in a natural population (Polak et al. 2004). Although no line is fitted because the data are nonindependent (the same genetic lines are represented at the two temperatures), this figure illustrates the emergence of negative covariation from the pooling of subsets of individuals with different thermodevelopmental histories.

DISCUSSION

The genetic data, indicating significant genotypic variation, corroborate a previous study showing significant isofemale heritability (h^2_{iso}) for size of each of the elements of the sex comb (Polak et al. 2004). The fact that hybrid lines often tended to fall intermediate between parental lines suggests that additive genetic activity underlies expression of the sex comb. However, considerable heterogeneity in the pattern of inheritance was revealed. For example, mean TC1 values in hybrids relative to parental lines differed dramatically between the replicate crosses. In replicate A, both F1

TABLE 2. Results of ANCOVA, with thorax length (tl) as the covariate, showing effects of replicate cross, line, temperature, and vial on TC2 (number of teeth in the second comb segment). All interactions were nonsignificant.

Source	df	MS	*F*	*P*
tl	1	6.62	3.12	ns
Cross	1	2.54	0.66	ns
Line	3	3.04	0.79	ns
Temp	1	511.86	142.36	<0.0001
Vial	16	3.97	1.90	0.02
Error	2050	2.09		

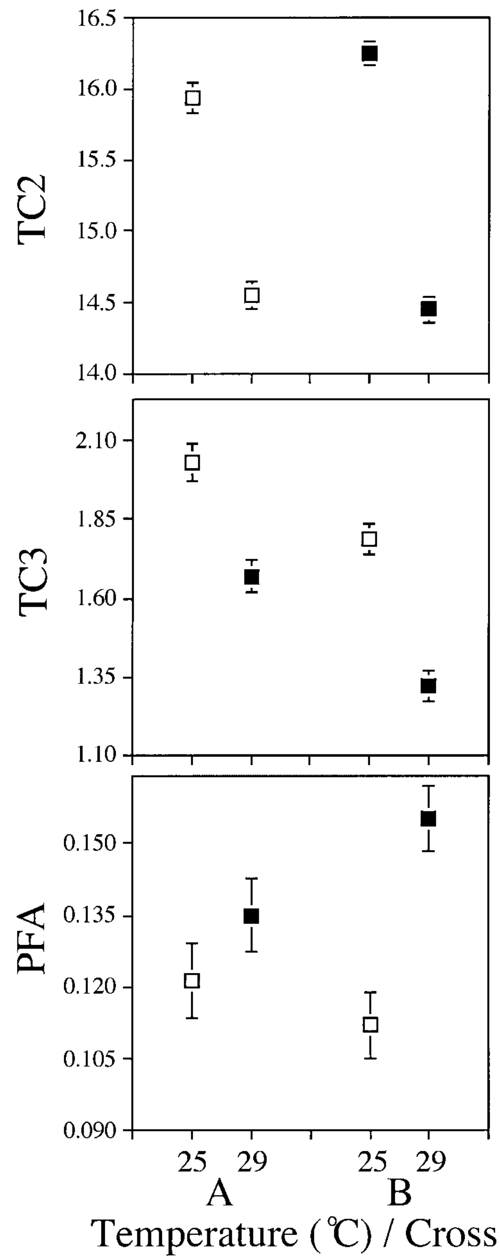

FIG. 3. Effects of temperature treatment on total tooth number in the second (TC2) and third (TC3) comb segments and on positional fluctuating asymmetry (PFA) in replicate crosses A and B. Error bars are ± 1 SE.

TABLE 3. Results of ANCOVA, with trait size (TC1,2) as the covariate, showing effects of replicate cross, line, temperature, and vial on comb PFA (positional fluctuating asymmetry). Nonsignificant interactions are not shown.

Source	df	MS*	*F*	*P*
Trait size	1	2.16	0.18	ns
Cross	1	5.59	0.79	ns
Line	3	3.97	0.56	ns
Temp	1	152.95	19.44	0.0001
Cross × temp	1	48.19	6.80	0.017
Vial	16	6.81	0.56	ns
Error	1050	12.19		

* Mean squares have been multiplied by 10^3.

and F1r means were nearly identical to the L line, suggesting dominance interactions among the relevant alleles. In contrast, hybrid lines in replicate B fell midway between the parental lines, indicating additive genetic activity in this case.

There are several possible explanations for these different patterns. One is that different alleles were present in the parental lines, which is likely because the replicate crosses were based on independent H and L lines; different genes are known to respond differently to changing environments (e.g., Merilä and Fry 1998). Epistatic interactions between the Y chromosome and loci elsewhere in the genome is another possibility, particularly in the case of TC3 for which Y-chromosomal effects were here detected, but which will require verification. For example, Chippindale and Rice (2001) showed that the effects of the Y on fitness in *Drosophila melanogaster* depended strongly on genetic background. Fitness reverted from high to low in different genetic backgrounds, indicating strong epistasis between Y-linked and X- or autosomal-linked genes (and see Carvalho et al. 1997).

