

Developmental stability and environmental stress in natural populations of *Drosophila pachea*

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We examined the relationship between developmental stress and fluctuating asymmetry in a natural population of *Drosophila pachea*, a cactophilic fruitfly. Cactus host variation was found to exert significant influence on the size of legs and of wings of emerging adults, but stressors associated with reduced size did not show the predicted increase in fluctuating asymmetry for either leg or wing length. These findings underscore questions raised by other investigators as to the broad utility of fluctuating asymmetry as a measure of environmental stress.

Keywords: Developmental instability; cactophilic; *Drosophila*; fluctuating asymmetry; stress

Introduction

Biologists need a means of assessing the impact of a wide range of environmental stressors on natural populations. Stressors of interest include pollutants to which organisms have never before been exposed as well as changes in natural environmental features such as temperature, humidity, density, and shifts in resource use induced by global warming or habitat destruction.

Fluctuating asymmetry, or FA, has been proposed to be a useful means of detecting the influence of all of these stressors (Parsons, 1990), even before their impact compromises the fitness of organisms of interest (Clarke, 1994). Fluctuating asymmetry – random deviations from anticipated bilateral symmetry – reflects the degree to which developmental noise, either environmental or genetic in origin, can perturb the programmed developmental trajectory (Palmer, 1994).

Numerous examples of increased FA in natural populations exposed to environmental stressors exist in the literature (reviewed in Parsons, 1990; Clarke, 1994). On the other hand, there are reports in which the anticipated increase in asymmetry was not found (Parsons, 1961; Clarke, 1993; Wardhaugh *et al.*, 1994), despite clear evidence of stress in the form of size reduction or morphological abnormalities. These apparent inconsistencies have led to the recommendation that the conditions under which increased FA is expected, be more completely understood (see Hedrick in Markow, 1994).

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Drosophila pachea is a cactophilic fruitfly endemic to the harsh Sonoran Desert of North America. It utilizes pockets of necrotic senita cactus (*Lophocereus schottii*) to feed and reproduce. The toxic alkaloids found in senita exclude other *Drosophila* species from utilizing this cactus as a host (Kircher *et al.*, 1967). During the course of necrosis, cactus tissue dries out and secondary plant compounds in the cactus become more concentrated (Fogleman, 1982; Kircher, 1982). Rot pockets in which *Drosophila* larvae grow become nutritionally modified by the presence of other organisms as well as by the larvae themselves (Etges and Heed, 1987), and emerging adult cactophilic *Drosophila* exhibit reduced body size in response to environmental stress during development (Heed and Mangan, 1986; Thomas, 1993). Furthermore, in the summer, populations of *D. pachea* undergo a sizeable reduction in numbers (Rockwood-Sluss *et al.*, 1973; Ward *et al.*, 1974; Pitnick, 1993; Breitmeyer, 1994), attributed to the extremely high temperatures. Thus flies of *D. pachea* appear to be living near their physiological tolerances and provide the opportunity to estimate the influence of natural environmental stressors on development as reflected in body size and fluctuating asymmetry. Below we ask if differences between individual host plants and within a single host over time can influence adult size in *D. pachea*, and, if so, are the associated stressors also reflected in increased FA?

Methods

Collection and handling of flies

Two necrotic arms of senita (*Lophocereus schottii*) were collected on 14 February 1995 and brought back to the laboratory to collect and measure eclosing *Drosophila pachea* adults. Both rots were collected at the site known locally as 'El Sahuaral', located ca 1.5 km east of the town of San José de Guaymas, municipality of Guaymas, Sonora, México (27° 50' N; 110° 53' W). This zone presents a columnar cacti community dominated by the senita, organ pipe (*Stenocereus thurberi*), and cholla (*Pachycereus pringlei*) and the site is the location of long term ecological studies on cactophilic *Drosophila* endemic to the Sonoran Desert.

When rots were transferred to the laboratory at ITESM Guaymas Campus, each was placed in its own sealed glass aquarium (60 litres), and kept at laboratory temperature (18–20 °C). Aquaria were monitored daily for emergence of flies. Collection of flies began immediately after the first fly appeared, and every other day afterwards until no more flies emerged. Flies from each aquarium were collected by means of mouth aspirators and kept in vials with either instant *Drosophila* medium (Carolina Biological) or banana medium with live yeast, at densities no greater than 20 flies per vial. Each vial was identified with the date and aquarium number. Cactus number 1 yielded flies for 19 days and cactus number 2 for 29 days. Flies were transferred to clean vials with new food every six days until they could be measured.

