

A primary role of developmental instability in sexual selection

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In evolutionary biology, fluctuating asymmetry (FA) is thought to reveal developmental instability (DI, inability to buffer development against perturbations), but its adaptive and genetic bases are being debated. In other fields, such as human clinical genetics, DI is being assessed as incidence of minor morphological abnormalities (MMAs) and used to predict certain fitness outcomes. Here, for the first time, we combine these complementary measures of DI in sexual selection and quantitative genetic studies of a natural population. Comprehensive multivariate analyses demonstrate that FA and MMAs in a condition-dependent sexual ornament, the male *Drosophila bipectinata* sex comb used in courtship, are sole significant targets of selection favouring their reduced expression in New Caledonia. Comb FA and MMAs are positively correlated, confirming that each are linked to a common buffering system. Ornament size and DI (as FA and MMAs) are positively correlated, genetically and phenotypically, contrary to theoretical expectation of negative size-FA scaling under the assumption that FA reveals overall genetic quality. There exists significant additive genetic variance for MMAs, demonstrating their evolutionary potential. Ornament DI in New Caledonia is markedly elevated compared with populations where such selection was not detected, suggesting that the increased population-level DI is capacitating adaptive evolution.

Keywords: fluctuating asymmetry; phenodeviance; sexual selection; developmental instability; heritability; evolutionary capacitance

1. INTRODUCTION

Secondary sexual traits are widespread among polygynous taxa and are among the most rapidly evolving classes of animal traits (Andersson 1994). Because they tend to be physiologically costly to produce and maintain, the degree of expression (e.g. size) of sexual traits used in courtship and mate competition is prone to reflect an organism's genetic quality (Johnstone 1995). It is thought that subtle departures from perfect bilateral symmetry (fluctuating asymmetry or FA) in sexual traits may be an especially sensitive indicator of genetic quality (Møller & Pomiankowski 1993; Tomkins & Simmons 2003), because FA reveals developmental instability (DI), the compromised ability to buffer development against perturbations (Van Valen 1962).

The role of FA in sexual selection, however, is being debated. Controversy stems in large part from the intuitive appeal of symmetry as a 'health certificate' on one hand (Møller & Swaddle 1997), juxtaposed against inconsistency across studies concerning the fitness consequences and genetic bases of FA on the other hand (Leamy & Klingenberg 2005; Van Dongen 2006). Indeed, the issues of FA/DI heritability, and of the significance of covariance between FA and ornament size, remain far from being adequately resolved (Simmons *et al.* 1995; Fuller & Houle 2003; Polak & Starmer 2005; Van Dongen 2006), despite their continuing prominent positions in the DI–sexual selection hypothesis (Møller & Pomiankowski 1993; Watson & Thornhill 1994; Møller & Cuervo 2003). One robust conclusion that has emerged, however, is that FA is

a reliable predictor of mating success and sexual attractiveness in humans, as well as in some insects, fishes and birds (Tomkins & Simmons 2003; Brown *et al.* 2005; Gross *et al.* 2007; Rhodes & Simmons 2007). Yet, the biological reasons for why FA predicts sexual success in some species, but not in others, remain virtually unknown (Møller & Thornhill 1998; Møller & Cuervo 2003; Swaddle 1999).

One general explanation may be that most species and populations simply do not harbour sufficient DI variation to sustain FA-mediated sexual selection. After all, populations generally are under canalizing selection to maintain robust buffering systems (Gibson & Wagner 2000). Exacerbating this problem is that FA is only weakly correlated with underlying DI (Houle 2000), eroding the effectiveness of FA to reveal DI differences among individuals.

A relationship between FA and mating success is therefore most apt to exist in populations with DI-enriched FA in particular traits, or more generally throughout the genome, owing to environmental and/or genetic destabilizing factors. Some secondary sexual traits may fall into such a category owing to their condition dependence, and the 'destabilization' of development they may be experiencing owing to the evolutionary loss of effective canalizing modifiers (Wagner *et al.* 1997) and/or allelic substitution at structural loci (Clarke & McKenzie 1987) resulting from a recent history of sustained directional selection (Møller & Pomiankowski 1993; Watson & Thornhill 1994).

