SHORT COMMUNICATION

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Larval density and developmental instability in the acoustically orienting parasitoid *Homotrixa alleni*.

Abstract The relationship between developmental stress caused by intraspecific larval density and fluctuating asymmetry (FA) was examined in the tachinid parasitoid *Homotrixa alleni*. Individuals reared in larger clutches were significantly smaller in body size when they emerged as adults. As predicted, smaller individuals were shown to have significantly higher levels of FA in their tibiae. This negative relationship was consistent across each pair of tibia and the magnitude of FA was also significantly correlated between tibiae. The exact nature of the observed relationship between larval density and FA in *H. alleni* remains speculative.

Key words Larval density · Body size · Fluctuating asymmetry · Environmental stress · Tachinidae

Introduction

In many insect species, increased larval density typically has deleterious effects on many aspects of adult fitness (Prinkkilä and Hanski 1995). Gregarious parasitoids, in particular, have been shown to exhibit reductions in body size (Waage and Ng 1984; Bai and Mackauer 1992; Adamo at al. 1995; Nakamura 1995), fecundity (Visser 1994) and longevity (Visser 1994) when completing development at high larval density. In these instances, the adverse effects of larval density are presumed to originate from larval competition because host size is fixed, which forces larvae to compete for limited nutritional resources during their development (Godfray 1994). How-

ever, a number of other factors have the potential to interact with the effects of larval density, which makes identifying the exact cause of fitness reductions in adults somewhat troublesome (Godfray 1994).

In this study we use fluctuating asymmetry (FA) as a measure to examine the impact that larval density has on the development of *Homotrixa alleni* (Diptera: Tachinidae) larvae. *H. alleni* is an acoustically orienting parasitoid fly that parasitises up to 87% of calling male *Sciarasaga quadrata* (Orthoptera: Tettigoniidae) in the field (Allen 1995a, b). The majority of male *S. quadrata* are multiparasitised with up to 16 fly larvae developing within hosts in the field (Allen 1995a). FA refers to the small, random deviations from perfect symmetry that exist in morphological traits and presumably arise because of genetic and/or environmental stressors that are experienced during development (Palmer and Strobeck 1986). Based on this it may be predicted that FA should increase with larval density. *H. alleni* provides an excellent opportunity to test this prediction as (1) parasitised hosts are abundant and readily collected in the field, (2) the number of larvae developing within a host can be accurately determined early in larval development so as to quantify larval density, and (3) larvae are easily reared through their successive developmental stages in the laboratory.

Methods

A total of 92 calling male *S. quadrata* were collected in the 1995–1996 field season (December 1995 to February 1996) within a 2-km area of coastal heath at Cape Naturaliste (33°33′S, 115°01′E) in the southwest of Australia. Parasitism of *S. quadrata* was determined by examining males for the presence of respiratory holes produced by the fly larvae by the 4th day of the 14 days of larval development within the host (Allen, 1995a). The number of these supernumerary spiracles present on the host provided an accurate measure of the number of larvae developing within a host and was used as our estimate of larval density (Allen, 1995a). Parasitised males were maintained in the laboratory under identical conditions following the methods of Allen (1995a). Upon completion of fly development in the host we recorded the number of fly larvae that successfully pupated and measured the weight of

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Table 1 The between-individuals *F*-ratio, intraclass repeatability estimates (*RE*), and measurement errors (*ME*) for mean trait size and fluctuating asymmetry (*FA*) in each of the morphological traits examined. The *F*-ratio demonstrates a significantly greater variance between individuals than within the repeated measures of the same individual. *ME* = the proportion of within-individual variation to total variation (Merilä and Björklund 1995) and *RE* = the intraclass correlation of the variance component of a repeated measures ANOVA

Morphological trait		$F_{(19,40)}$	RE	ME(%)
Fore tibia	Size	1956.57**	0.99	0.15
	FA	28.23**	0.90	8.56
Mid tibia	Size	1155.84**	0.99	0.26
	FA	16.62**	0.84	12.13
Hind tibia	Size	3321.73**	0.99	0.10
	FA	16.96**	0.87	10.39
Head width	Size	2035.91**	0.99	0.19

**P<0.0001

each pupa within 48 h of emergence. Flies that emerged from pupae were frozen prior to morphological measurements.

For each fly we measured head capsule width and the length of its left and right fore, mid, and hind tibiae. Legs were mounted on slides in Hoyer's solution prior to measurement. Tibiae were measured with an eyepiece graticule at 30× magnification under a binocular microscope with a 0.007-mm accuracy, and head width was measured at 20× magnification with a 0.01-mm accuracy. Head width was used as an index of body size.