Measurements of flies

Two bilateral characters were measured on both sides of each fly. One was the length of tibia on the first pair of legs (Coyne, 1985) and the other was the distance from the anterior crossvein to the wing margin (Markow and Ricker, 1992). Because neither of these traits change in size over the course of the life of an adult fly, they can be employed to assess earlier developmental processes.

The front legs and the wings were removed from the flies with fine tweezers under a dissecting microscope. Wings and legs were mounted separately on glass microscope slides using double-sided transparent tape. The glass slides holding the wings were covered with a strip of transparency.

Length of the tibia was measured using an ocular micrometer in the eyepiece of the dissecting microscope at 45X. Each leg was measured three times in order to estimate measurement error. Slides with the wings were projected onto white paper taped to a wall, covered with paper for measuring, using a Leitz-Wetzler Prado 500 slide projector with 1:2.5/90 mm lenses. The length measured was from the anterior crossvein to the end of the second longitudinal vein (Fig. 1) and was in arbitrary units.

Analysis of fluctuating asymmetry

Fluctuating asymmetry is usually assessed by subtracting the left from the right and calculating the mean of these values for a given sample. However, certain features of the distribution of these scores may require FA to be statistically analysed using a different index. In order to determine the appropriateness of using $|R-L|$ scores, we first ruled out the existence of confounding factors (Palmer, 1994). There was no significant directional asymmetry and no correlation between the degree of asymmetry and character size for either trait. Measurement error was very low. Of 379 legs measured three times each (1137 measures), different values were obtained on only six occasions.

Results

Leg and wing sizes for female and male *D. pachea* are presented in Table 1. No directional asymmetries were observed between the right and left sides for either trait in flies emerging from either necrotic cactus.

A two by two factorial ANOVA revealed that significant differences exist for mean tibial length ($F_{3,183} = 6.79$, $p = 0.0002$) that can be attributed to cactus ($F = 17.53$, $p = 0.0001$) but not sex ($F = 2.74$, $p = 0.0997$). Mean wing length differences were also highly significant ($F_{3,147} = 19.18$, $p = 0.0001$) and attributable to both cactus ($F = 17.74$, $p = 0.0001$) and sex ($F = 39.57$, $p = 0.0001$). Flies emerging from cactus 1 were

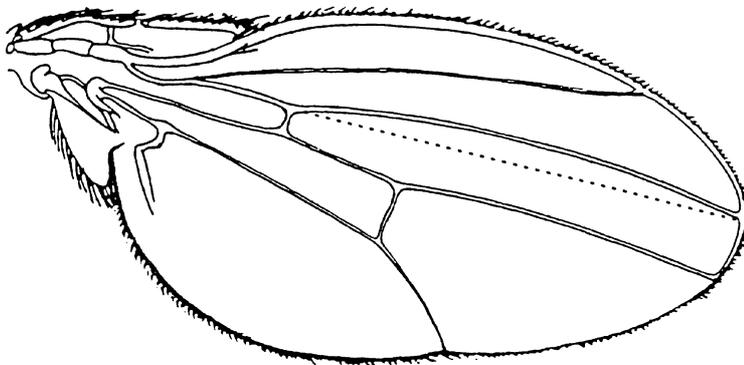


Fig. 1. Wing length was measured from the anterior crossvein to the wing margin (Markow and Ricker, 1992).

Table 1. Mean (\pm SE) wing and leg lengths in *Drosophila pachea* from two different necrotic cacti

Character	Cactus	Females (n)	c.v.	Males (n)	c.v.
Left wing	1	18.53 \pm 0.16 (15)	3.36	17.62 \pm 0.12 (40)	4.34
Right wing	1	18.72 \pm 0.16 (15)	3.28	17.74 \pm 0.12 (40)	4.11
Left leg	1	5.11 \pm 0.10 (20)	8.86	5.11 \pm 0.03 (55)	5.74
Right leg	1	5.23 \pm 0.07 (19)	5.66	5.05 \pm 0.05 (54)	6.88
Left wing	2	17.72 \pm 0.11 (49)	4.63	16.90 \pm 0.12 (47)	5.06
Right wing	2	17.81 \pm 0.12 (49)	4.73	17.03 \pm 0.12 (47)	5.05
Left leg	2	4.93 \pm 0.04 (56)	6.28	4.91 \pm 0.04 (61)	6.78
Right leg	2	4.98 \pm 0.04 (55)	5.98	4.88 \pm 0.04 (59)	6.88

Wing lengths are in arbitrary units, leg lengths are in mm.

larger than flies from cactus 2. Females from both cacti had longer wings than did males.

