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Fluctuating asymmetry, call structure and the risk of attack from phonotactic parasitoids in the bushcricket *Sciarasaga quadrata* (Orthoptera: Tettigoniidae)

Abstract The acoustically orienting parasitoid *Homotrixa alleni* (Diptera: Tachinidae) is a significant mortality factor of calling male *Sciarasaga quadrata* (Orthoptera: Tettigoniidae), with less than 10% of males escaping parasitism within the duration of the calling season. This study examined fluctuating asymmetry (FA) (small, random deviations from perfect symmetry) in five morphological traits in *S. quadrata* and its relation to the survival of calling males in the field. A significant relationship was demonstrated in only a single trait; across three consecutive field seasons and among each of the collections per season, parasitised males were shown to have significantly more asymmetric hind tibia than unparasitised males. Since the morphological development of males is complete prior to parasitoid attack, this relationship is not caused by the activities of the parasitoid. Further experimentation revealed that there was no relationship between the level of FA in the hind tibia and the success of parasitism, with males artificially parasitised in the laboratory showing no difference in FA from those where parasitism was unsuccessful. Instead, FA was shown to be negatively related to chirp length, an aspect of call structure that significantly affects the survival of calling males under parasitism risk in the field. We explore the possible reasons why more asymmetric males produce calls of shorter chirp length and hypothesise that the difference in hind tibia asymmetry seen between parasitised and unparasitised males in the field is related to host location in *H. alleni* rather than the inability of *S. quadrata* to defend itself against parasitoid attack.

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Introduction

Organisms generally produce bilateral structures or organs and in the majority of cases stabilising selection maintains bilateral symmetry, giving an a priori case for expecting symmetry to be the ideal state (Van Valen 1962; Palmer and Strobeck 1986; Watson and Thornhill 1994). Since both sides of a bilaterally symmetrical character are the products of expression of a single genome, departures from bilateral symmetry presumably reflect the inability of the genome to coordinate development precisely (Waddington 1957; Soulé 1982; Soulé and Cuzin-Roudy 1982; Van Valen 1962). Fluctuating asymmetry (FA) measures these random deviations from perfect symmetry in morphological traits (Palmer and Strobeck 1986).

Severe stress applied at certain critical developmental stages has been shown to greatly increase the variability of development, producing abnormal phenotypes and high levels of FA (Waddington 1957; Emlen et al. 1993). Both environmental and genomic stressors of sufficient intensity have been shown to generate higher levels of FA (see reviews by Parsons 1990a; Møller 1993). Since fitness often depends upon the precise functioning of morphological traits it is understandable that high FA is often associated with a reduction in individual fitness (e.g. Packer and Pussey 1993; Naugler and Leech 1994; Ueno 1994; Møller 1996) and/or reproductive success (e.g. Markow and Ricker 1992; Thornhill 1992; Simmons 1995; Allen and Simmons 1996; Møller 1996).

One stressor that has been shown to adversely effect both fitness and developmental stability is parasite infestation (see review by Polak 1996). By directly competing with their host for available nutrients, parasites have been shown to impair host metabolism, growth rates and development (Sykes and Coop 1977;

Thompson 1983; Schall et al. 1982; Goater et al. 1993), ultimately elevating the degree of host FA. A number of correlational (Kieser et al. 1986; Liveshits et al. 1988; Nilsson 1994; Bonn et al. 1996) and experimental (Sciulli et al. 1979; Parsons 1990b; Møller 1992, 1995; Polak 1993; Folstad et al. 1996) studies provide support for a causal link between parasitism and FA. This appears to be especially true for secondary sexual traits that are often expressed close to their physiological limits due to intense sexual selection (Polak 1993).

However, in many instances, parasite-host interactions are restricted to the adult stage of the host's life-cycle (see review by Clayton 1991) where ontogeny has ceased and the activities of the parasite are no longer able to influence host FA. A non-causal link between FA and parasitism might be expected under at least two conditions. Firstly, parasitism and FA may be associated if high FA reflects the inability of the host's immune system to defend itself against parasite attack. A direct relationship between high FA and an increased susceptibility to parasitism has been convincingly demonstrated in humans (*Homo sapiens*) (Shapiro 1983), the western fence lizard (*Sceloporus occidentalis*) (Schall 1995) and the house fly (*Musca domestica*) (Møller 1996). Moreover, numerous studies have shown that the stressor elevating FA may also independently augment susceptibility to parasitism (O'Brien and Evermann 1988; Moulia et al. 1991; Clarke 1993). Secondly, parasitism and FA may be related if individuals with low FA are more successful in avoiding parasitism, either through direct or indirect means (Bonn et al. 1996). For example, individuals may indirectly avoid parasitism by preferentially residing in parasite free habitats or may directly avoid parasitism via behaviours such as grooming or preening (e.g. Baker and Smith 1995). Swaddle (1997) has recently demonstrated the importance of FA in an analogous system, showing that houseflies (*Musca domestica*) with more asymmetric tibia were more heavily predated by yellow dungflies (*Scathophaga stercoraria*) in the laboratory.

