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Evaluating the Developmental Instability–Sexual Selection Hypothesis in the Fruit Fly, Drosophila bipectinata (Diptera: Drosophilidae)

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Evaluating the Developmental Instability–Sexual Selection Hypothesis in the Fruit Fly, *Drosophila bipectinata* (Diptera: Drosophilidae)

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Abstract

Because developmental instability is the outcome of an individual’s inability to buffer against environmental perturbations, the phenotypic outcomes of DI, fluctuating asymmetry and phenodeviance, are thought to be indicators of individual genetic quality. The developmental instability–sexual selection hypothesis posits that sexual selection favors reduced DI in secondary sexual traits because reduced DI reveals the genetic quality of potential mates or sexual rivals. The present study evaluates this hypothesis by examining the genotypic and phenotypic effects of developmental instability on fitness using isofemale lines of the fruit fly *Drosophila bipectinata* exhibiting divergent levels of phenodeviance. I found significant genetic variation for phenodeviance among lines, and confirmed the stability of these differences across multiple generations. These results support the hypothesis that genotypic effects underly developmental instability in the studied population. I then tested flies from these genetic lines for several measures of fitness: preadult survivorship, male mating success, and egg hatch rate. Genetic lines differed in levels of preadult survivorship, but these differences were unrelated to level of developmental instability in each line. Initial mating success assays showed no effect of DI on mating success, but later field-mimic assays showed significant effects of both genotypic and phenotypic developmental instability on mating success. Mating latency was significantly higher for high-developmental instability males in both assays. Finally, I found no significant effects of phenotypic or genotypic paternal DI on egg hatch rate. Although effects of DI were heterogeneous in this study, these results generally fail to support the DI-sexual selection hypothesis as originally formulated. Furthermore, several predictions of the hypothesis have been weakly supported across other studies. Therefore, the DI-sexual selection hypothesis is in difficulty and may not be a sound explanation for the negative relationship between DI and sexual fitness noted in this and other species.
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List of Abbreviations

C1  First Segment of Sex Comb
C2  Second Segment of Sex Comb
DI  Developmental Instability
FA  Fluctuating Asymmetry
FA1 Fluctuating Asymmetry in Comb Segment C1 (Absolute Value)
FA2 Fluctuating Asymmetry in Comb Segment C2 (Absolute Value)
MMA Minor Morphological Abnormality (or phenodeviance)
MMA1 Minor Morphological Abnormality (or phenodeviance) in Comb Segment C1
MMA2 Minor Morphological Abnormality (or phenodeviance) in Comb Segment C2
1 Introduction

In organisms with bilateral symmetry, separate body sides of an individual typically share the same genome and should develop identically (Waddington 1957). Therefore, any deviation from perfect symmetry may indicate a developmental error or accident, and may be an indicator of the sensitivity of a developmental program to environmental or genetic stress (Waddington 1957; Möller and Swaddle 1997). Developmental instability (DI) is the compromised ability of an organism to buffer against random errors during the course of development (Van Valen 1962). Deviation from perfect bilateral symmetry, or fluctuating asymmetry (FA), has been considered an attractive measure of the amount of developmental instability experienced by an individual (Ludwig 1932, Waddington 1957).

Although FA is ubiquitous as a measure of DI (Palmer and Strobeck 1986), there is another, less-studied measure of DI. Phenodeviance is the incidence of minor morphological abnormalities (MMAs) occurring at low frequency (less than 4 percent) in the population (Jones 2006). Phenodeviance is a suggested measure of DI because it is thought to be the result of random developmental accidents in the development of a trait (Graham et al. 1993, Hoyme 1993, Polak and Taylor 2007).

The hypothesized causes of elevated DI are various, including both environmental factors, such as nutritional stress, temperature stress, or pesticides, and genetic factors, such as inbreeding, hybridization, and mutation (reviewed in Palmer and Strobeck 1986, Möller and Swaddle 1997). One prevailing hypothesis for the cause of elevated DI at the genetic level is the genomic coadaptation theory (reviewed in Clarke 1993). Genomic coadaptation is the outcome of the selective process that increases the concentration of “harmoniously cooperating genes” in a population’s gene pool (Dobzhansky 1951). Coadapted gene complexes consist of multiple well-integrated genes that tend to be selected in combination, such that individuals with disrupted gene complexes may be of lower relative fitness and at a selective disadvantage (Mather 1973, Clarke 1993). In other words, combinations of genes that function well together should be favored by selection (Dobzhansky 1951). Under this
theory, DI is suspected to result from disruption of such coadapted gene complexes, either by hybridization, inbreeding, or through changes in an organism’s genome, for example by mutation or gene flow (Dobzhansky 1951, Clarke 1993). Any genomic change that alters these gene complexes may disrupt complex developmental pathways and therefore perturb development, leading to FA or phenodeviance in the organism (Dobzhansky 1951, Batterham et al. 1996).

1.1 Developmental instability and fitness

The ability to maintain precise development and produce a predetermined optimum phenotype is expected to place an individual at a selective advantage (Waddington 1957, Clarke 1993). Therefore, developmental stability, the processes that reduce phenotypic variation in the face of developmental accidents, may be seen as a component of individual fitness (Zhakarov 1989, Clarke 1993). Any genotypic disruption causing imprecise development may be expected to reduce the fitness of the individual (Clarke 1993). Likewise, organisms that are best able to develop their “target phenotype” with minimum error in the face of environmental or genetic stress should be the most fit (Clarke 1993, Nijhout and Davidowitz 2003).

Perhaps one of the best understood examples of the mechanisms underlying a link between genes, DI, and reduced fitness comes from the evolution of pesticide resistance in the Australian sheep blowfly, *Lucilia cuprina*. Between 1958 and 1980, the most widely used pesticide against *L. cuprina* was the organophosphorous pesticide diazinon (Batterham et al. 1996). In response to the use of this pesticide, a resistance allele arose in the natural population at the gene *Rop-1*, located on chromosome IV (Batterham et al. 1996). The product of this resistance allele is a modified membrane-bound carboxylesterase with increased phosphatase activity, responsible for the detoxification of the pesticide (Hughes and Raftos 1985, Russell et al. 1990, Parker et al. 1991).

This resistance allele has pleiotropic effects, conferring resistance against diazinon on the
one hand, whereas also reducing relative fitness and increasing asymmetry of frontal head
bristles on the other hand (McKenzie et al. 1982, McKenzie and Clarke 1988, Batterham et
al. 1996). It has been hypothesized that this increase in asymmetry is due to disruption of
a coadapted gene complex by the change in the Rop-1 gene (Clarke 1993). In the absence
of pesticide, resistant genotypes were shown to be at a selective disadvantage relative to
susceptible genotypes (McKenzie et al. 1982). In response to this fitness reduction produced
by the resistance allele, L. cuprina was later shown to have evolved a modifier gene, located
on chromosome 3, which ameliorated the negative fitness effects of the resistance allele and
restored the relative fitness and asymmetry levels of resistant flies to the levels of suscep-
tible flies (Clarke and McKenzie 1987, McKenzie and O’Farrell 1993). Both the resistance
allele and the modifier gene have been hypothesized to moderate cell adhesion properties
in the nervous system, impacting the development of bristle cells, and therefore leading to
bristle asymmetry (Batterham et al. 1996). Therefore, the case of L. cuprina represents a
well-documented example in which the negative association between specific genetic factors,
asymmetry and relative fitness is well understood (Palmer 1996, McKenzie 2003).

Although the precise causes of a negative DI-fitness association are not always as well
understood as in the case of L. cuprina, a number of other studies have also demonstrated
fitness correlates of DI. For example, FA is correlated with reduced fecundity in humans,
yellow fever mosquitoes (Aedes aegypti), and gemsbok (an African antelope; Oryx gazella);
with reduced egg hatchability in Drosophila melanogaster; and with fewer fledged offspring
of asymmetrical parents in zebra finches (Taeniopygia guttata) (reviewed in Møller 1997). A
negative relationship between asymmetry and survival has also been found in such species
as the house fly, the common shrew, the house mouse (mus musculus), and a viviparous
lizard (reviewed in Møller 1997). More recently, a negative association between FA and
fitness correlates have been found in, for example, ejaculate quality in humans (Firman et
al. 2003), general health in chimpanzees (Sefcek and King 2007), and male body condition
in red deer (Concha et al. 2008).
A negative correlation has also been demonstrated between phenodeviance and fitness in several species. Increased phenodeviance has been found to be associated with reduced growth rate and survival in various fish (Kirpichnikov 1981) and with reduced survival in humans (Shapiro 1992). Much research on phenodeviance, also referred to as “minor physical anomalies” (MPAs), has focused on correlations between DI and human psychological conditions. For example, increased prevalence of MPAs is associated with both schizophrenia (e.g. Gualtieri et al. 1982, O’Callaghan et al. 1991) and autism (e.g. Waldrop et al. 1968, Campbell et al. 1978, Rodier et al. 1997, Miles and Hillman 2000). However, the inverse relationship between DI and fitness may not be a general trend (Markow et al. 1996, Tomkins and Simmons 1998), and there is a large amount of heterogeneity among studies (Møller 1997).

1.2 The developmental instability–sexual selection hypothesis

If there are fitness costs of imprecise development, selection should be expected to occur for reducing DI. In particular, in cases where DI reduces vigor and/or attractiveness, we would expect sexual selection to act to reduce DI. This hypothesis is known as the DI–sexual selection hypothesis (reviewed in Polak 2008). The DI–sexual selection hypothesis proposes that sexual selection favors reduced DI in secondary sexual traits because reduced DI reveals the genetic quality of potential mates or sexual rivals (Møller and Pomiankowski 1993, Møller 1997, Polak 2008).

1.3 Predictions of the DI–sexual selection hypothesis

The DI–sexual selection hypothesis makes several testable predictions: the four which have received the most attention in the literature (review in Polak 2008) are:

1. DI has a genetic basis;

2. DI is negatively correlated with reproductive success;
3. DI reflects overall genetic fitness;

4. DI is condition dependent.

The first prediction of the DI-sexual selection hypothesis is that DI should have a genetic basis (Polak 2008). This requirement is crucial because if DI does not have a heritable genetic basis, then FA and phenodeviance cannot have evolutionary potential, and thus it is unlikely that preferences for symmetry as an indicator of individual genetic quality would evolve (Lande 1981, Fuller and Houle 2003, Polak 2008).

