RESEARCH ARTICLE

Mesosternal bristle number in a cosmopolitan drosophilid: an X-linked variable trait independent of sternopleural bristles

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Abstract

Mesosternal (MS) bristles in *Drosophila* are a pair of machrochaetae found at the sternal end of the sternopleural (STP) microchaetae, and are thought to be invariable. In a closely related drosophilid genus, *Zaprionus*, their number is four and, in contrast to *Drosophila*, they show interspecific and intraspecific variability. The genetic basis of MS bristle number variability was studied in *Z. indianus*, the only cosmopolitan species of the genus. The trait responded rapidly to selection and two lines were obtained, one lacking any bristles (0-0) and the other bearing the normal phenotype (2-2). Other symmetrical phenotypes, (1-1) and (3-3), could also be selected for, but with lesser success. By contrast, STP bristle number did not vary significantly between the two lines (0-0) and (2-2), revealing its genetic independence from MS bristle number. Reciprocal crosses between these two lines showed that MS bristle number is mainly influenced by a major gene on the X chromosome (i.e. F_1 males always resembled their mothers) with codominant expression (i.e. heterozygous F_1 females harboured an average phenotype of 2 bristles). However, trait penetrance was incomplete and backcrosses revealed that this variability was partly due to genetic modifiers, most likely autosomal. The canalization of MS bristle number was investigated under different temperatures, and the increased appearance of abnormal phenotypes mainly occurred at extreme temperatures. There was a bias, however, towards bristle loss, as shown by a liability (developmental map) analysis. Finally, when ancestral and introduced populations were compared, the latter were far less stable, suggesting that genetic bottlenecks may perturb the MS bristle number canalization system. MS bristle number, thus, appears to be an excellent model for investigating developmental canalization at both the quantitative and the molecular level.

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Introduction

The sternopleural (STP) mechanosensory bristle system is a very well studied quantitative character in *Drosophila melanogaster* (Mackay 2004; Mackay and Lyman 2005). This system consists of a pair of macrochaetae on the katepisternum, and a series of microchaetae extending along the sternopleuron down to the mesosternum. The two macrochaetae are variable in size among different species (the anterior being small, or sometimes completely reduced) and are used in *Drosophila* systematics (Sturtevant 1942; Wheeler 1981; Grimaldi 1990). The microchaetae, on the other hand, are variable in number, and, thus, they have been widely used to document the genetic architecture of the trait using directional selection (Clayton and Robertson 1957; Nuzhdin *et al*. 1999) and QTL mapping (Breese and Mather 1957; Thoday 1979; Dilda and Mackay 2002). Besides this main genetic interest, STP bristles have also been used to analyze geographic variations (latitudinal clines: Capy *et al*. 1993) as well as phenotypic plasticity in response to temperature (Moreteau *et al*. 2003) and various other stresses (Bubliy *et al*. 2000; Dworkin 2005; Mackay *et al*. 2005; Milton *et al*. 2005; Petavy *et al*. 2006). Other drosophilid species have been less investigated, but geographic clines have been

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found in at least three other species: *D. simulans* (Gibert *et al*. 2004), *D. kikkawai* (Karan *et al*. 1998) and *Zaprionus indianus* (Karan *et al*. 2000).

Going down the microchaetae series, we find, after a gap, a pair of fairly large bristles, close to the median line of the episternum. In *D. melanogaster*, these two bristles are considered as invariant in number (strongly canalized) and are referred to as mesosternal (MS) bristles (sternals, *sensu* Demerec 1965). Due to their invariance, the genetic architecture of the MS bristles has never been studied (T. F. C. Mackay, personal communication) and they have never been used in any systematic survey (D. Grimaldi, P. O'Grady, personal communication).

