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Fluctuating asymmetry in pyrethroid-resistant and -susceptible populations of the maize weevil (*Sitophilus zeamais*)

A.S. Corrêa¹, J.T. Santos¹, E.M.G. Cordeiro¹, R.N.C. Guedes^{1,*}

Abstract

Two populations of the maize weevil (*Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), one resistant and another susceptible to pyrethroids, were used to assess the fluctuating asymmetry (FA) associated with insecticide resistance. Two independent measurements were carried out in wing veins and tibiae encompassing a total of 12 traits. The results indicate significant differences in asymmetry between the insecticide resistant (Jacarezinho) and susceptible (Sete Lagoas) populations with the Sete Lagoas population showing significantly higher asymmetry than the Jacarezinho. The results also allowed the analysis of FA between sexes (male and female) and significant differences were observed with males showing lower asymmetry than females, a potential consequence of sexual selection. These results suggest a higher fitness of the individuals from the resistant population compared with the susceptible one, probably due to the long period of exposure of the Jacarezinho population to insecticides. This extended selection probably led to the evolution of fitness modifier genes improving the performance of the resistant genotype and leading to its fixation in the population. Insects from the resistant population may have higher dispersal ability since they show higher symmetry in the locomotion appendages (wings and legs). The fixation of the resistance trait and the lack of fitness disadvantage in the

resistant population, aided by its potentially higher dispersal ability, make its management more difficult and emphasize its importance to prevent this genotype dispersal to other regions.

Keywords: Insecticide resistance, developmental instability, pyrethroid insecticides, fitness, adaptive cost.

Introduction

Fluctuating asymmetry (FA) can be defined as the random non-directional deviation from perfect bilateral symmetry with a normal distribution around a mean of zero (Møller and Swaddle, 1997). FA of bilateral traits is a measure of developmental instability (or reduced developmental homeostasis) suggested as a monitoring tool for environmental pollution with potential consequences for stress and fitness (Lens et al., 2002; Leamy and Klingenberg, 2005). Such suggestion is the consequence of several studies indicating that FA increases when organisms develop under stressful conditions, as is the case with insecticide use (e.g., Hardersen, 2000; Hoffmann et al., 2005). Studies associating insecticide resistance, FA and fitness are however limited to the Australian sheep blowfly, *Lucilia cuprina* (McKenzie and Clarke, 1988; McKenzie and O'Farrell, 1993; Clarke et al., 2000), certainly deserving more attention.

Responses to selective agents may have

¹ Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil

* To whom correspondence must be addressed [tel. (+55) (31) 3899-4008; Fax (+55) (31) 3899-4012; e-mail: guedes@ufv].

pleiotropic effects influencing development and phenotype (McKenzie and Batterham, 1994; Leamy and Klingenberg, 2005), what has yet to be examined for stored product insecticides and insect-pests. Environmental stress imposed by insecticide applications in genetically variable populations may result in selection of certain genes that ameliorate the immediate stress, but that themselves disturb the developmental processes and result in increased asymmetry. This situation was observed in populations the Australian sheep blowfly that have developed insecticide resistance and also exhibited increasing levels of FA compared with susceptible populations (McKenzie and Clarke, 1988; McKenzie and O'Farrell, 1993). The relative fitness of the resistant flies is lower than that of the susceptible ones in the absence of insecticides when resistance initially develops, however the continued selection with insecticide selected for a modifier gene that ameliorates this effect and also reduces FA (McKenzie and Batterham, 1994; Clarke et al., 2000).

The maize weevil (*Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae)) is a key pest of stored cereals, particularly maize, whose infestations may start in the field before harvest and extends throughout the storage period (Rees, 1996). The over-reliance on insecticides for its control caused insecticide resistance development in Brazilian populations of this pest species sparking environmental and economical concerns in the country (Guedes et al., 1995; Ribeiro et al., 2003).

The major genes responsible for the adaptation to insecticide-treated environments are usually associated with an adaptative cost determined by resource allocation affecting metabolic or developmental processes, and decreasing reproductive potential (Coustau et al., 2000; Berticat et al., 2002). Insecticide resistance in maize weevil is associated with such costs in some strains, but seems mitigated in others (Fragoso et al., 2005; Guedes et al., 2006). These strains therefore, may allow establishing the potential association between insecticide resistance, fitness disadvantage and FA as earlier

observed for the Australian sheep blowfly. Higher levels of FA are expected in the insecticide resistant population of maize weevil, if carrying fitness cost associated with resistance, but not so in resistant strains where such costs were mitigated. In addition, sex differences in FA among strains were also assessed since FA may also be an important target of sexual selection (e.g., Møller and Thornhill, 1998), what can be potentially important for the evolution of insecticide resistance (Arnaud and Haubruge, 2002).