We also found that comb size is remarkably temperature-stress labile, decreasing with increasing developmental temperature independently of body size. Specifically, an increase of 4°C sharply decreased peg number in TC2 and TC3 by 10% and 22%, respectively, although curiously, TC1 was insensitive to this temperature treatment. Comb positional fluctuating asymmetry (PFA) also exhibited strong temperature-dependent plasticity, *increasing* overall by a dramatic 23%, and supporting the hypothesis that asymmetry in a sexual ornament can be indicative of reduced developmental stability arising from environmental stress (Parsons 1990, 1995; Møller and Pomiankowski 1993; Hunt and Simmons 1997; Hoffmann and Woods 2003). The experimental temperatures used in the present study are within the range experienced by the flies in nature, so the phenotypic plasticity (Stearns 1989) observed for these features of the sex comb is likely to be manifested in field populations.

The present study therefore demonstrates that a significant portion of the phenotypic variation sustaining sexual selection in natural populations likely originates from variation in the species' thermal environment. Previously we showed that both TC2 and PFA were under sexual selection in a natural Australian population of this species (sexual selection was not detected for TC1 and TC3): mated males had higher TC2 and lower PFA than single individuals sampled from field substrates across 12 reproductive episodes (Polak et al.

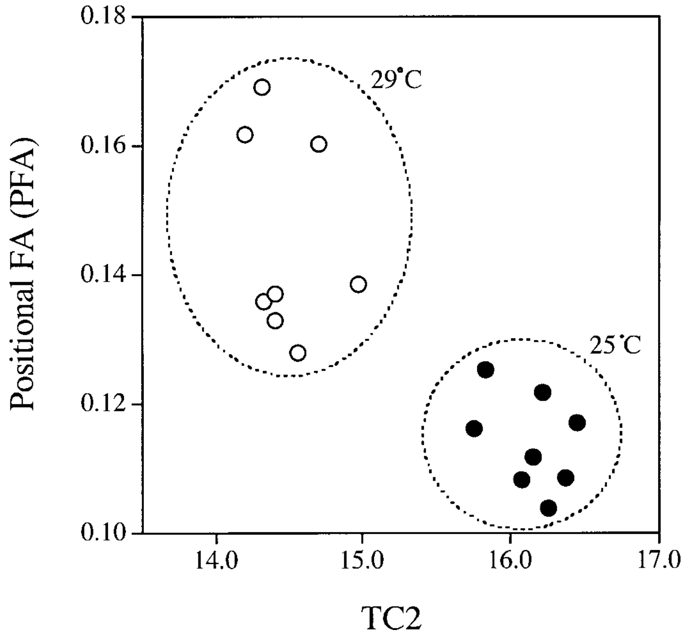

FIG. 4. Mean values of positional fluctuating asymmetry (PFA) and trait size (TC2) in eight genetic lines (four from each replicate A and B) reared at 29°C (empty circles) and 25°C (filled circles). The figure illustrates how negative size-fluctuating asymmetry covariation arises in a population composed of subsets of individuals that experienced different levels of environmental stress during development, as predicted by the environmental heterogeneity (EH) hypothesis.

2004). Although significant narrow-sense heritability was found for TC2 in the laboratory (h^2 of PFA was nonsignificant) (Polak et al. 2004), the expected consequence of the elevated environmental variance present in the field, arising from the effects of temperature and temperature-by-genotype interactions, would be to lower the field heritability and diminish short term response to selection (Houle 1992; Endler 2000; Hoffmann 2000). A consideration of the effects of mean temperature, and regional temperature fluctuations, might warrant incorporation into evolutionary models of comb diversification across Australasian populations and related taxa (Kopp and True 2002; West-Eberhard 2003; Polak et al. 2004).

Because high developmental temperature may have detrimental effects on a variety of fitness traits in *Drosophila*, such as body size and condition (e.g., Oudman et al. 1991; Crill et al. 1996), an enlarged sex comb could therefore signal a superior male quality, and also possibly reflect other features of males such as enhanced social dominance, mating success and ejaculate potency (Hoffmann 1987; Partridge et al. 1987; Pitnick and Garcia-Gonzalez 2002), all of which are known to be correlated positively with environmentally-induced shifts in male body size in flies.

A Critique of the FA-Sexual Selection Hypothesis

According to the FA-sexual selection hypothesis, deviations from perfect bilateral symmetry reveal differences in the health or quality among individuals (Møller and Pomiamkowski 1993; Møller and Swaddle 1997; Tomkins and

Simmons 2003). In fact, it has been suggested that FA ''. . . in secondary sexual traits may honestly signal the ability of individual males to cope with environmental conditions during growth and development of the ornament'' (Møller 1990, p. 1187). A key prediction of this hypothesis is the existence of negative phenotypic correlations between size and FA for secondary sexual traits that are condition-dependent (Ridley 1992; Møller and Pomiankowski 1993; Rowe et al. 1997; Watson and Thornhill 1994; Blanckenhorn et al. 1998; Tomkins and Simmons 2003). Such a pattern is predicted because if trait size is proportional to the costs of trait production, then only high quality individuals should be able to afford to produce sexual traits that are both large *and* symmetrical (Møller 1990; Møller and Pomiankowski 1993; Tomkins and Simmons 2003; Møller and Cuervo 2003). We refer to this hypothesized mechanism for negative size-FA correlations, which posits inter-male differences in genetic quality to be their cause, as the ''quality heterogeneity'' (QH) hypothesis.

