

Fluctuating asymmetry of meristic traits: an isofemale line analysis in an invasive drosophilid, *Zaprionus indianus*

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Abstract Metric (e.g., body size) and meristic (e.g., bristle number) traits are of general use in quantitative genetic studies, and the phenotypic variance is subdivided into a genetic and a non-genetic environmental component. The non-genetic variance may have two origins: a common garden effect between individuals and a developmental instability within the same individual. Developmental instability may be studied by considering the fluctuating asymmetry (FA) between the two sides of the body. The isofemale line technique is a convenient method for investigating the architecture of natural populations but has been rarely implemented for investigating FA. In this paper, we use this experimental design for analyzing four meristic traits in eight populations of the cosmopolitan *Zaprionus indianus*. A study of the correlation between left and right side of each line revealed that almost 90% of the variability was due to a developmental noise, while a much higher correlation among the means of the lines from the same

population was observed. A slight trend toward a directional asymmetry was observed: more thoracic bristles on the left side. Four kinds of indices, scaled or non-scaled to the mean were used for comparing the different traits. Unscaled values (mean absolute values or standard deviation of each line) revealed a linear increase with the means. Interestingly the results of ovariole number were included in the same regression. With the scaled indices (mean absolute divided by each individual value or standard deviation divided by the mean), the differences among traits were considerably decreased, but still remained significant. The mean FA of the various traits were not correlated, suggesting that each trait harbors its own developmental stability. The CVs of FA were high with a magnitude similar to those of the trait themselves, slightly less than 10%. Finally, even with the isofemale line design, which is a powerful means for unravelling slight genetic variations, we did not find any clear indication of a genetic component of FA under the optimal environmental conditions used in this study.

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Introduction

In the evolutionary history of animals, a major step has been the appearance of Bilateria, a phylum which is characterized by a bilateral symmetry and which encompasses most extant multicellular taxa (Maynard Smith and Szathmari 1995). The way in which this symmetry has been established is a long debated problem among developmental biologists (Waddington 1957; Brown and Wolpert 1990; McManus 2002; Levin and Palmer 2007; Nakamura and

Hamada 2012; Vandenberg and Levin 2013). Even for normally symmetric traits, the two sides of the body are generally not absolutely identical, revealing some imprecision in the developmental process: this is generally described as resulting from developmental noise or developmental instability, while the reciprocal process, that ensures phenotypic consistency among body sides, is called developmental stability. During the last decades, a renewed interest has appeared for such lateral variations that are designated as fluctuating asymmetry (FA) (Polak 2003). Many ways have been proposed and explored for describing and analyzing such variations (Palmer and Strobeck 1986, 2003).

It is well known that different quantitative traits are more variable than others. For example, in *Drosophila* grown under the best conditions of the laboratory, wing and thorax length have a coefficient of variation (CV)=2.5, (David et al. 2006) while the number of bristles on the thorax or abdomen have CVs around ten (Araripe et al. 2004; Chakir et al. 2007; Yassin et al. 2009). Both kinds of traits are, however, paradigms for quantitative genetic analyses (Falconer and McKay 1996; Lynch and Walsh 1998). All have high heritability and respond rapidly to selection (Roff and Mousseau 1987). Other studies (Coayne and Beecham 1987; Gibert et al. 1998; Woods et al. 1998; Yassin et al. 2009; Chakir et al. 2007) have shown that in wild living flies, the phenotypic variability of size was much higher (CVs in the range 7–9), while that of bristle number was almost not increased (CVs around 11). These observations reveal that in nature, wild individuals develop under very heterogeneous environmental conditions mostly due to variation in feeding resources (Woods et al. 1998; Yassin et al. 2009). Size is very plastic and very sensitive to environmental heterogeneity, while bristle number exhibits a high intrinsic variability (high within line CV), i.e., a low developmental stability, but accompanied by a high resilience/canalization against environmental effects.

These observations seem somehow contradictory, and it was interesting to consider another approach for studying developmental stability, that is fluctuating asymmetry (FA). Many studies have been done on FA of metric traits in *Drosophila* (e.g. Markow and Ricker 1992; Imasheva et al. 1997; Klingenberg and Zaklan 2000; Santos 2001; Debat et al. 2006, 2008, 2009, 2011; Trotta et al. 2005; Soto et al. 2008; Carter et al. 2009; Kurbalija et al. 2010) but they are always affected by the problem of measurement error (Palmer and Strobeck 2003). Anyway, a general conclusion seems that for metric traits, which are always related to size, the level of FA is low, (typically around or lower than 1% of trait size) (Palmer 1996, 1999). FA of meristic traits has also been investigated in many papers (Graham et al. 1993; Imasheva et al. 1999; Bourguet 2000; Indrasamy et al. 2000; Woods et al. 2002; Pétavy et al. 2006; Vishalakshi and Singh 2008; Chakir et al. 2007). However, in most

published papers on meristic traits, such as bristle numbers or ovariole number, FA is not considered and only the total number is studied.