Material and methods

Insect strains

Two Brazilian populations of *S. zeamais* were used in this study, one susceptible and one resistant to insecticides. The standard susceptible population was obtained from the Embrapa Milho e Sorgo (Sete Lagoas, MG, Brazil), where it has been maintained for over 15 years without insecticide exposure and its susceptibility to pyrethroids and organophosphates is known (Guedes et al., 1994, 1995; Ribeiro et al., 2003). The second population is resistant to DDT and pyrethroids and was collected from infested maize in Jacarezinho County (State of Paraná, Brazil) in the early 1980s; its high resistance levels to pyrethroids (> 1,000-fold) was earlier reported and the inheritance of deltamethrin resistance in this population is sex-linked (Guedes et al., 1994, 1995). The resistant population from Jacarezinho does not exhibit fitness cost based on demographic studies (Fragoso et al., 2005; Guedes et al., 2006). Each population was established in laboratory from at least 500 individuals. All populations were reared in whole maize grains free of insecticides and maintained at controlled conditions (25 ± 2 °C and 70 ± 5 % r.h.).

Measurements of fluctuating asymmetry

One hundred insects from each sex and

population were used for the measurements of FA to avoid biased estimates, particularly regarding sex differences. Random samples of insects were obtained from culture jars, the insects were frozen-killed and had their hind wings and legs excised at the point of attachment to the thorax and then mounted on slides (Hardersen, 2000; Hoffmann et al., 2005). The venational pattern is polyphagan and the following veins were measured: *radius* (R) and its continuation *radius 1* (R_1), *media* (M) and its branch *media 1* (M_1). The distance between the branching of the radial and median veins was also measured (D_{BR-bM}), as well as the distance from the terminal end of R_1 and the tip of the wing – the terminal end of M_1 ($D_{R_1-M_1}$). In addition, tibia length and width (measured as the distance between spurs near the tarsi attachment) were also measured for each leg (fore, mid and hind legs) in a total of 12 morphological traits. The measurements were carried out on a stereomicroscope Leica MZ 7.5 equipped with a Leica Qwin Lite image capture system (Leica, Göttingen, Germany). All measurements were blindly taken twice to allow assessment of the measurement error (Møller and Swaddle, 1997; Palmer and Strobeck, 2003).

Analysis of fluctuating asymmetry

Analysis of FA regarding its conceptual assumptions followed guidance given by Palmer and Strobeck (2003). The FA data of each sex and population was subsequently subjected to multivariate analysis of variance and canonical variate analysis (CVA) using the procedure CANDISC from SAS, with the DISTANCE statement (SAS Institute 1997). The significance of the separation among sexes and strains of *S. zeamais*, indicated by the ordination through CVA, was determined by the pair-wise comparison of each combination of sex and strain using the approximate F-test ($p < 0.05$). The traits most relevant in explaining the variance observed were subsequently subjected to univariate analysis of variance and Fisher's LSD test ($p < 0.05$), if appropriate (PROC GLM;

SAS Institute 1997).

Results

FA data validation

A two-way, mixed model analysis of variance [sides (fixed) x strains (random)] with repeated measurements of each side used to assess the significance of measurement error showed no-significant differences on both blind measurements ($p > 0.05$) and significant non-directional asymmetry ($p < 0.05$) indicating negligible contribution of measurement error on the asymmetry recorded. In addition, directional asymmetry was also not significant ($p > 0.05$) according with the mixed model, therefore no side showed consistently larger size than the other in the present study.

Frequency distributions of differences between right and left sides for each trait significantly departed from normality only for the wing veins M and R and the distance between branching of R and M (D_{BR-bM}) (Shapiro-Wilk statistics (W) at $p < 0.05$); these traits were not further considered in the present study. Size-dependence was not observed among samples ($p > 0.05$ for regression slope), nor within samples ($p < 0.05$ for regression slope). Therefore correction for size-dependence was not required and the FA index used was $FA = |\text{right} - \text{left}|$.

