Parasitol Res (2007) 101:1289–1294
DOI 10.1007/s00436-007-0635-3

ORIGINAL PAPER

Sex differences in fluctuating asymmetry of body traits in chewing lice *Docophorulus coarctatus* (Phthiraptera: Ischnocera)

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Received: 10 May 2007 / Accepted: 12 June 2007 / Published online: 23 June 2007

Abstract Fluctuating asymmetry (FA) that reflects randomly directed deviations from bilateral symmetry has been shown to increase in organisms exposed to environmental and/or genetic stress. We studied fluctuating asymmetry in head and prothorax of chewing lice *Docophorulus coarctatus*, a parasite of the great grey shrike *Lanius excubitor*, to investigate associations between parasite body size and fluctuating asymmetry. Samples of ten individual lice (five females, five males) were randomly collected for measurements from 32 shrikes. Relative FA (scaled to trait size) was estimated for head and prothorax. Sex and trait differences in FA were very distinct (all differences significant at $P < 0.001$). However, relative FA of head was not a predictor to relative FA of prothorax in either sex. Moreover, relative FA measurements of females and males from the same host were not significantly correlated, contrary to expectation if hosts imposed similar selection pressures on parasites of the two sexes. Relative FA of a trait was negatively related to its size, except for the relationship between relative FA of prothorax and prothorax in females. Differences in FA between traits may be explained by time when host condition affects louse developmental biology, with the head developing in a very short time during larval and nymph stages. The sex difference in asymmetry was probably related to different selection pressures by hosts on the sexes of the parasite, with females generally being under more intense selection.

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Introduction

An environment constituted by a host is crucial for the life of a parasite (Møller 1996; Poulin 1996; Tschirren et al. 2007). Host behaviour and/or immune system affect population sizes of parasites living on or within the body of the host and also affect body size and shape of parasites (Poulin 1996; Clayton et al. 1999; Tryjanowski et al. 2007). However, not only effects of hosts influence parasite body size, but so does the density of conspecifics (Tryjanowski et al. 2007). In a previous study (Tryjanowski et al. 2007), we found that body size of the chewing louse *Docophorulus coarctatus* (Scopoli 1763) is positively related to host size and negatively related to population size of lice of its avian host, the great grey shrike *Lanius excubitor*. Moreover, we showed negative interactions between host size and louse population density, which may be interpreted as an outcome of conspecific competition for available resources such as space and/or food. Therefore, we hypothesised that environmental stressors (here population density and host condition) may affect the population of lice not only by influencing body size of the parasite but also its morphological asymmetry, as has been shown in previous studies on fluctuating asymmetry (FA; e.g. Clarke and McKenzie

1992; Møller 1992; Palmer 1994; Møller and Swaddle 1997; Radwan 2003). Fluctuating asymmetry represents the non-directional kind of asymmetry for otherwise bilaterally symmetric traits caused by developmental errors during ontogeny. Such asymmetries are usually assumed to impose a handicap on organisms simply because symmetry in most cases constitutes the optimal functional design. We paid special attention to variation in sex differences in asymmetry of body traits of lice because female parasites are subject to more intense selection than males (Tschirren et al. 2007; Tryjanowski et al. 2007). Finally, we hypothesised that if stress factors affect body size of lice (through host size or crowding by other lice; Tryjanowski et al. 2007), then smaller lice—that are assumed to be more stressed—should also be characterised by a greater level of asymmetry.

A study of asymmetry of parasites is also interesting from a different perspective. It is well known that parasites influence host reproductive effort and many life history traits, including fluctuating asymmetry of the host especially for sexually selected traits (Møller 1992, 1996; Thomas et al. 1998). In contrast, there is, to the best of our knowledge, not a single study investigating how the environment offered by a host (and cospecifers) affects fluctuating asymmetry of its parasites.

There is controversy as how to study FA (Van Dongen et al. 1999; Palmer and Strobeck 2003; Söderman et al. 2007). To reduce potential sampling errors, parasites should be sampled in equal numbers for both sexes from a number of host individuals. Moreover, to avoid pseudoreplication and/or work on marginal measurements, only average values for local lice populations (separately for females and males) should be correlated with phenotypic traits of the host. For the present paper, we collected the data according to this strict protocol (see also Tryjanowski et al. 2007).