The Austrosagine *Sciarasaga quadrata* (Orthoptera: Tettigoniidae) is a large, brachypterous, univoltine bushcricket found within a restricted range of coastal heath in south western Australia (Rentz 1993; Allen 1995a, b). Adult males calling in the field are subject to significant mortality from the acoustically orienting ormiine fly *Homotrixa alleni* (Diptera: Tachinidae), with parasitism levels increasing over the calling season and peaking at 87% by the end of the 3- to 4-month calling season (Allen 1995a). Males die within 2 weeks of being parasitised, leading to a rapid decrease in the number and density of adult males in the field as flies continually cull calling males from the general population (Allen 1995a). The synchronous and non-overlapping phenology of *S. quadrata* permits a direct assessment of the characteristics of surviving males and how these relate to escaping parasitoid attack.

The aim of this study was to examine the relationship between FA and parasitism in male *S. quadrata* to ascertain if differences in developmental stability can predict which males are most likely to escape parasitism within a calling season. To achieve this, FA was quantified across three successive field seasons and comparisons were made between the parasitised and unparasitised males collected within each season. Upon establishing a relationship between FA and parasitism, two possible reasons underlying this association were examined. The first examined the relationship between FA and success of parasitism by parasitising males under controlled conditions and comparing the success of the developing larvae with host FA. The second examined the relationship between FA and call structure because differences in call structure have been shown to be relevant to host location in other ormiine flies (Walker 1993; Wagner 1996).

Methods

Collection of animals

Field collections of calling male *S. quadrata* were undertaken over 3 successive years within a 2-km² area of coastal heath at Cape Naturaliste (33°33′, 115°01′) in the southwest of Western Australia. Typically, males begin calling during the 2nd to 3rd calendar week of November, with surviving males continuing to call until mid to late February (Allen 1995b). Five separate collecting trips were made during the 1992–1993 and 1994–1995 calling seasons, and six separate collecting trips were made during the 1993–1994 calling season, spanning the entire length of the 3- to 4-month calling season (see Allen 1995a, 1998 for exact dates). On the morning following collection, each male was examined in the laboratory for the presence and number of small holes visible on their abdomen. These holes are produced through the actions of the fly larvae 3–4 days after entry into the host and serve as the respiratory outlet, thereby providing an accurate means to assess parasitism and the developmental status of the fly larvae (Allen 1995a). Parasitism rates were ultimately determined by maintaining males in the laboratory for at least 14 days until fly larvae emerge to pupate (Allen 1995a).

All field collected animals were housed in individual 10 × 8 × 8 cm plastic containers within a constant temperature room maintained at 20 ± 1°C and a 14:10 L:D cycle. This selected temperature is the long-term November–February mean at Cape Naturaliste and fell within the range (10.6–24.2°C) experienced by *S. quadrata* in the field when calling (Allen 1995a). Males were fed daily on a mixed diet of commercial bee pollen, fresh apple and water.

Upon death males were frozen, except for those males from the 1992–1993 season which were stored in 70% ethanol. The number of fly larvae to emerge and successfully pupate from each parasitised host was recorded, allowing the interaction between FA and the number of fly larvae successfully developing in hosts to be further examined. If FA and parasitism success are related, it is expected that males with high FA would experience a greater number of larvae developing within them. Unparasitised males in each of the seasons were used in parasitism experiments and males from the 1994–1995 season were used to record and analyse call structure.

Parasitism experiments

Gravid female flies were collected in the field using artificial chirper units broadcasting the synthesised calls of *S. quadrata* (see Allen 1998a). In the laboratory, flies were anaesthetised using carbon dioxide and dissected under a binocular microscope to expose their highly coiled uterus holding the developing first instar larvae (see Ferrar 1987). The uterus was removed from the abdomen and the larvae dispersed in insect physiological saline. The larvae

within the uterus vary in age with the oldest being at the distal end of the uterus. Older larvae are characterised by the development of more prominent, darker intersegmental plates and only these larvae were used in subsequent experiments. Under a dissecting microscope, a fine-tip paint brush was used to remove individual larvae from solution and deposit them on the abdomen of unparasitised males. All larvae moved rapidly when deposited on the host's abdomen and upon reaching the intersegmental membrane a probe was used to lift the intersegmental plate, thereby ensuring entrance into the host. The number of larvae used to parasitise each male ranged between two and four larvae per host and a total of 33 males were parasitised in the experiment. This range was consistent with the mean number of fly larvae per host observed in the field (Allen 1995a). Artificially parasitised males were maintained under identical conditions to all field-collected animals. Parasitism success was recorded 7 days after infestation by counting the number of respiratory holes visible on each male. Upon death, each of the experimental hosts were frozen prior to morphological measurements.