Overall trend of FA

The multivariate analysis of variance for FA of maize weevil indicated significant differences among sexes and strains (Wilks' lambda = 0.83; $F = 2.69$; $df_{num/den} = 27/11,338$; $p < 0.0001$). Among the three canonical axes generated, only two were significant ($p < 0.0001$ and $p = 0.05$) accounting for 91 % of the total variance explained. The wing veins R_1 , the distance from the terminal end of R_1 and the terminal end of M_1 ($D_{R_1-M_1}$) and the length of the mid tibia (TL_{MT}) were the main contributors for the divergence provided by the 1st axis (canonical loadings of 0.99, - 0.93 and 0.90 respectively). In contrast,

the length of the fore and hind tibiae (TL_{FT} and TL_{HT}) and the width of the hind tibia (TW_{HT}) were the main contributors for the divergence provided by the 2nd axis, based on their canonical loading (between canonical structure; 0.90, 0.80 and 0.82 respectively).

The ordination diagram derived from the CVA indicated significant differences between insecticide susceptible and resistant populations, mainly due to the 1st canonical axis (Figure 1). No significant differences in FA between sex in the susceptible populations was observed, but the FA levels of the resistant males were significantly different from the resistant females, basically due to the 2nd axis (Figure 1).

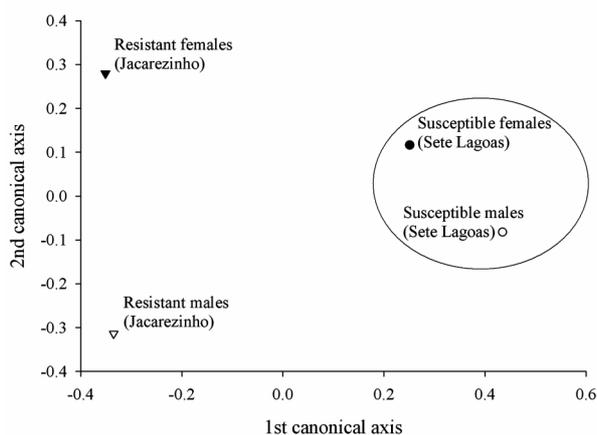


Figure 1. CVA ordination diagram showing the discrimination between both sexes of one insecticide-susceptible and one insecticide-resistant population of the maize weevil (*S. zeamais*) based on their fluctuating asymmetry. The symbols are centroids of treatments and represent the class mean canonical variates. Large circles indicate clusters of treatments that are not significantly different by the approximated F-test ($p < 0.05$), based on the Mahalanobis distances (D^2) between class means.

FA of individual traits

Univariate analyses of variance for FA of the traits subjected to CVA indicated significant differences only for the wing vein R_1 ($F_{1,396} >$

33.90 , $p < 0.0001$), the length of the mid and hind tibiae (TL_{MT} and TL_{HT}) ($F_{1,396} = 3.46$, $p = 0.01$ and $F_{1,396} = 3.49$, $p = 0.01$ respectively), and the width of the hind tibia (TW_{HT}) ($F_{1,396} = 2.45$, $p = 0.04$). The interactions population-sex were not significant in any of these traits ($p > 0.05$), but there was significant effect of population for the FA of wing vein R_1 ($F_{1,396} = 99.84$, $p < 0.0001$) and the length of the mid and hind tibiae (TL_{MT} : $F_{1,396} = 8.84$, $p = 0.003$ and TL_{HT} : $F_{1,396} = 4.56$, $p = 0.03$) with the resistant insects always showing lower asymmetry than the susceptible ones (Figure 2). Significant effect of sex was observed for tibia length and width of the hind legs (TL_{HT} : $F_{1,396} = 4.71$, $p < 0.03$ and TW_{HT} : $F_{1,396} = 7.10$, $p < 0.008$), with males showing lower asymmetry than females, regardless of the population (Figure 3).

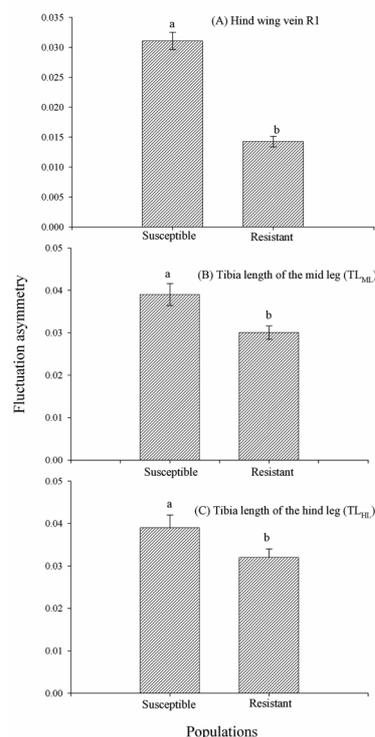


Figure 2. Fluctuating asymmetry (\pm standard error) of the length of the hind wing vein R_1 and tibia length of the mid and hind legs from an insecticide-susceptible (Sete Lagoas) and an insecticide-resistant population (Jacarezinho) of the maize weevil *S. zeamais*. Histogram bars with the same letter do not significantly differ by Fisher's LSD test ($p < 0.05$).

