

Inbreeding not stress increases fluctuating asymmetry in the bulb mite

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ABSTRACT

In this study, I examined the effects of inbreeding and stress on fluctuating asymmetry and body length in the bulb mite, *Rhizoglyphus robini*. Mites were subjected to six generations of brother–sister mating and inbred lines were then crossed to obtain inbred and outbred progeny. At the larval stage, both the inbred and outbred progeny were divided between two treatments: the control was reared at a stable temperature of 22°C, whereas the stressed lines were kept under oscillating temperatures of between 10°C (night) and 28°C (day) until reaching adulthood. Fluctuating asymmetry, quantified by means of procrustean analysis based on five landmarks, and body length were measured for one male from each line. Fluctuating asymmetry increased with inbreeding but not with stress. In contrast, body length decreased with stress but not with inbreeding. There was no significant interaction between stress and inbreeding in their effect on either fluctuating asymmetry or body length.

Keywords: developmental stability, fluctuating asymmetry, heterozygosity, inbreeding depression, *Rhizoglyphus robini*.

INTRODUCTION

Fluctuating asymmetry is a measure of small, random deviations from bilateral symmetry (Van Valen, 1962; Palmer and Strobeck, 1986). Such deviations are thought to increase as a result of environmental perturbations during development and fluctuating asymmetry, therefore, is considered to be a useful measure of environmental stress (Parsons, 1992; Møller and Swaddle, 1997). However, the generality of this association is debated (Bjorksten *et al.*, 2000; Møller, 2000).

The amount of fluctuating asymmetry is thought to reflect developmental stability (or homeostasis) – that is, the ability of an organism to resist stress (Lerner, 1954; Zakharov, 1992; Debat and David, 2001). Developmental stability was hypothesized to increase with heterozygosity (Lerner, 1954; Mitton, 1995), but a recent review by Vøllestad *et al.* (1999) found only weak support for the hypothesis. Evidence from recent studies continues to be

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mixed. Some studies have reported positive associations (Gomendio *et al.*, 2000; Debat *et al.*, 2001), whereas others have not (Hosken *et al.*, 2000; Carchini *et al.*, 2001; Kark *et al.*, 2001; Radwan and Drewniak, 2001; Taylor, 2001) or have been inconclusive (Gillian *et al.*, 2000; Alves *et al.*, 2001).

One reason for the inconsistency of these results may be the interaction between stress and developmental stability, such that stress affects fluctuating asymmetry more when developmental stability is decreased, for example due to inbreeding (Lens *et al.*, 2000). In this study, I examined the effects of inbreeding and temperature stress on fluctuating asymmetry in the bulb mite, *Rhizoglyphus robini*.

Lerner (1954) proposed that possessing alleles with the optima under different environmental conditions allows heterozygotes to cope with a wider range of environments. However, this means that application of stress at only one end of the environmental range may in fact favour homozygotes, with the optimum closer to that end rather than heterozygotes, thus obscuring the interaction between stress and developmental stability. In this study, I examined the effect of stress caused by the temperature oscillating on a daily basis between values that were much lower or higher than optimal. Temperature stress was chosen because it has been effective in increasing fluctuating asymmetry in many studies (e.g. Imasheva *et al.*, 1997; Bublly *et al.*, 2000; Clarke *et al.*, 2000; Hosken *et al.*, 2000; but see Bjorksten *et al.*, 2001).

Bjorksten *et al.* (2000) suggested that because of the inconsistency of fluctuating asymmetry studies, measures as simple as body size may be better indicators of stress or inbreeding, a view challenged by Møller (2000). Therefore, in addition to measuring fluctuating asymmetry, I also looked at the consequences of inbreeding and stress on body length.

One source of the inconsistency of results between studies of fluctuating asymmetry may be that they usually measure asymmetry of single traits. Such fluctuating asymmetry is expected to be much more weakly associated with developmental stability than aggregate measures based on several traits (Gangestad and Thornhill, 1999; Leung *et al.*, 2000). Another way to increase the sensitivity of measures of fluctuating asymmetry has been suggested by Polak and Starmer (2001). They proposed that positional asymmetry may be a better measure of stress than fluctuating asymmetry of metric or meristic traits because position, reflecting two-dimensional interactions between different morphogen gradients, may be more vulnerable to environmental disturbances than trait size. In this study, I used a morphometric method for comparing shapes, procrustean analysis, which combines both of these improvements by comparing positions of several landmarks on the left and right side of the body (Bookstein, 1991; Smith *et al.*, 1997; Klingenberg and McIntyre, 1998). In mice dentition analysis, such methods have been shown to be better than traditional linear measures (Auffray *et al.*, 1996). The results reported below indicate that such sensitive measures of fluctuating asymmetry can indeed be useful indicators of inbreeding, but question the generality of the association between fluctuating asymmetry and stress.

