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Cite this article: Polak M, Tomkins JL. 2013

Developmental selection against developmental instability: a direct demonstration. *Biol Lett* 9: 20121081. <http://dx.doi.org/10.1098/rsbl.2012.1081>

Received: 19 November 2012

Accepted: 18 December 2012

Subject Areas:

evolution

Keywords:

developmental selection, strength of natural selection, developmental instability, phenodeviance

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2012.1081> or via <http://rsbl.royalsocietypublishing.org>.

Evolutionary biology

Developmental selection against developmental instability: a direct demonstration

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Developmental selection, the non-random elimination of offspring during development, is hypothesized to alter the opportunity for selection on a given trait at later stages of the life cycle. Here, we provide a direct demonstration of developmental selection against developmental instability, assessed as the incidence of minor, discrete phenotypic abnormalities in the male sex comb, a condition-dependent secondary sexual trait in *Drosophila bipectinata*. We exposed developing flies from two geographically separate populations to increasing levels of temperature stress, and recovered the males that died during development by teasing them out of their pupal cases. These dead males, the so-called ‘invisible fraction’ of the population, were more developmentally unstable than their surviving counterparts, and dramatically so under conditions of relatively high temperature stress. We illustrate that had these dead juvenile flies actually survived and entered the pool of sexually mature adult individuals, their mating success would have been significantly reduced, thus intensifying sexual selection in the adult cohort for reducing developmental instability. The data suggest that without accounting for developmental selection, a study focusing exclusively on the adult cohort may unwittingly underestimate the net force of selection operating on a given phenotypic trait.

1. Introduction

Natural selection, the mechanism of adaptive evolution, is widely accepted to be the principal force shaping phenotypic diversity in natural populations [1]. A great deal of effort over recent decades has been aimed at quantifying the strength and direction of selection, enabling a number of major syntheses of this literature [2–4]. One general pattern that has become evident is that whereas the strength of directional selection can be quite strong, there exists a great deal of variability across selection estimates [2–4]. This result is aptly reflected in the fact that absolute values of linear selection typically are approximately exponentially distributed, such that large values form a long ‘tail’ and that small values are most common, clustering around zero [2,3]. Discussion continues to focus on potential bias in the selection data [3–5], such as the possibility that estimates of weak selection based on small sample sizes may be underrepresented in the literature owing to publication bias.

Here, we suggest a biological source of potential bias on selection estimates, stemming from the Darwinian ‘struggle for existence’ [6]: the ubiquitous truth of nature that parents produce many more offspring than ever survive to reproduce. If, as Darwin supposed, these offspring die during development not entirely at random, but in relation to a heritable trait, this developmental selection [7] could attenuate the strength of selection in the surviving fraction of the population. Because studies of natural selection tend to limit their analysis to how traits affect survival and reproduction at the adult stage [3], developmental