The genus *Zaprionus*, which harbours about 50 Afrotropical and Oriental species (Chassagnard and Tsacas 1993), is closely related to the subgenus *Drosophila* (Throckmorton 1975; Grimaldi 1990; Robe *et al*. 2005). One domestic species, *Z. indianus*, has a strong colonizing capacity. It was introduced in India about four decades ago, and more recently, in 1998, in Brazil (Vilela 1999). Its colonizing capacity is such that it is now established in Florida (van der *et al*. 2006). During a geographic survey comparing old world and new world populations (David *et al*. 2006a,b), we noticed that *Zaprionus*, unlike most drosophilids, had four MS bristles instead of two, and that this number was variable in both wild-collected or laboratory-grown flies, ranging from three to five bristles. This variability was investigated at the genetic level with the isofemale line method (David *et al*. 2005). Moreover, the identification of lines with extreme phenotypes favoured further studies of directional selection. Starting with a line from Madagascar, selection was undertaken to reduce the number, and a strain with no MS bristles was obtained quite rapidly. Reciprocally, an isofemale line from Rio de Janeiro, with supernumerary bristles, was used for selection because of increased MS bristle number. A positive response was obtained, although it was not possible to obtain a pure strain with six MS bristles.

The strain without MS bristles (0-0 phenotype) was used for a genetic analysis in a cross with the normal wild type *Zaprionus* (2-2 Phenotype), and the results have revealed a major X chromosome effect. In all investigations, the STP bristles were not affected by selection on MS bristle number. Our results demonstrate that, in drosophilids, the MS bristle number may be variable, either within species or among species, and that its genetic basis is independent of STP bristle number, although they are located on the same segment of the thorax.

Materials and methods

Survey of other Zaprionus species and drosophilid genera

To examine the evolutionary status of the four bristle phenotype, MS bristle number as investigated in various *Zaprionus* species and other related genera. For *Zaprionus*, a number of species belonging to the Afrotropical subgenus *Zaprionus s. str.*, reared at our laboratory in Gif, were surveyed. Some of these species are new and are as yet not described. The other subgenus is *Anaprionus* and it represents an oriental radiation. We investigated two species of the Oriental subgenus *Anaprionus: Z. bogoriensis* and *Z. obscuricornis*. Species belonging to other genera were sampled in an hierarchical manner according to their phylogenetic relationship to the genus *Zaprionus*. Grimaldi (1990) erected the *Zaprionus* genus group to include genera *Zaprionus, Phorticella* and *Samoaia*, which were already known to be closely related to the *D. immigrans* species group (Throckmorton 1975). Hence, the character was also surveyed in *Phorticella flavipennis, Samoaia leonensis* and *D. immigrans*.

Phenotypic plasticity among isofemale lines

A natural population of *Z. indianus* was collected with fermented fruit baits from Rio de Janeiro (Brazil) in December 2003 and used to establish isofemale lines. Special larval culture cautions specific to *Zaprionus* were undertaken, following David *et al*. (2006a,b). Ten lines of the first laboratory generation were then drawn at random and allowed to oviposit at room temperature (21–22°C) in culture vials containing a high nutrient, killed-yeast medium. Egg-containing vials were then transferred to one of six constant experimental temperatures (15, 17, 21, 25, 28 and 31°C). All these temperatures fall within the viable thermal range of *Z. indianus* (Karan *et al*. 1999; Araripe *et al*. 2004). After emergence, ten pairs of flies were taken randomly from each line at each temperature and the number of MS bristles was counted on each of them.

Selection experiments

An isofemale line isolated from a population of Antananarivo, Madagascar (see David *et al*. 2006a,b), and reared at 25°C, exhibited a high frequency of phenotypes with three or two bristles instead of four. This line was used to initiate directional selection for decreasing the MS bristle number. A strong and rapid response to selection was observed, and after a few generation, phenotypes with one or no bristles started to appear. The selection was continued and, after ten generations, the selected line was almost monomorphic for no bristles, with only a few flies harbouring one or two bristles.

