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# Fluctuating asymmetry in an insect host: a big role for big parasites?

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## Abstract

Parasites are expected to be associated with host developmental instability because developmentally unstable hosts may be more susceptible to, or more frequently exposed to, parasitic infections compared with developmentally stable ones, or because parasites may directly disrupt host development. In this study, we analysed the relationship between developmental stability in the weta *Pleiopectron simplex* (Orthoptera) and infections by hairworm and mermithid parasites. These parasites have a long development in their host and the size of adult worms exceeds the length of the host by a considerable amount (15–20 cm). For one character (femora) we found that fluctuating asymmetry was significantly higher in parasitized individuals compared with unparasitized ones, in each of two samples collected in different years. Because no relationship was observed between the level of fluctuating asymmetry and the parasite size or number, however, infection by hairworm and mermithid parasites could be more a consequence of developmental instability than a cause. For other characters (tibia, external and internal spines of femora), the level of fluctuating asymmetry between parasitized and unparasitized individuals was not significantly different. Our results are discussed in relation to ecological constraints met by hairworm and mermithid parasites to complete their life cycle.

## Keywords

Fluctuating asymmetry, Mermithidae, Nematomorpha, Orthoptera, parasitism.

## INTRODUCTION

In theory, bilaterally symmetrical characters should be expressed identically on both the right and the left sides of the body, because morphological characters on each side are the product of the same genotype (Polak 1993; Palmer 1996). Deviations from perfect symmetry, however, are frequently observed and have been interpreted as indicators of developmental instability (Parsons 1990; Palmer 1996). A common measure of developmental instability is fluctuating asymmetry (FA), a measure of the small and random differences between the right and the left values of an otherwise symmetrical morphological character (Van Valen 1962; Palmer & Strobeck 1986). A wide range of environmental and genetic factors are known to lead to elevated levels of developmental instability (see Møller & Swaddle 1997 for a review). Recently, there has been a growing interest in understanding the relationships between parasitic infections and host FA, especially in the context of conservation biology (see Møller 1996a for a review). Numerous studies showed that parasites are associated with elevated develop-

mental instability in their host (Møller 1992, 1995; Polak 1993; Saino & Møller 1994; Escos *et al.* 1995; Folstad *et al.* 1996; but see Polak 1997). This positive correlation is expected to result from at least three main phenomena (Møller 1996a). First, developmentally unstable individuals may be immunologically more susceptible to parasite infections compared with developmentally stable ones (Møller 1992; Polak 1993). Second, developmentally unstable hosts may be more often exposed to parasites than developmentally stable ones. A third reason is that parasites may be a direct cause of instability when they disrupt the development of their host (Møller 1995, 1996a).

Recent studies have suggested that not all parasites are expected to be directly responsible for developmental instability. Indeed, high levels of FA are generally a direct and significant cause of a decrease in fitness (see Møller 1997 for a review). From the parasite perspective, what happens to the host as a consequence of its exploitation may in return affect its own fitness. For instance, it has been suggested that venereal pathogens should not

increase host FA because host mating success, which result in opportunities of transmission for venereal diseases, generally correlates negatively with FA (Møller 1996a). Recently, it has also been demonstrated that vertically transmitted parasites are not associated with high levels of FA compared with horizontally transmitted ones (Agnew & Koella 1997). Parasites transmitted purely vertically from parent to offspring have their reproductive success explicitly linked to that of their hosts, and thus any cost to host reproductive success affects the parasite's own success. In many situations, it has also been demonstrated that FA is responsible for a decrease in survival (e.g. Møller 1996b; see Møller 1997 for a review). Host longevity following infection may be a crucial parameter for many parasites, especially those that require a long time to develop before reaching the adult stage or before becoming infective for a next host. It is thus expected that there should be a negative relationship between the time required by a parasite to develop in its host and FA it induces.

The weta *Pleiolectron simplex* Hutton (Orthoptera: Rhaphidophoridae) is a cave insect naturally infected with the hairworm parasite *Gordius dimorphus* Poinar (Nematomorpha: Gordioidea), and an undescribed nematode of the genus *Mermis* (Nematoda: Mermithidae). Wetas become infected with hairworms when they ingest parasitic larvae in dead paratenic insect host (Poinar 1991a, b). Both these parasites develop inside the weta over several months, a time that includes periods of host moulting and growth. Individual wetas are usually hosts to a single worm, although two worms are occasionally found in the same host. During their long development, these parasites grow from a microscopic cyst to a large worm whose size exceeds the length of the host by a considerable amount. Indeed, when the parasitic development has been completed, the worm (between 10 and 20 cm) occupies all the host space with the exception of the head and the legs. Worms are only ready to emerge once they reach this stage. They then alter the behaviour of wetas, making them commit "suicide" by entering water where the adult worm must emerge (Poinar 1991a, b; Vance 1996).