For each pair of legs, FA was calculated by subtracting the left from the right side. The repeatability and extent of measurement error in measures of trait size and FA were examined using a random subset of 20 animals measured blindly over 3 consecutive days. All measurements were performed by one author (J. Hunt) to prevent inter-observer measurement error and measured blindly with regard to larval density. All measures were highly repeatable and demonstrated low to moderate measurement errors (Table 1). Furthermore, the signed asymmetry values across all flies examined were normally distributed (Filliben correlation coefficient: fore tibiae: *r*=0.996, *n*=121, *P*>0.25; mid tibiae: *r*=0.992, *n*=128, *P*>0.25; hind tibiae: *r*=0.989, *n*=123, *P*>0.10) around a mean of zero (one-sample *t*-test: fore tibiae: 0.002±0.001 mm, *t*=1.757, *df*=120, *P*=0.08; mid tibiae: −0.001±0.001 mm, *t*=0.911, *df*=126, *P*=0.36; hind tibiae: −0.001±0.001 mm, *t*=1.15, *df*=115, *P*=0.25) and hence exhibited the statistical properties of FA (Palmer and Strobeck 1986; Swaddle et al. 1994).

Since it is the magnitude of deviation from perfect symmetry that is of importance rather than the direction of the deviation, all analyses involving FA were performed on the absolute values (Palmer and Strobeck 1986). Non-parametric statistics were used to account for the resultant half-normal frequency distribution (Swaddle et al. 1994). Regression analysis was used for data not involving FA. When correlating the level of FA with body size and between tibia, only animals that possessed all tibiae were used, thus accounting for the reduction in sample size.

Results

The mean weight of emerging pupae per clutch size decreased rapidly with clutch size (*t*=5.97, *r*=0.91, *n*=8, *P*=0.0001) (Fig. 1) and pupal weight was shown to correlate directly with adult body size (*t*=12.07, *r*=0.84, *n*=61, *P*=0.0001). Thus, increases in larval density constrain adult body size.

As predicted, levels of FA increased significantly with larval density, generating a consistent negative relationship between tibiae FA and body size (Spearman rank correlation: fore tibiae: r_s=−0.46, *z*=−4.60,
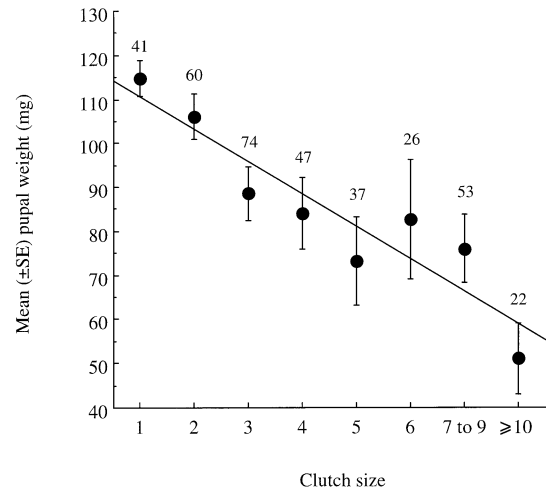

Fig 1 Relationship between mean pupal weight and clutch size in *Homotrixa alleni*. Sample sizes are given above each data point

P=0.0001; mid tibiae: r_s=−0.56, *z*=−5.64, *P*=0.0001; hind tibiae: r_s=−0.31, *z*=−3.12, all *n*'s=102, *P*=0.0018) (Fig. 2a–c, respectively). The level of FA in the tibiae of *H. alleni* was positively correlated across all tibia (Spearman rank correlation: fore vs. mid tibiae: r_s=0.39, *P*=0.0001; fore vs. hind tibiae: r_s=0.39, *P*=0.0001; mid vs. hind tibiae: r_s=0.36, *P*=0.003; all *n*'s=102). Thus, an increase in larval density consistently generates higher levels of FA across each pair of tibiae in *H. alleni*.