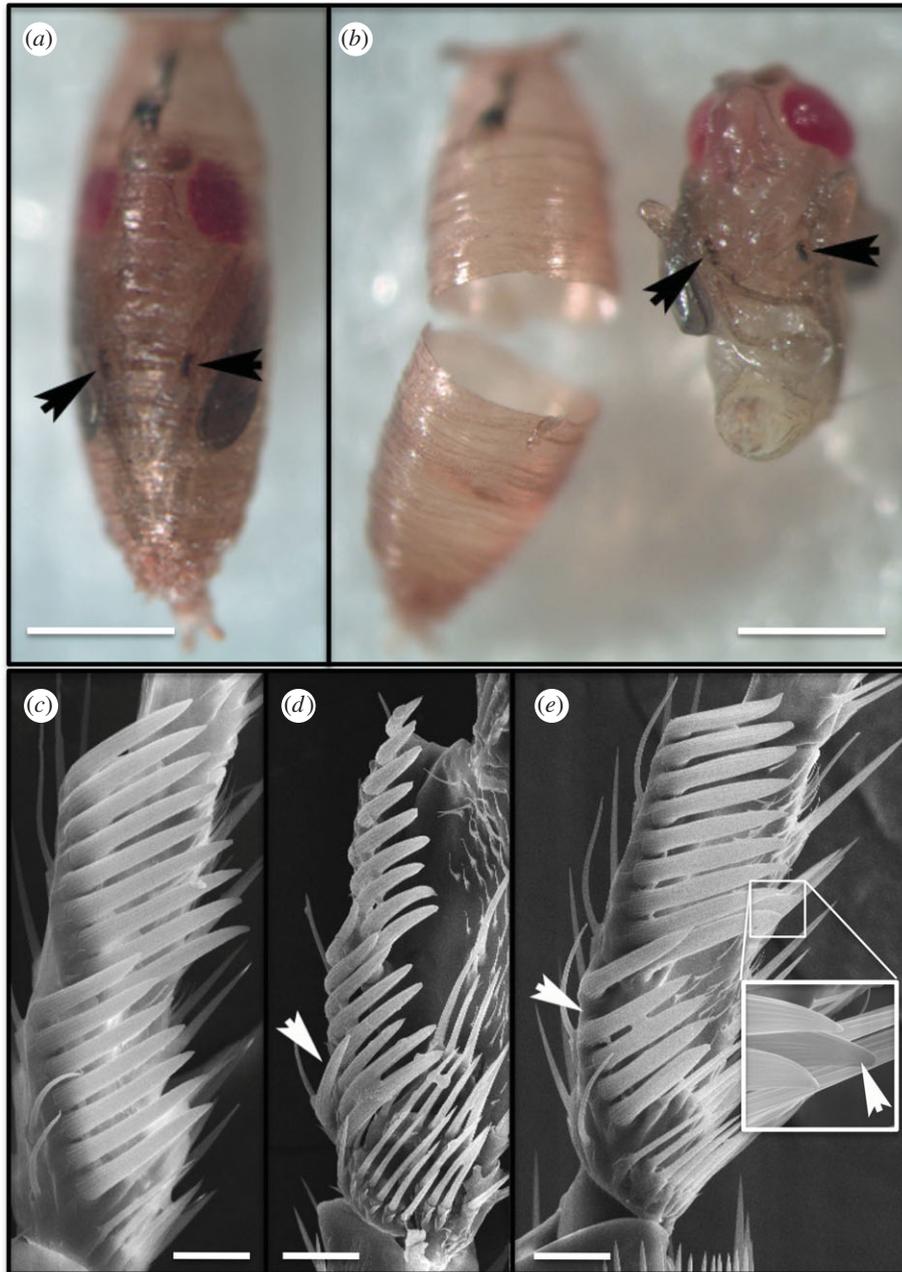


Figure 1. Scanning electron micrographs of (a) a dead pupal male, and (b) the same male after extraction from its pupal case; arrows in (a,b) point to the sex combs, which exhibit considerable phenodeviance. (c) Shows an intact comb, and (d,e) show phenodeviant combs. Arrow in (d) points to a misplaced tooth emerging anterior to row 1; arrows in (e) indicate a gap between teeth and a misplaced tooth posterior to C2. Scale bars (a,b) 0.5 mm, and (c–e) 20 μ m. (Online version in colour.)

selection may be an overlooked source of downward bias on selection estimates.

We demonstrate developmental selection on a trait that, importantly, is both heritable and under selection among adults, by showing just how remarkably different the individuals that die young can indeed be compared with those surviving the developmental gauntlet. The trait is developmental instability [8], assessed as the incidence and number of minor, discrete morphological abnormalities or phenodeviations [8,9], in the male sex comb of *Drosophila bipectinata* Duda (Diptera: Drosophilidae). In *D. bipectinata*, sex comb developmental instability is a target of pre-copulatory sexual selection in a natural New Caledonian population, because males with reduced developmental instability enjoy higher mating success [10].