Studies have generally shown the heritability of FA ($h_{FA}^2$) to be small and not significant (Polak 2008). Estimates of $h_{FA}^2$ from 21 studies reviewed by Fuller and Houle (2003) had a mean of 0.026 (SE = 0.015), and of these, all estimates of $h_{FA}^2$ for sexually selected traits (N=4) were nonsignificant. Nevertheless, $h_{FA}^2$ is thought to underestimate the heritability of DI, and $h_{DI}^2$ has been calculated to be as high as 0.55 using mathematical models relating FA to DI (Whitlock 1996, Gangestad and Thornhill 1999, Houle 2000). Despite the evidence that DI may be heritable, the generally small estimates of $h_{FA}^2$ are problematic for the DI-sexual selection hypothesis because potential mates or sexual rivals cannot assess DI directly, and must use FA or phenodeviance as a proxy (Polak 2008). Therefore, additional studies are called for to resolve the still open question of whether there is genetic variation underlying measures of DI, a key requirement for the evolutionary potential of DI.

A second important prediction of the DI–sexual selection hypothesis is that DI is expected to be negatively correlated with measures of reproductive success, such as mating success, perhaps by mediating mate selection or intrasexual competition (Polak 2008). A number of studies spanning various species have demonstrated mate preferences for symmetrical individuals of the opposite sex (reviewed in Møller 1993, Møller and Swaddle 1997, Tomkins and Simmons 2003, Polak 2008). For example, female barn swallows prefer to mate with symmetrical males (Moller 1990). In humans, both men and women prefer symmetrical faces (e.g. Jones and Hill 1993, Grammer and Thornhill 1994, Mealey et al. 1999, Penton-Voak et al. 2001, Little and Jones 2003). Human women have been shown to be more attracted
to males other than their own partner during their fertile phase when their partner has high developmental instability (Gangestad et al. 2005). In other taxa, zebra finch (*Taeniopygia guttata*) females display a preference for males with symmetrical leg bands (Swaddle and Cuthill 1994, but see Rohde et al. 1997). *Drosophila melanogaster* males that mate have more symmetrical sternopleural bristles than do single males (Markow 1987). Female Trinidadian guppies (*Poecilia reticulata*) prefer to mate with males with more symmetrical carotenoid pigmentation (Gong and Gibson 1996). Preference for symmetrical sexual organs is even seen in pollinator preferences for symmetrical flowers (Møller 1995, Møller and Eriksson 1995).

Experimental manipulations of fluctuating asymmetry can provide a strong test of the role of FA in sexual selection, because in such cases all other traits of an individual are held constant (Tomkins and Simmons 2003). This approach is necessarily more powerful than the alternative, statistical methods in correlative studies (Tomkins and Simmons 2003). Several studies have provided evidence of female preference for symmetry in experimentally manipulated males. In poecilid fishes (*Xiphophorus cortezi* and *Poecilia latipinna*), females show preference for symmetry of male body bars when body bars are manipulated by experimenters (Morris 1998, Morris and Casey 1998, Schlüter et al. 1998). Female zebra finches (*Taeniopygia guttata*) and bluethroats (*Luscinia svecica svecica*) show preferences for symmetrical leg bands on males (Swaddle and Cuthill 1994, Swaddle 1996, Jennions 1998, but see Tomkins and Simmons 2003).

In contrast to these findings, a number of studies have also detected no significant relationship between DI and sexual fitness (reviewed in Polak 2008). For example, Eggert and Sakaluk (1994) found no relationship between male wing FA and spermatophore size in decorated crickets (*Gryllodes sigillatus*), Tomkins and Simmons (1998) found that female earwigs (*Forficula auricularia*) showed no preference for males with symmetrical forceps, Van Dongen et al. (1999) found no association between tibial FA and copulation probability in the winter moth (*Operophtera brumata*), and Cooley (2004) found that FA of several
body traits in a periodical cicada (*Magicicada septendecim*) was not correlated with mating success. Thus, it appears that effects of DI on reproductive success are highly heterogeneous (Polak 2008).

A third prediction of the DI–sexual selection hypothesis is that DI reflects overall genetic fitness of an individual. The level of DI in a secondary sexual trait has been proposed to reflect the ability of the individual to contend with genetic and environmental stresses (Palmer and Strobeck 1986, Parsons 1990). If DI in a secondary sexual trait is a physiological outcome due to reduced genetic quality, then traits that have developed with little FA or phenodeviance may be an advertisement for the “good genes” of that individual (Kodric-Brown and Brown 1984). To put it another way, only individuals with the best inherent ‘quality’ or genes may be able to direct energy and resources towards the precise development the trait in question. Therefore, sexual selection favoring the reduction of DI in secondary sexual traits may be driven by the fact that DI in these traits is a reliable indicator of the genetic quality of the individual (Kodric-Brown and Brown 1984). For instance, if a relatively low level of DI in a secondary sexual trait in a male indicates relatively high genetic fitness, then females may preferentially mate with low-DI males in order to accrue genetic benefits for their offspring (Kodric-Brown and Brown 1984).

A fourth prediction of the DI–sexual selection hypothesis is that DI should be condition dependent. Condition refers to the amount of limited resources that an organism has available to direct towards traits related to fitness (Tomkins *et al.* 2004). Developmentally unstable individuals are expected to be less well adapted to their environment and less fit, and therefore are expected to have reduced condition (Tomkins and Simmons 2003). If such individuals have reduced condition, this is expected to be manifested as increased asymmetry or phenodeviance of secondary sexual traits, since such traits generally have heightened condition dependence (Møller and Pomiankowski 1993, Andersson 1994, Tomkins and Simmons 2003). Thus far, studies attempting to link body condition to DI have produced conflicting results, and showing a causative link between condition and DI has been even more
difficult (reviewed in Polak 2008). The lack of evidence for condition dependence of FA in secondary sexual traits is potentially a serious problem for the DI–sexual selection hypothesis as currently formulated.

1.4 Research objectives

The main goal of this project was to evaluate three key predictions of the DI–sexual selection hypothesis in a laboratory population of *D. bipectinata* under experimentally controlled conditions. My focus is on expressions of DI in the male sex comb, a secondary sexual trait in this species (see below for further details). These predictions are: 1) that DI has a genetic basis; 2) that DI is correlated with reduced reproductive success, as mating success; and 3) that DI reflects components of genetic fitness other than reproductive success, such as juvenile survivorship.

To test these predictions, I utilize two complementary measures of DI, fluctuating asymmetry and phenodeviance. This approach is expected to be a more sensitive assay of the relationship between DI and fitness, compared to using only a single metric of DI. I also employ an isofemale line approach, which is a standard approach used by geneticists to extract genotypic variation from a population. Typically, isofemale lines are genetic lines initiated with a single fertilized female each. In the present case, virgin *D. bipectinata* females were mated to just one male each, and genetic lines with high and low levels of phenodeviance were established. Thus, each isofemale line in my study represents the combination of only two parental genotypes.

To evaluate the first prediction, that DI has a genetic basis, I test for significant differences among isofemale lines in their degree of phenodeviance, and test for the stability of any such differences over multiple generations of laboratory culture.

To test the second prediction of the DI–sexual selection hypothesis, that DI is negatively correlated with reproductive fitness, I test for differences in male mating success among males from isofemale lines with divergent levels of phenodeviance.
Finally, to test the third prediction, that DI is negatively associated with overall genetic fitness, I test for a relationship between DI and two individual fitness components, preadult survivorship and egg hatch rate, in the isofemale lines.

1.5 Experimental system

_Drosophila bipectinata_ Duda (Diptera, Drosophilidae) is a fruit fly distributed throughout the Australasian biogeographic zone (Bock 1978). Polak and Taylor (2007) identify this as a natural system in which both elevated fluctuating asymmetry and phenodeviance are associated with reduced mating success in the field. At dawn, males and females of this species gather on rotting fruit to feed and copulate (Polak et al. 2004, Polak 2008). Females oviposit on fruit, after which larvae develop and then pupate in the fruit or nearby surfaces (Polak 2008). Males aggressively seek out and court females, as well as engage in agonistic encounters with competing males for access to females (Polak 2008).

Males have a prominent sexual ornament, the male sex comb, which is condition-dependent (Polak and Starmer 2005). This comb is undergoing rapid evolutionary diversification across populations (e.g., islands in the South Pacific) (Polak et al. 2004). The sex comb is found on the first and second tarsal segments of the male front legs and consists of three rows of enlarged, hardened bristles, or “teeth” (Bock 1971). In my scheme, a normal sex comb contains three rows of teeth in straight lines, while a phenodeviant sex comb contains at least one tooth that is out of line (either in front of or behind the comb) (Figure 1). An asymmetrical fly is one whose combs have different numbers of sex comb teeth on the left and right sides of the body.

Although Polak and Taylor (2007) have shown significant sexual selection for reducing both FA and phenodeviance of the sex comb in New Caledonia, the exact function of the comb is not yet known. During courtship, the male approaches a female from behind and attempts to position his abdomen to align the genitalia (Polak et al. 2004). As he mounts the female, the sex combs come into contact with both sides of her abdomen; thus, it is
possible that females receive tactile cues about male quality during courtship (Polak and Starmer 2005). Recent evidence indicates that the sex comb is used to grasp the female abdomen during close-contact courtship and help maintain contact while the male attempts to initiate copulation (Polak, personal communication).

There is significant additive genetic variance for phenodeviance of the comb (as minor morphological abnormalities, MMAs) (but not for FA), confirming that these instances of phenodeviance have evolutionary potential (Polak and Taylor 2007). In addition, FA and MMAs are correlated with reduced mating success in the field, with single males having higher FA and MMA values than mated males (Polak and Taylor 2007). Polak and Taylor’s (2007) study provides some of the most compelling evidence to date for the importance of phenodeviance as a measure of developmental instability.

2 Methods

2.1 General methods

A base laboratory population of D. bipectinata was established in January 2006 from wild-caught flies collected approximately 80 m from La Baïe de Anse Vata in Noumea, New Caledonia (Polak and Taylor 2007). Females were collected from the surface of fallen mangoes while in copula, and an additional 100 males were collected up to 30 m from the site by use of an insect net. Flies were cultured in 8 240-mL glass milk bottles with 12g Instant Drosophila Medium (Carolina Supply Co., Burlington, NC), 47mL water, and 8–10mL of banana-live yeast slurry (50g of banana, 100mL water, and 3g active yeast). In each generation, bottles were seeded with ~40 adults randomly selected from the previous generation.