Morphological phenodeviants, defined as minor morphological abnormalities (MMAs) occurring at low frequency in the population, are also an expression of DI

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(Graham *et al.* 1993; Hoyme 1993; Jones 2006). This is because, like asymmetry, an MMA is thought to be a product of random accidents of development within the developmental trajectory of a particular trait. Whereas a link between MMAs and sexual selection has been predicted (Møller & Thornhill 1998), no such relationship has yet been demonstrated. In one study of barn swallows (*Hirundo rustica*) at Chernobyl, Ukraine, Møller (1993) found that males with aberrant morphology ('brushy and transparent' outermost tail feathers) reproduced later in the breeding season, suggesting female avoidance of males with aberrant tails. This aberrant phenotype, however, appears to be caused by sex-limited mutation(s) resulting from radiation exposure and not DI. The fact that a molecular mechanism (the Hsp90 buffering system) linking MMAs to DI is now known (Rutherford & Lindquist 1998; Queitsch *et al.* 2002) underscores the exciting potential of using MMAs to provide a much-needed confirmatory test of the hypothesized link between DI and sexual selection.

Here, we test for the importance of DI in sexual selection by evaluating the effects of FA, phenodeviance and their interaction on mating success in a natural New Caledonia population of *Drosophila bipectinata* Duda. This species, distributed in the Australian and Oriental biogeographic zones (Bock 1978), is undergoing inter-population diversification in a secondary sexual trait, the male sex comb (Polak *et al.* 2004). The comb on either leg comes into contact with the female during late stages of courtship (mounting from behind), as the male's foretarsi and combs are pressed against the sides of the female's abdomen (Cooperman *et al.* 2007). It is at this stage that the female may be receiving tactile cues concerning features of the sex comb. In failed copulation attempts, the male dismounts apparently owing to his inability to overcome female resistance to mate. We focused on this island population because trait size and levels of DI are high relative to another population thus far surveyed in Australia (Cape Tribulation, Queensland; Polak *et al.* 2004), a condition of destabilized development expected *a priori* to engender a link between DI and sexual selection (see above).

We also performed a quantitative genetic analysis of this same New Caledonia population, permitting a test of key predictions of the DI–sexual selection hypothesis, namely that FA and phenodeviance are heritable, and that these measures of DI are negatively genetically correlated with ornament size (Møller & Pomiankowski 1993; Polak & Starmer 2005). The results challenge predictions concerning significant FA heritability and refute existing expectations of negative scaling between ornament size and DI.

2. MATERIAL AND METHODS

(a) Field study of sexual selection

A field study of sexual selection was conducted on 8–12 January 2006, in Noumea, New Caledonia following the procedures of Polak *et al.* (2004). On each morning for 5 consecutive days, between 04.55 and 05.45 hours, copulating and single male *D. bipectinata* were sampled from the surface of fallen mangoes under parent trees approximately 80 m from La Baie de Anse Vata. Fruits were continuously monitored for copulating pairs. When a pair was spotted, one to five single

flies in the immediate vicinity (within 5 cm) of the pair were aspirated into a 35 ml vial. The pair was then immediately aspirated into a separate vial. Within 2 hours of collection, all male flies were preserved in 95% ethanol. Females were placed in culture vials. All male flies were characterized within 14 days of collection by one researcher (M.P.). Flies were individually extracted from the alcohol, hydrated by immersion into water for a minimum of 1 min and placed on a microscope slide. Thorax length was measured using an ocular micrometer, and sex comb teeth were counted under an SZX12 Olympus stereomicroscope. The teeth of the sex comb occur in three distinct segments (1, 2 and 3, with 1 being the most proximal) on the first and second tarsal sections of the front legs of males. C1, C2 and C3 refer to the number of teeth (trait size) in segments 1, 2 and 3, respectively. Likewise, FA1, FA2 and FA3 refer to FA (unsigned, right-minus-left values) in these segments. Measurement error in tooth counts is negligible; no counting errors were made across 25 independent replicate counts of teeth in all segments (Polak *et al.* 2004). A normal comb is defined as one in which the teeth in each segment line up in a straight row (figure 1a). A comb segment was phenodeviant if it possessed a minor abnormality, of which three kinds were observed. In 82% of MMAs, a tooth was misplaced such that it was positioned in front of a segment (figure 1b,c). In 11% of MMAs, the tooth was positioned behind the row (figure 1c, white arrow). Finally, 7% of MMAs were breaks in the segment, defined by a gap between any two teeth of a size greater than or equal to the basal diameter of an individual tooth. MMA1 and MMA2 refer to an abnormality in segments 1 and 2, respectively.

(b) Trait diagnostics and population contrasts

For traits exhibiting FA, the sample distribution of signed right-minus-left (R–L) should be approximately normal or leptokurtotic (depending on the magnitude of inter-individual DI variation in the population, Van Dongen 2006) and the mean should equal 0 (Palmer & Strobeck 1986). Mean, skewness and kurtosis (Sokal & Rohlf 1995) for signed right-minus-left values were calculated for all comb segments in both field and laboratory samples of males. To check for size dependence, absolute (i.e. unsigned) asymmetry values were regressed on trait size. Unless otherwise stated, FA refers to absolute R–L values.