In the present study, we exposed fly pupae to three thermal environments resulting in differential levels of mortality; we focused on pupae because the sex comb appears during

this stage of development. We acquired both males that survived, and by teasing them out of their pupal cases, also the males that died during development. We were thus able to directly contrast the incidence and number of sex trait phenodeviations between the flies that died, the so-called ‘invisible fraction’ [11] and the survivors across increasing levels of environmental stress.

2. Material and methods

We used two geographically separate populations of *D. bipectinata* initiated in the laboratory in 2006; one population was from New Caledonia and the other from Taiwan. Collection localities and methods, fly culturing and temperature treatments are described in detail elsewhere [12]. In November 2006, pupae were exposed to low (constant 25°C), intermediate (constant 29°C), and high stress (cycling between 18 h at 29°C and 6 h at 34°C) treatments based on their relative effects on mortality (see fig. 1 in Polak & Tomkins

Table 1. (A) Results of logistic regression on incidence of phenodeviance in the sex comb as a whole. The population \times mortality ($\chi^2 = 1.714$, d.f. = 1, $p = 0.190$) and the population \times temperature ($\chi^2 = 0.306$, d.f. = 2, $p = 0.86$) interactions were non-significant, so they were excluded from the model. Model fit to the data was good ($\chi^2 = 1.902$, d.f. = 4, $p = 0.75$). (B) Results of ANOVA on $\log_{10}(y + 2)$ -transformed residual number of sex comb phenodeviations. The population \times mortality ($F_{1,428} = 0.54$, $p = 0.46$) and the population \times temperature ($F_{2,428} = 0.15$, $p = 0.86$) interaction terms were likewise non-significant. Model fit to the data was good ($F_{1,418} = 0.006$, $p = 0.94$).

source	d.f.	χ^2	p	
(A) logistic regression				
population	1	1.404	0.24	
temperature treatment	2	23.118	< 0.0001	
mortality status	1	0.0732	0.79	
temperature \times mortality	2	11.399	0.0033	
source	d.f.	mean square	F	p
(B) ANOVA				
population	1	0.4229	11.70	0.0007
temperature treatment	2	0.5304	14.67	< 0.0001
mortality status	1	0.2780	7.69	0.0058
temperature \times mortality	2	0.2037	5.64	0.0038
error	431	0.0362		

[12]). When all adult emergences had ceased, adult male flies that had emerged from their pupal cases were characterized (see below) under a stereomicroscope. All males with discernible phenotypic traits that died prior to emergence were gently teased out of their pupal cases (figure 1*a,b*) in a drop of water on a depression slide, and characterized. For surviving ($n = 407$) and dead ($n = 31$) males, the total number of teeth in comb segment 1 (TC1) and comb segment 2 (TC2) were counted on each foreleg (figure 1*c*), and all phenodeviations, i.e. deviations from normal phenotype [10] in the sex comb recorded; phenodeviations are diagnostic of developmental instability [8,9,13]. Here, a phenodeviation occurred as either a misplaced tooth (figure 1*d,e*) or a break (figure 1*e*) in a row of teeth [10]. Total comb size (TC12) was calculated as TC1 + TC2. The raw data are provided in the electronic supplementary material.

We used multiple logistic regression [14] to test for the effects of population, temperature treatment and mortality status on whether or not the sex comb contained at least one phenodeviation. In an alternative approach, we regressed the total number of phenodeviations (range 0–14, $n = 438$ flies) on TC12 (slope (s.e.) = 0.1649 (0.03780), $F_{1,436} = 19.03$, $p < 0.0001$), and then $\log_{10}(y + 2)$ transformed the residuals. Transformed residuals were subjected to factorial analysis of variance (ANOVA) using the same terms noted above. But, because the transformation failed to adequately normalize the residuals (Shapiro–Wilk $W = 0.93$, $p < 0.001$), we also tested non-parametrically (Wilcoxon signed-rank test) for differences between dead and live flies separately at each temperature.