Call analysis

To prevent any potential interference of neighbouring calling males on recordings, unparasitised males found calling in the laboratory were relocated into separate constant temperature rooms maintained at $20 \pm 1°C$ for sound recording. Recordings were restricted to the first 3 h of scotophase to minimise any variation that may occur to call structure over the duration of the evening. For each recording, a microphone was secured 10 cm above the calling male and 3 min of continuous calling recorded using a Sony Professional Walkman (model WM-D6C). All calls were analysed using the "Canary" software package.

The call of male *S. quadrata* is composed of an incessant and uninterrupted chirp (Rentz 1993), with each chirp being produced by a single wing closure (Fig. 1) (Allen, unpublished work). Five parameters relating to call structure were examined: chirp length (CL), interchirp length (IC), peak frequency (PF), duty cycle [DC = CL/(CL + IC)] and chirp rate (CR). Chirp rate was estimated by averaging chirp rate across three 30-s segments of the recording, whereas the other four parameters were averaged from ten 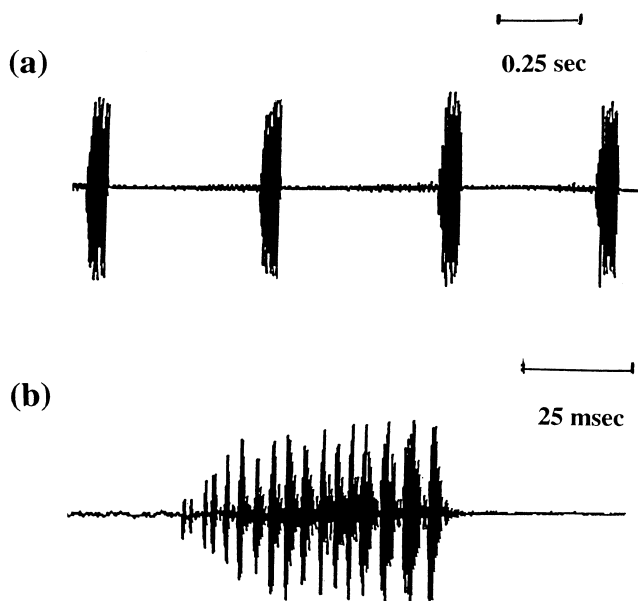calls taken at random within the recording. All five call parameters have been shown to be significantly repeatable in the short term [within a 3-min recording period: range of r values = 0.57 (PF)–0.88 (CL)] and in the long term [consecutive nights: range of r values = 0.27 (PF)–0.83 (CR); over lifespan: range = 0.16 (PF)–0.43 (CL)] (see Allen, unpublished work).

Morphological measurements and fluctuating asymmetry

It is generally accepted that multiple traits are preferred over single traits when testing for differences in developmental stability between samples (Leary and Allendorf 1989; Palmer 1994; Dufour and Weatherhead 1996). Consequently, FA was measured across five different morphological traits in male *S. quadrata*. These were the maxillary palps, hind wings, fore tibia, fore tibial ears and hind tibia. Only hind tibia was measured in the 1992–1993 season. Each trait was carefully dissected and removed from the body for measuring. The maxillary palps and hind wings were mounted on slides in Hoyer's solution for measuring. The length of the penultimate distal segment of the maxillary palps and the maximum width of the hind wings were measured with an eyepiece graticule at $8 \times$ magnification under a binocular microscope.

The fore and hind tibia were dissected at the joint from the femur and tarsi and excess body tissue removed to expose a well defined cuticular measuring landmark. The fore tibia was measured under a binocular microscope using an eyepiece graticule at $8 \times$ magnification and the aperture length of the tibial ear was measured at $15 \times$ magnification. All microscope measurements were made to the nearest quarter of a graticule unit. Hind tibia length and maximum head width were measured using Mitutoyo digital calipers, re-calibrated at the beginning of each measure. Head width was measured as an index of body size.

In each trait FA was calculated by subtracting the left from the right side and trait size was calculated as the average of the right and left sides. The repeatability and degree of measurement error (ME) in measures of trait size and FA were assessed using a random subset of 20 animals measured "blindly" over 3 consecutive days. Measures were accepted as being repeatable if there was a significantly greater variance between individuals than within the repeated measures of the same individual. Repeatability estimates of these measures were based on an intraclass correlation of the variance component of a repeated measures ANOVA. ME was calculated according to Eq. 1 in Merilä and Björklund (1995), being estimated as the proportion of within individual variation to total variation. All measurements were performed by J.H. to prevent any inter-observer ME. Repeatability estimates and ME for measures of trait size and asymmetry are provided in Table 1.

