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Temperature and genotypic effects on life history and fluctuating asymmetry in a field strain of *Culex pipiens*

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Fluctuating asymmetry (FA) has been proposed as a tool to measure levels of stress experienced by populations of organisms during development. To be of value as a biomarker to highlight conditions at particular sites, it is important that variation in FA is due to environmental (eg pollution) variation and not genetic variation among populations and families, in other words heritability for FA should be very close to zero. A full-sib design was set up in which families of *Culex pipiens* mosquitoes collected from the field were reared at three different developmental temperatures. The effects of temperature and family on developmental rate, egg to adult survival and four wing morphological measures were assessed. There was both a temperature and a family effect on development rate and survival. Temperature affected all four wing traits, but an influence of family was only evident in two of the wing traits. Two separate measures of FA for each of the wing traits were obtained. The mean estimates of FA were mainly around 1% of the value of the character measured. There was evidence of an increase in FA with increase in temperature stress. Heritability was estimated for the wing traits and wing trait FA's using restricted estimation maximum likelihood. The estimates of heritability for the wing traits were small and, individually, did not differ significantly from zero. There was also no evidence of heritable genetic variation for any of the wing trait FA's. The results are discussed in relation to other studies where FA heritabilities have been estimated and in relation to the use of FA as an indicator of environmental stress.
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Keywords: *Culex pipiens*; heritability; wing morphology; fluctuating asymmetry; environmental stress

Introduction

Fluctuating asymmetry (FA) has been proposed as a tool to measure the developmental stability of organisms (Zakharov, 1992). The basis of the importance of this measure is the assumption that both sides of a character are under the control of the same genes and that any deviation from the normal bilaterally symmetrical phenotype would be due to perturbation of environmental or genetic origin during ontogeny. A number of studies have found an association between increasing FA and environmental or genetic stress (reviewed in Allendorf and Leary, 1986; Palmer and Strobeck, 1986). Although many studies support this, some failed to find any association between FA and environmental stress (eg Rabitsch, 1997; Dobrin and Corkum, 1999; Bjorksten *et al*, 2000; Mpho *et al*, 2000) or genetic stress (Møller, 1992; Fowler and Whitlock, 1994; Sheridan and Pomiankowski, 1997; Hunt and Simmons, 1997; Woods *et al*, 1998; Floate and Fox, 2000). The disproportional representation of positive to negative association in the literature could be because many negative results are not reported.

FA has been reported to be negatively correlated with fitness in a wide range of organisms (Møller, 1996; reviewed in Møller, 1997). This has led to the formulation of the hypothesis that FA may provide a direct measure of genetic quality, with genetically superior individuals being more symmetric than individuals of lower genetic quality (Møller and Pomiankowski, 1994). However, the relationship between FA and fitness is not always consistent (Markow and Ricker, 1992; Ueno, 1994), with some studies reporting a negative relationship (eg Thornhill and Sauer, 1992; Møller and Thornhill, 1997; Møller and Zamora-Munoz, 1997) whereas others did not (Hunt and Simmons, 1997, 1998; Goulson *et al*, 1999; Tomkins and Simmons, 1999; Bjorksten *et al*, 2000).

The genetic quality hypothesis assumes that FA has a heritable genetic component. The existence of significant levels of genetic variation for FA is the most contentious area of developmental stability studies. Møller and Thornhill (1997) recently reported significant mean heritability of FA. This was met with scepticism and disputes on grounds that many of the studies on heritable variation were flawed (Leamy, 1997; Markow and Clarke, 1997; Whitlock and Fowler, 1997) and that the analyses used were inappropriate (Leamy, 1997; Markow and Clarke, 1997). Although some studies have detected significant heritable variation for FA (eg Thornhill and Sauer, 1992) many more have reported low and non-significant heritability estimates of FA (Hunt and Simmons, 1997, 1998; Tomkins and Simmons, 1998; Woods *et al*, 1998; Windig, 1998; Leamy, 1999; Van Dongen *et al*, 1999; Bjorksten *et al*, 2000). Due to these inconsistencies, clearly more research work still needs to be done. For FA to be of any evolutionary significance, it must be a reflection

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of genetic quality and must display heritable genetic variation (Goulson *et al*, 1999).