FAs in New Caledonia and Cape Tribulation were contrasted using ANCOVA, with thorax length entered as a covariate to control for ornament scaling with body size; the homogeneity of slopes assumption was met for FA in both segments 1 and 2 ($p=0.96$ and 0.70 , respectively). Incidence of MMAs in New Caledonia and Cape Tribulation populations (presence/absence) was modelled using logistic regression (PROC LOGISTIC, SAS Institute 2001), again with thorax length entered as a covariate. Regression coefficients (α) and standard errors (s.e.) are provided.

(c) Estimation of the strength of sexual selection

Logistic regression was used to quantify the strength of precopula sexual selection simultaneously on multiple phenotypic traits of males in the New Caledonia population. An initial full model was constructed containing 10 traits: tooth number in each of the three comb segments, asymmetry in each segment, comb positional fluctuating asymmetry (PFA, $|(rightC1(rightC2) - (leftC1(leftC2)))|$), presence/absence of MMA in segment 1 (MMA1) and 2 (MMA2) and thorax length as an estimate of body size. The interaction terms

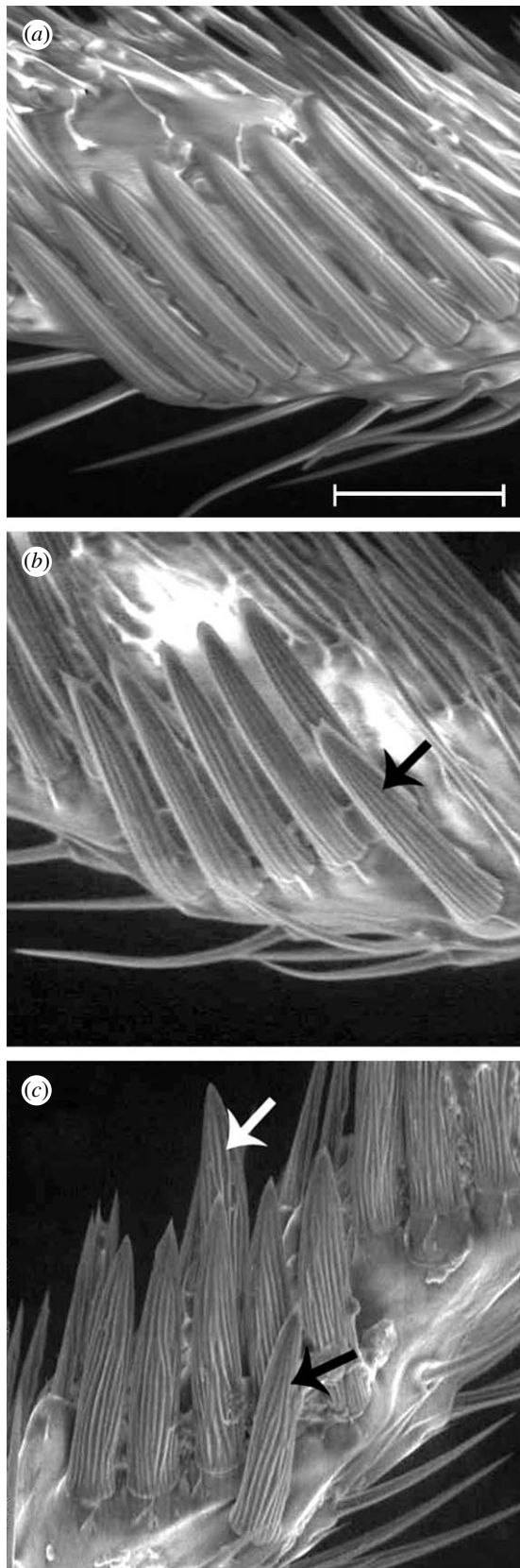


Figure 1. Scanning electron micrograph (1500 \times) of (a) normal and phenodeviant sex combs of *Drosophila bipectinata*. Misplaced teeth (arrows) growing from a position either (b,c) anterior or (c) posterior to the longitudinal axis of C1. Whereas (a) and (b) are flies from Noumea, New Caledonia, (c) is a fly from a mutant laboratory strain originally from Taiwan (kindly provided to us by A. Kopp) illustrating the deviant positions of teeth encountered in Noumea. The scale bar represents 20 μ m and applies to all panels.

FA1 \times MMA1s and FA2 \times MMA2s were of interest to explore how complementary measures of DI might interact, and so were included. A backward elimination procedure removed variables from the model in sequential order not meeting a retention criterion of 0.05 (SAS Institute 2001; the same variables were retained with 0.01 or 0.1 criteria).