Flies from isofemale lines (see “Isofemale lines” below) used in experiments were maintained in 35-mL polystyrene vials with 5 mL cornmeal agar food substrate at a density of 15-20 adults per vial. Culturing vials and bottles in all experiments were held in temperature-controlled incubators at 24°C Day: 22°C Night with a 12 h light: 12 h dark photo-period to
ensure near-identical environmental conditions across all vials and bottles, unless otherwise stated.

2.2 Morphological traits

Several important morphological traits of males were measured throughout this study. Thorax length (TL) is the distance between the anterior end of the thorax and the tip of the scutellum, and is used here as a measure of male body size (Robertson and Reeve 1952). As previously described, the male sex combs are located on the front legs and each consist of three segments of stout bristles (Bock 1971). These segments are referred to as C1, C2, and C3. FA1 is fluctuating asymmetry in segment C1, calculated as the absolute value of the difference in the number of teeth between the left and right C1 segments. (For example, if a male’s sex comb contained 6 teeth in the C1 segment on the right leg sex comb, and 8 teeth in the C1 segment on the left leg sex comb, the value of FA1 would be equal to 2.) Likewise, FA2 is the absolute value of the difference in the number of teeth between the left and right C2 segments.

A MMA (minor morphological abnormality, or phenodeviance) is defined as an abnormal sex comb, such that either: (a) a tooth is located in front of the main row of teeth in the comb, (b) a tooth is located behind the main row of teeth, (c) a tooth is short relative to others in the comb, such that its length is no more than two-thirds the length of nearby teeth, or (d) a gap occurs between two teeth greater than or equal to the width of one tooth (developed and expanded from Polak and Taylor 2007). Figure 1 illustrates abnormalities as misplaced teeth. MMA1 refers to phenodeviance in segment C1, whereas MMA2 refers to phenodeviance in segment C2.

2.3 Isofemale lines

Males and females from the base laboratory population were collected on the same day, during the period of peak emergence, within 4 hours of eclosion to ensure virginity. The sexes were
held separately in cornmeal vials for three days. Active yeast was added to vials containing females. Males were then scored for phenodeviance in each sex comb under anesthesia with an Olympus SZX12 stereomicroscope [1.6X objective lens, 20X eye-pieces, 6.4X zoom setting on a 1.6X magnification display ring]. Twenty-five males with low phenodeviance (no MMAs) and 50 males with high phenodeviance (one or more MMAs) were selected. Since the phenodeviance level of offspring from a given male can vary greatly depending on the genotype of the male, as well as the genotype of the female mated to him, I initiated a larger number lines from phenodeviant males in order to increase the likelihood of obtaining some isofemale lines with relatively high levels of offspring phenodeviance.

Each selected male was placed into a cornmeal agar vial with 0.1 mL yeast slurry containing 0.14 g active yeast suspended in tap water, along with one randomly selected virgin female. Thus, 75 isofemale lines were initiated, each representing the combination of one paternal and one maternal genotype. After three days, both adults were removed from each vial and discarded. After F₁ offspring had emerged, each line was expanded into three replicate vials, each seeded with five females and five males randomly selected from the F₁ offspring in each line. Adults were removed after three days. Lines continued to be cultured in this manner at each subsequent generation. Excess males during each generation that were not used for seeding the next generation were preserved in 70% alcohol for later characterization.

Preserved F₅ males were later removed from alcohol and rehydrated in small dishes with water. Ten males from each of two replicate vials per line were scored for phenodeviance and thorax length with an Olympus SZX12 stereomicroscope [1.6X objective lens, 20X eye-pieces, 6.4X zoom setting on a 1.6X magnification display ring]. Foretarsi of flies were removed using fine-tipped forceps and placed on the surface of double-sided transparent tape. The numbers of teeth in each sex comb segment of each male were recorded, as well as the thorax length of each male. Each male was assigned a score of 0 for no MMA, or 1 for any MMA present. (Males with 1, 2, or more instances of phenodeviance, regardless of location on the sex combs, were all assigned a score of 1.) Generations F₈, F₉, F₁₄, and F₂₆ were characterized in the
The proportion of males that were phenodeviant was calculated for each genetic line at $F_5$, and lines were sorted by level of phenodeviance. The five lowest- and five highest-proportion phenodeviance lines were selected to be used for future experiments. The five low phenodeviance lines are hereafter referred to as “low lines” and the high phenodeviance lines referred to as “high lines.” This classification (high or low) is referred to as the “phenodeviance category” of each line. All other lines were discarded.

**Data analysis.** To ascertain whether differences in levels of male phenodeviance among the ten chosen lines at $F_5$ were statistically significant, I used binary logistic regression on male phenodeviance with genetic line as a factor. Replicate vial and thorax length were initially included as factors but later removed because they were nonsignificant (vial $P = 0.22$, thorax length $P = 0.97$). Because logistic regression does not accommodate nested factors, a second binary logistic regression was used with phenodeviance category as a factor without including line as a factor.

Because experiments were conducted using flies from multiple generations, I also tested whether differences in levels of phenodeviance across multiple generations were stable over the course of the study. I performed an additional analysis one generation after the completion of all experiments, including phenodeviance data from five generations ($F_5$, $F_8$, $F_9$, $F_{14}$, and $F_{26}$). Thus, this analysis in combination with the previous analysis encompasses all experiments conducted by testing for differences in levels of phenodeviance at generations spanning the start of all experiments until after the completion of the last experiment. Binary logistic regression was performed on male phenodeviance with genetic line, generation, and the genetic line $\times$ generation interaction as factors. Replicate vial and thorax length were initially included as factors but later removed because they were nonsignificant (vial $P = 0.81$, thorax length $P = 0.32$). A final binary logistic regression was used with phenodeviance category, generation, and the phenodeviance category $\times$ generation interaction as factors.
Note, when the level of phenodeviance in each isofemale line was scored at generation 14, one of the high phenodeviance category lines was found to have a reduced level of phenodeviance, such that the level of phenodeviance in that line was in the range of the levels in the low phenodeviance lines. For this reason, this line and a paired line in the low phenodeviance category were discarded and not used for further experiments. Therefore, prior to generation 14, all experiments (including the vial mating success assay, preadult survivorship assay, and egg hatch rate assay) utilized five high phenodeviance lines and five low phenodeviance lines, while the experiment after that time (the field-mimicking mating success assay) used only four high phenodeviance lines and four low phenodeviance lines.

**FA–phenodeviance correlation.** If FA and phenodeviance are both expressions of DI, I expect them to be phenotypically correlated. To test this, I examined sex comb data from a random sample of males (N = 66) chosen from all isofemale lines at F7. I used analysis of covariance (ANCOVA) on FA1 with MMA1 as a factor (i.e., phenodeviant or not) and with TL and size of C1 as covariates. If FA and phenodeviance are expressions of DI, phenodeviant males should on average express significantly high FA than nonphenodeviant males.

### 2.4 Mating success assay: vials

In order to test the second prediction of the DI-sexual selection hypothesis, that DI is negatively correlated with reproductive success, I competitively assayed the copulation success of high-DI and low-DI males with virgin females. I predicted that phenodeviant and asymmetric males would have lower mating success than their normal counterparts, and that males from high-DI lines would have lower mating success than males from low-DI lines.

Prior to this experiment, first-instar larvae were collected and allowed to develop to adulthood under conditions of controlled larval density and temperature (see “Preadult Survivorship assay,” below). After juveniles had developed to adulthood, males were collected within 4 hours of eclosion to ensure virginity, and then moved to cornmeal vials for 3 days.
On the same day that males emerged, virgin females were collected at the period of peak emergence from the laboratory base population of *D. bipectinata* within 4 hours of eclosion. Females were held in 35-mL cornmeal agar vials with active yeast for 3 days. Note, females were collected from a different source population than any of the males.

One day prior to the mating success assay, males were scored for phenodeviance under anesthesia with an Olympus SZX12 stereomicroscope. From the genetic lines, both phenodeviant and non-phenodeviant males were collected. On the evening before the assay, 40 35-mL vials with 5-mL grape juice agar substrate were lined up along the edge of a table. Into each vial, one non-phenodeviant male and one phenodeviant male, from different genetic lines, were gently aspirated. Phenodeviant males were taken from high-phenodeviance lines, and non-phenodeviant males were taken from low-phenodeviance lines. At dawn on the following morning, laboratory lights were turned on and one virgin female from the base population was gently aspirated into each vial. Thus, this experiment consisted of competing a phenodeviance male against a non-phenodeviance male for access to a virgin female.

Vials were observed closely until copulation occurred or until 2h had elapsed. As each copulation occurred, the start time was recorded and the single (unsuccessful) male was carefully removed from each vial and preserved in 70% alcohol. Mating latency was calculated as the time elapsed from the introduction of the female until a male obtained copulation. The copulating pair was left undisturbed until copulation ended. The successful male was then preserved in 70% alcohol for characterization, and the female was moved into a fresh grape juice agar vial for a subsequent experiment (see “Egg hatch rate assay,” below). Copulated and single males were rehydrated in small dishes of water for 60s and then scored for phenodeviance, FA, and thorax length with an Olympus SZX12 stereomicroscope, as described above.

**Data analysis.** Mating success was scored on a binary scale, as whether or not a male mated, and analyzed using binary logistic regression, with genetic line, MMA1, MMA2, FA1,
FA2, the MMA1 × FA1 interaction, and the MMA2 × FA2 interaction as factors and the size of C1 and size of C2 (calculated as the average number of teeth in segments C1 and C2 on the left and right sex combs) as continuous variables. Thorax length and block were entered as factors initially but later removed because the coefficients were not statistically significant (thorax length $P = 0.94$, block $P = 0.77$).

Because nesting of factors is not possible in logistic regression, an additional analysis was employed to test for the effects of phenodeviance category on mating success. Binary logistic regression was used with phenodeviance category added as a factor, and genetic line removed. All other factors and covariables were the same as above.

Mating latency: The effect of male phenodeviance and FA on mating latency was analyzed using analysis of variance (ANOVA) with FA1, FA2, MMA1, MMA2, category, and line as factors and size of C1 and size of C2 as covariates. Line (nested in category) was treated as a random factor. Thorax length and block were entered as factors initially but later removed (thorax length $P = 0.28$, block $P = 0.84$).