Such was the case in a study of many populations of the fly *Zaprionus indianus* which, native to tropical Africa, recently invaded South America (David et al. 2006a). It was found that size traits exhibited latitudinal clines in long established populations from Africa or India, but not in the recent American populations. For the number of thoracic bristles, however, no latitudinal trend was observed, but a significant heritability, 0.24 in female and 0.16 in male. These studies were implemented with the isofemale line technique, a convenient method for analyzing the phenotypic and genetic characteristics of natural populations (David et al. 2006). This method has rarely been used for the analysis of FA (Pétavy et al. 2006; Chakir et al. 2007) and we considered that the *Zaprionus* study was an opportunity to fill this gap. The bulk of the data concerns thoracic, sternopleural bristles (STP), measured on a total of 103 lines from 8 natural populations. Here we present a detailed analysis of FA of STP bristles in these populations. Moreover, on some populations, three other meristic traits were measured, in females only: the bristles on the tergite and sternite of the 7th abdominal segment, and the number of ovarian tubules, or ovarioles, in the ovaries.

Besides a quantification of FA of several meristic traits in a drosophilid, the aims of this work were twofold: (1) use a powerful but not widespread method, the isofemale line technique, for measuring FA at three levels (individuals, lines and populations) and look for associated genetic variations; (2) compare FA of several meristic traits and analyze the relationship with the mean values of the traits.

We evidenced a slight directional asymmetry for STP bristles, favoring the left side, a very low left–right correlation within lines suggesting that developmental noise is responsible for almost 90% of the phenotypic variability, a strong positive linear correlation across traits between FA and the mean of the traits, something which could be corrected only in part by using an absolute scaled index. Concerning the possibility of a genetic variability among lines, we did not find evidence for a significant line effect.

Materials and methods

Populations investigated

We investigated four different populations from tropical Africa, which is the native place of *Zaprionus indianus*, and four Brazilian populations probably originating from a single propagule accidentally introduced in 1998 (Vilela 1999). In two cases, the same population was investigated

twice on different years. Details on these populations may be found in David et al. (2006b), and also in Table 1.

Experimental design

Wild collected *Z. indianus* were brought to the laboratory and females isolated in single vials to establish isofemale lines. The progeny of each female (ten pairs) were used as the parents of the experimental flies which correspond to a second laboratory generation. After sexual maturity, these parents were transferred to vials containing a medium in which a high proportion of killed yeast prevents larval crowding effect (Karan et al. 1999). Development occurred at 25 °C and in all cases the number of emerging adults from a vial was less than 100. The total number of investigated lines is 55 for Brazilian populations and 48 for African ones. For each isofemale line, ten males and ten females were used for a total sample of 2060 flies, for STP.

Traits measured

For all individuals, sternopleural bristles (STP) were counted on each side of the thorax. In some of these populations, other meristic traits were also measured, as indicated in Table 1. Bristles were counted on both sides of the 7th segment of female, i.e., the dorsal part or tergite and the ventral part or sternite. In addition, the number of ovarioles was counted after dissection of mature females (4–5 days). For the latter trait, only five females were dissected in each line.

Data analysis

The advantage of meristic traits is that there are no measurement errors so that their analysis is facilitated. *Z. indianus* was a favorable material for counting the bristles because it has a big size and an overall light color so that the dark bristles are easily seen and counted without any error. As already mentioned (Palmer and Strobeck 2003; Palmer 1994) many statistics and indices can be used for describing FA. In the present work, we first considered the correlation between the two sides. The experimental design permits to calculate such correlations among the flies of each line which share a common origin, i.e., the same grandmother, and also among the means of the lines from the same population. We also investigated FA by considering the difference between the left and right side using several indices at the line and the population levels. First, the signed asymmetry, $(L-R)$, which has an expectation of zero and provides some information on a possible directional asymmetry. Second, the absolute value, $|L-R|$ which is always positive. Third, the scaled absolute value in which each individual difference is divided by $(L+R)/2$. Fourth, we calculated the variance and the standard deviation for the ten flies (or five in case of ovarioles) of each line. Finally, this standard deviation was scaled to the mean number of each line, and the ratio multiplied by 100, providing a coefficient of variation (CV), convenient for comparing different traits with different values. The isofemale line design is interesting because for a given trait, calculations can be done at two different levels, lines and populations. In this respect, the scaled absolute value is especially interesting because it permits to estimate a coefficient of