METHODS

Rearing

Rhizoglyphus robini infests the subterranean structures of plants and is commonly found on bulbs of onions, garlic and other members of the Liliaceae. They also infest stored food

products (Diaz *et al.*, 2000). The mites used in this study came from a stock culture derived from a colony of about 100 individuals found on onions in a garden near Cracow, Poland, in 1998. They were kept in the laboratory as a large population at 22–26°C and >90% humidity; they were fed a 3:1 mixture of powdered yeast and wheat germ. Average generation time under these conditions is about 2 weeks. Thus, the mites were reared in the laboratory for about 50 generations before this study began.

Beginning 3 months before starting inbred lines and throughout the six generations of inbreeding, mites were maintained at a stable temperature of 22°C. During the experiments, individual and paired mites were kept in 50 ml Eppendorf vials filled to a third with solidified plaster of Paris mixed with 10% powdered charcoal for better visualization of the whitish mites on the background, and soaked with water. Vials were closed with non-absorbent cotton wool and food was provided *ad libitum*.

The experiment began with random pairings of virgin females and males. Ten offspring of each pair were isolated individually at the larval stage to obtain virgin females. One inbred line was started from each family by mating a randomly chosen brother and sister. Each of these 155 established lines was split in two replicates in the subsequent generation by mating each of two female offspring with randomly chosen brothers. This established a total of 310 inbred lines for study. The lines were then propagated through brother–sister matings for the next five generations. By the sixth generation of sib-mating, nearly half of all lines were lost, mainly because pairs failed to produce progeny (J. Radwan, unpublished). In the sixth generation, two virgin females were collected from each of the 77 surviving lines (when both replicates of the same line survived, one replicate was selected at random). One female was paired with one of her brothers (chosen at random) and one to an unrelated male. Twelve newly emerged larvae from each inbred and outbred family were divided into two treatments: unstressed mites were maintained at 22°C until reaching adulthood, whereas stressed mites were kept at 28°C between 06.00 and 10.00 h and at 10°C between 10.00 and 06.00 h. These temperatures were close to the limits under which these mites are capable of growth and reproduction (*c.* 5–32°C; Bielska, 1983; Gerson *et al.*, 1983).

One randomly selected male from each family was mounted on a slide in Berlese medium (50 ml distilled water, 50 g chloral hydrate, 20 ml glycerine, 30 g gum arabic; Hughes, 1976) 2 days after emergence. Males were chosen for the analysis because of the ease with which they can be mounted on slides; their body is much flatter than that of females.

Not all crosses produced progenies (10 inbred pairs and 9 outbred pairs failed) and a large proportion (about 30%) of mounted specimens were impossible to measure because preparation artefacts obscured the selected landmarks on their bodies (Fig. 1). As a result, only 2 of 77 surviving inbred lines were represented by all inbreeding $\times$ treatment combinations, so it was not possible to enter lines as a factor into the analysis. Therefore, I included only one male from each line in the subsequent analyses.

There are two male morphs in this species: heteromorphic males with thickened legs and homeomorphic males with unmodified legs (Radwan, 1995). As many families contained only heteromorphic males, and this morph was represented in most lines in at least one inbreeding–stress combination, only the data for this more common morph were analysed. In lines that had a measurable heteromorphic male in more than one inbreeding–stress combination, the specimens to be analysed were selected so as to ensure the maximum balance of the data. This was achieved by retaining only a specimen belonging to the treatment with the fewest measurable males: stressed inbred mites were thus most likely to be retained, followed by stressed outbred mites, control outbred mites and control inbred mites.