In this paper, using FA as a measure of developmental instability, we analysed the relationship between infection by hairworm and mermithid parasites and FA in the weta *P. simplex*. If developmentally unstable wetas are more susceptible or more frequently exposed to infection, parasitized individuals should display a higher level of FA compared with unparasitized individuals. In addition, if the parasitic development is itself a cause of developmental instability, the level of FA in parasitized hosts should be correlated with the total worm length.

## MATERIALS AND METHODS

### Measurements

Two samples (S1 and S2) of the weta *P. simplex* were collected from the clay banks of a small stream at Cass (43°02'S, 171°45'E), near Arthurs Pass, in the South Island of New Zealand during November 1995 and January 1997, respectively. We obtained 64 (S1, 38 males, 26 females) and 178 (S2, 126 males, 52 females) wetas. Right and left hind femora of the third leg pair were detached from the body after the subjects had died naturally or had been sacrificed using chloroform. Measurements of all femora were taken from identical points on the leg. Three repeated measurements were made of each femur using a graticule on a dissecting microscope with 10× magnification and an accuracy of 0.05 mm. Measurements in S1 and S2 were made by two different observers and were taken prior to the dissection of the weta so that the observers had no knowledge of the infection status of individual insects. For the S2 sample, we also counted external and internal spines occurring in rows on femora and, with the same methodology as for femora, we measured the right and left tibias on the first pair of legs. All measurements were made on intact characters only. For the number of external and internal spines there was no measurement error (ME). For other characters, the repeatability of measures and their asymmetry was very high and statistically significant (correlations,  $r \geq 0.99$ ,  $P < 0.001$  for all characters), suggesting that the measurements were sufficiently precise to allow analyses of FA (e.g. Møller 1996b). When no significant FA difference was observed between two groups, however, we conducted a comparison of the mean FA considering the two most extreme samples possible when taken into account the ME (Thomas *et al.*, in press). For this, we first estimated the ME as the proportion of within-individual variation to total variation (Merila & Bjorklund 1995). Then, when the mean FA of the first sample was not significantly higher than the mean FA of the second sample, the comparison was repeated but after having increased by ME all the values in the first sample and having decreased by the same percentage those in the second sample. Because this procedure is really conservative, it can be concluded if the test remains nonsignificant that ME is not responsible for the absence of a significant difference between the two samples. When a weta was infected, the worm was straightened without stretching and measured to the nearest millimetre. Except for femora in S1 (one sample *t* test,  $P < 0.001$ ), characters did not demonstrate directional asymmetry because mean signed FA values did not deviate significantly from zero (one sample *t* tests,  $P > 0.05$  in all cases). Characters did not demonstrate anti-

symmetry since the frequency distributions of signed FA did not deviate significantly from normal distributions (one-sample Kolmogorov–Smirnov tests,  $P > 0.05$  in all cases). Absolute FA was estimated as the unsigned left-minus-right character value. Because interpretation of FA may be confounded by a significant relationship between absolute FA and the size of the character, we checked for size dependency by regressing absolute FA on character size calculated as [(value of left + value of right)/2]. Absolute FA on femur size was not correlated with size in both S1 ( $r = 0$ ,  $N = 64$ ,  $P = 0.89$ ) and S2 ( $r = 0.11$ ,  $N = 137$ ,  $P = 0.10$ ); absolute FA on the number of spines was not related with the mean spines number (S2, external spines,  $r = 0.08$ ,  $N = 140$ ,  $P = 0.38$ ; internal spines,  $r = 0.06$ ,  $N = 140$ ,  $P = 0.44$ ); however, absolute FA on tibia size was positively and significantly correlated with the size of tibia (S2,  $r = 0.20$ ,  $N = 164$ ,  $P = 0.006$ ). Thus, for tibia analyses, we used relative asymmetry estimated as absolute asymmetry divided by the mean left and right tibia size (Palmer & Strobeck 1986), as no size dependency was observed after this procedure (S2,  $r = 0.001$ ,  $N = 164$ ,  $P = 0.84$ ).