Table 1 Mean values of morphometric traits in natural populations of South America and Africa investigated with the isofemale technique

Population	n	Sternopleural bristles		Ovarioles	Sternite 7	Tergite 7
		Female	Male			
South América						
Rio, 1999	23	19.58±0.12	18.60±0.12	39.07±0.30	–	–
Salvador, 1999	10	19.79±0.18	18.42±0.15	35.44±0.69	–	–
Rio, 2000	11	19.49±0.23	18.36±0.18	40.90±0.43	–	–
Belém, 2002	11	19.27±0.18	18.31±0.18	–	23.08±0.18	56.79±0.44
Mean		19.52±0.08	18.46±0.08	38.93±0.25		
Africa						
São Tome, 2001	11	19.44±0.24	17.99±0.18	38.90±0.41	–	–
São Tome, 2003	15	20.08±0.19	18.57±0.17	–	20.06±0.18	50.56±0.50
Benin, 2000	10	20.54±0.19	19.66±0.81	40.10±0.50	–	–
Cape Town, 2002	12	19.50±0.20	18.39±0.21	–	20.18±0.20	54.70±0.53
Mean		19.89±0.10	18.45±0.09	39.38±0.31	20.11±0.13	52.21±0.38
Total Mean		19.69±0.06	18.46±0.06	39.07±0.19	20.97±0.13	53.53±0.31
Intercontinental comparison (<i>t</i> test)		2.72**	0.10 ns	1.11 ns	12.88***	7.79**

intraclass correlation which is akin to heritability (David et al. 2005).

Results

Mean traits values

The results are summarized in Table 1. For STP number, 103 lines from eight natural populations were available, taken from Brazil and tropical Africa. Females have more bristles than males, in spite of the fact that their sizes are almost identical (see David et al. 2006). A small but significant difference exists between Brazilian and African populations, but in females only, the African flies having more bristles than the American ones. For ovariole number, only 65 lines were investigated; there is no difference between the two geographic regions and the overall mean number is 39.07. For the bristles on segment 7, still a lesser number of lines (38) were investigated. There seems to exist a big difference between Brazil and Africa. However, since only one Brazilian population was investigated, this difference should be confirmed by more extensive studies. Altogether, there are large differences in mean numbers of the various traits, from 18.45 (STP) up to 53.53 (tergite 7), the ovariole number laying in-between (39.07 ± 0.31).

FA analysis: the L × R correlation

Traits on both sides of a fly are expected to be identical because they share both the same developmental environment and the same genotype. A correlation coefficient lesser than one reveals the occurrence of a developmental noise. Indeed, a null correlation would rule out any control of the symmetry at the individual level. In the present study, we were able to calculate the correlations at two levels: first among ten individuals of the same line, i.e., the within line correlation; and second, among the mean values of the lines from the same population, i.e., the between line correlation. The values of r coefficients are given in Table 2. A striking observation is that the within line correlations, although on average greater than zero, are quite small, while the mean values at the population level are much higher. This phenomenon is illustrated in Fig. 1. The within line coefficient has an overall unimodal distribution but a broad variability, from -0.5 up to 1. This variability illustrates the fact that the sampling error of a correlation is very big when calculated on only ten observations. The between line correlation in the same population exhibits as expected a much narrower distribution. But it is also remarkable by its much higher mean value, close to 0.8. This effect is likely attributable to the existence of genetic variation for the number of bristles across lines, which

triggers the between sides correlation among lines. It does not reveal any genetic variability of FA. Table 2 shows that correlation were consistently higher in African populations and very significantly so in females. In this case, a genetic difference between continents seems to occur, developmental noise being higher in recent populations.

In conclusion, the L *R correlations shows that the developmental noise is extremely important in determining the number of bristles. A correlation of 0.30 gives a square value, $R^2=0.09$, meaning that 91% of the bilateral variability is due to developmental instability.

For the three other traits, the correlations are also given in Table 2. The same phenomenon is observed in each case, i.e., a lower within line correlation (average 0.33 for the three characters) and a significantly much higher value for the among line correlation (average 0.71).