Traits were accepted as displaying true FA if the signed asymmetries were normally distributed around a mean value of zero or if they did differ from normality they did so as a result of leptokurtosis and not platykurtosis or skewness (Palmer and

(a)

0.25 sec

(b)

25 msec

Fig. 1 The call structure of *Sciarasaga quadrata*, **a** showing 4 successive chirps and **b** showing the fine structure of the single chirp (one wing closure) broken down into a series of tooth impacts as the plectrum on the right tegmen scrapes over the teeth of the left tegmen

Table 1 The between-individuals F ratio, intraclass repeatability estimates and measurement errors (ME) for mean trait size and fluctuating asymmetry (FA) in each of the morphological traits examined

Morphological trait		$F_{(19,40)}$	Repeatability	ME(%)
Maxillary palps	size	48.98**	0.98	5.50
	FA	17.63**	0.94	14.61
Fore tibia	size	142.72**	0.99	2.02
	FA	15.91**	0.94	23.08
Fore tibial ears	size	25.18**	0.96	16.68
	FA	25.84**	0.96	15.02
Hind wings	size	338.76**	0.99	0.86
	FA	285.51**	0.99	0.88
Hind tibia	size	677.60**	0.99	0.36
	FA	28.87**	0.97	10.17

**$P < 0.0001$

Table 2 Statistical properties of signed FA measures in the bilateral traits of male *Sciarasaga quadrata*. The Filliben correlation coefficient shows significant deviations from normality and the Spearman rank correlation coefficient (r_s) shows the relationship between the absolute asymmetry in a trait and the mean trait size. The Spearman rank correlation coefficient is corrected for ties in the data set

Morphological trait	Filliben coefficient	Mean FA ($\pm$ SE)	r_s	n
1992–1993				
Hind tibia	0.9899	0.007 $\pm$ 0.014	0.113	81
1993–1994				
Maxillary palps	0.9872	−0.002 $\pm$ 0.003	−0.146	68
Fore tibia	0.9929	−0.007 $\pm$ 0.006	0.005	79
Fore tibial ears	0.9932	−0.001 $\pm$ 0.002	0.110	79
Hind wings	0.9939	0.014 $\pm$ 0.014	0.201	62
Hind tibia	0.9921	0.007 $\pm$ 0.014	0.201	82
1994–1995				
Maxillary palps	0.9898	−0.002 $\pm$ 0.003	0.181	83
Fore tibia	0.9900	0.007 $\pm$ 0.007	0.028	109
Fore tibial ears	0.9890	0.001 $\pm$ 0.002	−0.034	109
Hind wings	0.9930	−0.012 $\pm$ 0.013	−0.024	89
Hind tibia	0.9938	−0.024 $\pm$ 0.017	0.008	106

Strobeck 1986). Normality was tested using a Filliben correlation coefficient which demonstrates above-average power in detecting deviations from normality due to long-tailed and/or skewed distributions (Filliben 1975). Departure from a mean FA of zero was tested using a one-sample *t*-test. All analyses are in agreement with the statistical recommendations of Swaddle et al. (1994). The statistical properties of our FA measures are given in Table 2 for each of the three sampling seasons.

Since it is the magnitude of deviation from symmetry that is of importance rather than the actual direction of this deviation, all comparisons of FA were performed on absolute values using non-parametric statistics to account for the half normal frequency distribution (Palmer and Strobeck 1986). Size-dependent FA has the potential to confound inferences made in studies of developmental stability so that the level of FA is often required to be scaled for the size of the trait being compared (Palmer 1994). To test for size dependence of FA, the absolute asymmetry of traits were plotted against the mean trait size and assessed using a Spearman rank correlation coefficient (Table 2). Since FA did not consistently demonstrate any size dependence, comparisons of FA within traits could be made directly using absolute FA. In comparisons of FA across different traits relative asymmetry (absolute asymmetry divided by trait size) was used to control for trait size (Swaddle et al. 1994). By summing the relative asymmetries for individuals possessing FA measures for each of the five morphological traits, an index of organism-wide developmental stability was assessed. This overall level of FA in morphology can be regarded as a health certificate of the individual (Zakharov 1992).

Results

Patterns of FA in field-collected animals

The degree of FA in the morphological traits of parasitised and unparasitised males did not significantly differ across the collection trips in each of the seasons examined (Kruskal-Wallis: parasitised = H_{corr} ranged from 0.54 to 5.55, unparasitised = H_{corr} ranged from 0.94 to 6.03, all Ps > 0.05). Subsequent comparisons were therefore made directly on the pooled data within each of the calling seasons. The one outstanding predictor of parasitism in male *S. quadrata* was the degree of FA in the hind tibia. The level of FA in the hind tibia of parasitised males was significantly greater than in unparasitised males for each of the seasons examined (Table 3, Fig. 2). In contrast, parasitised and un-

Table 3 Mann-Whitney test comparing the level of absolute FA in each of the morphological traits between parasitised and unparasitied male *S. quadrata*. *Z* values are corrected for ties in the data set

Morphological trait	Z	n
1992–1993		
Hind tibia	−5.91**	81
1993–1994		
Hind tibia	−4.85**	82
Maxillary palps	−0.17	68
Hind wings	−1.08	62
Fore tibia	−1.93	79
Fore tibial ears	−0.82	79
Overall asymmetry index	−1.55	48
1994–1995		
Hind tibia	−2.06*	106
Maxillary palps	−1.30	83
Hind wings	−0.59	89
Fore tibia	−0.90	109
Fore tibial ears	−1.16	109
Overall asymmetry index	−0.11	70

**P < 0.0001, *P < 0.05

parasitised males did not significantly differ in the level of FA expressed in the other four morphological traits examined (Table 3, Fig. 3a–d). Likewise, the overall asymmetry index did not significantly differ between parasitised and unparasitised males (Table 3, Fig. 3e).