### Statistical analysis

Because assumptions of parametric analyses are generally violated with data on absolute and relative FA (Palmer & Strobeck 1986), we use sampled randomization tests that do not make distributional assumptions and are often more powerful than traditional non parametric statistics (Sokal & Rohlf 1981; Manly 1991; Adams & Anthony 1996; Thomas & Poulin 1997). Thus, in order to compare FA between parasitized and unparasitized wetas, we considered (successively for femora, tibias, and external and internal spines) the observed set of data as one of many but equally possible outcomes that could have arisen by chance. We then formed 20,000 possible outcomes by randomly allocating data values to the two groups of data, and we calculated the mean difference for each iteration. By performing this procedure, a distribution of possible outcomes is generated. We then compared the observed sample mean difference with the distribution obtained by randomly allocating data values. The significance of the test was calculated as the probability of obtaining a more extreme value than the observed mean difference (Thomas & Poulin 1997). Assuming that a large parasite could be more or less damaging according to the size of the host, we also conducted a multiple linear regression with FA values of parasitized individuals as the dependent variable (Y) and both the size of the parasite (total worm length) and the size of the host (mean femora length) as regressors. The significance levels of the regression coefficients were

determined by randomizations on the order of the Y values (20,000 iterations). For all analyses, we pooled males and females together since the mean FA were not significantly different between sexes (randomization tests:  $P > 0.05$  for all characters). Wetas parasitized with both types of parasites were also lumped together because we were unable to do a precise determination of non adult parasite stages. In addition, the two parasite species are similar with respect to life cycle, adult size, and effects on host lifespan (Poinar 1991b).

Throughout the paper, values given are mean  $\pm$  SD. All tests were two-tailed. Results were considered significant at the 5% level and significances were Bonferroni adjusted (Rice 1989). Randomization analyses were performed using RT 2.0 (B. Manly, University of Otago, New Zealand).

### RESULTS

Dissection revealed that parasitic prevalences were 12% in S1 and 11% in S2, which is not statistically different (Fisher's exact test,  $P = 0.85$ ). Parasitized and unparasitized wetas were not significantly different in size (estimated by the mean femora length,  $t$  test,  $P \geq 0.15$  in both S1 and S2). In both S1 and S2, four wetas harboured two worms each, whereas other parasitized wetas harboured a single parasite. Mean total parasite length per infected wetas was  $151.7 \text{ mm} \pm 60.1$  in S1 ( $n = 9$ ) and  $130.6 \text{ mm} \pm 93.7$  in S2 ( $n = 19$ ) (Mann–Whitney  $U$  test,  $U = 239$ ,  $P = 0.43$ ). Results show that absolute FA in femora size was significantly higher for parasitized individuals compared with unparasitized individuals in both S1 and S2 (Table 1). No significant difference between parasitized and unparasitized wetas was observed for relative FA in tibia size (Table 1). This absence of significant difference was not due to the 9.5% of ME on this character because the previous comparison remained nonsignificant even when FA data in the parasitized group were increased by 9.5%, and those of the unparasitized group decreased by the same percentage (randomization test,  $P = 0.21$ ). Although there was no ME for the number of spines, parasitized and unparasitized individuals also did not differ in the absolute FA in these traits (Table 1). Wetas infected by a single worm were not more asymmetric than those infected by two worms (randomization tests,  $P > 0.05$  for all characters). There was no significant relationship between worm length and host size (linear regressions,  $r = 0.5$ ,  $P = 0.09$  and  $r = 0.03$ ,  $P = 0.91$ , for S1 and S2, respectively). FA of parasitized individuals also did not correlate with the total worm length they harboured, even when controlling for the host size effect (multiple linear regressions,  $P > 0.05$  in all cases). There was no significant pairwise relationship

between the asymmetry in the different traits (correlations,  $P > 0.05$  for all comparisons), suggesting that individuals with large asymmetry in femur do not have a large asymmetry on other traits.