This study investigates the effect of rearing temperature on life history and phenotypic variation of wing morphological characters in a British field strain of *Culex pipiens* mosquitoes, with special reference to the heritability of wing trait size and wing trait FA. The design followed allowed testing for the effect of rearing temperature on level of asymmetry, ie whether FA increases with increase in temperature stress. It also enabled the estimation of genotype (family) performance within and across a range of developmental temperatures. Significant genotype × environment interaction effects would indicate variation among families attributable to genetic differences. Consistent rank of level of FA among families across environments would indicate that FA is a good measure of genetic quality, whereas a random pattern might be an indication that FA is purely environmentally determined (Bjorksten *et al*, 2000). The more variation in FA reflects variation in environmental conditions, the greater the potential of FA as a biomarker of environmental quality.

Materials and methods

Establishment of families

Culex pipiens lays eggs in boat-like rafts, which permits easy collection of single families. Egg rafts were collected from a garden water butt in Reading (Berkshire, England) in June 1998. To make sure that eggs were laid within 12 h of each other, egg rafts were removed the night before a morning collection of freshly laid rafts. In the laboratory individual rafts were placed in 1 L plastic tubs with 500 ml of tap water and reared as a family. Fish food (Trouw, UK) was used as the standard larval diet. The larvae were counted at the second instar larval stage and only those families that had more than 225 progeny were kept for use in the subsequent experiments. Larvae and adults from each family were taken to confirm species identity using a key to British mosquitoes (Cranston *et al*, 1987).

Experimental design and rearing of families

A full-sib design was used to test for the effects of rearing temperature on the life history and FA of wing traits. Seven full-sib families were each divided into three and each part raised at a different temperature (25°C, 30°C and 37°C) representing different levels of stress. Three replicates of 25 larvae per family were set up and reared to adult stage at each of the respective temperatures in 140 ml water in plastic cups.

Dissection and measurement of wing morphological characters

The measurement of wing morphological characters using image analysis followed the method detailed in Mpho *et al* (2000). Four traits were measured: wing length (WL), measured as the linear distance from the distal end of the alula to the peripheral tip of the R_3 vein; D6, the linear distance from the junction of the radio-medial cross vein and medial vein to the peripheral tip of the R_{4+5} vein; D8, the linear distance from the junction of the radio-medial cross vein and medial vein to the peripheral tip of the R_2 vein; wing area (WA); the surface area of the wing (excluding the fringe) to the bend of the trailing edge of the alula and joining the subcosta with a perpendicular line. All linear and area measurements were measured to an accuracy of 0.01 mm and 0.01 mm^2, respectively. Each wing character was measured four times, without reference to the previous set of measurements.

Environmental and genotypic effects on life-history, character size and FA

Environmental and genotypic effects on life-history, character size and FA phenotypes were tested using two-way mixed model analysis of variance, with 'temperature' as a fixed factor and 'family' as a random factor. The analyses were performed separately for each sex. The linear model for the analysis was specified as follows:

$$Y_{ij} = \mu + T_i + F_j + TF_{ij}$$

where Y_{ij} is the mean phenotype of the trait under consideration, μ is the population mean, T_i is the effect of the ith temperature, F_j is the effect due to the jth family and TF_{ik} is the effect due to the interaction of the temperature and the family.

Effects of temperature on life history parameters

Development time and survival (measured from egg hatch to adult eclosion) were measured for each family at the three temperatures. Pupae from each replicate were isolated daily and placed in a separate cup and then placed at the respective temperature for adult eclosion. Adults that emerged daily were collected, placed in sample tubes and stored in a −20°C freezer until further analysis.