Estimated selection gradients (β_s), which have the same interpretation and utility for predicting microevolutionary change as multiple linear regression estimates (Lande & Arnold 1983), and their standard errors were calculated by transforming the logistic regression coefficients (Janzen & Stern 1998). Briefly, the method requires calculating $W(z)$ (the expected fitness) for each individual characterized by a set of traits (z,s) using the estimated logistic regression coefficients. The average of $W(z)[1 - W(z)]$ was then calculated and multiplied with each multiple regression coefficient to obtain the selection gradient. Selection gradients were calculated for each sampling date for FA1 and MMA1s and displayed graphically to visualize selection pressure separately across the five episodes of sexual selection. FA1 was further analysed using ANCOVA, with trait size (C1) as the covariate, and day (1–5) and copulation status (copulating versus single) as factors. A separate analysis evaluated the significance of the interaction between factors. Slopes of functions relating FA1 and C1 were statistically homogeneous across days and copulation status.

(d) Estimation of genetic parameters

A standard half-sib design (Falconer & Mackay 1996) was used to estimate genetic parameters. The base population was derived from females that had been collected in copula during the field component of the study, described above, plus an additional 100 males from a general collection acquired by sweeping an insect net over mangoes up to 30 m from the site of the study after sampling mated and single on the last day of the study. Flies were cultured en masse for two generations at $24 \pm 1^\circ\text{C}$, 60–70% RH and a 12 hours light : 12 hours dark photoperiod prior to commencing the quantitative genetics experiment at Macquarie University, Sydney, Australia. Virgin flies were harvested within 6 hours of emergence and held in vials containing banana-agar medium and active yeast. Eighty-eight sexually mature virgin dams were then individually placed into food vials with one sire. Each sire was transferred sequentially to two other vials each containing one virgin female. Thus, the half-sib families were created by mating each sire to three different, randomly selected dams.

Females were allowed to oviposit for 2 days into a 35 ml food vial and were then transferred to a fresh vial for another 2 days to ensure that adequate numbers of progeny were produced by each dam. The larval food substrate consisted of 1.7 g instant *Drosophila* medium (Carolina Biological, North Carolina, USA), 8 ml H₂O and 1 ml of crushed banana-yeast slurry. All progeny were harvested from vials and preserved in ethanol in a labelled vial. A total of five male progeny were randomly selected from each vial, extracted from the alcohol and characterized as above.

Genetic parameters for FA1 were calculated both with and without correcting for trait size. To correct for trait size, FA1 was regressed on C1 and the residuals were $(y_i + 2.1)^{0.35}$ transformed (selected from among a series of tried transformations) to help achieve normality; FA uncorrected for size was $(y_i + 5 \times 10^{-5})^{0.33}$ transformed. MMA1s were corrected for size also by regression, but in this case the residuals were $\log_{10}(y_i + 1)$ -transformed. Uncorrected MMA1 values were

Table 1. Logistic regression coefficients and selection gradients estimating the strength of sexual selection on phenotypic traits of *D. bipunctata* in Noumea.

variable	α^a	s.e.	p	β^b	s.e.
FA1	-0.832	0.236	0.0004	-0.337	0.0955
MMA1	-1.449	0.548	0.008	-0.317	0.120
MMA1 \times FA1	1.152	0.411	0.005	0.377	0.135

^a Multiple logistic regression coefficients.

^b Selection gradients obtained by transforming regression coefficients.

retained on a binary (1, 0) scale. Trait size (C1) was corrected for thorax length by regression, and the raw, untransformed residuals were used to estimate genetic parameters.

Observational variance components were estimated with the restricted maximum likelihood (REML) method using PROC MIXED in SAS (SAS Institute 2001). Causal components, namely the additive genetic variance (V_A) and the total phenotypic variance (V_P), were calculated from these observational components (Falconer & Mackay 1996). The standard error (s.e.) of V_A was calculated as four times the standard error of the sire variance (σ_S^2), whereas the standard error of V_P as the square root of the sum of all the elements of the asymptotic covariance matrix (Fry 2004). Tests of significance evaluating $H_0: V_A > 0$ were done with the likelihood ratio test (one-tailed p -values). The heritability was calculated as $h^2 = V_A/V_P$ (Falconer & Mackay 1996). The standard error of h^2 was calculated by the delta method for variance estimation (Hogg & Craig 1995).

Incidence of phenodeviance was also modelled as a threshold trait (Swain 1987; Falconer & Mackay 1996). The threshold model assumes that binomial variation is determined by an underlying, continuously distributed variable called the 'liability', influenced by polygenic and environmental factors. The heritability computed on a binary (1, 0) scale was converted to heritability on the liability scale (h_l^2) (Roff 1997, pp. 52–55).