To predict adult male mating probability (π) we used the following equation [14]:

$$\pi = \frac{e^{\alpha + \beta_1(\text{FA1}) + \beta_2(\text{Pheno1}) + \beta_3(\text{FA1} \times \text{Pheno1})}}{1 + e^{\alpha + \beta_1(\text{FA1}) + \beta_2(\text{Pheno1}) + \beta_3(\text{FA1} \times \text{Pheno1})}}$$

where α is the intercept, β_s are regression coefficients [10], and FA1 and Pheno1 are fluctuating asymmetry and incidence of phenodeviance in comb segment 1, respectively. We used this equation in an exercise to illustrate the potential effect of developmental selection on mating probabilities and by extension, on sexual selection. π was first calculated using average phenotypic predictors from the Noumea field population studied previously [10], and then after substituting mean incidence of phenodeviance (0.762) of the dead males from the highest stress category for the field value (0.222).

3. Results

Logistic regression on the incidence of phenodeviance in the sex comb revealed a significant effect of temperature treatment, but not of population (table 1A), consistent with a previous report [12]. Importantly, the analysis also revealed a significant interaction between temperature treatment and mortality status (table 1A), reflecting the elevated incidence of phenodeviance among dead males relative to surviving males at the intermediate and high temperature treatments compared with the low temperature treatment (figure 2*a*).

Factorial ANOVA on transformed *number* of phenodeviations revealed strongly significant effects of mortality status and the temperature treatment \times mortality status interaction (table 1B and figure 2*b*). When the populations were analysed separately, the effect of mortality status remained strongly significant in each case (New Caledonia: $F_{1,223} = 17.37$, $p < 0.0001$; Taiwan: $F_{1,211} = 32.63$, $p < 0.0001$). Non-parametric testing revealed non-significant differences between dead and surviving males at low ($z = -0.454$, $p = 0.65$) and intermediate temperatures ($z = 0.897$, $p = 0.37$), and a highly significant difference at the high temperature ($z = 4.651$, $p < 0.0001$). This pattern of separation across levels of temperature was similar to that demonstrated by *post hoc* testing subsequent to parametric analysis of variance (figure 2*b*).

The predicted mating probability of New Caledonia field males (π_{field}) based on average phenotypic values was 0.429, whereas that for males that died during development ($\pi_{\text{high-stress}}$) the estimate was 0.255, representing a 41 per cent decrease in predicted mating probability.

4. Discussion

We have demonstrated a remarkable increase in disparity in developmental instability between live and dead males with increasing thermal stress. Indeed, under greatest stress, males that died carried more than twice as many phenodeviations as the surviving ('visible') fraction of the population. We also