Morphological traits often differ in the degree to which they are canalised during development such that those traits most important to survival are expected to show the lowest levels of FA (Møller 1993). The hind wings of *S. quadrata* had the highest level of relative FA of all the morphological traits examined (Table 4). The level of relative FA did not significantly differ between the maxillary palps, fore tibial ears and fore tibia nor between the maxillary palps, fore tibial ears and hind tibia (Table 4). However, the magnitude of relative FA was significantly greater in the hind tibia than the fore tibia (Table 4). There were no significant correlations between the levels of FA in these traits, therefore demonstrating that an individuals FA for a given trait is a poor predictor of asymmetry for any other trait (Table 5).
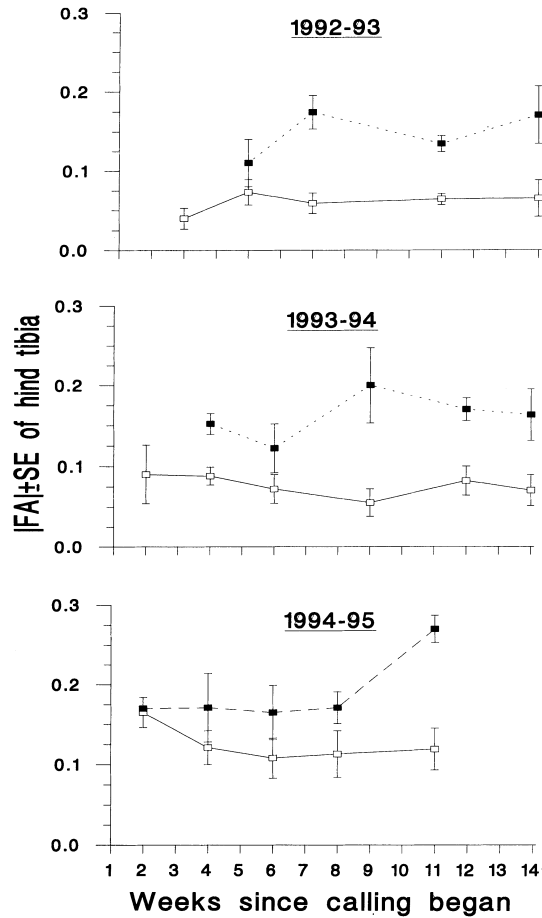
Fig. 2 The mean (±SE) absolute fluctuating asymmetry (FA) in the hind tibia of parasitised and unparasitised male *S. quadrata* collected over three successive calling seasons. The sample sizes are: 1992–93: parasitised = 4, 11, 18, 4 and unparasitised = 10, 4, 8, 18, 4; 1993–94: parasitised = 5, 6, 6, 5, 6 and unparasitised = 5, 13, 8, 8, 12, 8; 1994–95: parasitised = 1, 10, 15, 15, 3 and unparasitised = 20, 15, 13, 7, 7. *Filled symbols* represent parasitised males and *empty symbols* represent unparasitised males
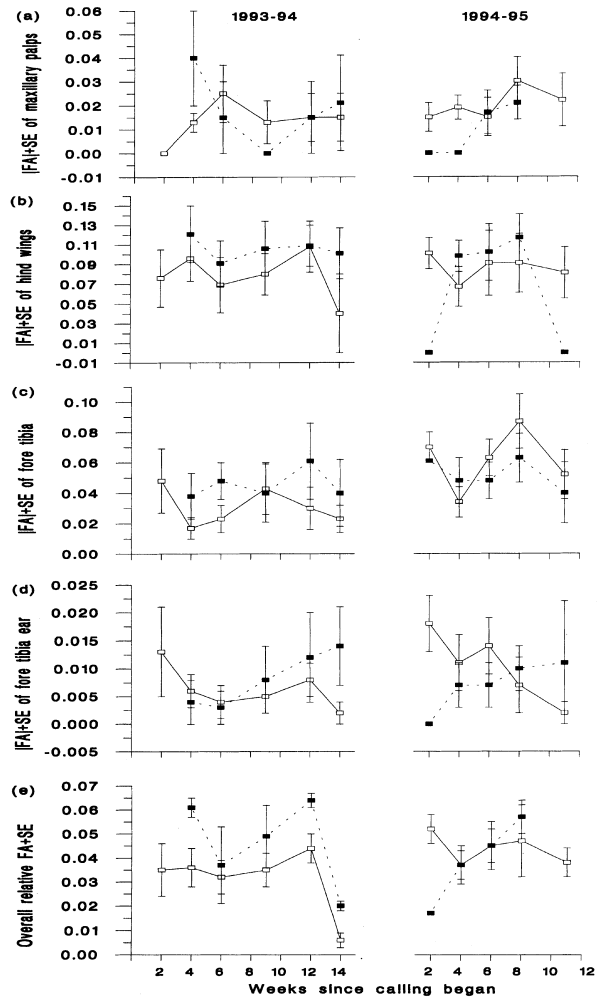