The additive genetic covariance (COV_A) was calculated as four times the observational among-sire covariance between two traits; the standard error of COV_A was taken as four times the standard error of the observational covariance. A likelihood ratio test evaluated $H_0: COV_A = 0$ with a two-tailed p -value because covariances in the theory can be less than or greater than 0. The additive genetic correlation was calculated as $r_A = \sigma_{S1,2}/(\sigma_{S,1}^2\sigma_{S,2}^2)^{1/2}$, where $\sigma_{S1,2}$ is the sire covariance between two traits, and $\sigma_{S,1}^2$ and $\sigma_{S,2}^2$ are the additive genetic variances for traits 1 and 2, respectively (Fry 2004).

3. RESULTS

(a) Trait diagnostics

For both field and laboratory samples, mean *signed* FA values for all comb segments were not significantly different from 0, and no significant skewness was detected in any *signed* FA distribution. Signed FA1 and FA3 in the laboratory population ($n=1200$) exhibited significant leptokurtosis ($g_2=0.339$ and 1.232 , $p<0.05$ and 0.001 , respectively) consistent with the presence of DI heterogeneity in the population (Van Dongen 2006).

(b) Population contrasts

FA and abnormalities present in C1 and C2 were contrasted between the Noumea population and that of a recently studied population at Cape Tribulation,

Australia (Polak *et al.* 2004), where significant pre-copula sexual selection was *not* detected for either FA or MMAs in any comb segment. Least-squares (LS) mean ($\bar{x} \pm$ s.e.) FA1 was significantly higher in Noumea (0.869 ± 0.047 , $n=230$) than at Cape Tribulation (0.746 ± 0.024 , $n=863$; $F_{1,1089}=5.52$, $p=0.019$). The variances of *signed* asymmetry values for this trait were 1.348 and 1.032 for Noumea and Cape Tribulation, respectively. There was a 173% greater incidence (I) of MMA1s in Noumea ($I=0.222$) than at Cape Tribulation ($I=0.0811$; α (s.e.) = 0.591 (0.10), $\chi^2=33.94$, $p<0.0001$).

Thus, the level of DI in C1 was considerably elevated in Noumea compared with the Cape Tribulation population. These Noumea field values did not differ significantly from their corresponding laboratory values (FA1, $F_{1,1427}=0.649$, $p=0.42$; MMA1s, α (s.e.) = 0.138 (0.113), $\chi^2=1.49$, $p=0.22$).

For C2, in turn, incidence of MMA2s was also significantly higher in Noumea ($I=0.0522$) than at Cape Tribulation ($I=0.0197$; α (s.e.) = 0.503 (0.19), $\chi^2=6.84$, $p=0.0089$). However, FA2 did not differ significantly between these populations (LS mean in Noumea: 0.732 ± 0.0464 ; Cape Tribulation: 0.735 ± 0.0240 ; $F_{1,1089}=0.003$, $p=0.96$). The variances of *signed* FA2 values for Noumea and Cape Tribulation were 1.117 and 1.016, respectively.

Thus, this sexual ornament, whose C1 and C2 are 23.4 and 10.6% larger in Noumea than at Cape Tribulation (Polak *et al.* 2004), respectively, also exhibits elevated DI in Noumea. This elevated DI is more pronounced for C1, paralleling the pattern of size divergence between the segments.

(c) Sexual selection

The full logistic regression model, describing the effects of the 10 phenotypic traits of males (plus interactions) on the probability of copulation, revealed significant effects of FA1 (α , s.e.: -0.845, 0.291, $p=0.0037$), MMA1s (-1.338, 0.589, $p=0.023$) and their interaction (1.095, 0.427, $p=0.010$). Corresponding selection gradients, β (s.e.), were -0.336 (0.116), -0.288 (0.127) and 0.352 (0.138), respectively. None of the other potential predictors of mating success were significant (all p values ≥ 0.24). The selection gradients for FA1 and MMA1s were both negative, indicating that asymmetry and phenodeviance are each independently under sexual selection for reduced expression. We emphasize that these effects are significant despite accounting for other potential predictors of reproductive fitness.