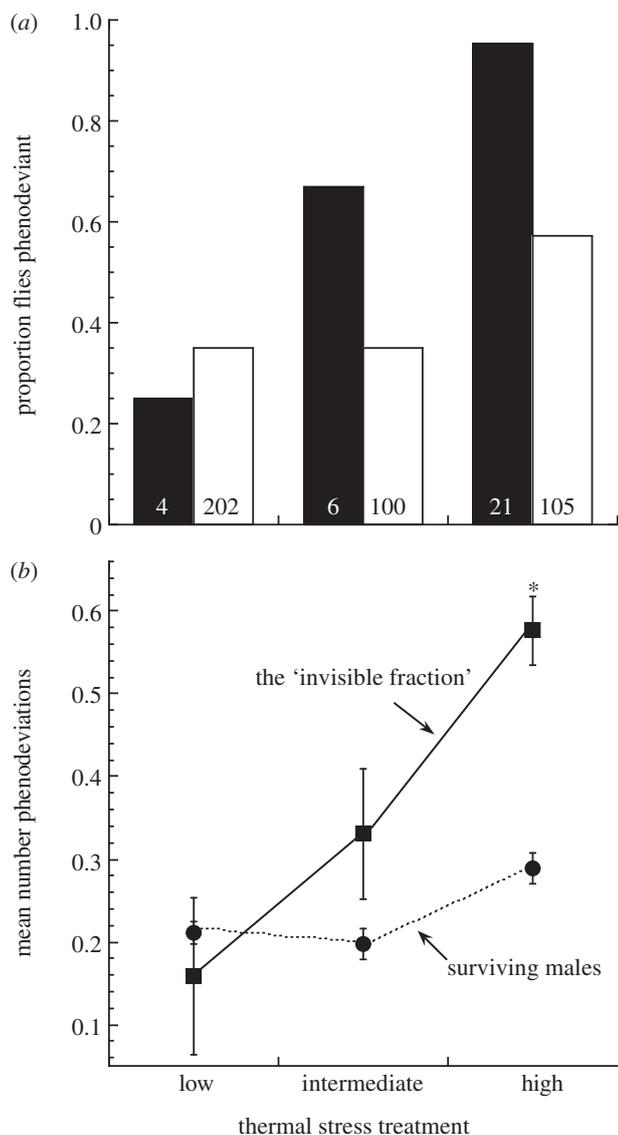


Figure 2. (a) Proportion of flies that carried at least one phenodeviation in dead (dark bars) and surviving (white bars) males across levels of thermal stress. Numerals within bars are sample sizes. (b) Mean (± 1 s.e.m.) residual number phenodeviations in dead (filled square) and surviving (filled circle) males. Asterisk indicates a significant difference ($p < 0.05$; Tukey test) between a pair of means.

demonstrated the action of developmental selection using the probability of phenodeviance (i.e. the probability of carrying at least one sex comb phenodeviation), which we found to be significantly elevated among dead males compared with the survivors at both intermediate and highest stress levels.

We incorporated these findings into what we know about selection on adult phenotypes in the wild. To do this, we used a previously published model predicting adult mating probability from phenodeviance, fluctuating asymmetry

and their cross-product in *D. bipectinata* [10]. We used this model to illustrate that had the males from the most stressful environment survived through to adulthood, they would have endured an estimated 41 per cent reduction in mating success compared with the average adult male. This result indicates that the presence of these males in the pool of sexually active adult individuals would have intensified sexual selection for reducing sex comb developmental instability. We note that in addition to reduced mating success, these hypothetical survivors would likely also have suffered decrements in other fitness traits (e.g. sperm production, adult lifespan) owing to the damaging effects of the thermal stress they experienced during development.

In our study, we measured a quantitative adult trait in a cohort of individuals that failed to survive their journey through development. Although such opportunities are likely to be rare (but see [7,15–18]), they demonstrate how developmental selection against pre-adult phenotypes could attenuate the strength of phenotypic selection operating at later developmental stages. We envision this dampening effect occurring as a result of developmental selection altering the properties of the phenotypic distribution of the survivors, primarily as a reduction of trait phenotypic variance, although genetic variance and covariance among traits could be affected also. Indeed, in the present study, developmental selection trimmed the phenotypic variance of the total (untransformed) number of comb phenodeviations by approximately 50 per cent (VAR of total population: 1.792; VAR of survivors: 0.817). Although our results are potentially applicable to phenotypic traits in general, they are of direct relevance to the field of developmental instability, where developmental selection has indeed been explicitly invoked (but rarely tested) to explain weak and statistically non-significant effects of developmental instability on sexual selection and of environmental stress on adult measures of developmental instability [19].

Darwin recognized how the asymmetry between stable population sizes and the overproduction of offspring meant that the struggle for existence would be a potent selective force. The challenges lie in identifying the traits that are under this developmental selection, and in quantifying developmental selection in free ranging populations because the traits of the dead will be difficult to measure. Despite these practical difficulties, the present study suggests that quantifying just how much developmental selection can explain sometimes paradoxical patterns of natural selection [3,20] will be a worthwhile task.

The research was partially funded by the National Science Foundation (DEB-1118599, M.P.), the Australian Research Council, and the McMicken College of Arts and Sciences, University of Cincinnati. We thank J. Kennington and L. Simmons for research support, and J. Alcock, G. Arnqvist, J. Kotiaho, A. Møller, T. Tregenza and the reviewers for comments.

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