2.5 Mating success assay: field-mimicking chambers

An additional mating success assay was performed in which experimental conditions were altered from the above vial experiment to more closely resemble those of the field (See “Experimental System” above). In this assay, we utilized a social environment with many males simultaneously involved in a vigorous contest (or “scramble competition”) (Thornhill and Alcock 1983) to locate and access non-virgin females. This design was expected to increase competition among males for females by reducing the number of receptive females available at any given time (i.e., because non-virgin females were used), while also increasing the potential for multiple-male competition interactions for a single female. In addition, we employed ripe mango as an ecologically appropriate food substrate, natural dawn sunlight, and increased air flow in mating arenas.

To carry out this assay, I procured virgin males from isofemale lines and procured females
from the laboratory base population. Females were obtained for this assay by collecting newly emerged male and female adults from the base population on the same day within a period of 8 hours, and holding them in cornmeal vials at a density of 10 males and 10 females per vial for three days. Thus, females were expected to be socially experienced, and non-virgin. Males were removed from vials after three days, and females were transferred to cornmeal agar vials with light active yeast. Polak and Simmons (2009) found that only 40% of females of this species remated after a period of 7 days following a copulation. Therefore, in order to ensure that some females would be receptive to mating in the assay, I held females for nine days after removing males before performing the assay.

To obtain virgin adult males from isofemale lines, I seeded six cornmeal vials for each line with five males and five plump, healthy females each. Adults were removed after three days, and offspring were allowed to mature. Emerging adult males were collected within 6-8 hours of eclosion to ensure virginity, and held in cornmeal vials. Males were moved to fresh vials every three days for twelve days prior to the mating success assay, so that males and previously described females would be of the same age.

In order to competitively assay males from low lines against males from high lines, I paired each low line with a randomly-chosen high line for this experiment. Because there were four low lines and four high lines, there were four pairs of lines in each block. The experiment was performed in three blocks, with each block being performed over the course of two days (see Figure 2). Two pairs of lines were assayed competitively on the first day of each block, with each pair competed in a single mating chamber, and the remaining two pairs were assayed competitively on the second day. In subsequent blocks, line pairings were changed such that the same two lines were not paired together twice.

Mating chambers used in this experiment were 11.5x12x24cm clear \( \frac{1}{4} \)-inch thick Plexiglas boxes, each containing sliding doors on either end of the chamber with 9.7-cm diameter mesh ventilation holes, and with fourteen 1–2cm diameter aspirating holes cut into the top and sides of each chamber, plugged with cotton (Figure 3).
One day prior to the competitive mating assay, each of the four low-developmental instability lines was chosen to be competed with one of the four high-developmental instability lines, as described above. In order to distinguish males from each line once they were placed into the mating arena, I clipped either the left or right scutellar bristle of each fly under anesthesia using micro dissecting scissors. One line from each line pair was randomly assigned to have the left bristle clipped, and the other pair was assigned to have the right bristle clipped. These designations were randomly assigned for each pairing in each block.

On the evening prior to each assay, the floor of each chamber was lined with several flat slices of ripe mango. One hundred sexually experienced females were placed into each chamber under anesthesia, along with 50 males from the chosen low-developmental instability line and 50 from the paired high-developmental instability line. Immediately afterward, chambers were wrapped loosely in clear plastic to prevent mango slices from drying out, and chambers were covered in dark cotton material to block out light.

At dawn on the morning of the experiment, the dark cotton coverings and plastic wrap were removed from each chamber. Chambers were situated in a room with a southeast-facing window. An oscillating fan on lowest setting was used to circulate air in the room. As copulating pairs formed, they were gently aspirated out of chambers through aspiration holes, and placed into labeled centrifuge tubes with 100% ethanol for later characterization. The start time of each copulation was recorded. Mating latency for each copulation was calculated as the time elapsed from the start on the experiment at dawn until the beginning of the copulation. After four hours had elapsed, remaining males were aspirated out of the chamber and also preserved in alcohol. These males were designated as single, or unsuccessful. Copulated and single males were rehydrated in small dishes of water for 60s and then scored for phenodeviance, FA, and thorax length with an Olympus SZX12 stereomicroscope.

**Data analysis.** Male mating success was analyzed using binary logistic regression, with genetic line, MMA1, MMA2, FA1, FA2, the MMA1 × FA1 interaction, and the MMA2
× FA2 interaction as factors and the size of C1, size of C2, block, and thorax length as continuous variables.

Because nesting of factors is not possible in logistic regression, a second analysis was used to test for differences in mating success between the two phenodeviance categories. Binary logistic regression was used with phenodeviance category added as a factor, and genetic line removed. All other factors and covariables were the same as above.

Mating latency: The effect of male FA on mating latency was analyzed using analysis of covariance (ANCOVA) with FA1, FA2, category, line, and block as factors and size of C1 and size of C2 as covariates. Line (nested in category) was treated as a random factor. Thorax length was entered as a factor initially but later removed ($P = 0.41$).

I also examined the effect of male phenodeviance on mating latency using an additional analysis of covariance (ANCOVA) with MMA1, MMA2, category, line, and block as factors and size of C1 and size of C2 as covariates. Line (nested in category) was treated as a random factor. Thorax length was entered as a factor initially but later removed ($P = 0.59$).

2.6 Preadult survivorship assay

**Harvesting larvae.** In preparation for the vial mating success assay, adults from genetic lines were collected after emergence and held in cornmeal vials with live yeast for at least 3 days. From each genetic line, 40 males and 40 plump, healthy females were randomly selected and gently aspirated into a mating chamber. Mating chambers were constructed from 225-mL plastic cups, containing one small (1 cm-diameter) hole plugged with cotton on the upper surface, inverted over 9.5-cm diameter glass Petri dishes lined with 30 mL grape juice agar substrate (Figure 4). Grape juice agar was used to increase visible contrast between dark substrate and the lighter larvae. From each line, two chambers were seeded with adults.

After 24h, adults were removed from chambers with CO$_2$ anesthesia. After an additional 24h, newly hatched first-instar larvae were gently removed from the surface of the agar
using sterile blunt-tipped probes. From each chamber, 40 larvae were placed into each of two cornmeal vials. Vials were maintained in incubators until all offspring had developed through to adulthood.

**Survivorship analysis.** The proportion of offspring surviving to adulthood in each vial was calculated as the number of emerged adults divided by 40. Proportion data were arcsine square-root transformed prior to analysis to normalize the data. Proportion preadult survivorship was examined using analysis of variance (ANOVA) with phenodeviance category, line, and block as factors. Line (nested in phenodeviance category) was treated as a random factor. Vial (nested in mating chamber) and mating chamber were initially entered as factors, but later removed (vial $P = 0.29$; chamber $P = 0.88$).

### 2.7 Egg hatch rate assay

During the previously described vial mating success assay, virgin females were each mated to one male from either a high or low phenodeviance genetic line (see “Mating Success Assay: Vials”). Immediately following the assay, females that had copulated were transferred to new 35-mL polystyrene vials with 5mL grape juice agar food. (Grape juice agar facilitated data collection by increasing visible contrast between the light eggs and dark substrate.) Each female was moved to a new vial every 24 hours, until at least 30 eggs were laid or until 5 days had elapsed. The number of eggs in each vial was counted prior to larval hatching, and the number of newly hatched larvae was counted every 24h until no remaining eggs hatched. The hatch rate was determined by dividing the number of larvae hatched by the number of eggs laid for each female. Clutches of less than ten eggs were removed from the subsequent analysis.

Egg hatch rate was analyzed using analysis of covariance (ANCOVA) with paternal MMA1, MMA2, FA1, FA2, phenodeviance category, and line as factors and size of C1 and size of C2 as covariates. Line (nested in phenodeviance category) was entered as a random
factor. The FA1 × MMA1 interaction and FA2 × MMA2 interactions were initially included, but later removed (FA1 × MMA1, \( P = 0.89 \); FA2 × MMA2, \( P = 0.48 \)). Proportion data were arcsine-square root transformed prior to analysis. Hatch rate was not normally distributed (Kolmogorov-Smirnov; \( P < 0.0100 \)), but also failed to be normally distributed after arcsine square-root transformation (Kolmogorov-Smirnov; \( P < 0.0100 \)).

In addition to examining the effects of paternal DI on offspring hatch rate, I also examined the relationship between the level of DI in a genetic line and the hatch rate of eggs sired by males from those lines. If DI is associated with decrements in egg hatch rate, I would expect that genetic lines with higher levels of DI would have lower egg hatch rates. To test this, I used least-squares regression to examine the relationship between proportion phenodeviance of each genetic line at F5 and egg hatch rate of clutches sired by males from each line.

3 Results

3.1 Isofemale Lines

Proportion phenodeviance for all genetic lines at F5 (the first generation scored, prior to the start of other experiments) was distributed approximately normally and ranged from 0.050 to 0.800 (mean = 0.302, std dev = 0.157, \( N = 50 \)) (Figure 5). For the ten genetic lines chosen for use in additional experiments, binary logistic regression at F5 showed significant differences in phenodeviance (line: \( \chi^2 = 77.7 \), DF = 9, \( P < 0.0001 \))(Figure 6). In addition, a second binary logistic regression confirmed a significant difference in phenodeviance between the two chosen phenodeviance categories (high versus low phenodeviance lines) at F5 (phenodeviance category: \( \chi^2 = 74.1 \), DF = 1, \( P < 0.0001 \)), where the high lines had higher levels of phenodeviance than did the low lines. These results confirm that genetic lines were successfully isolated which differed in levels of phenodeviance.

I also tested whether genetic lines remained significantly different in levels of phenodeviance throughout the duration of all experiments by performing an additional analysis.
including phenodeviance data from five generations spanning from before the first experiment until after the completion of the last experiment (generations F_5, F_8, F_9, F_{14}, and F_{26}). Binary logistic regression showed a significant effect of genetic line on phenodeviance, indicating that lines significantly differed in levels of phenodeviance across all generations scored (genetic line: \(\chi^2 = 122.9, \text{DF} = 7, P < 0.0001\)). I also found a significant effect of generation on phenodeviance, indicating generational effects on overall levels of phenodeviance in the lines (generation: \(\chi^2 = 24.2, \text{DF} = 4, P < 0.0001\)). However, there was no significant interaction between generation and line (generation \(\times\) line: \(\chi^2 = 37.6, \text{DF} = 26, P = 0.066\)), meaning that all lines changed approximately equally over generations.