FA analysis

The FA analysis followed the method detailed in Mpho *et al* (2000). Two FA indices were obtained: FA1, the mean absolute difference between the left and the right sides of a character $[(\Sigma|R_i - L_i|)/N)$ and FA10, the measurement error corrected variances between the sides (Palmer, 1994). A third composite asymmetry index, sumFA, was obtained by summing FA values across three traits (WL, D6 and D8), after standardizing the individual unsigned difference between sides by subtracting the sample mean from each datum (Bennett and Hoffmann, 1998; Leamy, 1999). Standardisation in this manner ensures that all traits contribute equally to the measurement of developmental stability (Bennett and Hoffmann, 1998) and tests for the overall organism-wide effect of the environment (Bjorksten *et al*, 2000).

Heritability estimation of wing character size and FA

Heritability of wing trait size and FA was estimated using the 'animal model' restricted estimation maximum likelihood (REML) (Meyer, 1997). An unbiased design was employed due to differential survival among temperature treatments. Individuals were taken from different replicate cups to minimize the effect of common rearing environment. The heritability of each trait was estimated separately for each of the developmental temperatures since a heritability estimate is specific to a given population in a given environment (Falconer, 1989). The nature of the experimental design (full-sib analysis) meant that the heritability estimates in this study could be inflated through maternal, common

environmental and non-additive gene effects (Falconer, 1989; Roff, 1997). Significance of the h^2 estimates from zero was tested using a simple z-test.

All data were analysed using Minitab Release 10.51, Genstat (4th edition for Windows) and Palmer's Microsoft Excel spreadsheet based on formulae and analyses in Palmer and Strobeck (1986) and Palmer (1994). The spreadsheet is available at http://gause.biology.ualberta.ca/palmer.hp/asymmetry.html.

Results

Effects of temperature on life-history parameters

Development time, measured as the mean number of days from egg hatch to adult eclosion, was influenced by genotype (family) ($F_{6,42} = 85.21$, $P < 0.001$) and rearing temperature ($F_{2,42} = 281.17$, $P < 0.001$). The mean development time was shortest at 37°C (14.98 ± 0.42 days), intermediate at 30°C (15.86 ± 0.46 days) and longest at the temperature of 25°C (18.24 ± 0.42 days). A multiple comparison test (Tukey HSD) revealed that the developmental duration at 25°C was significantly longer than the developmental durations at the other two temperatures, but there was no significant difference in developmental duration between 30°C and 37°C. There was no interaction between genotype and rearing environments ($F_{12,42} = 0.58$, $P = 0.844$).

The survival data were transformed into proportions and then normalised for ANOVA by arcsine transformation. Survival was variable among families ($F_{6,42} = 10.52$, $P < 0.001$) and across the temperatures ($F_{2,42} = 39.39$, $P < 0.001$). Survival was highest at 30°C (mean proportion of adults surviving in each family: 0.43 ± 0.039), intermediate at 25°C (0.39 ± 0.033) and lowest at 37°C (0.19 ± 0.020). A multiple comparison test of all temperature means showed a significant difference between 37°C and the other two temperatures (25°C and 30°C) but no significant difference between 25°C and 30°C. The genotype-environment interaction was significant ($F_{12,42} = 3.11$, $P = 0.003$) indicating that family rank varied with temperature. If survivorship is used as a measure of the magnitude of the stress experienced by the organisms, it can be clearly seen that 37°C was the most stressful developmental temperature to the mosquitoes.

The effects of rearing temperature on wing morphological characters are presented in Table 1. Analysis of variance of the morphological characters showed that temperature and sex were the main sources of variation ($P < 0.001$ in both cases). All the morphological characters significantly decreased in size with increase in temperature (Table 1, $P < 0.001$ in all cases). The family effect was significant for D6 and D8 ($P < 0.01$ in both cases), indicating genetic variability among families for these characters, but not for WL and WA. No significant temperature × sex interaction was observed for WL, but it was significant for D6, D8 and WA ($P < 0.05$ at least in all cases). There was also a significant temperature × family effect for the characters WL, D6 and D8 ($P < 0.05$ at least in all cases) indicating phenotypic plasticity for these characters in relation to developmental temperature.