Directional asymmetry: signed value of the L – R difference

The L – R difference can be calculated for each individual and then averaged at the line level. A significant departure from zero will reveal a directional asymmetry (one side greater than the other). Results are given in Table 3. A surprising observation is a significantly positive value in both sexes for STP in Brazilian samples (females $m=0.141 \pm 0.054$; $p=0.009$; males, $m=0.113 \pm 0.050$, $p=0.016$), meaning there are on average more bristles on the left side. In the African populations the directional asymmetry is marginally significant in females only ($m=0.118 \pm 0.057$; $p=0.039$). The other traits do not exhibit any trend toward a directional asymmetry. There are some differences among traits, the most variable being the sternopleural bristles and ovariole number the least. However, these variations are not significant. Altogether, this directional asymmetry, which is difficult to interpret, is small and does not precludes the use of classical indices of FA, as done in the following sections.

FA analysis: unsigned asymmetry and heritability

A commonly utilized method for estimating the magnitude of FA is to consider the unsigned asymmetry, i.e., the absolute value $|L - R|$, or the same value scaled to the mean of each individual, we call scaled absolute value. If we assume that the signed difference has a Gaussian distribution, the absolute value will exhibit a truncated shape, which prevents convenient statistical comparisons (Palmer and Strobeck 2003; Moreteau et al. 2000; Moller and Swadlow 1997; Pétavy et al. 1997). This is illustrated in Fig. 2. On the other hand, if such indices are calculated on groups of flies, the variance between groups will be reduced and the distribution, according to the central limit theorem, will

Table 2 Correlations coefficients between the left and right sides at the within (W) and between (B) lines level

Population	n	Sternopleural bristles				Ovarioles				Sternite 7				Tergite 7			
		F		M		B		W		B		W		B		W	
		B	W	B	W	B	W	B	W	B	W	B	W	B	W		
Brazil																	
Rio, 1999	23	0.85	0.17 ± 0.07	0.84	0.24 ± 0.06	0.63	0.43 ± 0.06	-	-	-	-	-	-	-	-	-	-
Salvador, 1999	10	0.71	0.07 ± 0.10	0.74	0.20 ± 0.09	0.41	0.31 ± 0.07	-	-	-	-	-	-	-	-	-	-
Rio, 2000	11	0.92	0.27 ± 0.08	0.94	0.47 ± 0.07	0.63	0.54 ± 0.08	-	-	-	-	-	-	-	-	-	-
Belém, 2002	11	0.91	0.08 ± 0.12	0.89	0.26 ± 0.12	-	-	0.81	0.25 ± 0.06	0.54	0.36 ± 0.09	-	-	-	-	-	-
Total		0.85 ± 0.05	0.17 ± 0.04	0.85 ± 0.04	0.28 ± 0.04	0.56 ± 0.04	0.43 ± 0.04										
Africa																	
São Tome, 2001	11	0.90	0.44 ± 0.06	0.88	0.19 ± 0.08	0.77	0.54 ± 0.02	-	-	-	-	-	-	-	-	-	-
São Tome, 2003	15	0.91	0.36 ± 0.11	0.68	0.36 ± 0.09	-	-	0.72	0.37 ± 0.05	0.90	0.57 ± 0.06	-	-	-	-	-	-
Benin, 2000	10	0.73	0.35 ± 0.09	0.78	0.48 ± 0.08	0.84	0.53 ± 0.03	-	-	-	-	-	-	-	-	-	-
CapeTown, 2002	12	0.85	0.36 ± 0.08	0.88	0.47 ± 0.07	-	-	0.84	0.45 ± 0.08	0.94	0.41 ± 0.07	-	-	-	-	-	-
Total		0.85 ± 0.04	0.38 ± 0.05	0.81 ± 0.05	0.37 ± 0.04	0.80 ± 0.02	0.53 ± 0.06	0.78 ± 0.04	0.41 ± 0.04	0.92 ± 0.02	0.49 ± 0.05						
Mean ± SE		0.85 ± 0.03	0.27 ± 0.02	0.83 ± 0.02	0.32 ± 0.03	0.68 ± 0.09	0.48 ± 0.05	0.79 ± 0.01	0.33 ± 0.02	0.73 ± 0.13	0.43 ± 0.02						
Intercontinental comparison	p	1.000	0.001**	0.799	0.073	0.085	0.096	0.705	0.024*	0.449	0.0954						