Fig. 3 The mean (±SE) absolute FA in the **a** maxillary palps, **b** hind wings, **c** fore tibia, **d** fore tibial ear and mean (±SE) relative FA in the **e** overall relative asymmetry, for the 1993–94 and 1994–95 calling seasons. Symbols as in Fig. 2

Table 4 Wilcoxon paired analysis tests for differences in the levels of relative FA in each of the morphological traits examined in the 1993–1994 and 1994–1995 seasons. The Z values are corrected for ties in the data set and the sample size is given in brackets (*HT* hind tibia, *MP* maxillary palps, *HW* hind wings, *FT* fore tibia, *FTE* fore tibial ears). Significance levels are Bonferroni adjusted, with a significance level of 0.005

	Maxillary palps	Hind wings	Fore tibia	Fore tibial ears
1993–1994				
Hind tibia	HT < MP Z = −0.455 (64)	HT < HW* Z = −4.493 (61)	HT > FT* Z = −4.257 (77)	HT < FTE Z = −0.533 (75)
Maxillary palps		MP < HW* Z = 2.832 (42)	MP > FT Z = −1.857 (45)	MP > FTE Z = −0.419 (34)
Hind wings			HW > FT* Z = −5.118 (49)	HW > FTE* Z = −3.277 (48)
Fore tibia				FT < FTE Z = −1.940 (51)
1994–1995				
Hind tibia	HT > MP Z = −0.591 (79)	HT < HW Z = −2.66 (87)	HT > FT* Z = −3.689 (106)	HT < FTE Z = −0.647 (105)
Maxillary palps		MP < HW* Z = −2.923 (60)	MP > FT Z = −1.336 (69)	MP > FTE Z = −0.544 (48)
Hind wings			HW > FT* Z = −4.803 (83)	HW > FTE Z = −1.346 (76)
Fore tibia				FT < FTE Z = −1.321 (87)

*Significant at the Bonferroni-adjusted probability of *P* < 0.005

Table 5 Spearman rank correlation coefficients testing for associations between the level of FA in each of the traits examined in the 1993–1994 and 1994–1995 seasons. The r_s values are corrected for ties in the data set. The sample size is given in parentheses. All values are not significant at the 95% confidence interval

	Maxillary palps	Hind wings	Fore tibia	Fore tibial ears
1993–1994				
Hind tibia	0.101(66)	0.152 (61)	0.087 (77)	0.085 (77)
Maxillary palps		0.115 (54)	0.121 (63)	0.054 (63)
Hind wings			0.123 (57)	0.080 (57)
Fore tibia				0.223 (75)
1994–1995				
Hind tibia	0.178 (81)	0.640 (88)	0.166 (106)	0.116 (106)
Maxillary palps		0.166 (72)	0.032 (83)	0.105 (83)
Hind wings			0.066 (90)	0.117 (90)
Fore tibia				0.014 (109)

Parasitism experiments

Out of all the males infected in the laboratory only 9% escaped parasitism. The number of larvae to successfully emerge and pupate from hosts was not significantly influenced by the initial number of larvae inserted into each host, suggesting that larval competition, within the range tested, is not influencing the success of parasitism (Mann-Whitney: $Z_{corr} = -0.89$, $n = 33$, $P = 0.38$). Therefore the level of FA in hind tibia was compared directly using pooled data across the range of larvae inserted. The mean level of hind tibia FA did not significantly differ between males that were successfully parasitised (0.114 ± 0.018 mm, $n = 30$) and those escaping parasitism (0.117 ± 0.009 mm, $n = 3$) (Mann-Whitney: $Z_{corr} = -0.94$, $n = 33$, $P = 0.35$).

Among field parasitised males, hind tibia FA was not significantly correlated with the number of larvae developing within them (range 1–16 larvae) (Spearman rank correlation 1992–1993: $r_s = -0.21$, $n = 33$, $P = 0.24$; 1993–1994: $r_s = 0.31$, $n = 24$, $P = 0.14$; 1994–1995: $r_s = 0.16$, $n = 44$, $P = 0.28$). This supports the laboratory findings that physiological differences are unlikely to explain the significantly higher levels of hind tibia FA found amongst field parasitised males.