FA analysis

Three characters conformed to the conditions required for FA analysis: WL, D6 and D8 (see Palmer, 1994). WA displayed significant directional asymmetry and skewness and was therefore excluded from the FA analysis. Since no significant differences in FA were observed across families, the results were pooled over all the families and then the temperature effect was tested. Table 2 summarises the mean levels of FA found. Mean FA was mainly around 1% of the value of the mean character value. A significant temperature effect on FA1 was observed in females for the characters WL ($F_{2,247} = 6.71$, $P = 0.001$) and D8 ($F_{2,247} = 4.20$, $P = 0.016$), but no effect was observed for the character D6 ($F_{2,247} = 2.33$, $P = 0.099$) (Table 2). Combining the asymmetry of the individual traits into a composite index, sumFA, did not result in a significant difference across temperatures (Table 2). In males, no significant effect of temperature was detected on any of the character FA values (Table 2).

There was a significant increase in FA10 with increase in temperature with the highest FA10 values recorded at 37°C for all the characters in both sexes (Figure 1). All the FA10 values of the three wing morphological characters varied significantly across developmental temperature in females (F_{max}, $P < 0.01$ in all cases), but only one character (D6) varied significantly in males ($P < 0.05$), although FA for the other characters also increased with temperature (Figure 1).

Estimation of heritability of wing traits and FA

None of the wing trait measures or FA displayed heritabilities deviating significantly from zero at any of the

Table 1 Mean ± s.e. of wing morphological traits (WL = wing length, D6 and D8 are linear measurements within the wing (see text for details) all measured in mm, WA = wing area measured in mm²) of a field population of *Culex pipiens* mosquitoes reared at different temperatures. *n* = sample size

	Trait	*25°C*		*30°C*		*37°C*	
		n	*Mean ± s.e.*	*n*	*Mean ± s.e.*	*n*	*Mean ± s.e.*
Female	WL	104	3.43 ± 0.028	101	3.23 ± 0.020	47	3.02 ± 0.028
	D6	104	1.52 ± 0.014	101	1.40 ± 0.009	47	1.29 ± 0.015
	D8	104	1.51 ± 0.015	101	1.39 ± 0.010	47	1.28 ± 0.016
	WA	104	2.61 ± 0.042	101	2.31 ± 0.029	47	2.01 ± 0.037
Male	WL	84	2.84 ± 0.022	92	2.76 ± 0.020	39	2.66 ± 0.024
	D6	84	1.16 ± 0.010	92	1.11 ± 0.010	39	1.05 ± 0.015
	D8	84	1.14 ± 0.010	92	1.09 ± 0.010	39	1.03 ± 0.015
	WA	84	1.66 ± 0.027	92	1.60 ± 0.026	39	1.48 ± 0.030

Table 2 FA1 of wing morphological traits of female and male adult *Culex pipiens* reared at different temperatures (WL = wing length; D6 and D8 = measure of distances within the wing measured in mm – see text for details; sumFA = composite asymmetry score – see text for details). Results of Levene's test for heterogeneity of variances are also shown. *P* = probability of no effect of temperature on the character value

Trait	*Females*					*Males*				
	Temperature					*Temperature*				
	25°C	*30°C*	*37°C*	*F*	*P*	*25°C*	*30°C*	*37°C*	*F*	*P*
WL	0.0111	0.0098	0.0177	6.71	0.001	0.0087	0.0082	0.0110	1.65	0.194
D6	0.0092	0.0082	0.0119	2.33	0.099	0.0069	0.0070	0.0095	2.31	0.102
D8	0.0105	0.0082	0.0132	4.20	0.016	0.0081	0.0076	0.0090	0.59	0.553
sumFA	0.0150	0.0046	0.0251	1.94	0.145	0.0048	0.0028	0.0036	2.70	0.106