Because both factors showed significant effects in the ANOVA, Pearson correlation analyses were performed separately by cactus and sex. Fly size was negatively correlated with necrosis age at the time of emergence, such that as the age of the cactus increased, emerging flies were smaller for both traits. This relationship achieved statistical significance in cactus 1 for female wing length, Pearson's $R = -0.31$ ($p = 0.02$) and male tibia length, $R = -0.33$ ($p = 0.03$), and in cactus 2 for female tibia length, $R = -0.29$ ($p = 0.03$) and for wings, $R = -0.44$ ($p = 0.001$).

After verifying the independence of FA and character size, we asked if FA differed between cacti or if it increased with necrosis age. If the same stressors responsible for reduced size increased developmental instability, flies from cactus 2 were predicted to show greater FA than those from cactus 1. This prediction was not supported by one tailed t -tests for either sex for either character. Neither tibial FA nor wing FA were correlated with necrosis age in males or females from either cactus. Correlation coefficients range from $R = -0.09$ to 0.07 . The stressors that underlie the decreasing sizes attained by developing flies did not cause any increase in developmental instability, as measured by FA (Table 2).

Discussion

Developmental environment was observed to exert significant influence on the size of two characters in *D. pachea*. This influence was detected between cacti as well as within a single cactus at different stages of necrosis. Flies emerging over the life of a cactus rot

Table 2. Mean (\pm SE) fluctuating asymmetry |R-L| in wing and leg length in *Drosophila pachea* from two different necrotic cacti

Character	Cactus	Females	Males
Leg	1	0.28 \pm 0.07 (19)	0.28 \pm 0.04 (54)
	2	0.20 \pm 0.02 (55)	0.22 \pm 0.02 (59)
Wing	1	0.20 \pm 0.03 (15)	0.18 \pm 0.02 (40)
	2	0.18 \pm 0.02 (49)	0.19 \pm 0.03 (41)

become smaller as the necrosis progresses. Our observations agree with those of Thomas (1993) in which larval nutrition was linked to adult body size in natural populations of the cactophilic *D. buzzatii*. A number of differences in the developmental environment of cactus rots have been linked to size of emerging adults in other cactophilic *Drosophila* including host chemistry (Fogleman, 1982), rot age, larval density, and microbial activity (Etges and Heed, 1987). These factors may be exacerbated for *D. pachea*, as the small stem diameter of senita cactus, compared to the large host cacti of the other desert flies, makes them more prone to rapid desiccation (Ward *et al.*, 1974).

For *D. pachea*, these necrosis features, both between plants and across time, were stressful enough to significantly influence the size of flies, but not developmental stability as measured by FA. Observations on other *Drosophila* species show that wings may exhibit significant levels of fluctuating asymmetry, making it unlikely that the characters we examined simply failed to show developmental instability (Markow and Ricker, 1992). The long term association between these desert flies and their host plants may underlie their apparent developmental stability under the range of conditions in the present study. When faced with atypical developmental stressors, an increase in FA may well be observed (Graham *et al.*, 1993). Evaluation of any natural population will only be able to assess FA in recoverable individuals, as in our study. Furthermore, the predicted effects of stress were clearly detectable for size.

Failure to observe an increase in fluctuating asymmetry under stressful conditions has been reported in several other studies. In most cases, the stressor was novel, not something that the organisms are typically exposed to in nature. For example, phenylthiourea, added to the diets of *D. melanogaster* larvae, significantly reduced adult weights but did not increase FA (Parsons, 1961). Bush flies of the species *Musca vetustissima* that had developed in the presence of the drug ivermectin showed no increase in wing vein FA despite a reduction in size compared to untreated control flies (Wardhaugh *et al.*, 1994). Clarke (1993) found that atmospheric pollution did not increase wing FA in the lacewing (*Chrysopa perla*), but he observed an increase in morphological anomalies. It is unclear if additional negative findings remain unpublished, but it is clear that if FA is to be employed as a monitor of exposure to stress, additional studies, across a wider range of taxa and stressors, must be performed in order to ascertain the conditions under which significant FA can be predictably and meaningfully observed.

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