Call analysis

The level of FA in the hind tibia of calling male *S. quadrata* did not significantly correlate with interchirp length, peak frequency nor chirp rate (Spearman rank correlation: interchirp length: $r_s = 0.22$, $P = 0.13$; peak frequency: $r_s = 0.07$, $P = 0.64$; chirp rate: $r_s = -0.17$, $P = 0.23$; all n's = 51). However, hind tibia FA did show a strong significant negative relationship with chirp length ($r_s = -0.37$, $n = 51$, $P = 0.009$) (Fig. 4). A weaker negative relationship was also shown with duty cycle ($r_s = -0.36$, $n = 51$, $P = 0.01$), which is expected due to the concomitant decrease in chirp length. Since chirp length is effected by male age and size (Allen, unpublished work), a multiple regression was used to analyse the relationship between hind tibia FA and chirp length. When male age and size were accounted for in the analysis, the significant neg-
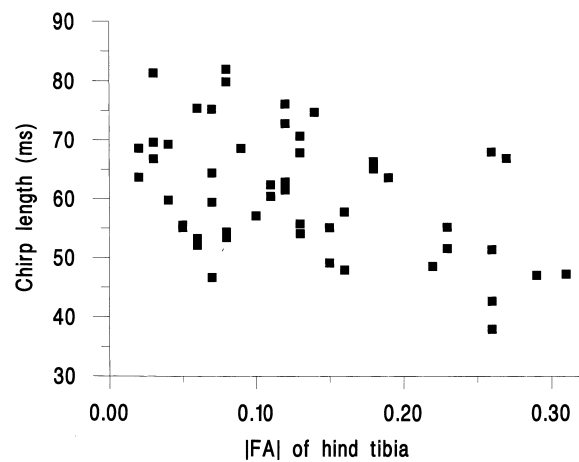

Fig. 4 The relationship between chirp length and absolute FA in the hind tibia of male *S. quadrata*

ative relationship between hind tibia FA and chirp length persisted ($F_{(2,47)} = 9.69$, $P = 0.0001$; age: 0.16 ± 0.06, $t = 2.86$, $P = 0.006$; body size: −7.79 ± 3.73, $t = 2.09$, $P = 0.04$; hind tibia FA: −41.39 ± 15.61, $t = 2.65$, $P = 0.01$). Since duty cycle does not correlate with male size (Allen, unpublished work), a multiple regression was only used to account for the effects of male age. When male age was taken into account the significant negative relationship between hind tibia FA and duty cycle remained ($F_{(2,48)} = 3.72$, $P = 0.03$; age: −0.05 ± 0.04, $t = 1.02$, $P = 0.31$; hind tibia FA: −22.99 ± 8.60, $t = 2.67$, $P = 0.01$).

Discussion

Our field results provide strong evidence for an association between elevated levels of FA in the hind tibia and the occurrence of parasitism in the bushcricket *S. quadrata*. In *S. quadrata* parasitism is restricted to calling adult males and hence morphological development is complete prior to parasitoid attack. This ensures that the level of FA expressed in the morphological traits of calling males is not influenced by the actions of developing fly larvae. Therefore, in contrast to the multitude of studies demonstrating a causal link between parasit-

ism and FA (see review by Polak 1996), the relationship observed in the hind tibia of *S. quadrata* is not caused by parasitism.

In the limited number of studies examining non-causal associations between parasitism and FA, individuals with high levels of FA experience an increased probability of parasitism because of the inability of their immune system to defend itself against parasite attack (Shapiro 1983; Schall 1995; Møller 1996). In the most convincing of these studies, Møller (1996) experimentally infected adult flies (*Musca domestica*) with the entomopathogenic fungus (*Enthomophthora muscae*) and showed that individuals with asymmetric wings were more susceptible to fungal infections, demonstrated lower mating success and were more frequently predated by the insectivorous barn swallow, *Hirundo rustica*. Contrary to these findings, we found no relationship in the laboratory between hind tibia asymmetry and susceptibility to parasitism in male *S. quadrata*. While Møller (1996) showed that approximately 37% of male and 47% of female flies were able to escape infection, here we demonstrate that *S. quadrata* is far less successful, with only 9% of males able to escape parasitism, a value closely resembling natural levels observed in the field towards the end of the calling season (see Allen 1995a). This suggests that the immune system of *S. quadrata* is poorly developed and/or the ability of *H. alleni* larvae to overcome host immune defences is well developed. Several aspects of the biology of *H. alleni* suggests that the latter is most likely.