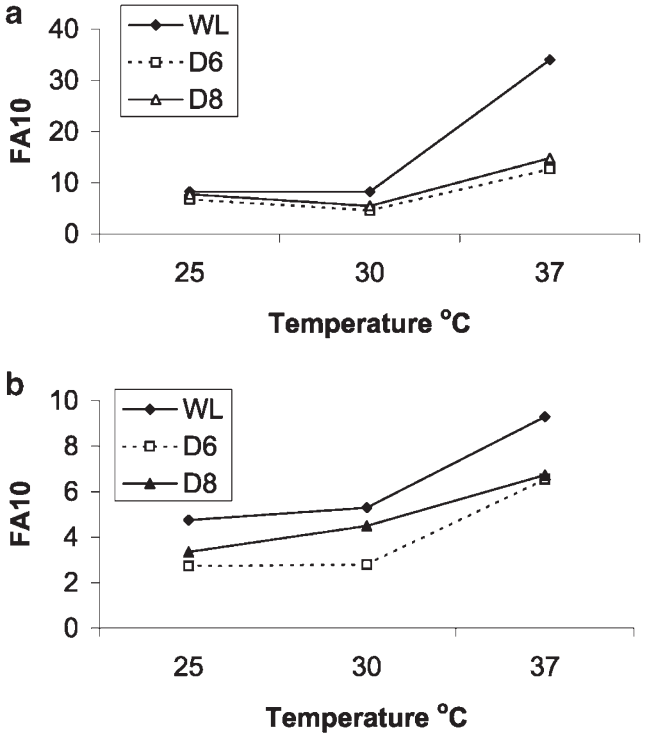

Figure 1 Relationship between FA10 values for a number of wing morphological traits and developmental temperature in (**a**) females and (**b**) males of a field population of *Culex pipiens* (WL = wing length; D6 and D8 = measures of distance within the wing – see text for details).

developmental temperatures in either sex (Table 3). Heritability estimates of FA were low and frequently less than 0.1 (average 0.083, range 0.002–0.323) with the exception of 37°C for both sexes. Heritability estimates of wing size were all positive and greater than 0.1 in most cases (average 0.181, range 0.043–0.33) but none were significantly greater than zero.

Discussion

This study investigated the effect of genotype and temperature stress on certain wing and life history characters and wing FA in a field strain of the mosquito *C. pipiens*. Temperature had a significant effect on all of the life history and wing character values. There was also a signifi-

Table 3 Heritability (h^2) (± s.e.) estimates for trait size and fluctuating asymmetry of wing morphological characters in females and males of a field collected population of *Culex pipiens* mosquitoes reared under different temperatures. (WL, D6 and D8 measured in mm; WA measured in mm^2; for definition of these characters see text)

	Female	*Male*
	h^2 ± *s.e.*	h^2 ± s.e.
25°C		
Wing trait size		
WL	0.291 ± 0.230	0.147 ± 0.153
D6	0.161 ± 0.182	0.212 ± 0.177
D8	0.203 ± 0.198	0.209 ± 0.175
WA	0.330 ± 0.244	0.210 ± 0.183
Wing trait FA		
WL	0.027 ± 0.103	0.055 ± 0.223
D6	0.088 ± 0.136	0.026 ± 0.253
D8	0.021 ± 0.088	0.144 ± 0.208
WA	0.051 ± 0.128	0.024 ± 0.226
30°C		
Wing trait size		
WL	0.055 ± 0.144	0.250 ± 0.216
D6	0.070 ± 0.175	0.186 ± 0.193
D8	0.063 ± 0.218	0.177 ± 0.186
WA	0.043 ± 0.169	0.186 ± 0.194
Wing trait FA		
WL	0.011 ± 0.183	0.008 ± 0.157
D6	0.029 ± 0.125	0.002 ± 0.550
D8	0.006 ± 0.285	0.016 ± 0.112
WA	0.099 ± 0.151	0.022 ± 0.135
37°C		
Wing trait size		
WL	0.153 ± 0.384	0.105 ± 0.295
D6	0.285 ± 0.378	0.170 ± 0.327
D8	0.212 ± 0.377	0.185 ± 0.319
WA	0.297 ± 0.379	0.144 ± 0.277
Wing trait FA		
WL	0.122 ± 0.227	0.068 ± 0.418
D6	0.007 ± 0.223	0.323 ± 0.415
D8	0.125 ± 0.349	0.222 ± 0.312
WA	0.222 ± 0.326	0.271 ± 0.427

cant genotype (family) effect on the values of the life history characters and two of the wing traits (D6 and D8). However, the heritability estimates for the wing traits were quite small, averaging 0.181, and none of the individual estimates deviated significantly from zero. There is little consensus in the literature on the level of heritability of wing traits. Some authors, as with the present study, have found relatively small, non-significant heritability values (eg Woods *et al*, 1998; Leamy, 1999; Tomkins and Simmons, 1999; Bjorksten *et al*, 2000), whilst others have reported higher levels of wing trait heritability (see Bjorksten *et al*, 2000).