F female, M male, N number of lines

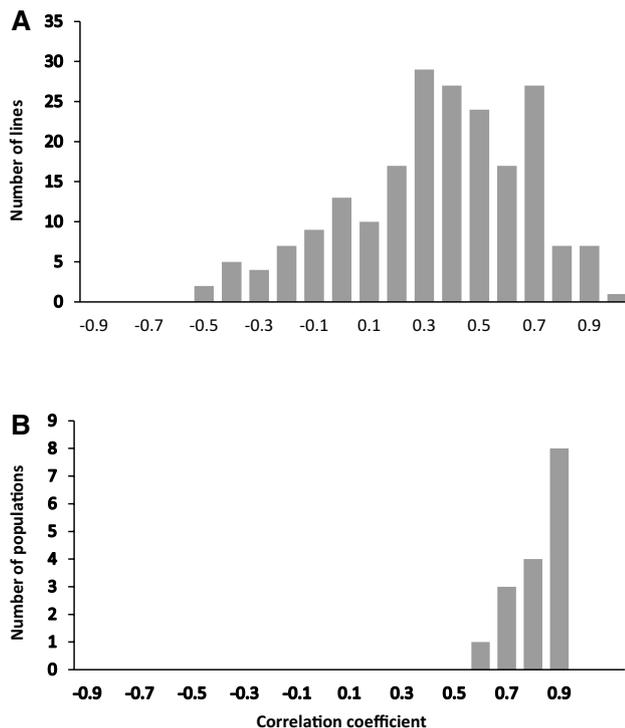


Fig. 1 Distributions of the L×R correlation coefficients of sternopleural bristle numbers, **a** coefficients calculated on each line (males and females pooled, 206 values). **b** coefficients calculated between lines for each population (16 values). Notice the major differences in variance and in the mean of the distributions ($r=0.33$ in **a** and $r=0.84$ in **b**)

approach a Gaussian curve. Since ANOVA is a robust statistical test, we performed a one way ANOVA on each population and calculated the intraclass correlation coefficient whose value is akin to a heritability (see David et al. 2005). The between line variance was never significantly greater

than the within line variance and, surprisingly, most of the intraclass correlations (not shown) had a negative value. We must therefore conclude that there is lack of heterogeneity between the lines of a given population, i.e., a lack of genetic variability for FA.

FA of different traits

In Table 4 we present the means of four different indices based on $|L-R|$ and used for describing FA of the four meristic traits. In each case, the Brazilian and African populations were compared by ANOVA, but no significant difference was observed between these two regions. The mean absolute value ranges from 0.95 for STP bristles up to 2.24 for the bristles on tergite 7. Heterogeneity between traits is highly significant and related to the mean of each trait (see Table 1). When the scaling correction is done as usually advised (Palmer and Strobeck 1992) the variability between traits is considerably reduced (F ratio decreasing from 186.1 to 9.4) but still remains significant. Two traits, ovariole and tergite 7 seems slightly less variables: these are the traits with the greater mean values.

For each isofemale line, we also calculated the variance (see Fowler and Whitlock 1994) and then the standard deviation (SD) whose average values are given in Table 4. It seemed interesting to compare these values to the mean of the traits (calculated for one side). The ratio of the SD to the mean is a coefficient of variation (CV) and the values are also shown in Table 4. The SD are very variable between traits, increasing with the mean values, and this phenomenon decreases when SD is scaled to the mean and transformed into a CV although it remains significant. It seemed interesting to analyze further the relationship between the unscaled indices (absolute values and SD) and the means of the traits. This is shown in Fig. 3. For the two

Table 3 The signed asymmetry L–R of various meristic traits

Population	Sternopleural bristles		Ovarioles	Sternite 7	Tergite 7
	Female	Male			
Brazil					
Rio, 1999	0.147±0.082	0.069±0.083	-0.183±0.181	–	–
Salvador, 1999	0.090±0.119	0.260±0.113	-0.040±0.404	–	–
Rio 2000	0.064±0.123	0.073±0.102	0.073±0.229	–	–
Belém, 2002	0.255±0.134	0.118±0.114	–	0.245±0.142	0.100±0.274
Mean Brazil	0.141±0.054	0.114±0.050	-0.086±0.143	0.245±0.142	0.100±0.274
Africa					
São Tome, 2001	0.191±0.113	0.027±0.121	0.291±0.263	–	–
São Tome, 2003	0.180±0.106	-0.040±0.103	–	-0.047±0.120	-0.207±0.215
Benin, 2000	-0.020±0.126	0.120±0.115	-0.100±0.225	–	–
Cape Town, 2002	0.092±0.117	-0.158±0.121	–	0.017±0.119	0.058±0.271
Mean Africa	0.118±0.057	-0.020±0.057	0.105±0.175	-0.108±0.084	-0.089±0.179
Total mean	0.131±0.032	0.051±0.038	-0.025±0.112	0.057±0.073	-0.034±0.144