An additional binary logistic regression confirmed that the two chosen phenodeviance categories (high lines versus low lines) also continued to be significantly different in phenodeviance (phenodeviance category: \(\chi^2 = 113.0, \text{DF} = 1, P < 0.0001\)). Once again, I found a significant effect of generation on phenodeviance (generation: \(\chi^2 = 20.7, \text{DF} = 4, P = 0.0004\)). I also found a significant interaction between generation and phenodeviance category (generation \(\times\) phenodeviance category: \(\chi^2 = 12.2, \text{DF} = 4, P = 0.016\)). This significant interaction reflects the fact that the proportion phenodeviance in “low” lines drifted upward in intermediate generations, with the result that there was some apparent loss in separation between high and low lines during these intermediate generations (see Figure 7). Nevertheless, despite the lessening separation between lines from the high and low category at intermediate generations, the high and low phenodeviance categories remained significantly different from each other at each generation. These results confirm the stability of genotypic effects on phenodeviance over time, and thus the first prediction of the DI–sexual selection hypothesis, that DI has a genetic basis, has been supported in this system.

**FA–phenodeviance correlation.** If FA and phenodeviance are both expressions of DI, then I predicted that males that were phenodeviant would also be more likely to also be asymmetrical. I found that in generation F_7, males that were phenodeviant in C1 had 91%
greater FA in C1 than non-phenodeviant males (ANOVA: $F_{3.62} = 8.64$, $P = 0.0046$) (Figure 8). Size of C1 was also significantly correlated with FA1 in this analysis ($P = 0.0087$), but thorax length was not ($P = 0.58$). These results support the idea that both FA and phenodeviance reflect errors in the same underlying developmental buffering system.

### 3.2 Mating success assay: vials

**Mating success.** In the vial assay, I detected no significant effect of genetic line on the probability of mating, nor did I detect a significant effect of any phenotypic measure of DI on mating success, including FA1, FA2, MMA1, and MMA2 (Table 1). All other factors were nonsignificant (all $P > 0.05$) (Table 1). An additional analysis found no significant effect of phenodeviance category on mating success ($P = 0.32$). All other factors were nonsignificant (all $P > 0.05$) (Table 2). Thus, these results show no significant genotypic or phenotypic effect of DI on male mating success in vials.

**Mating latency.** Males that were phenodeviant in C1 took more than twice as long to obtain copulations than non-phenodeviant males (ANOVA: $F_{15.66} = 12.9$, $P = 0.0006$) (Figure 9). FA1 also had a significant effect on latency, but in the opposite direction predicted: more asymmetrical males mated faster (ANOVA: $F_{15.66} = 4.52$, $P = 0.037$). In contrast, MMA2, FA2, line, category, size of C1, and size of C2 did not significantly affect mating latency (all $P > 0.05$)(Figure 10). Thus, phenodeviance in C1 and FA in the same comb segment had opposite effects on mating success in vials.

### 3.3 Mating success assay: field-mimicking chambers

**Mating success.** In the field-mimicking chambers, genetic line significantly predicted male mating success. Consistent with my prediction, individual male phenodeviance (as MMA1) significantly decreased the probability of mating ($P = 0.041$) (Table 3). However, further analysis showed that the effect of MMA1 on mating success was highly dependent on the
inclusion of the MMA1 × FA1 interaction term in the model; when the interaction term was excluded, MMA1 was no longer found to be significant ($P = 0.35$). Additional measures of male phenotypic DI (as MMA2, FA1, and FA2) did not significantly predict mating success. I found significant effects of C1 size, time block, and thorax length on mating success, but not of C2 size (Table 3). Significant interaction effects were found between FA1 and MMA1, but not between FA2 and MMA2 (Table 3). An additional analysis showed that contrary to prediction, males from the high phenodeviance category lines had higher mating success than males from the low phenodeviance category lines (Table 4, Figure 11).

**Mating latency.** Males that were phenodeviant (as MMA1 or MMA2) did not significantly differ from non-phenodeviant males in speed of obtaining a copulation (Table 5). Males that were asymmetrical in C2 were slower to mate than symmetrical males, and asymmetry in C1 had no significant effect on mating latency (Table 6). Genetic effects were detected as a significant effect of line on mating latency, such that the time elapsed before males obtained copulations significantly differed among all genetic lines (Table 4). However, the category of each genetic line (high versus low phenodeviance) did not significantly predict mating latency.

### 3.4 Preadult survivorship assay

ANOVA on proportion preadult survivorship showed that genetic lines significantly differed in levels of preadult survivorship (ANOVA: $F_{8,108} = 2.09$, $P = 0.043$)(Table 7, Figure 12). However, there was no significant effect of phenodeviance category (high or low) of the genetic lines, showing that differences in survival of larvae and pupae among lines were unrelated to phenodeviance category (ANOVA: $F_{1,108} = 0.42$, $P = 0.52$) (Figure 13). Block also had a significant effect on preadult survivorship, with larvae and pupae having a significantly higher survivorship during the second block than during the first or third blocks (Table 7). Thus, these results do not support the prediction that preadult survival is associated with
increased DI.

3.5 Egg hatch rate assay

ANCOVA showed no significant effect of paternal MMA1, MMA2, FA1, FA2, genetic line, phenodeviance category (high versus low phenodeviance lines), size of C1, or size of C2 on egg hatch rate (all \( P > 0.10 \)) (Table 8). Likewise, least-squares regression showed that proportion phenodeviance of each line at F5 did not significantly predict egg hatch rate (proportion phenodeviance: slope = 0.0082, t = 0.07, \( P = 0.94 \))(Figure 14). Thus, these results do not support the prediction that reduced egg hatch rate is associated with increased DI.

4 Discussion

Developmental instability (DI) is thought to reflect the inability of an organism to buffer the development of its phenotype against random, disruptive events during the course of development (Van Valen 1962). DI is thought to be reflected phenotypically as fluctuating asymmetry, deviation from perfect bilateral symmetry (Ludwig 1932), or as phenodeviance, the incidence of minor morphological abnormalities in a trait (Jones 2006). Because the ability to produce an optimum phenotype during development is expected to be selectively advantageous, imprecise development is expected to reduce the fitness of an individual (Waddington 1957, Clarke 1993). Indeed, DI has been shown to be negatively correlated with numerous fitness correlates spanning various taxa, including mating success or correlates thereof, but these results are highly heterogeneous across species and populations (Leamy and Klingenberg 2005, Van Dongen 2006, Polak 2008).

The DI–sexual selection hypothesis proposes that sexual selection favors reduced DI in secondary sexual traits because reduced DI reveals the genetic quality of potential mates or sexual rivals (Møller 1997, Polak 2008). A number of studies have supported this hypothesis
by demonstrating reduced reproductive success for individuals having asymmetrical traits, but there also exist many studies showing either no association between FA and reproductive fitness or even a reverse association between FA and fitness (reviewed in Polak 2008).

A previous field study has shown that the fruit fly *Drosophila bipectinata* is undergoing sexual selection for reducing FA and phenodeviance in Noumea, New Caledonia (Polak and Taylor 2007). In the field, copulating males were shown to have significantly lower FA and phenodeviance than single flies (Polak and Taylor 2007). The present study expanded on Polak and Taylor’s (2007) work by evaluating additional key predictions of the DI–sexual selection hypothesis in carefully controlled laboratory conditions. By integrating two measures of DI, FA and phenodeviance, my study provides a sensitive approach to assaying the role of DI in sexual selection in this system.

### 4.1 The genetic basis of developmental instability

The first prediction of the DI–sexual selection hypothesis that I tested is that DI (as measured by phenodeviance) has a genetic basis. I successfully isolated isofemale lines (genotypes) that differed significantly in levels of phenodeviance in a secondary sexual trait, the male sex comb of *D. bipectinata*. These differences persisted across 26 generations, demonstrating the stability of genotypic effects of developmental instability as phenodeviance over time in my study system.

Although the present study did not estimate the additive genetic variance of phenodeviance, the fact that significant genetic effects of phenodeviance were found across generations is generally consistent with Polak and Taylor’s (2007) findings, which showed significant additive genetic variance for phenodeviance in the field population of this species. Indeed, the heritability of phenodeviance in C1 was estimated to be 0.149 (s.e., 0.080) (Polak and Taylor 2007). Because phenodeviance is thought to be an expression of DI, the first prediction of the DI–sexual selection hypothesis has been supported in this system in terms of phenodeviance. However, in contrast to phenodeviance, the heritability of FA in this system has not been
shown to be significantly different from zero, meaning that FA is unlikely to reveal genetic quality of males, and therefore it is unlikely that FA could be decreased evolutionarily by sexual selection (Polak and Taylor 2007). Indeed, estimates of the heritability of FA across multiple studies generally are lower than 5% and are generally not significantly different from zero (Fuller and Houle 2003, Polak 2008). Therefore, I conclude that phenodeviance may be the only phenotypic expression of DI that has significant evolutionary potential as a signal of genetic quality in this population.

I also found a significant positive correlation between FA and phenodeviance, where males that were phenodeviant in C1 had 91% higher FA than non-phenodeviant males. This result agrees with the findings of Polak and Taylor (2007), which showed that in the field, FA1 and MMA1 are strongly phenotypically correlated. Similar positive correlations between FA and morphological abnormalities were reported by Leary et al. (1984) in trout and by Bailit et al. (1970) in human teeth. The present findings suggest that both FA and phenodeviance are linked to the same buffering system, and that both are measures of DI (Klingenberg 2003, Polak and Taylor 2007).

4.2 Developmental instability and mating success

A second testable prediction of the developmental instability–sexual selection hypothesis is that developmental instability of secondary sexual traits should be negatively correlated with mating success (Polak 2008). The present study used two experimental designs to evaluate this prediction by examining the relationship between male mating success and two measures of developmental instability in the male sex comb, phenodeviance and FA.

Mating success assay: vials. In the first test of this prediction, I compared the mating success of males from isofemale genetic lines with divergent levels of developmental instability, by placing one virgin female from the base laboratory population with two males in vials: one male from a high-DI line and one male from a low-DI line. If sexual selection is acting
to reduce DI in this system, I predicted that males from high-DI lines would have reduced mating success compared to males from low-DI lines. Contrary to prediction, I found no significant effect of genetic line on mating success. I also found no significant effect of category of genetic line (high versus low phenodeviance) on mating success. In addition, I found no significant effect of any phenotypic measure of individual male DI on mating success, including MMA1, MMA2, FA1, and FA2, while controlling for the effects of genetic line, category of line, and male thorax length. Although these results do not lend support for the DI–sexual selection hypothesis in this system, it is possible that important environmental and social cues necessary to bring about differential mating success were absent in this vial assay. Therefore, I next attempted to assay mating success in a setting that more closely resembled the field situation.