Reducing this full model to three terms using backward elimination strengthened the effects of FA1 and MMA1s (table 1). Figure 2 shows magnitudes of directional sexual selection across sequential sampling days. For FA1, the sign of the selection gradients was negative on 4 of these 5 days. Interestingly, the deviant selection gradient (day 2)

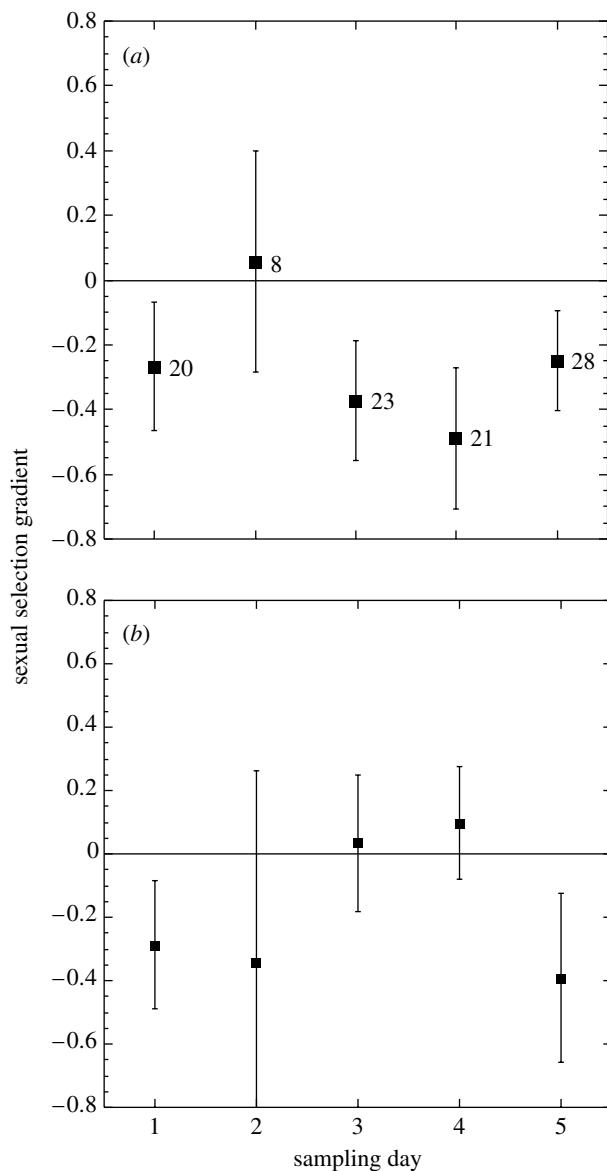


Figure 2. Sexual selection gradients ($\beta \pm \text{s.e.}$) for reducing (a) FA and (b) MMA in C1 across five episodes of selection in the field. Numerals are numbers of copulation males, which apply to both panels.

is associated with the least degree of confidence, as it is based on less than half of the regular sample of copulating males (figure 1a); a tropical weather front moved through the study area at the time of sampling, dramatically curtailing mating activity. The significant FA1 \times MMA1 interaction (table 1) may be understood as follows: when the data are split by phenodeviant category, among non-phenodeviant males the negative effect of FA1 on copulation is strongly significant (α , s.e.: $-0.832, 0.236$, $p < 0.0005$). By contrast, among phenodeviant males, this effect is absent ($0.320, 0.34$, $p > 0.3$).

ANCOVA revealed that single males had 39% greater mean FA1 compared with mated males (least squares \pm s.e. for copulating males, 0.713 ± 0.0786 , $n = 100$; and for singletons, 0.995 ± 0.0692 , $n = 130$; $F_{1,223} = 7.30$, $p = 0.0074$) confirming the results of the above multivariate evaluation. There were no significant effects of day ($F_{4,223} = 0.21$, $p = 0.93$), interaction between day and copulation status ($F_{4,219} = 0.58$, $p = 0.68$) or trait size ($F_{1,223} = 0.69$, $p = 0.41$).

Males that were phenodeviant for C1 had a 40% greater mean FA1 (\pm s.e. = 1.12 ± 0.107) than non-phenodeviant males (0.799 ± 0.0569 ; ANOVA: $F_{1,228} = 6.96$, $p = 0.009$). Neither C1 nor thorax length were significantly associated with FA1 when entered as covariates in this analysis (p values = 0.74 and 0.45, respectively).

(d) Estimation of genetic parameters

Genetic parameters were estimated for traits identified as significant targets of selection to further appraise their evolutionary potential. The additive genetic variance (V_A) calculated from REML observational components was not significant for either FA1 or size-corrected FA1 (FA1c; table 2). The heritability (h^2) estimate for FA1 was 4.4% and approximately seven times less than this value for FA1c. Thus, additive genetic effects contribute little to FA.

For MMA1s, V_A was significant (table 2). Heritability estimates were considerably larger for MMA1s than for FA1 (table 2). The heritability of MMA1s on a liability scale, h_1^2 (s.e.), was 0.104 (0.0556).

Estimates of V_A for both uncorrected and body size-corrected ornament size were similar and strongly significant; heritability estimates fell between 45 and 50% (table 2).