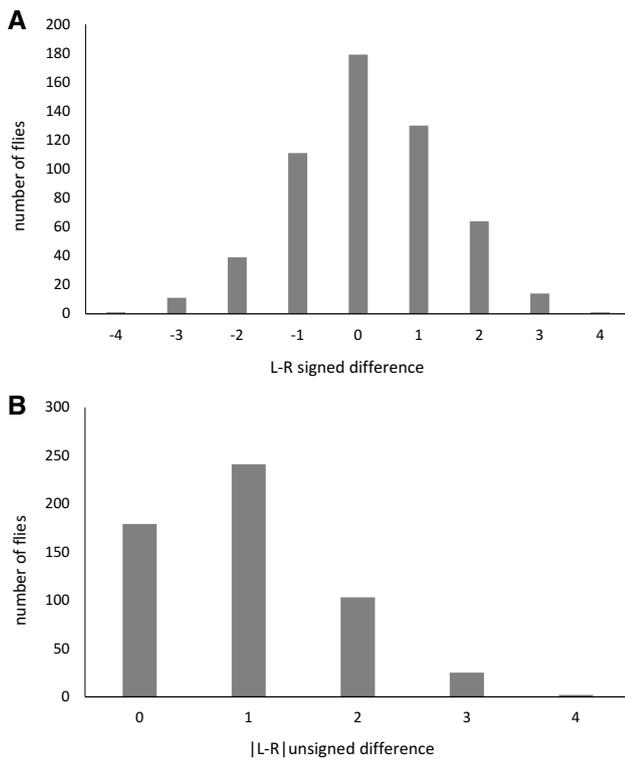


Fig. 2 Distribution of the L–R values for STP number at the individual level (data of both sexes of Brazilian populations). **a** signed differences: an overall bell shape curve is observed, with a mean value close to zero; **b** absolute value of the difference. Notice the truncated shape of the distribution

indices, linear relationships are observed, but the slopes are slightly different. Note that this effect did not appear within trait, where no correlation between trait means and trait asymmetry was detected (calculations not shown).

Correlation among indices of different traits

If developmental instability is a property expressed at the organism level, we expect that individuals with a general lesser stability will produce higher FA indices for different traits. In other words, these indices calculated in the same individual should be positively correlated. In the present work, the various traits were often measured on different flies of the same line so that the correlations could not be calculated within each line. The only possibility was to use the means of the lines for each pair in relevant populations. Due to the experimental design, only three different correlations could be calculated (see Table 1). The mean correlations averaged over populations are the following: STP—ovarioles: $r = -0.063 \pm 0.059$ ($n = 5$ populations); STP—sternite 7: $r = 0.021 \pm 0.051$ ($n = 3$); STP—tergite 7: $r = 0.015 \pm 0.152$ ($n = 3$).

There is a clear conclusion: correlations are nil. FAs of the different characters are completely independent, at least at the between line within population level.

If some lines are more symmetrical than others, the two sexes should be more similar than if they were taken at random from a single bivariate distribution. In other words, male and female FA indices should be positively correlated.

Correlations calculated in each population were very variable, ranging from -0.63 to 0.74 (details not shown).

Table 4 Values of different estimates of FA for the four meristic traits investigated

FA estimates					
Trait and Population	n	AV	SAV	SD	CV
STP					
Brazil	55	0.95 ± 0.03	0.10 ± 0.01	0.82 ± 0.02	8.37 ± 0.19
Africa	48	0.94 ± 0.03	0.09 ± 0.01	0.82 ± 0.02	8.49 ± 0.18
Ovarioles					
Brazil	44	1.60 ± 0.09	0.06 ± 0.01	1.19 ± 0.08	6.66 ± 0.53
Africa	21	1.40 ± 0.10	0.05 ± 0.01	1.06 ± 0.09	5.48 ± 0.44
Sternite 7					
Brazil	11	1.18 ± 0.08	0.07 ± 0.01	0.88 ± 0.06	7.65 ± 0.57
Africa	27	1.09 ± 0.05	0.07 ± 0.01	0.81 ± 0.04	8.10 ± 0.39
Tergite 7					
Brazil	11	2.24 ± 0.16	0.05 ± 0.01	1.70 ± 0.13	5.97 ± 0.48
Africa	27	2.23 ± 0.10	0.06 ± 0.01	1.60 ± 0.08	6.14 ± 0.31
ANOVA					
Between traits		$F = 186.13$	$F = 9.43$	$F = 128.40$	$F = 25.60$
		$p = 0.00001$	$p = 0.00002$	$p = 0.00000$	$p = 0.00001$