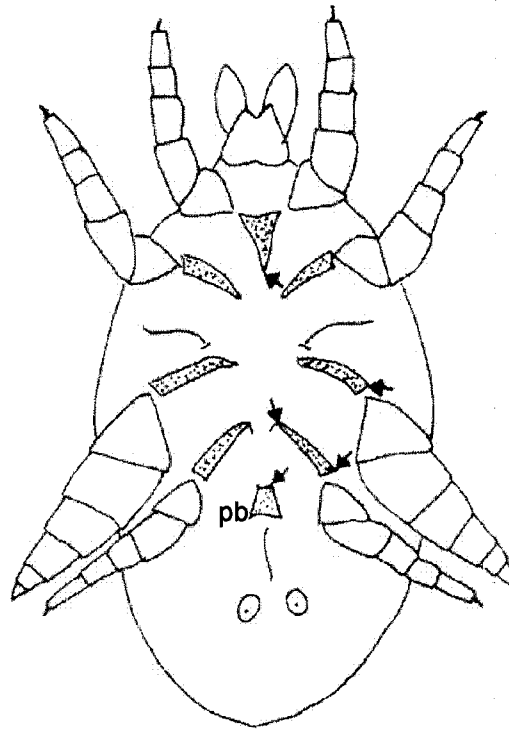


Fig. 1. Schematic representation of the ventral side of a male mite showing the positions of the landmarks (arrows) located on the edges of the sclerotized epimera or penis base (pb).

This procedure resulted in the retention of 21 heteromorphs in the stressed inbred group, 23 in the stressed outbred group, 24 in the control inbred group and 24 in the control outbred group. For each male, I measured idiosoma length (i.e. whole body without mouth-parts) and the procrustean distance between body sides based on five landmarks (Fig. 1).

Procrustean analysis

The analysis was based on five landmarks on the ventral side of males (Fig. 1) that were previously used to quantify fluctuating asymmetry in the founding generation. A procrustean analysis of variance (Klingenberg and McIntyre, 1998) was conducted for 22 males measured twice and revealed a highly significant ($P < 0.001$) side $\times$ individual interaction, indicating that fluctuating asymmetry can be discerned from measurement error, but there was no significant directional asymmetry (J. Radwan, P. Watson, J. Farslow and R. Thornhill, unpublished). Landmark coordinates were located under 120 $\times$ magnification using Scion Image software. The coordinates for the right side and mirror images of the left side were then entered into the GRF-NS software (Slice, 1994) to obtain coordinates of optimally superimposed landmark configurations of the left and right sides scaled to a centroid size of 1.

Procrustean distances are calculated as square roots of sums of squared differences between coordinates of all landmarks on the left and right sides of the body (see details in

Klingenberg and McIntyre, 1998). The procrustean distance is equivalent to the absolute left–right difference with overall size controlled by initial standardization of all coordinate sets to the same centroid size. The procrustean distances were log-transformed to improve the normality of the distribution. The directional component of asymmetry was calculated as a square root of the sum (over landmarks) of squared mean differences between landmark locations for the left and right sides (Smith *et al.*, 1997; Klingenberg and McIntyre, 1998).

RESULTS

The directional component of asymmetry accounted for only 1.7% of the total asymmetry, confirming previous findings (Radwan *et al.*, unpublished) that directional asymmetry is insignificant. Examination of the scatter plots of vectors corresponding to right–left differences for each landmark revealed no distinct clumping in any experimental group – that is, there was no indication of anti-symmetry (Debat *et al.*, 2001).

There was no significant association between male size and fluctuating asymmetry in any of the groups (unstressed inbred: $r = -0.097$, $t_{21} = -0.44$, $P = 0.65$; unstressed outbred: $r = -0.002$, $t_{22} = 0.12$, $P = 0.99$; stressed inbred: $r = -0.058$, $t_{18} = 0.24$, $P = 0.81$; stressed outbred: $r = -0.17$, $t_{21} = -0.83$, $P = 0.41$). The slopes and intercepts of regressions of fluctuating asymmetry on body length did not differ significantly between groups (slopes: $F_3 = 0.21$, $P = 0.89$; intercepts: $F_3 = 1.87$, $P = 0.14$), which allowed me to test for the association between male size and fluctuating asymmetry using the pooled data. However, again it was non-significant ($r = -0.127$, $t_{88} = -1.203$, $P = 0.232$).

Two-way analysis of variance revealed a significant effect of inbreeding but not stress on fluctuating asymmetry (Fig. 2a, Table 1). There was no significant interaction between the factors. In contrast, an analysis of variance on body length revealed a significant effect of stress but not of inbreeding (Fig. 2b, Table 1). Again, the interaction was non-significant.

DISCUSSION

In this study, I found a significant effect of inbreeding on the fluctuating asymmetry but not on the body length of male bulb mites. The results suggest that more sensitive measures of fluctuating asymmetry, such as those based on trait position (Polak and Starmer, 2001), may be better indicators of inbreeding than traditional measures based on size of single traits, which tend to show inconsistent and, on average, weak effects (reviewed by Vøllestad *et al.*, 1999). More studies based on positional asymmetry and using multi-trait indexes are required to confirm the generality of this finding. While averaging over several traits may obscure interesting trait-specific patterns (e.g. Clarke *et al.*, 2000; Indrasamy *et al.*, 2000; Andersen *et al.*, 2002), multi-trait indexes may still be more powerful tools to scrutinize organism- or population-wide claims about fluctuating asymmetry, such as its association with the degree of inbreeding or outbreeding (e.g. Auffray *et al.*, 1996; this study) or with fitness (e.g. Hewa-Kapuge and Hoffmann, 2001).