**Mating success assay: field-mimicking chambers.** In a second test, I again compared the mating success of males from low-DI and high-DI genetic lines. However, in this experiment, I modified the experimental design by placing flies in field-mimicking chambers. These chambers imitated field conditions by including ripe mango as a substrate, allowing air flow throughout the chamber, using non-virgin females, and including a large number of flies in each chamber (100 females and 100 males).

Non-virgin females were used for several reasons. First, females of this species that have already copulated show a lower rate of mating (Polak and Simmons 2009). Therefore, the use of non-virgin females was expected to produce a lower ratio of receptive females to courting males than in the previous experiment, hypothetically increasing the potential for significant male-male competition for access to females. Indeed, I did observe that males must court for longer durations of time before securing a copulation. Second, females that have already copulated are experienced and may show increased ability to assess male quality, therefore increasing the potential for female choice in this experiment. The importance of previous experience with males in female mate choice decision making has been demonstrated in sev-
eral species, for example, in mottled sculpins (*Cottus bairdi*) (Brown 1981), pied flycatchers (*Ficedula hypoleuca*) (Dale et al. 1990), sticklebacks (*Gasterosteus aculeatus*) (Milinski and Bakker 1990), zebrafish (*Taeniopygia guttata*) (Collins 1995), and the Amazon molly (*Poe- cilia formosa*) (Marler et al. 1997). In fruit flies, Dukas (2005) demonstrated that previous experience with *D. melanogaster* males had a significant impact on female mate choice, where the size of males that virgin females mated with influenced the size of males that they mated with a day later, implicating the importance of experience on mate choice in *Drosophila*. For all these reasons, it may be expected that the relationship between male quality and mating success should be more apparent in field-mimicking chambers than in the previous vial setup.

In the field-mimicking chamber assay, I found a significant effect of phenodeviance category on the probability that a male mated, but this difference was in the opposite direction predicted by the DI–sexual selection hypothesis. Males from the high-DI category lines had a significantly higher probability of mating than males from low-DI lines.

In contrast, I did find a significant effect of individual male phenotypic expressions of developmental instability on mating success, where males that were phenodeviant in comb segment C1 had significantly lower mating success than non-phenodeviant males. However, this result must be treated with caution, because the statistical significance of the effect of MMA1 on mating success was dependent on the inclusion of an interaction term in the model, indicating that this result is probably not robust. However, this result nevertheless does suggest a possible negative effect of phenodeviance on mating success, which would support the DI–sexual selection hypothesis.

In contrast to the present study, Polak and Taylor (2007) found that in the field, copulating males of this species had significantly lower FA and phenodeviance than single males, and the effects of FA1 and MMA1 on copulation probability were consistent across days. However, the field study could not measure or account for potential differences in levels of developmental stress experienced by each male. Therefore, it is possible that differences in
mating success for these males may be entirely attributable to variation in individual quality due to developmental conditions, such as nutritive or heat stress.

Unlike in the field study, the present study carefully controlled for levels of environmental stress in mating success assays by rearing larvae under conditions of controlled larval density, temperature, and food availability. Because I found no significant effect of FA on mating success in conditions of experimentally controlled, low stress levels, the present study fails to account for the field association between mating success and low DI. However, I did find a significant reduction in mating success for males that were phenodeviant in C1, consistent with Polak and Taylor’s (2007) findings.

If DI is condition dependent, such that the correct development of a morphological trait depends on a limited quantity of resources available to be directed towards fitness-related traits (Tomkins et al. 2004), then it is also possible that stressful developmental conditions are necessary to reduce the pool of resources available to a male and thus limit his ability to undergo correct development of the sex comb. This may occur because he must direct resources into physiological processes necessary to survive under stress. Without stress, male FA or phenodeviance may not be a reliable indicator of male quality, since even low-quality males could potentially have enough energy to direct into correct development of the sex comb. In other words, under stressful conditions, individuals of lower genetic quality may experience greater difficulties and become ‘unmasked’ (Lens et al. 2002, Woods et al. 2002, Hendrickx et al. 2003). It is possible that the experimental design used in the present study, where larvae had access to optimal temperatures during development, may have obfuscated any differences among males in inherent quality.

Numerous studies across various taxa have demonstrated a positive correlation between FA in morphological traits and environmental stresses during development, including extreme temperature stress. For example, Parsons (1962) found that *D. melanogaster* raised at 30°C had significantly higher FA of sternopleural bristles than flies raised at 25°C. Similarly, Imasheva et al. (1997) reared two species of fruit fly, *D. melanogaster* and *D. buzzati*, under
stressful high temperatures and found significant increases in FA of wing length and FA of the number of arista branches in both species. Vishalakshi and Singh (2008) found a general increase in a multiple-trait composite FA in *D. ananassae* when reared under both high- and low-temperature stress. However, the response of FA to environmental stress appears to be inconsistent and specific to the trait, species, and type of stress (Bjorksten *et al.* 2000).

It remains unclear whether DI is condition-dependent; correlational studies in several species (e.g. Solberg and Saether 1993, Blanckenhorn *et al.* 1998) have shown a negative correlation between FA and body condition, while others (e.g. Ketola *et al.* 1997, Côté and Festa-Bianchet 2001) have failed to find such a relationship. Stronger evidence for the condition-dependence of DI should be expected from experimental studies, but such studies have generally failed to report significant condition-dependence of FA in secondary sexual traits (reviewed in Polak 2008).

**Mating latency.** In addition to comparing males with differing levels of DI in terms of absolute mating success, I also compared them in terms of mating latency, or the time elapsed before a male obtained a copulation in the presence of females. I predicted that males from high-DI genetic lines would be slower to mate. I also predicted that males with phenodeviant and asymmetrical sex combs would be slower to obtain copulations. Two previous studies have shown a negative relationship between FA and mating latency. Radesäter and Halldórsdóttir (1993) exposed virgin female earwigs to males with symmetrical and asymmetrical cerci, and found that mating latency was significantly higher for asymmetrical males. Similarly, Polak and Stillabower (2004) compared the mating latency of *Drosophila immigrans* males from genetic lines with high and low levels of sternopleural bristle FA, and found that males from high-FA lines were significantly slower to mate. Individual male positional fluctuating asymmetry also positively predicted mating success, with asymmetrical males being slower to mate (Polak and Stillabower 2004).

In this study, I found that neither genetic line nor phenodeviance category significantly
predicted mating latency in the vial assay. However, in the complex social environment of the field-mimicking chambers, genetic lines did significantly differ in mating latency, and as specifically predicted, males from high-DI lines were significantly slower to mate. Thus, genotypic effects appear to have been important to mating latency only in the complex micro-environment that mimicked the field situation.

I predicted that phenodeviant males would be slower to obtain copulations in the presence of females. Although I did find that males that were phenodeviant in C1 were slower to mate in the vial assay, I found no other significant effect of sex comb phenodeviance on mating latency in the vial assay or the field-mimicking chamber assay. I also predicted that males with asymmetrical sex combs would be slower to mate, but I obtained conflicting results. In the vial assay, I found that males that were asymmetrical in C1 were actually faster to mate than their symmetrical counterparts, contrary to prediction. However, I did find that males that were asymmetrical in C2 were slower to mate in the field-mimicking chamber assay, consistent with previous studies.

The conflicting nature of these results makes explanation difficult. It is unclear why asymmetric males had an apparent advantage in speed of obtaining copulations in the vial assay. However, I cautiously interpret the negative effect of FA2 on latency in the field-mimicking chambers as a disadvantage for high-DI males in terms of ability to obtain copulations in a complex social arena, consistent with the DI-sexual selection hypothesis. Shorter copulation latencies could potentially lead to a greater number of lifetime reproductive partners for low-DI males, driving selection to reduce DI of the sex comb.

4.3 Fitness consequences of developmental instability

A third prediction of the DI–sexual selection hypothesis that I evaluated was that if developmental instability is important for sexual selection, because it is a signal of underlying genetic quality or 'good genes,' then FA and phenodeviance of a secondary sexual trait should be negatively correlated with individual quality indicators (Polak 2008). To test this prediction,
I compared isofemale genetic lines with divergent levels of developmental instability in terms of two components of individual fitness at different life stages. The first test compared the survivorship of juveniles from different genetic lines, spanning from the first instar stage until adulthood. If DI is negatively correlated with offspring survival in this system, I expected potential decrements in preadult survivorship for flies from high-DI genetic lines. The second test compared the hatch rates of eggs sired by males from different genetic lines. Reduced egg hatch rate could potentially reflect poor individual quality in two ways: it could indicate a lower quality of paternal ejaculate such that fewer eggs were fertilized, or it could indicate reduced embryonic offspring performance, which could be a reflection of the contributions of a sub-optimal paternal genotype.

**Preadult survivorship.** In the preadult survivorship assay, I raised juveniles from the first instar stage into adulthood and compared survivorship among genetic lines from high and low phenodeviance categories. Although significant differences in survivorship were found among genetic lines, the phenodeviance category (high versus low phenodeviance) of these lines did not significantly predict survivorship. These results indicate that the genotypic differences underlying DI do not influence survivorship of juveniles in this species. Therefore, these results do not support the prediction that reduced juvenile survivorship is a fitness cost associated with DI.

**Egg hatch rate.** In the egg hatch rate assay, I examined the hatch rate of eggs sired by males from high and low phenodeviance genetic lines. Females were randomly sampled from a general base population, so this experiment tested for sire effects unconfounded by potential maternal effects associated with DI. I found no significant effect of genetic line, phenodeviance category of each line, paternal FA, paternal phenodeviance, or any other factor on egg hatch rate. Therefore, these results do not support the hypothesis that reduced egg hatch rate is a fitness cost associated with DI in this system. These results also suggest that sperm from males from high-DI and low-DI lines do not differ in fertilization
success, at least under non-sperm competitive conditions. Several studies have demonstrated a significant negative relationship between FA and ejaculate quality, including number of sperm and motility of sperm (e.g. Roldan et al. 1998, Manning et al. 1998, Farmer and Barnard 2000, Firman et al. 2003). These results may contrast with the present study, since ejaculate quality should significantly affect egg hatch rate, and the present study showed no difference in egg hatch rate between high-DI and low-DI males.