## DISCUSSION

The results of this study support the hypothesis that parasitic infection is associated with instability in host development, as measured by absolute FA in hind femur length. Parasitized wetas had a higher mean FA than unparasitized wetas and this result was consistent in the two samples S1 and S2. This phenomenon could indicate that infection *per se* is responsible for developmental instability; however, no positive relationship is observed between the level of FA and the total worm size as would be expected if the parasitic development was the direct cause of developmental instability. This may be due also to the low sample size of parasitized individuals and/or these parasites intervening with host development only shortly after the infection. A more important consideration is that the host needs to be developing for the parasite to induce developmental instability. Unfortunately, little is known concerning growth in *P. simplex*. We also found no evidence that double infection results in a larger asymmetry for the host. These results thus suggest that although hairworm and mermithid parasites are responsible for important damage in their weta host, they are able to develop without increasing the level of host FA. Numerous studies have shown that an elevated level of FA may be a direct cause of reduced survival (see Møller 1997 for a review). Given the long development of hairworm and mermithid parasites in wetas, it may be expected that the use of resources allocated to the maintenance of the host developmental stability could result through time in a high probability of host mortality, and therefore in a reduced probability of successful transmission for the parasites. It is thus likely that a strong selection occurs on these parasites to minimize any costs on host survival during their development.

Although the parasitic development is not associated with developmental instability, parasitized wetas are more asymmetric in femora size than unparasitized ones. This result is consistent with two hypotheses. First, individual wetas may develop an asymmetric phenotype because of genetic or environmental stresses, and such individuals may be less efficient at mounting an immune response to defend themselves against parasites. Second, asymmetric wetas may be more frequently exposed to parasitic infection compared with symmetric wetas. Wetas become infected with hairworms when they ingest parasitic larvae in dead paratenic insect host (Poinar 1991a, b). There is evidence that prevalence of infection in paratenic host populations can be extremely high (e.g. 100%; Poinar 1991a). The risk of infection is thus likely to be high for wetas feeding on dead insects. Although wetas can be omnivores, they are primarily vegetarians (Richards 1973; Meads 1990). Dead insects could thus be a food of second choice more frequently consumed by developmentally unstable individuals. Further experiments, however, are necessary to analyse the relationship between feeding behaviour, immuno-competence, and individual quality.

No difference was found between parasitized and unparasitized wetas for FA in tibia size and number of spines. This could indicate that these characters are developmentally canalysed (Waddington 1957) and buffered against the effects of environmental perturbations. However, whether asymmetry in tibia or spines is more costly for hosts than asymmetry in femora is not known in our case. Several other hypotheses can explain these results. For instance, it has been suggested recently that if selective mortality is sufficiently high, the levels of instability among a parasitized and an unparasitized group will converge over time (Campbell & Emlen 1996). When the parasite fitness is closely related to the host survival, however, a significant selective mortality is also expected to strongly select against individual parasites responsible for high developmental instability. For this reason, we consider this explanation inappropriate in the present

**Table 1** Difference between parasitized and unparasitized wetas in FA measured on five characters [ $P$  value comes from randomization test; results on femora remain significant after Bonferroni adjustment (Rice 1989) as  $\alpha = 0.025$ ; sample sizes differ because only intact characters are analysed]

Character	$[\Sigma(R \pm L)/2]/n$ $\pm$ SE (mm)	$\Sigma(R-L)/n$	VAR of ( $R-L$ )	Parasitized			Unparasitized			$P$
				Mean	SD	$N$	Mean	SD	$N$	
Femur (S1)	$10.37 \pm 0.22$	0.033	0.005	0.100	0.033	9	0.056	0.043	55	0.003
Femur (S2)	$10.60 \pm 0.17$	0.006	0.005	0.064	0.093	14	0.026	0.057	123	0.018
Tibia	$5.63 \pm 0.12$	0.002	0.011	0.0086	0.0099	17	0.0065	0.0118	147	0.49
External spines	$28.46 \pm 0.41$	0.12	3.389	1.231	0.832	13	1.425	1.218	127	0.63
Internal spines	$29.07 \pm 0.40$	-0.064	3.932	1.462	1.450	13	1.433	1.360	127	0.92

situation. Alternatively, genes for the immune system may have pleiotropic effects on or be genetically linked to developmental stability of certain characters only. Finally, when the mean FA in a host population is much larger than the threshold value of FA associated with infection and when that parasite has a low prevalence, parasitized individuals are not expected to be more asymmetric than unparasitized ones.

In conclusion, this study suggests that a full understanding of parasite-induced developmental instability also requires that we take into account the constraints imposed on parasites by the successful completion of their life cycle. Host instability is not expected to correlate with the level of harm induced by parasites when host survival is a crucial parameter for the parasite fitness. The precise origin of the association between developmental instability in wetas and infections by hairworms and mermithids remains to be determined.

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## BIOSKETCH

Frédéric Thomas' research interests include every aspect of the ecology and the evolution of host-parasite relationships, especially the influence of parasites on host behaviour.