In this paper, we describe patterns of asymmetry for two body traits (head and prothorax) in *D. coarctatus* collected from their avian host, the great grey shrike. We paid special attention to variation in louse FA in relation to parasite size, sex and traits chosen for analysis.

Materials and methods

Study species and populations

D. coarctatus (Phthiraptera: Ischnocera) is the most numerous species (present in 93.5% of hosts) of lice in the great grey shrike (Szczykutowicz et al. 2006). Lice were collected by T. Weisz and/or by taxidermists from recently killed birds by direct inspection for ectoparasites without using special methods such as fumigation. All lice were preserved in 75% ethyl alcohol (more details in Szczykutowicz et al. 2006). After removal from alcohol, all lice were cleaned and mounted on microscope slides for examination and identification.

Measurements

Only well-preserved lice (with both characters required for measurement, with both left and right side preserved in good condition) were used in morphometric analyses. We measured lice from 32 shrike individuals (20 females, 10 males, 2 undetermined) from which a minimum of five males and five females of *D. coarctatus* in good condition could be sampled at random by a laboratory assistant who had no knowledge of the hypotheses to be tested (for details on procedure, see Tryjanowski et al. 2007).

Two characters were measured for the study of asymmetry: head length and prothorax shield (taken at the widest point; Fig. 1). Lice were coated with gold in a Balzers SPC 050 ion coater and were observed, analysed and measured in Zeiss Evo 40 scanning electron microscope (Tryjanowski et al. 2005). To reduce potential measurement error, all measurements were made by the same person (ZA; see also below). To establish repeatability of body traits, the same observer measured the randomly selected part of previously measured individuals (n=29 for head and n=37 for prothorax) once again after 7 months from the first measurements, and a laboratory assistant had blindly made the previous measurements. Both traits had high and statistically significant repeatability (repeatability r was estimated as the intra-class coefficient: head, r=0.997 and prothorax, r=0.949, P<0.0001 in both cases).

Fluctuating asymmetry

Right (R) and left (L) values of both body traits of 320 lice (from 32 hosts) were measured. FA values from each

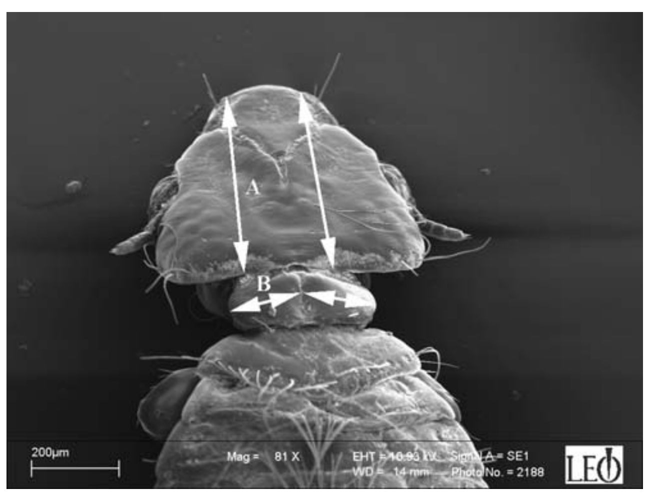

Fig. 1 Morphological trait values for which FA was calculated in the louse species *Docophorulus coarctatus*: *a* left and right side of head length and *b* left and right prothorax shield width

Table 1 Descriptive statistics of the studied traits and their FA in relation to parasite sex (n=32 populations of five males and five females of *Docophorulus coarctatus*)

Trait	Sex	Size (mean±SE)	FA (mean±SE)	FA skewness	FA kurtosis
Fluctuating asymmetry					
Head	Male	424.904±13.166	1.703±0.142	1.964	5.407
	Female	457.393±13.776	4.872±0.340	0.986	0.926
Prothorax	Male	89.389±3.956	5.989±0.379	0.455	−0.360
	Female	138.197±4.636	8.294±0.671	0.442	−0.378
Relative fluctuating asymmetry[a]					
Head	Male	424.904±13.166	0.421±0.039	1.482	2.943
	Female	457.393±13.776	1.118±0.098	1.519	3.194
Prothorax	Male	89.389±3.956	4.890±0.389	1.253	3.038
	Female	138.197±4.636	6.059±0.491	0.695	0.027