4.4 Evaluating the DI–sexual selection hypothesis

I evaluated three key predictions of the DI–sexual selection hypothesis in this study system. The first prediction, that DI has a genetic basis, was confirmed by the successful isolation of isofemale genetic lines that differed in levels of phenodeviance across multiple generations.

The second prediction, that there should be a negative association between DI and mating success, was generally not supported by my findings in terms of either FA or phenodeviance, with one exception: I found that males that were phenodeviant in C1 had significantly lower probability of mating in field-mimicking mating chambers. However, I did find a suggestion of a significant reduction in mating latency for high-DI males, where males that were phenodeviant in C1 took longer to mate than non-phenodeviant males in vials, and males with asymmetry in C2 took longer to mate than symmetric males in chambers. These differences in mating latency could potentially lead to differences in lifetime levels of mating success among males in the field. However, these results are tempered by the fact that I also found that males that were asymmetric in C1 were actually faster to mate in vials.

A reduction in mating latency for males that were phenodeviant in C1 in the vial assay may reflect reduced male-male competitive ability in this system, potentially caused by a mechanical disadvantage for such males in obtaining copulations. Since the sex comb plays a crucial role in the ability of males to grasp females, any structural irregularity may negatively impact the mechanical ability of sex combs to anchor the male in the proper position for copulation in a competitive context. Indeed, a preliminary study indicated that males with
sex combs surgically removed showed a complete inability to obtain copulations (Rashed, personal communication; Polak, personal communication). This possibility is consistent with my findings of significantly higher mating latency for males that are phenodeviant in C1 in both mating success assays. A mechanical hindrance in grasping females could easily lead to these males being slower to obtain a copulation, which could drive the reduction of DI in the population, especially when competition for receptive females is high and the window of mating opportunity is small. FA or phenodeviance may also reduce the combative ability of sex combs if they are used as weapons against rival males (Tomkins and Simmons 2003).

The third prediction, that DI should reliably indicate individual quality, was not supported by the present findings. I found that egg hatch rate, a potential indicator of paternal ejaculate quality, was not negatively associated with DI. I also found no association between DI and preadult survivorship, a measure of juvenile survivorship. However, I tested only two potential measures of individual quality, and it is possible that FA or phenodeviance may reliably indicate some other individual fitness trait in this system, for example, body condition, longevity or vigor of high-DI males or their offspring.

Another prediction of the DI–sexual selection hypothesis not tested in the present study is that phenotypic expressions of DI should be assessed by mates or rivals (Polak 2008), but it is unclear whether females in this species possess sufficient sensory abilities for sensitive detection of asymmetry or phenodeviance of the sex comb. Because males primarily court and mount females from the sides and rear, it is unlikely that females use visual cues to assess male asymmetry or phenodeviance of the sex comb. Indeed, visual assessment of morphological asymmetry by females is thought to be rare in most systems because of physiological limits to female perceptual capabilities and costs of detection (such as lost time) (Gangestad and Thornhill 2003, Polak 2008). However, it is possible that females may receive tactile cues about male sex combs while male foretarsi contact the female abdomen during copulation attempts (Polak and Taylor 2007).

If asymmetry and phenodeviance of the sex comb are “cryptic,” meaning that females or
competing males are unable to directly detect or assess phenodeviance or asymmetry of the
sex comb, a final possibility is that asymmetry and phenodeviance of the comb may simply
be phenotypically linked with some other aspect of male quality, such as reduced vigor or
body condition (Polak 2008). If this is the case, we cannot conclude that developmental
instability itself is a target of sexual selection (Polak 2008).

A possible explanation for sexual selection to reduce DI of sex combs, shown by Polak
and Taylor (2007) in the field population, that is not examined in the present study is a
postcopulatory competitive disadvantage for developmentally instable males in situations
where females remate and ejaculates from multiple males may be present within a female.
Some experimental evidence thus far suggests an important role for post-copulatory sexual
selection in this species. Polak and Simmons (2009) showed a positive correlation between
sex comb size and competitive fertilization ability in *D. bipectinata*, although this difference
could also be attributable to cryptic female choice. In contrast to Polak and Simmons’ (2009)
study, I evaluated the fertilization success of males who were the only potential father to a
clutch of eggs, such that no sperm competition would have been possible.

Rashed and Polak (2009) compared the fertilization success of *D. bipectinata* genetic
lines differing in sex comb size in a noncompetitive environment and found that despite
a significantly higher incidence of insemination failure for small sex comb lines, the two
categories of sex comb size did not differ in egg hatch rate of sired eggs. Given that the
sex comb is condition dependent, it is possible that males of higher inherent quality or
condition may better be able to direct resources into both the development of the sex comb
and also into ejaculate quality. However, differences in ejaculate quality may only be of
importance in competitive contexts. Therefore, it is possible that sexual selection to reduce
developmental instability of the sex comb may be occurring due to decreased *competitive*
fertilization success in high-DI males of this species when females mate with multiple males.
Further studies investigating the relationship between sex comb FA and phenodeviance on
competitive fertilization success would be worthwhile for evaluating this possibility.
To summarize, the present study evaluated three predictions of the DI–sexual selection hypothesis in a study system shown by Polak and Taylor (2007) to be undergoing significant sexual selection for reducing DI in the field. However, only one of these predictions, that DI has a genetic basis, was confirmed in the laboratory under controlled conditions. Perhaps the most basic prediction of the DI–sexual selection hypothesis is that DI must be associated with reduced mating success. However, I failed to consistently support this prediction in the laboratory, where I obtained heterogeneous results, depending on the measure of male sex comb DI examined and the specific micro-environment in which flies were observed. In addition, I did not find support for the third prediction, that DI is associated with reduced individual quality. Therefore, this study has generally failed to lend support to the viability of the DI–sexual selection hypothesis.

Is the DI–sexual selection hypothesis still viable? In a recent review, Polak (2008) makes a strong case that it is not. Although researchers have been demonstrating reduced sexual success for asymmetric individuals across numerous species since the early 1990s, and there is also considerable evidence that sexual mates and rivals in many cases may directly assess measures of DI, several other important predictions of the hypothesis remain poorly supported by studies to date (Polak 2008). Perhaps most significantly, there is little evidence that FA in secondary sexual traits is heritable, with most reliable estimates for average $h^2_{FA}$ falling below 5% (reviewed in Polak 2008). If measures of DI are not heritable, then they cannot reflect a potential mate’s genetic quality to be imparted to offspring (Polak 2008). In addition, support has been weak and inconsistent for the predictions that measures of DI should be condition dependent and should be negatively correlated with sexual ornament size (Polak 2008).

Although the present study was not exhaustive in its scope, the findings here presented do not lend sufficient support to the DI-sexual selection hypothesis as an explanation for why Polak and Taylor (2007) observed significant sexual selection for reducing sex comb DI in the field population of D. bipectinata in Noumea. A major problem for the interpretation
of Polak and Taylor’s (2007) findings is that developmental conditions experienced by males in the field were unknown. Therefore, it is quite possible that both reduced mating success and sex comb DI were responses to relatively poor developmental conditions and exposure to common environmental stresses experienced by those males.

At any rate, my results do not support the hypothesis that this particular system is under sexual selection as originally formulated by the DI-sexual selection hypothesis. Whether the DI-sexual selection hypothesis is a good explanation for the negative associations between DI and reproductive fitness in other species and populations is beyond the scope of this work.

An important question yet to be answered, especially in light of the waning support for the DI-sexual selection hypothesis as originally formulated (Møller 1990, 1992; Møller and Pomiankowski 1993), is why a number of species (e.g. humans, zebra finches, sticklebacks, wolf spiders, and barn swallows) have seemingly independently evolved sexual preferences for symmetry (Polak 2008). Long-term, in-depth experimental studies using species well adapted for controlled experimentation are called for to help discover the mechanisms working to keep sexual selection against asymmetry and phenodeviance active in many populations. In particular, studies utilizing experimental manipulation of secondary sexual trait asymmetry and phenodeviance may be exceptionally useful in helping to settle this twenty year-long debate among evolutionary biologists.

5 Summary and General Conclusions

The present study evaluated three key predictions of the developmental-instability hypothesis in *Drosophila bipectinata*. The results can be summarized as follows:

1. The first prediction is that DI (as measured by phenodeviance here) has a genetic basis. Ten isofemale lines were characterized in terms of phenodeviance of the male sex comb and found to differ significantly in phenodeviance across multiple generations, confirming the stability of genotypic effects on phenodeviance over time in my study.
2. The second prediction is that DI of a secondary sexual trait should be negatively correlated with mating success.

(a) In the vial mating success assay, neither the phenodeviance category of a male’s genetic line nor the individual male’s phenotypic DI (as phenodeviance and FA) significantly predicted mating success, contrary to prediction.

(b) In the field-mimicking chamber assay, I found conflicting results: phenodeviance in C1 was associated with reduced mating success, while males from high-DI lines had higher mating success than those from low-DI lines, contrary to prediction.

3. The third prediction of the DI–sexual selection hypothesis is that high DI should be associated with reduced individual quality.

(a) Survivorship of preadults (larvae and pupae) was not significantly predicted by the phenodeviance category of the genetic line, contrary to prediction.

(b) Egg hatch rate was not significantly predicted by the phenodeviance category of the genetic line or by the paternal phenotypic DI (as FA and phenodeviance), also contrary to my prediction.

4. General conclusions about the DI–sexual selection hypothesis

(a) The present findings do not lend substantial support to the DI-sexual selection hypothesis as an explanation for the observed sexual selection for reducing DI in the field population.

(b) Generally weak support across many studies for several important predictions of the hypothesis put its future in doubt.

5. Suggestions for future directions
(a) In this system, additional studies are called for that examine the relationship between DI and post-copulatory sexual selection, especially sperm competition. In addition, the importance of stress, especially during development, should be carefully assayed to determine the role that stress plays in sexual selection for reducing DI.