STP sternopleural bristles, ovarioles number of ovarian tubules, Sternite 7 bristle number on segment 7 sternite, tergite 7 bristle number on segment 7 tergite, AV absolute value $|L - R|$, SAV scaled absolute value: $|L - R| / [(L + R) / 2]$, SD standard deviation of L – R in each isofemale line, CV SD/mean $[(L + R) / 2]$

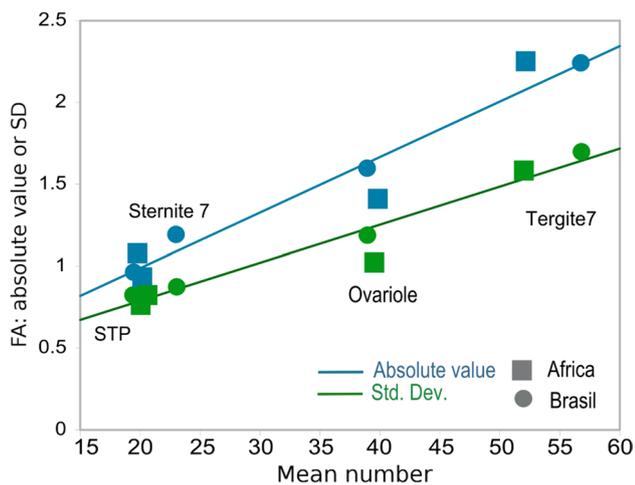


Fig. 3 Linear relationship between mean trait value and the two unscaled FA indices. In both cases a linear regression was applied. The slopes are slightly different (see text)

The overall mean correlation was very close to zero ($r=0.03 \pm 0.16$), revealing a lack of correlation and thus no genetic component.

Discussion and conclusions

As stated before, the isofemale line design has rarely been implemented for analyzing FA. A most significant paper is Pétavy et al. (2006) which investigated STP in both sexes of 33 lines of *D. melanogaster* submitted to different thermal stresses.

The left \times right correlation

The L \times R correlation is rarely considered in FA studies (Palmer 1994) assuming that this coefficient does not provides a significant information. (Palmer and Strobeck 1992; Swain 1987) In fact, this coefficient may provide some interesting conclusions when a large amount of data is available. In *Zaprionus*, we evidenced for the 4 traits a low but, on average positive correlation. An average value of 0.30 (Table 2) gives a coefficient of determination R^2 of 0.09, meaning that 91% of the variability is due to developmental noise. A similar analysis was done in *D. melanogaster* by Pétavy et al. (2006) who found correlation coefficients of 0.54 ± 0.05 and 0.53 ± 0.05 at 17 and 25 °C, respectively. These values, although low, are significantly greater than those found here in *Zaprionus*, suggesting that developmental noise is lower in *D. melanogaster*.

Several studies have found that stressful conditions could increase FA (Delpuech et al. 1995; Woods et al. 1998, 1999). A similar conclusion was obtained by Pétavy

et al. (2006) by considering the L \times R correlation. This study showed that when the cultures were subjected to a daily thermal stress, a significant decrease of the correlation was observed, and especially a very low value after a cold stress ($r: 0.37 \pm 0.04$).

Another interesting observation in *Zaprionus* concerns the difference between African and American populations. It has been found (Table 2) that American populations exhibited a lower correlation (in females only) and a directional asymmetry. Such a difference, which suggests a genetic basis, might be the consequence of the invasive process in America. The invasion of Brazil was probably done by a small propagule, followed by a huge demographic expansion. Such a phenomenon, called a founder-flush effect (Giddings et al. 1989), could in some cases result in a rapid genetic change of the introduced population, and in our case a greater developmental instability. Another interesting observation was made on two other *Zaprionus* species (Allemand et al. 1977). In the invasive *Z. tuberculatus*, the L \times R correlation was 0.25, while it was 0.71 in the related African endemic *Z. sepsoides*. Such values, obtained on 100 flies in each case, are highly different.