Data on size through the table and figures are presented in microliters.
[a] Standardised for trait size (see "Materials and methods")

individual was recalculated to the absolute deviation of $R-L$ ($|R-L|$) for further analyses. Based on these results, size corrected and unsigned FA index (FA $=|R-L|/$mean $[(R+L)/2]$) was recalculated according to Palmer (1994) where the unsigned difference was divided by the sample mean of the average trait size. Because this relative asymmetry index assumes isometry, which may be rare, we also computed residuals from a regression of asymmetry on size. However, such residuals were strongly positively correlated with relative FA expressed in the traditional way ($r>0.8$ and $P<0.001$ in all cases), and therefore, we decided to present data in this classical way.

Measurements of left and right sides were highly repeatable (head: $F_{28,29}=840.3$, $P<0.0001$; prothorax: $F_{27,28}=7.00$, $P<0.01$). Similarly, repeatabilities of asymmetries were also highly statistically significant (head: $F_{28,29}=3.34$, $P<0.001$; prothorax: $F_{36,37}=10.05$, $P<0.0001$).

To establish FA at the population scale, for each population of lice living on an individual host, all relative FA individual measurements were averaged (separately for five females and five males) and, in that case, one value (for each sex and measured trait) was obtained. Therefore, further analysis was done on FA (expressed as a residual from regression line of asymmetry on size) at the level of the parasite population.

Because some authors suggested (Clayton et al. 1999; Johnson et al. 2005) that bill size (measurements, see Hromada et al. 2003) affects the ability of avian hosts to control their lice populations, we additionally undertook a separate analysis on this trait. We did not find a significant relationship between bill width or height and relative or absolute FA of lice ($r<0.30$ and $P>0.1$ in all comparisons; after correction for multiple tests $P>0.2$ in all comparisons).

All values reported are means±SE. Statistical analyses were performed using SPSS 11.0 for Windows. Because in

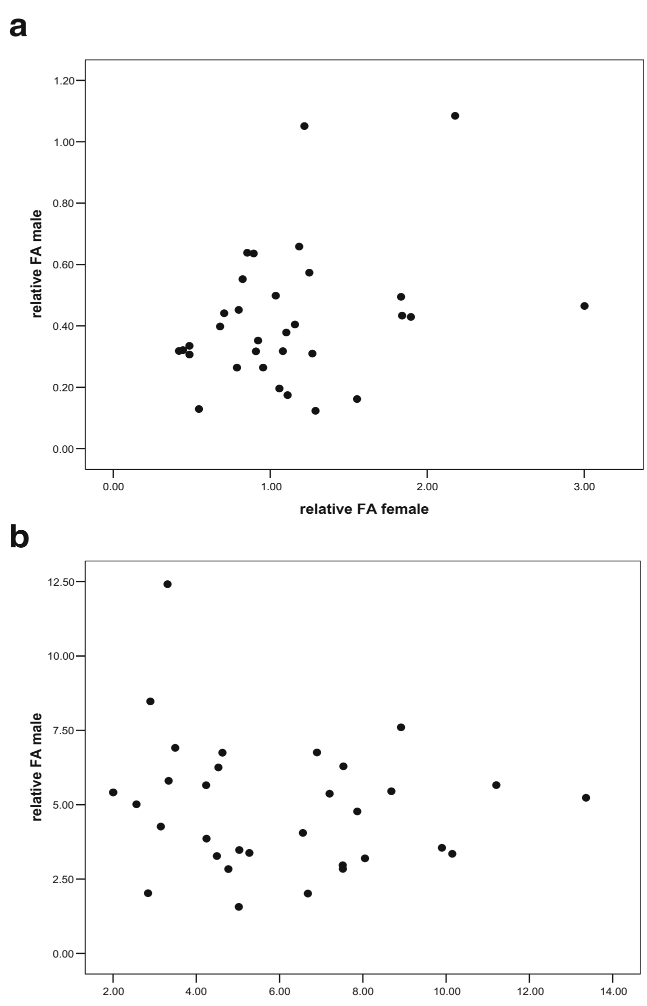

Fig. 2 Relationship between relative fluctuating asymmetry of **a** head and **b** prothorax of male and female *D. coarctatus* obtained from the same 32 avian host individuals (r=0.308, P=0.086 and r=−0.144, P=0.431, respectively)

the paper we made some multiple comparisons, significance levels were corrected by the false discovery rate (the proportion of false discoveries), a method proved to better compromise between type I and II errors (Benjamini and Hochberg 1995; Waite and Campbell 2006).