Firstly, by retaining live first instar larvae in their uterus, *H. alleni* does not expose the vulnerable egg and early larval stages to the host's immune system. Secondly, upon entry into the host, many tachinid larvae are able to actively overcome the host's immune response by positioning themselves in regions of the host less vulnerable to immune attack (Belshaw 1994). Changes in larval position do occur in the ormiine *Ormia ochracea* where the first instar larvae embed themselves in the host's muscle for the first 3 days and then migrate to the abdomen (Adamo et al. 1995). Surface properties of segmental plates of *H. alleni* larvae may also resist encapsulation by the host's immune response, as has been demonstrated in the larvae of the parasitoid wasp, *Cardiochiles nigriceps* (Stoltz and Vinson 1979; Strand 1986). Thirdly, the puncturing of the host's integument to produce a respiratory hole upon reaching the second instar stage may help overcome the likelihood of asphyxiation, one of the most prominent immune defences utilised by hosts (Belshaw 1994). Finally, *H. alleni* is able to develop in a wide variety of host species, with at least two other tettigoniid species being known field hosts and a further eight species of ensiferan Orthoptera being physiologically suitable hosts in the laboratory (Barraclough and Allen 1996). Therefore it appears unlikely that intraspecific differences in a male's susceptibility to parasitism can adequately explain the difference in hind tibia FA between parasitised and unparasitised males.

Here, we establish a correlation between hind tibia asymmetry and the length of the chirp component of a male's call. Individual males exhibiting higher levels of FA in their hind tibia were found to produce calls with a reduced chirp length, suggesting that chirp length may be an important determinant of parasitism in the field. Indeed, chirp length has been shown to be one of the primary call parameters influencing the survivorship of calling male *S. quadrata* in the field (Allen, unpublished work). On average, males that survive later into the calling season produce significantly longer chirps, implying that flies select calls of shorter chirp length. Chirp length is important to host location in at least one other ormiine parasitoid *Ormia ochracea* which favours longer chirp lengths in the call structure of its host *Gryllus lineaticeps* (Wagner 1996). However the call structure of *S. quadrata* is far simpler than the call of *G. lineaticeps* since chirps are made up of single rather than multiple wing closures. The preference for shorter rather than longer chirp lengths in *H. alleni* may be the result of an underlying sensory bias in the fly's hearing enabling it to locate and parasitise a wide range of hosts in the field. Polyphagy in *H. alleni* is apparent late in the calling season of *S. quadrata* at Cape Naturaliste where it may be found parasitising other tettigoniid species, by its wide geographic range that extends beyond that of *S. quadrata*, and by the broad frequency tuning of its ear (Lakes-Harlan et al. 1995; Barraclough and Allen 1996).

The nature of the relationship between hind tibia asymmetry and chirp length in male *S. quadrata* remains speculative. One possibility may relate to the metabolic costs of calling. Calls with longer chirp lengths have been demonstrated to be more energetically expensive in Anurans (Ryan 1985) and Orthoptera (Bailey et al. 1993; Prestwich 1994), so that only males with greater energy reserves expressing low FA may be able to afford these costs. Another explanation may relate to the mechanics of calling in *S. quadrata*. In *S. quadrata,* each chirp is composed of a series of discrete syllables that are formed when the plectrum of the right wing engages the teeth of the file on the left wing during wing closure (see Fig. 1). The number of teeth successfully contacting the plectrum ultimately determines the length of the chirp. Males with more asymmetric hind tibia may engage less teeth during each wing closure, as a result of imprecise coupling of wings during closure, therefore producing calls of shorter chirp length. In gryllid crickets, Simmons and Ritchie (1996) have shown that the magnitude of directional asymmetry in the sound resonating structures ("harps") of male *Gryllus campestris* is directly related to the level of FA expressed in the hind tibia. However, the file and plectrum of tettigoniids perform markedly different functions from those in gryllids (Bailey 1991) and the two structures may possibly be governed by independent genomes.

Why, then, if males with asymmetric hind tibia are being selected by flies does the mean level of FA not decrease over the calling season? If the chirp length preference of *H. alleni* includes a fixed upper and lower

limit to attractiveness, then any alteration to chirp length may shift a male's call outside of this threshold. Temperature certainly alters chirp length in tettigoniids with chirp length typically decreasing linearly with increasing ambient temperature (Bailey 1991). Ambient temperatures during the calling season of *S. quadrata* ranges from 10.6 to 24.2°C and may drop by up to 10°C within a given night (Allen 1995b). Unless the hearing template of *H. alleni* is temperature coupled, this may result in a high level of plasticity in the dynamics of the host-parasitoid system.

Finally, it is important to recognise that the levels of FA exhibited by the different morphological traits significantly varied in relation to parasitism. Out of the five different morphological traits examined, only FA in the hind tibia of males was significant in relation to parasitism. Since differences in FA are expected to be most pronounced in those traits that are most vital to survival (Palmer 1994), it suggests that the hind tibia in *S. quadrata* should be subject to the strongest regime of stabilising selection. However, this is inconsistent with the levels of relative FA shown in the traits of *S. quadrata* (see Table 3). Furthermore, the level of FA in any one particular trait was a poor predictor of the level of FA for any other trait. The results of this study add to the increasing number of studies demonstrating weak concordance in the levels of FA amongst traits (Brakefield and Breuker 1996; Dufour and Weatherhead 1996; Whitlock 1996) and clearly demonstrates that a better understanding of developmental processes is required before it can be assumed that all traits should respond in the same manner when subjected to a uniform stress (Emlen et al. 1993).

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