Finally, it is interesting to recall that the L \times R correlation increases considerably when calculated between the means of the different isofemale lines from the same population. This observation made repeatedly (Pétavy et al. 2006; Chakir et al. 2007) is not an indication of a genetic variability of FA, but likely a consequence of the existence of a significant genetic variance for the mean of the trait itself.

The relationship between the mean of the trait and FA

Searching for a possible relationship between the mean of the trait and FA should be generally done when analyzing the data. If a positive relationship is found, the FA value should be scaled to the mean, but only in this case (Palmer 1994). We did not find any correlation within each trait, presumably because the variations of the mean were too small, but highly significant correlations were evidenced when different traits were compared.

In Table 4, we presented, for comparing the four traits, two unscaled values (the absolute value of the L–R difference, and the standard deviation of FA calculated in each line). These two indices have the same order of magnitude and this seems to be a general property for meristic traits in different taxa (Whitlock 1996). We analyzed the relationship FA as a function of the mean (Fig. 3) and found, in each case, a very high correlation and a linear relationship. The regression coefficients (slopes) were significantly different, with values of (0.034 for the absolute value and 0.023 for the standard deviation; $t=8.6$ $dft=11.2$, $p<0.001$). This linear increase seems very interesting and

should be investigated in more traits for a developmental purpose. The fact that three traits concerning bristle numbers follow the same rule seems quite logical if we consider that the development of the bristles on various parts of the body implements similar genetic pathways (Simpson et al. 1999). Finding the ovariole number in the middle of the bristle traits is unexpected and should be of interest to developmental geneticists. The relationship between trait variability and FA could be extended to other cases, for example, when the mean of the same trait varies between different related species. We are aware of a single case in which such data are available, which is the ovariole number in the *obscura* group of *Drosophila* (Moreteau et al. 2000). The variation of FA as a function of ovariole number was analyzed in 18 species and evidenced a very significant correlation, $r=0.78$ and a slope of 0.06 which is twice those illustrated in Fig. 3. Clearly the relationship between means and absolute FA needs more extensive studies.

We presented also in Table 4 two scaled indices, namely FA divided by the mean and SD divided by the mean. In both cases scaling clearly decreased the variability among traits (Table 4, ANOVA) but a significant heterogeneity persisted among them; some meristic traits seem less symmetrical than others. We interpret this as the persistence of a slight genetic difference among the traits. It is interesting to note that the values of the CVs of FA (last column in Table 4) have a mean value of 7.1. For the traits themselves, the CVs of the means were 8.32 ± 0.82 , 6.70 ± 1.30 , 8.32 ± 0.87 , and 6.32 ± 1.69 , for STP, ovarioles, sternite 7 and tergite 7 respectively, with an average of 7.4, almost identical to that of FA. For the scaled absolute value (SAV) index, in which each $|L-R|$ difference is divided by the value of the trait, the overall mean is much less, 0.069. We may however point out that a CV is a ratio (SD/mean) multiplied by 100, while the SAV index is only a ratio. We suggest that this ratio should also be multiplied by 100 for comparative purpose: the value of 6.9 has the same order of magnitude than the other values. This operation could be done when comparing the FA of very different traits, for example metric and meristic. We made such calculations on the FA of wing length of *Drosophila pseudobscura* (data published by Kurbalija et al. 2010) and obtained FA indices ranging between 0.6 and 0.8, suggesting that FA of wing might be ten times less than that of bristles.

In conclusion, meristic traits, which are often paradigms for genetic and evolutionary studies, appear very interesting for investigating developmental instability. The fact that meristic traits often appear as more variable, less canalized than metric traits, requires more extensive studies in metric traits, especially for those in which measurement errors are small. The isofemale lines method is a convenient and easy way to estimate genetic variation and (broad-sense) heritability, that can be applied to various traits. For FA, and

other trait akin to variances, for which complex breeding designs are impractical, its broad use should thus be promoted to increase the number of available estimates, and allow comparisons across studies.

Finally, it is worth stressing that estimates of quantitative genetic variation are environment dependent. This is of particular importance for FA, as developmental stability is known to be sensitive to environmental stress (e.g., Palmer and Strobeck 1992). It is conceivable that $G \times E$ interactions could lead to significant genetic variation for FA under adverse conditions (Gomez and Nowry 2012). The lack of genetic variation for FA reported in this study might thus not extend to suboptimal environmental conditions.

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