(b) In other systems, long-term, in-depth experimental studies using species well adapted for controlled experimentation are called for to help discover the mechanisms working to keep sexual selection against asymmetry and phenodeviance active in many populations.
References


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Figure 1. Scanning electron micrograph (1500 X) of A) normal *Drosophila bipectinata* male sex comb with teeth in a straight line, B) phenodeviant male sex comb with abnormally positioned tooth (indicated by arrow) in front of comb, and C) phenodeviant male sex comb with abnormal teeth (indicated by arrows) in front of and behind comb. Scale bar represents 20 µm.
Figure 2. Experimental design for field-mimic mating success chamber assay. Each chamber was lined with fresh mango slices and contained 100 experienced females from the base population, 50 virgin males from a low-developmental instability line, and 50 virgin males from a high-developmental instability line.
Figure 3. Field-mimic competitive mating success chamber used for the observation of mating behavior.
Figure 4. Mating chambers used for harvesting larvae, constructed from 225-mL plastic cups inverted over 9.5-cm diameter glass Petri dishes, lined with 30 mL grape juice agar substrate.
Figure 5. Distribution of proportion phenodeviance of 50 isofemale lines at F₅ isolated from a base laboratory population of *Drosophila bipectinata*. 
Figure 6. Proportion phenodeviance for males in ten genetic lines selected in two categories from all F$_5$ genetic lines.
Figure 7. Pooled proportion phenodeviance of males from genetic lines with divergent levels of phenodeviance across multiple scored generations.
Figure 8. Least-squares mean FA1 for phenodeviant and non-phenodeviant males. Phenodeviant males had 91% greater FA1 than non-phenodeviant males. Error bars represent ±1 standard error.
Figure 9. Least-squares mean latency to mate for males that were or were not phenodeviant in comb segment C1 in vial mating success assays. Error bars represent ±1 standard error.
Figure 10. Least-squares mean latency to mate for males that were or were not phenodeviant in comb segment C2 in vial mating success assays. Error bars represent ±1 standard error.
Figure 11. Effects of phenodeviance category (high versus low phenodeviance lines) on predicted probability of mating success in field-mimic mating success assays.
Figure 12. Proportion survivorship of *D. bipectinata* preadults (larvae and pupae) from ten genetic lines with divergent levels of phenodeviance. Proportion data were arcsine square-root transformed. Error bars represent ±1 standard error.
Figure 13. Proportion survivorship of *D. bipectinata* preadults (larvae and pupae) from two divergent categories of genetic lines (high versus low phenodeviance). Proportion data were arcsine square-root transformed. Error bars represent ±1 standard error.
Figure 14. Mean egg hatch rate of eggs sired by males from ten genetic lines with divergent levels of phenodeviance plotted against proportion phenodeviance of each line at F5. Clutches of less than ten eggs were excluded. No significant relationship existed (proportion phenodeviance: slope = 0.0082, t = 0.07, P = 0.94).
Table 1. Binary logistic regression on male mating success in the vial mating success assay.

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Table 2. Binary logistic regression on male mating success in the vial assay, including phenodeviance category as a factor.

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Table 3. Binary logistic regression for mating success of males during the field-mimic mating success assay.

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<td>0.041</td>
</tr>
<tr>
<td>MMA2</td>
<td>-0.29</td>
<td>0.16</td>
<td>-1.8</td>
<td>0.066</td>
</tr>
<tr>
<td>FA1</td>
<td>-0.05</td>
<td>0.11</td>
<td>-0.44</td>
<td>0.66</td>
</tr>
<tr>
<td>FA2</td>
<td>-0.16</td>
<td>0.11</td>
<td>-1.4</td>
<td>0.17</td>
</tr>
<tr>
<td>FA1 × MMA1</td>
<td>0.21</td>
<td>0.11</td>
<td>1.9</td>
<td>0.060</td>
</tr>
<tr>
<td>FA2 × MMA2</td>
<td>0.17</td>
<td>0.11</td>
<td>1.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Size of C1</td>
<td>-0.36</td>
<td>0.11</td>
<td>-3.2</td>
<td>0.0016</td>
</tr>
<tr>
<td>Size of C2</td>
<td>-0.06</td>
<td>0.10</td>
<td>-0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>Block 1</td>
<td>-0.14</td>
<td>0.11</td>
<td>-1.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Block 2</td>
<td>0.30</td>
<td>0.10</td>
<td>2.9</td>
<td>0.0034</td>
</tr>
<tr>
<td>Thorax Length</td>
<td>0.10</td>
<td>0.026</td>
<td>4.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>N</td>
<td>989</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Binary logistic regression on mating success of males during the field-mimic mating success assay, analyzing the effects of phenodeviance category.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−4.7</td>
<td>2.0</td>
<td>−2.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Phenodeviance category</td>
<td>0.33</td>
<td>0.081</td>
<td>4.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMA1</td>
<td>−0.24</td>
<td>0.14</td>
<td>−1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>MMA2</td>
<td>−0.23</td>
<td>0.16</td>
<td>−1.4</td>
<td>0.14</td>
</tr>
<tr>
<td>FA1</td>
<td>−0.05</td>
<td>0.11</td>
<td>−0.45</td>
<td>0.63</td>
</tr>
<tr>
<td>FA2</td>
<td>−0.15</td>
<td>0.11</td>
<td>−1.4</td>
<td>0.18</td>
</tr>
<tr>
<td>FA1 × MMA1</td>
<td>0.20</td>
<td>0.11</td>
<td>1.8</td>
<td>0.076</td>
</tr>
<tr>
<td>FA2 × MMA2</td>
<td>0.15</td>
<td>0.11</td>
<td>1.4</td>
<td>0.16</td>
</tr>
<tr>
<td>C1size</td>
<td>−0.20</td>
<td>0.10</td>
<td>−2.0</td>
<td>0.055</td>
</tr>
<tr>
<td>C2size</td>
<td>−0.083</td>
<td>0.10</td>
<td>−0.83</td>
<td>0.41</td>
</tr>
<tr>
<td>Block 1</td>
<td>−0.13</td>
<td>0.11</td>
<td>−1.2</td>
<td>0.22</td>
</tr>
<tr>
<td>Block 2</td>
<td>0.28</td>
<td>0.10</td>
<td>2.8</td>
<td>0.0054</td>
</tr>
<tr>
<td>TL</td>
<td>0.085</td>
<td>0.024</td>
<td>3.5</td>
<td>0.0004</td>
</tr>
<tr>
<td>N</td>
<td>989</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Results of analysis of covariance showing effects of fluctuating asymmetry on mating latency after field-mimic mating success assays. Male body size was not significant and was excluded.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>1</td>
<td>$7.991 \times 10^5$</td>
<td>0.04</td>
<td>0.8413</td>
</tr>
<tr>
<td>FA2</td>
<td>1</td>
<td>$1.086 \times 10^6$</td>
<td>5.46</td>
<td>0.0203</td>
</tr>
<tr>
<td>Line (phenodeviance category)</td>
<td>6</td>
<td>$6.121 \times 10^7$</td>
<td>3.08</td>
<td>0.0063</td>
</tr>
<tr>
<td>Phenodeviance category</td>
<td>1</td>
<td>$3.454 \times 10^7$</td>
<td>1.74</td>
<td>0.1888</td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>$6.458 \times 10^7$</td>
<td>3.25</td>
<td>0.0405</td>
</tr>
<tr>
<td>Size of C1</td>
<td>1</td>
<td>$1.460 \times 10^7$</td>
<td>0.73</td>
<td>0.3925</td>
</tr>
<tr>
<td>Size of C2</td>
<td>1</td>
<td>$2.214 \times 10^7$</td>
<td>1.11</td>
<td>0.2925</td>
</tr>
<tr>
<td>Error</td>
<td>256</td>
<td>$1.989 \times 10^7$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f., degrees of freedom
Table 6. Results of analysis of covariance showing effects of phenodeviance on mating latency after field-mimic mating success assays. Male body size was not significant and was excluded.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA1</td>
<td>1</td>
<td>$4.953 \times 10^6$</td>
<td>0.24</td>
<td>0.6218</td>
</tr>
<tr>
<td>MMA2</td>
<td>1</td>
<td>$9.529 \times 10^5$</td>
<td>0.05</td>
<td>0.8287</td>
</tr>
<tr>
<td>Line (phenodeviance category)</td>
<td>6</td>
<td>$5.709 \times 10^7$</td>
<td>2.81</td>
<td>0.0115</td>
</tr>
<tr>
<td>Phenodeviance category</td>
<td>1</td>
<td>$2.607 \times 10^7$</td>
<td>1.28</td>
<td>0.2582</td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>$6.429 \times 10^7$</td>
<td>3.17</td>
<td>0.0438</td>
</tr>
<tr>
<td>Size of C1</td>
<td>1</td>
<td>$8.315 \times 10^6$</td>
<td>0.41</td>
<td>0.5228</td>
</tr>
<tr>
<td>Size of C2</td>
<td>1</td>
<td>$9.028 \times 10^6$</td>
<td>0.44</td>
<td>0.5055</td>
</tr>
<tr>
<td>Error</td>
<td>256</td>
<td>$2.030 \times 10^7$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f., degrees of freedom
Table 7. Analysis of variance on the proportion survivorship of preadults (larvae and pupae) from genetic lines of high and low phenodeviance. Proportion survivorship was arcsine square-root transformed.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenodeviance category</td>
<td>1</td>
<td>0.0078</td>
<td>0.42</td>
<td>0.52</td>
</tr>
<tr>
<td>Line (phenodeviance category)</td>
<td>8</td>
<td>0.038</td>
<td>2.09</td>
<td>0.043</td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>0.089</td>
<td>4.86</td>
<td>0.0096</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f., degrees of freedom
Table 8. Analysis of covariance on hatch rate of eggs sired by males from high and low phenodeviance genetic lines. (Hatch rate was calculated only for clutches of 10 or more eggs.)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA1</td>
<td>1</td>
<td>0.029</td>
<td>0.40</td>
<td>0.53</td>
</tr>
<tr>
<td>MMA2</td>
<td>1</td>
<td>0.18</td>
<td>2.56</td>
<td>0.12</td>
</tr>
<tr>
<td>FA1</td>
<td>1</td>
<td>0.016</td>
<td>0.22</td>
<td>0.64</td>
</tr>
<tr>
<td>FA2</td>
<td>1</td>
<td>0.15</td>
<td>2.12</td>
<td>0.15</td>
</tr>
<tr>
<td>Phenodeviance category</td>
<td>1</td>
<td>0.095</td>
<td>1.33</td>
<td>0.26</td>
</tr>
<tr>
<td>Line (Phenodeviance category)</td>
<td>8</td>
<td>0.045</td>
<td>0.63</td>
<td>0.75</td>
</tr>
<tr>
<td>Size of C1</td>
<td>1</td>
<td>0.00044</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>Size of C2</td>
<td>1</td>
<td>0.0021</td>
<td>0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td>0.07</td>
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<td></td>
</tr>
</tbody>
</table>

d.f., degrees of freedom