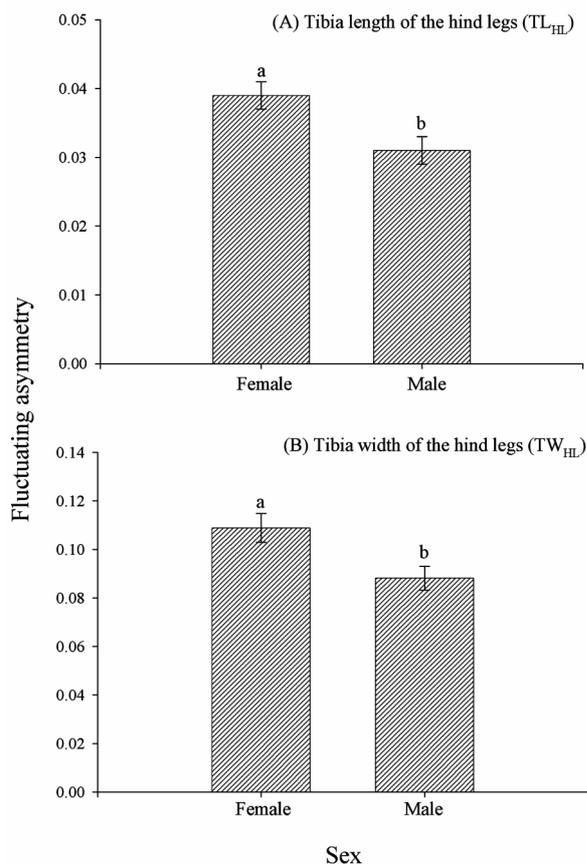


Figure 3. Fluctuating asymmetry (\pm standard error) of the length of the tibia length and width of the mid legs from females and males of the maize weevil *S. zeamais*. Histogram bars with the same letter do not significantly differ by Fisher's LSD test ($p < 0.05$).

Discussion

The insecticide resistant population from Jacarezinho, which was subjected to longer selection pressure for insecticide resistance in seed stores, showed the lower levels of FA as expected based on the results earlier obtained with insecticide resistant populations of the Australian sheep blowfly (McKenzie and Clarke, 1988; McKenzie and O'Farrell, 1993; Clarke et al., 2000). The studies with the sheep blowfly indicated that single autosomal genes induce resistance to diazinon and malathion in this species, which increases FA and reduce fitness when newly introduced into genome in the absence of insecticide selection (McKenzie and Batterham, 1994; Clarke

et al., 2000). Continued use of insecticide may lead to the selection of fitness modifier genes that ameliorate the cost of insecticide resistance increasing fitness and FA of the resistant individuals (McKenzie and O'Farrell, 1993; Clarke et al., 2000).

The past exposure of the Jacarezinho population to pyrethroid insecticides was extensive reflecting typical insecticide overuse in Brazilian seed stores until the early 1980's (Guedes et al., 1994; Fragoso et al., 2003). As a consequence, the evolution of cost mitigation mechanisms of insecticide resistance took place for the resistant population from Jacarezinho, what finds support in recent demographic and physiological studies with both strains (Guedes et al., 2006).

The FA asymmetry differences observed between sexes is suggestive of FA as a potential target of sexual selection (Møller and Thornhill, 1998). The large number of individuals used in the present study minimizes the potential bias in detecting FA differences between sexes and provides support for the potential involvement of FA on sexual selection in maize weevils, probably added by body weight/size (Holloway and Smith, 1987). Males especially from Jacarezinho are more symmetrical than males from the susceptible population and previous study also reports that such males are also heavier (Guedes et al., 2006), what may favor them when competing for females either from resistant or susceptible strains. Such pattern was already reported in red flour beetle (*Tribolium castaneum* (Coleoptera: Tenebrionidae)) resistant to the organophosphate insecticide malathion (Arnaud and Haubruge, 2002), although the sexual selection mechanism remains unknown.

The better potential mating competitiveness of insecticide resistant males favors the spread of this trait in the population. Furthermore, insecticide resistance is a fixed (or nearly so) genetic trait in some populations that does not exhibit fitness disadvantage in insecticide-free environments, what indicates that resistance management strategies based on the temporary suppression of insecticide use are of little use. In addition, the lower levels of FA observed in wings and legs of insecticide resistant insects may

benefit their dispersal favoring the spread of insecticide resistance to other areas, what certainly deserves and will be object of future attention.

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