Results

The directional component of asymmetry accounted for less than 1.5% of the total asymmetry, confirming findings that directional asymmetry is insignificant ($F_{1,56}$=0.154 and $F_{1,54}$=0.108 with P=0.70 and P=0.74 for head and prothorax, respectively). Furthermore, component of potential antisymmetry for all variables was very low (skewness values for considered traits, see Table 1). Fluctuating asymmetry of head and prothorax of a total of 320 adult *D. coarctatus* from 32 shrike individuals was measured. Descriptive statistics based on averages for individual avian hosts (see "Materials and methods") showed that both sexes, as well as the measured traits, are very distinct in terms of FA (Table 1, $F_{3,124}$=76.068, all differences significant at P<0.001; interaction sex × trait was non-significant at P=0.46). Interestingly, the correlations between relative FA of measured traits (head and prothorax) were very low and non-significant both in males and females (r=0.147, P=0.422 and r=−0.066, P=0.718, respectively). Therefore, relative FA of head was not a predictor of relative FA of prothorax in either sex at the population level.

Relative FA measurements of females and males from the same host were not significantly correlated (Fig. 2a,b), with Pearson r values 0.308 (P=0.086 for head) and −0.144 (P=0.431 for prothorax, N=32 in both cases).

Relative FA of a measured trait was negatively related to the size of that trait (Fig. 3), except for the relation between relative FA of prothorax and prothorax size in females (all three significant correlations were also significant after correction for multiple comparisons, N=32 in all cases). In the case of the head, we did not find significant differences between sexes in the relationship between relative FA and trait size (t test between slopes, P=0.37).

Discussion

This study has shown that fluctuating asymmetry in two body traits of the louse *D. coarctatus* is negatively related to size of the parasite. Similar patterns of negative correlation between asymmetry and body size have also been recorded in other insects such as the winter moth *Operophtera brumata* (Van Dongen et al. 1999), but not in the bulb mite *Rhizoglyphus robini* (Radwan 2003). Because a previous