By increasing homozygosity, and thus exposing recessive genes, inbreeding may improve the effectiveness of selection against deleterious mutations, both within inbred families and through extinction of inbred lines (Hedrick, 1994; Roff, 2002). This phenomenon of ‘purging of inbreeding depression’ is especially effective in the case of genes of major effect, such as recessive lethals and sub-lethals (Hedrick, 1994; Willis, 1999). If such genes affected

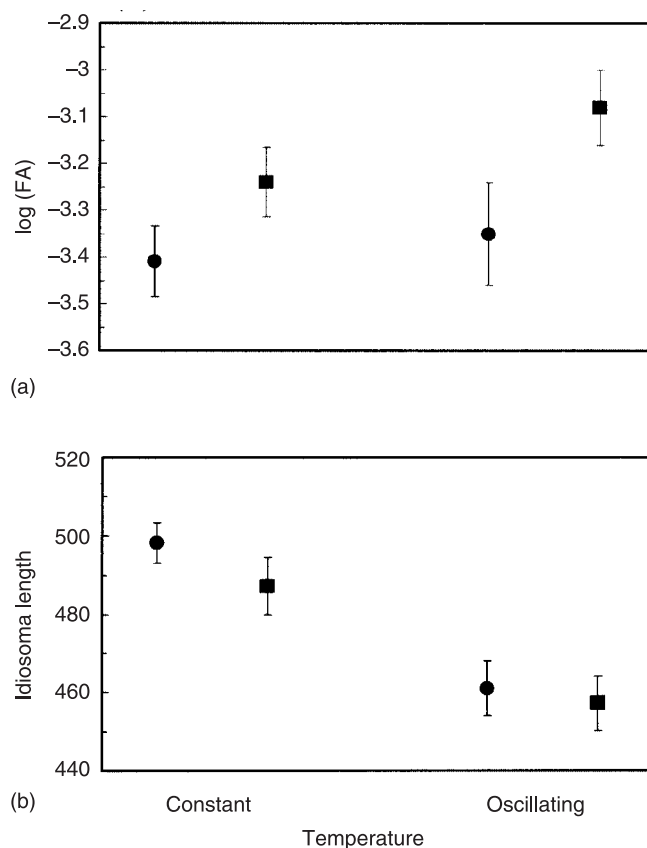


Fig. 2. The effect of inbreeding and stress (oscillating temperature) on (a) fluctuating asymmetry (FA) and (b) body length (μm). ●, outbreds; ■, inbreds. Error bars = standard error.

Table 1. Analysis of variance with log(FA) or body length as the dependent variable

Source	log(FA)				Body length			
	d.f.	MS	F	P	d.f.	MS	F	P
Inbreeding	1	1.134	6.75	0.011	1	1246	1.27	0.263
Stress	1	0.251	1.49	0.224	1	25266	25.74	<0.001
Inbreeding $\times$ stress	1	0.044	0.26	0.609	1	286	0.29	0.591
Error	88	0.168			86	981		

Abbreviation: FA = fluctuating asymmetry.

the developmental stability of the bulb mite, they might have been lost during the six generations of inbreeding that preceded the measurements of fluctuating asymmetry. Nevertheless, inbreeding depression for fluctuating asymmetry was still significant.

I did not find a significant effect of oscillating temperature stress on fluctuating asymmetry. The lack of statistical significance may reflect limited power of the test, but

it should be noted that the sample size was large enough to allow detection of other significant effects. The effect size (expressed in term of Pearson's correlation coefficient; Rosenthal, 1991) for the association between stress and fluctuating asymmetry in the present study was 0.12, less than the average of 0.16 found in a recent meta-analysis of 20 studies (N. Cadee, cited by Møller, 2000). The temperature oscillations in the present study were indeed stressful, as evidenced by their significant effects on male length. These effects were similar to the effects of food stress: highly restrictive cellulose also decreases male body mass (Radwan, 1995). Thus, unlike body size, fluctuating asymmetry measured as an integrated, multi-trait index does not appear to be an efficient indicator of arbitrary stress. Rather, specific types of stress may increase the fluctuating asymmetry of specific traits (Clarke *et al.*, 2000; Indrasamy *et al.*, 2000).

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