The levels of heritability found for wing trait FA were also low and, again, no heritability estimates were individually significantly different from zero. Even though these heritability estimates for FA are small they are likely to be exaggerated as a result of the full-sib design followed which could have inflated the estimates by including factors such as epistasis, maternal and dominance effects. These results contrast with the contentious claim by Møller and Thornhill (1997) that FA has a measurable heritable genetic basis. It has been argued that FA should have very low levels of genetic variation because the developmental process is genetically programmed to produce identical symmetrical phenotypes in bilaterally symmetrical organisms. It is only when the developmental process is overwhelmed by stress that deviation from the programmed trajectory occurs, leading to asymmetry. Consequently, heritability for FA should be zero or close to zero because any deviation from ideal symmetry is environmentally induced (Palmer, 1994).

However, it should be borne in mind that the population under study was derived from the field and placed under laboratory conditions. Genotype × environment interactions could have been quite large as a result of the transfer and could have interfered with the expression of heritable variation normally expressed under field conditions (Woods *et al*, 1998). On the other hand, it is well known that novel environmental conditions are likely to inflate levels of heritable genetic variation and hence estimates of heritability (Holloway *et al*, 1990). Drawing all of the evidence together: (1) the average FA heritability estimate was only 0.083, (2) a full-sib design was employed which yields a broad sense heritability estimate, and (3) the animals were reared under novel environmental conditions, hence it is likely that genetic variation for wing FA in *C. pipiens* is very small indeed.

It has been maintained by some authors (eg Møller, 1999) that FA is a condition-dependent asymmetry that increases under stressful conditions. However, this is not universally accepted and other studies have failed to reveal such a relationship (eg Fowler and Whitlock, 1994). In this study, wing FA was observed to increase with increase in temperature stress. However, female wing traits seem to be more responsive to temperature treatment than their male counterparts. In females, all the wing characters analysed were significantly larger at 37°C relative to 25°C and 30°C, whereas in males only D6 changed significantly. Nevertheless, all other male wing characters showed an increase in FA with increase in temperature, albeit not significantly. Results of the present study contrast with previous studies using a laboratory strain of *C. pipiens* where increased temperature did not significantly increase wing trait FA (Mpho, 2000; Mpho *et al*, 2000, 2001). The laboratory strain is maintained at reasonably high numbers but could have experienced periods of bottlenecks in the past, resulting in an elevated level of inbreeding. It is conceivable that inbreeding is a form of genetic stress which could raise levels of FA thus obliterating the effects of experimental treatments. Inbreeding is known to affect developmental stability in some experimental systems (Clarke *et al*, 1986; Deng, 1997; Eldridge *et al*, 1999; Roldan *et al*, 1998). A comparison of the FA of the two *Culex* strains revealed that the wing FA of the laboratory strain was consistently higher than that of the field strain at 25°C and 30°C in both sexes, which may support the assertion made above. The field strain is implicitly assumed to be an outbred strain. The high level of FA displayed by the laboratory strain may be close to the limit of viable load.

It has been proposed that FA may have value as an indicator of environmental stress induced by factors such as pollutants. If FA has a significant heritable component, then its use as a tool for biological monitoring of environmental stress would be limited because it would be difficult to distinguish between genetically determined FA and that which is induced by the environment. The results of this study indicate little heritable variation in FA of wing morphological traits in a field strain of *C. pipiens* and therefore suggest that FA may have value as an indicator of environmental condition, since FA is unlikely to be an indicator of genetic quality. However, FA should only be used as an indicator of environmental conditions with caution since there are many conflicting results in the literature and, if possible, should be complemented with other indicators, such as life history responses.

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