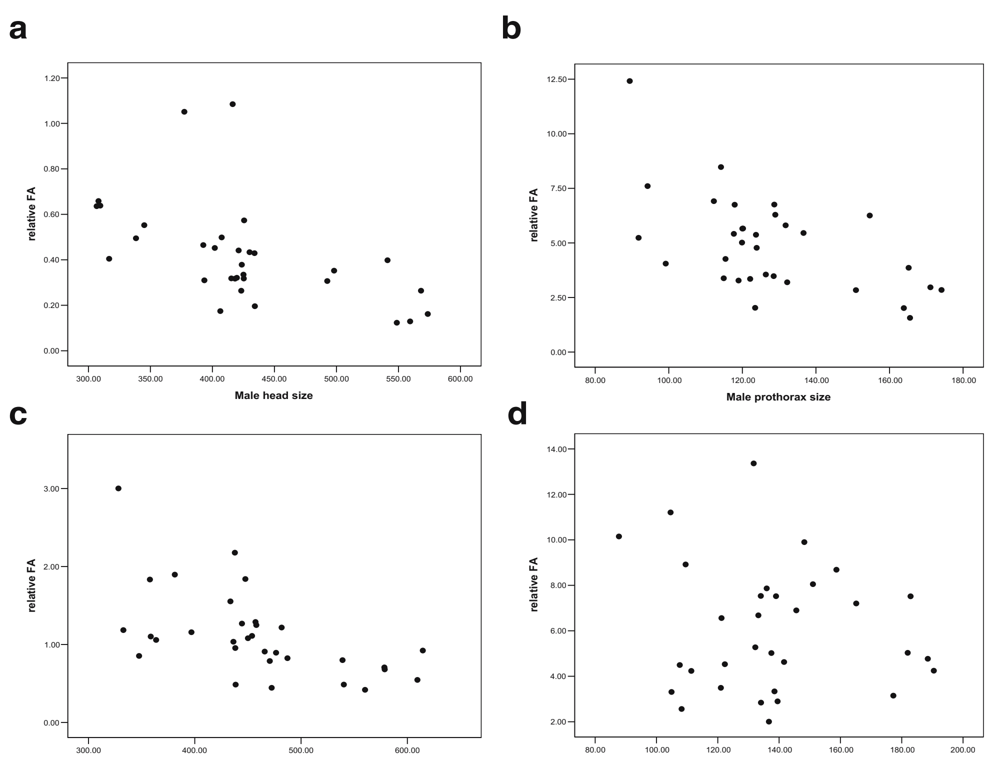

Fig. 3 Relationship between fluctuating asymmetry of head and prothorax of male (**a**, **b**) and female (**c**, **d**) *D. coarctatus* obtained from the same 32 avian host individuals (r=−0.540, P=0.001 and r=−0.573, P<0.001, r=−0.574, P<0.001, r=−0.111, P=0.545)

study of the same host–parasite system (Tryjanowski et al. 2007) showed that the size of *D. coarctatus* is strongly positively related to host size and local louse population density on individual hosts, the results on fluctuating asymmetry presented here may be interpreted as being determined during development.

We found differences in the degree of asymmetry between traits, with the head having lower asymmetry than the prothorax. This could be explained by the duration that host conditions can affect developmental biology of the louse. Generally, the head develops during a very short period (early during larval and nymphal stages), while the prothorax continues to grow up until the last imago stage (Złotorzycka 1994). Therefore, the prothorax is exposed for longer to environment influences than the head (both host and conspecific effects, see Tryjanowski et al. 2007). On the other hand, the difference in FA of the studied traits is not affected by host preening activity, and we did not find any relationships between shrike bill parameters and FA of lice or with body size of the host as was shown in the previous paper (Tryjanowski et al. 2007).

Which potential mechanism can explain the negative association between parasite FA and body size, especially the sex difference? When lice are transferred to a new host individual, they encounter novel environmental conditions for development, reproduction and growth. If conditions are beneficial (expressed for example by a large overall host body size where large hosts are superior competitors, and thus, gain access to more resources that can be exploited by parasites), lice may achieve larger size, and also—because they obtain more food during development time—develop a lower degree of FA. Sex differences in parasite phenotype are consequences of the different effects of hosts on body size of male and female parasites (see also Tryjanowski et al. 2007). In contrast to some previous studies where males were more asymmetric than females (Møller 1992; Møller and Swaddle 1997), here, we report that females are more asymmetric than males. This effect may arise from hosts exerting stronger selection pressures on female parasites than on males because females, in addition to resources required for their own maintenance, also need resources for reproduction.

Although FA is negatively correlated with body size of the parasite, we are unsure whether FA provides a reliable measure of the influence of the immune system of the host and/or the suitability of the host for the parasite. First, interpretation of the obtained finding is strongly limited by information on developmental processes of the louse species. Secondly, due to technical problems, it is much easier to count parasites, measure their size and then later study fluctuation asymmetry. However, under experimental conditions, FA may be a better estimator of environmental effects than other variables (Clarke and McKenzie 1992; Van Dongen et al. 1999; Vilisics et al. 2005; Söderman et al. 2007; but see also Stige et al. 2006).

To conclude, sex and body size of parasites were related to fluctuation asymmetry of lice.

Acknowledgements We thank the staff of Natural History Department, Šarišské Museum Bardejov, M. Hromada and T. Jazsay for their general help. We are also indebted to A.P. Møller, P. Zduniak, P. Ręk and V. Takacs who helped us with critical comments on a previous version of the manuscript and, particularly, to A. Szczykutowicz for lice identification. We would also like to add an ethical note that great grey shrike individuals were shot for reasons other than parasitological studies (museum collections), and legal regulations are now completely different from the past when the material was collected.

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