There was a significant positive genetic correlation (r_A) between ornament size and FA (table 3). Thus, genotypes producing a larger ornament produce one that is also more asymmetric. Both the laboratory and field-based phenotypic correlations, which were not significantly different from each other ($Z = 0.304$, $p > 0.5$), were also positive, although only the laboratory-based correlation differed significantly from zero (table 3).

MMA1s were not significantly genetically correlated with either trait size (C1) or FA1 (table 3), but corresponding phenotypic correlations were strongly positive (table 3). Laboratory and field phenotypic correlations did not differ significantly from each other for either pair of traits ($Z = 0.349$ and 0.072 , respectively, both p values > 0.5).

4. DISCUSSION

Our study is the first to demonstrate a role of ornament MMA1s in sexual selection. Remarkably, FA and MMA1s were the sole identified targets of sexual selection: despite accounting for eight other potential predictors of reproductive fitness, FA and MMA1s were the only significant predictors of mating probability; even ornament size was statistically unlinked to sexual selection, despite its significant heritability (table 2) and condition dependence (Polak & Starmer 2005). These results demonstrate that DI is the primary target of pre-copulatory sexual selection in the studied population. The sign and magnitude of the selection gradients reveal that sexual selection is directional for reducing both DI traits, but with the force of selection being notably stronger, and more consistent, for FA across the sequential episodes of selection we quantified.

FA and MMA1s are each recognized to reveal DI (Palmer & Strobeck 1986; Graham *et al.* 1993; Sangster *et al.* 2004) but their use has been the enterprise of traditionally separate fields, undoubtedly impeding their unification in evolutionary studies (Hoyme 1993; Møller & Swaddle 1997; Jones 2006). Our finding that FA and MMA1s are positively correlated phenotypically, and

Table 2. REML estimates of genetic parameters (V_A , V_P and h^2) for ornamental traits of males.

trait	V_A (s.e.)	p^a	V_P (s.e.)	h^2 (s.e.)
FA1	0.0117 (0.0158)	0.22	0.264 (0.0108)	0.0445 (0.0600)
FA1c ^b	0.000192 (0.00163)	0.45	0.0296 (0.00121)	0.00650 (0.0550)
MMA1	0.0310 (0.0169)	0.020	0.207 (0.00919)	0.149 (0.0802)
MMA1c	0.00386 (0.00254)	0.050	0.0304 (0.00128)	0.127 (0.0856)
C1	0.245 (0.0710)	<0.00001	0.544 (0.0253)	0.450 (0.121)
C1c	0.259 (0.0714)	<0.00001	0.533 (0.0250)	0.486 (0.123)

^a One-tailed p -value calculated by the likelihood ratio test, evaluating $H_0: V_A > 0$.

^b The 'c' for this trait and MMA1 indicates that they are statistically corrected for trait size or body size in the case of C1.

Table 3. The additive genetic covariance (COV_A) and additive genetic (r_A) and phenotypic (r_P) correlations for each of three trait combinations. (* $p < 0.01$; ** $p < 0.0001$ (all p values two-tailed).)

trait pair	COV_A (s.e)	r_A	p^a	r_P	
				laboratory ($n = 1200$)	field ($n = 230$)
FA1 versus size	0.0470 (0.0244)	0.876	0.040	0.0810*	0.0591
MMA1 versus size	0.0440 (0.0261)	0.504	0.069	0.371**	0.349**
FA1 versus MMA1	0.00778 (0.0117)	0.408	0.53	0.177**	0.172*

^a Two-tailed p calculated by the likelihood ratio test, evaluating $H_0: r_A = 0$.

that each is correlated with reproductive fitness, confirms that variation in these traits indeed reveals, at least in part, common buffering processes (Klingenberg 2003). This underscores the potential utility of their joint deployment to enhance the sensitivity of tests of DI–fitness relations. By contrast, the non-significant genetic correlation between FA and MMAs suggests that the genetic inputs to these phenomena are different (and see Milton *et al.* 2003).

The curious FA \times MMA interaction may be understood as reflecting heterogeneity in the effect of FA between phenodeviance categories: among males with structurally intact combs, FA effects on mating success were strongly negative, but among males with phenodeviant combs this effect was not significant. This result suggests that the advantages of symmetry are most strongly realized when the comb is also structurally intact, perhaps because there are multiplicative costs associated with attaining symmetry and structural integrity simultaneously.