Negative relationships between size and FA have often been found for traits subject to mate choice and intrasexual selection (Møller 1990, 1992b, 1993a, 1994; Møller and Höglund 1991), as well as for floral traits involved in pollinator attraction (Møller and Eriksson 1994; Møller 1995; Møller and Shykoff 1999), which have been used to support the QH hypothesis. However, several studies have been unable to uncover the predicted negative size-FA correlations in condition-dependent sexual ornaments (e.g., Møller 1992b; Evans et al. 1995; Hunt and Simmons 1997, 1998; Blanckenhorn et al. 1998), whereas others have suggested that such correlations may arise, not because individuals differ in genetic quality, but because individuals producing the largest traits in the population are constrained for mechanical and/or aerodynamic reasons to produce more symmetrical ornaments (Balmford et al. 1993; Evans 1993; Evans and Hatchwell 1993; cf. Møller and Cuervo 2003).

Furthermore, we previously pointed out that the QH hypothesis implicitly assumes that males within a population experience similar kinds and magnitude of environmental stress during trait development (Polak et al. 2004), an assumption unlikely to be biologically realistic because natural environments are anything but constant for different individuals: Abundant environmental fluctuations occur at both spatial or temporal scales (Davis 1986; Evans 1991; Endler 2000). For this reason, it was suggested that the negative association between size and FA predicted by the QH hypothesis should be restated in terms of a *genetic correlation* (Polak et al. 2004), because the QH hypothesis is predicated on the existence of covariation between size and asymmetry driven by differences in quality among *genotypes* within populations (e.g., Møller 1993b).

Here we propose the ''environmental heterogeneity'' (EH) hypothesis as an alternative mechanism generating negative size-FA correlations in natural populations. This hypothesis states that negative size-FA correlations arise because of environmental heterogeneity: the greater the magnitude of variation in stress experienced by different subsets of individuals, the stronger the negative size-FA correlation is expected to be. According to the EH hypothesis, negative relationships arise because individuals developing in a high-stress environment will, as adults, exhibit decreased size and increased asymmetry of secondary sexual traits, whereas individuals developing in a low-stress environment will, as adults, exhibit larger, and relatively more symmetrical ornaments. Within a given environment, the size-FA relationship may take any form (e.g., flat, negative, U-shaped etc.), but the *overall* population will tend toward exhibiting a negative size-FA correlation, as long as the among group stressor(s) simultaneously decreases size and increases asymmetry of the ornament.

Møller's quality heterogeneity (QH) hypothesis predicts that negative size-FA correlations are more likely to occur when the ornament is very costly to produce and reflects body condition (Møller 1993a). The EH hypothesis makes exactly this same prediction because costly traits are also those whose size and symmetry are expected to be most sensitive to environmental stress (see Introduction). Therefore, the mere existence of negative size-FA correlations in natural populations cannot discriminate these competing models. But because the EH hypothesis may require fewer, more realistic, conditions to work, it may in fact generate negative size-FA correlations in natural populations more frequently. Thus, the apparent support that the QH hypothesis has to date enjoyed may largely be unjustified because many of negative size-FA correlations that have been used to support it are derived from field samples (Møller 1990, 1992b, 1993a, 1994, 1995; Manning and Hartley 1991; Møller and Höglund 1991; Møller and Eriksson 1994).

In a previous study (Polak et al. 2004), a *positive* genetic correlation between sex comb size and FA was found, which is contrary to the QH hypothesis because it predicts a negative sign to this correlation. In this previous study, we raised flies under common environmental conditions, and in replicate bottles so that the environmental component of variation could be minimized, estimated, and partitioned from overall trait variability prior to calculating the genetic coefficient. In contrast, among field-caught males ($n = 863$), the phenotypic correlation was in the opposite direction, being significantly *negative* (Polak et al. 2004). The present study demonstrates that this negative correlation could have been generated by means of heterogeneous exposure to thermal stress: both ornament size and symmetry decreased with increasing temperature within the range experienced by the species in nature. Figure 4 illustrates how these effects would generate a negative size-FA correlation in the overall population; note that the same genetic lines are represented at the two experimental temperatures, meaning that the negative association in this figure could not have arisen by way of the mechanism postulated by the QH hypothesis (i.e., genetic differences in quality). Thus, the results suggest that the genetic correlation detected previously could be entirely masked by phenotypic plasticity across a heterogeneous environment. Our conclusions are that the EH hypothesis is plausible, and that size-FA correlations in nature should be interpreted cautiously.

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