Discussion

The findings of this study are of importance to fluctuating asymmetry theory in two major ways. Firstly, this study provides evidence that stress encountered during development, due to larval density, elevates levels of FA in morphological traits (Sciulli et al. 1979; Kieser et al. 1986, Nilsson, 1994). However, the correlational nature of this study places limitations on the interpretation of the results and thus we are unable to ascertain directly
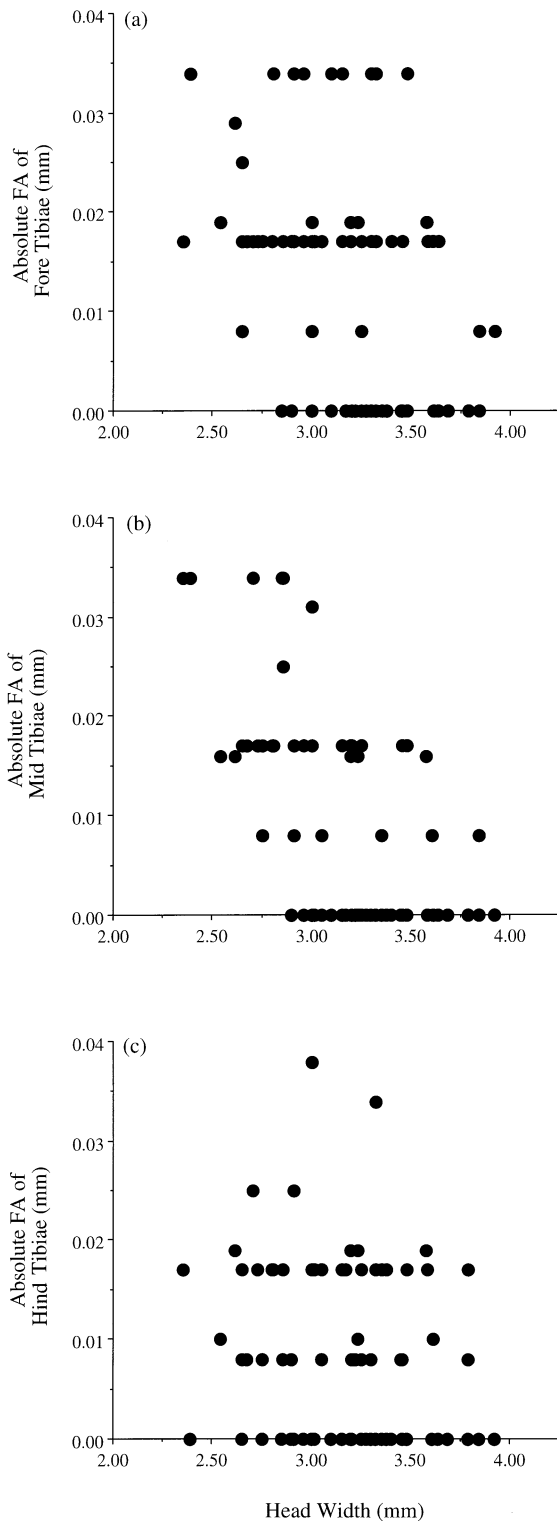
Fig. 2 Relationship between the absolute fluctuating asymmetry (FA) in (**a**) fore tibiae, (**b**) mid tibiae, and (**c**) hind tibiae and body size in *H. alleni*. Since the relative FA of tibiae (absolute FA / mean tibiae size) did not significantly differ between the sexes, male and female data were pooled (Mann–Whitney: fore tibiae: $z=-1.12$, $P=0.27$; mid tibiae: $z=1.12$, $P=0.26$, hind tibiae: $z=-1.18$, $P=0.24$; all n's=102)

the nature and intensity of the factors generating FA in *H. alleni*. The observed relationship between larval density and FA may potentially be caused by (1) larval competition due to a reduction in resources as larval density increases, (2) factors that interact with or are generated by larval density per se, and/or (3) external factors that act independently of larval density. External factors that generate FA may be genetic or environmental in derivation (Palmer and Strobeck 1986). In this study the majority of external environmental factors were controlled for with all larvae being reared under homogenous conditions in the laboratory, and host size is typically unrelated to larval density (Allen 1995a). However, there is the potential that genetic differences in both host and larval quality may confound the observed results, particularly if poor-quality larvae develop at higher larval densities. Likewise, there are a number of factors that may potentially interact with larval density to explain the observed results. For example, as larval density increases the developing larvae themselves may alter the environment they develop in, generating FA. However given that larval defense against super- and multiparasitism in gregarious parasitoids is typically mounted through resource preemption rather than by altering the environment, this appears unlikely (Godfray 1994). Finally, the observed relationship between larval density and FA may be caused by the effects of larval competition. It is well established in a number of other gregarious parasitoids that larval competition places nutritional stress on developing larvae, leading to reductions in adult body size (Waage and Ng 1984; Bai and Mackauer 1992; Adamo et al. 1995; Nakamura 1995). However, a controlled laboratory experiment is necessary to differentiate between these factors in *H. alleni*.

Secondly, this study demonstrates a consistent correlation in tibial asymmetry between traits. In *H. alleni*, the level of FA in the tibiae was positively correlated across each pair of tibia. While theory predicts that an individual of inferior quality should possess high levels of FA in all morphological traits, studies providing convincing evidence for this are rare. Most studies examining levels of FA in multiple traits have revealed only a weak concordance in the levels of FA amongst traits within an individual, somewhat questioning the applicability of FA studies (Dufour and Weatherhead 1996; Whitlock 1996; Hunt and Allen 1998). However, the high correlation between levels of FA in *H. alleni* may be expected if a single genome governs the development of legs from their imaginal discs. This appears to be the case in the fly genus *Drosophila* where the genetics of leg development has been studied extensively (Mardon et al. 1994; Adamson and Shearn 1996).

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