One key requirement for FA itself to reveal genetic quality to potential mates or rivals is that FA is heritable. However, a recent review yielded a non-significant mean FA heritability of 0.026 across 21 studies (Fuller & Houle 2003). Our estimate of 0.044 is comparable to this average value, and also it is not significant. However, the additive genetic correlation (r_A) between FA and comb size was large (0.88) and significant, indicating that some additive genetic variance exists for FA (Falconer & Mackay 1996), and by extension for underlying DI. But because estimates of h_{FA}^2 chronically underestimate h_{DI}^2 (Van Dongen 2007), we can infer that ornament h_{DI}^2 in the present system is likely to be considerably larger than 0.044. However, even if we were to convert our h_{FA}^2 estimate to h_{DI}^2 by conventional methods (Whitlock 1996; Fuller & Houle 2003), this value would be imprecise. The reason is that even in the face of high genetic variation in DI, sample sizes much greater than we used here (e.g. greater than 5000) are required to gain accurate and meaningful estimates of h_{DI}^2 (Van Dongen 2007). Despite the absence of an accurate estimate of the actual magnitude of h_{DI}^2 , the results nevertheless appear to indicate that DI

possesses evolutionary potential, and that females should be receiving indirect benefits in the form of superior (trait-specific) buffering capacity from the differential mating we documented. But what remains perhaps most remarkable is the indication of *strong and repeatable directional selection for reducing FA* despite its very weak heritability. These results are intriguing because they demonstrate strong linkage between largely non-heritable developmental variation and adaptive processes in a natural population. Whether female choice or male–male competition is responsible for this selection is remains unknown.

The 'good genes' hypothesis of sexual selection for reducing DI also explicitly predicts a *negative* phenotypic correlation between FA and trait size (Møller & Pomiankowski 1993). But because such correlations can be driven purely by environmental factors (Simmons *et al.* 1995; Polak & Starmer 2005), this prediction has been recast in terms of a genetic correlation (Polak & Starmer 2005). Thus, the *positive* genetic correlation between FA and trait size, and despite trait expression being condition-dependent (Polak & Starmer 2005), clearly contradicts the DI–sexual selection hypothesis. The evidence suggests that FA and phenodeviance in the sex comb of *D. bipunctata* reflect compromised *trait-specific* developmental buffering (Polak & Stillabower 2004) rather than overall genetic quality (Møller & Pomiankowski 1993).

The sex comb in New Caledonia is significantly more developmentally unstable than in other populations surveyed so far across the species range, *viz.* in north-eastern Australia (Polak *et al.* 2004) and Taiwan (M. Polak & Y. Hsu 2006, unpublished data). Pre-copula sexual selection for reducing either FA or MMAs in these two populations was *not* detected, despite sample sizes greater than two times the present study. Thus, this emerging pattern suggests the intriguing possibility that elevated DI in the New Caledonia population is fuelling the adaptive processes we detected.

What might be the cause(s) of elevated DI in New Caledonia? Whereas environmental stress is a possibility

(Polak & Starmer 2005), the present evidence points to genetic factors for the main reason that FA and MMA levels were similar in the field and laboratory. One possible genetic factor is elevated homozygosity owing to inbreeding. An alternative possibility is the recent evolution of genes at structural loci of the ornament. We favour this evolutionary model, in the light of the positive genetic correlation we uncovered between trait size and FA. Furthermore, comb size in Noumea is significantly greater than at Cape Tribulation (Polak *et al.* 2004), indeed indicating the probable recent evolution of alleles conferring large ornament size in New Caledonia, which may be destabilizing trait-specific networks (Clarke & McKenzie 1987; Sangster *et al.* 2004). This model assumes that New Caledonian *D. bipectinata* is derived from mainland Australia (or Southeast Asia), which is reasonable (Kopp & Barmina 2005; Schug *et al.* 2007).

In a provocative paper, Rutherford & Lindquist (1998) suggested that elevating DI can have profound evolutionary consequences. Under conditions of destabilized development, otherwise cryptic genetic variation is thought to become expressed and exposed to selection, in turn fuelling morphological evolution (Sangster *et al.* 2004). In support of this hypothesis, Rutherford & Lindquist (1998) have shown that when *Drosophila* Hsp90, a molecular chaperone conferring buffering capacity, is impaired, many selectable MMAs in adult flies and other species (Queitsch *et al.* 2002) can be induced in the laboratory, depending on genetic background. No study, however, has shown that such ontogenetic variants reflecting DI can be selected in nature. Our study appears to deliver this missing component of the DI-as-capacitor-of-evolution hypothesis.

The research was supported by the NSF (grant DEB-034990) and the Department of Biological Sciences, University of Cincinnati, to M.P. and Research Development funds from the Centre for the Integrative Study of Animal Behaviour, Macquarie University, to P.W.T. We thank J. Deddens, J. Fry, J. Kotiaho and S. McEvey for their statistical advice and stimulating discussion and N. Kaval for help with SEMs.

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