Starting at generation five, another selection was initiated for the intermediate phenotype (1–1) and was also successful. From the beginning of the experiment, the wild-type phenotype of the same line was also submitted to normalizing selection, that is abnormal phenotypes, generally less than 10%, were eliminated.

In another set of isofemale lines, established from Rio de Janeiro in December 2004 (see David *et al*. 2006a,b), and reared at 25°C, a line was observed to have an average bristle number greater than four. Selection was initiated on this line to increase the number of MS bristles. A positive response to selection was observed, and the proportion of phenotypes

with five or six bristles increased. It was not possible, however, to obtain a line with a high percent of symmetrical flies with six bristles.

Genetic analysis between line crosses

As indicated above, the fastest selection response was observed in the no-bristle line. We chose this line for a genetic analysis by crossing it with the wild-type line that originated from the same isofemale line from Antananarivo, and which presumably harboured the same genetic background. We investigated the progeny phenotypes from the two reciprocal F_1 crosses. Since a major difference between the F_1 males was observed, a backcross generation was also investigated, crossing the F_1 females with parental males of the 0–0 and 2–2 lines.

Analysis of geographical variation

Z. indianus is a recent cosmopolitan species and we have earlier compared morphometrical characters between five Afrotropical and five South American populations of this species using the isofemale line technique (David *et al*. 2006a,b). We also investigated MS bristle number for three of these populations, namely Belem (Brazil), Montevideo (Uruguay) and Sao Tome (Sao Tome and Principe), but the data were not published. In addition, we have recently investigated six isofemale lines from Brazzaville (Congo) and six from Alexandria (Egypt). All these populations were reared at 25°C, and thus the data from the 25°C lines of Rio de Janeiro used in the thermal plasticity experiment were also used in the analysis. The two Afrotropical populations (Sao Tome and Brazzaville) may be regarded as ancestral, while all the other populations were recently introduced, including that of Alexandria (Yassin and Abou-Youssef 2004). Our aim was, thus, to investigate the effect of introduction—which typically involves a genetic bottleneck—on the phenotypic stability of MS bristle number.

Statistical analysis

Basic statistics were estimated using STATISTICA 5.5 software (StatSoft 1999). MS bristle number is a threshold trait that can be subcategorized into three distinct character states: bristle loss (< 4), wild-type (4) and bristle gain (> 4). Each phenotype can be regarded as a function of its liability (i.e. its underlying variation). The rescaling of the liability value into the phenotypic value is called the developmental map, D_m (Waddington 1949; Sheldon *et al*. 1964). Developmental maps are usually defined using probit analysis as it is known that untransformed frequencies can give a misleading picture of the strength of canalization (Rendel 1959; Lynch and Walsh 1998). Thus, the liability can be deduced from the probit analysis of the width of the canalization zone from the equation

$$D_m = x_m - x_{m-1},$$

where x_m is the probit-scale value associated with the cumulative frequency to (and including) class m, and x_{m-1} the probit scale of the preceding class. The sampling variance of the estimator D_m is

$$\sigma^2(D_m) = \frac{1}{n}\left\{\frac{q(m-1)[1-q(m-1)]}{[p(x_m)]^2} + \frac{q(m)[1-q(m)]}{[p(x_{m-1})]^2} - 2\frac{q(m-1)[1-q(m)]}{p(x_m)p(x_{m-1})}\right\},$$

where n is total number of flies, q is the cumulative frequency to (and including) the phenotypic class, and $p(x) = \exp(-x^2/2)/\sqrt{2\pi}$ is the unit normal density.

Results

Phylogenetic status of mesosternal bristle number

Figure 1 shows the different MS bristle number phenotypes mapped on a phylogenetic tree representing the sampled *Zaprionus* species and related genera. The two bristle phenotype appears to be the ancestral (plesiomorphic) state as it is shown in *Drosophila* and *Samoaia*. However, the four bristle genotype started to appear in *Phorticella* and *Zaprionus*, but in a different manner. While in *Zaprionus* it consists of two pairs of equal size, in *Phorticella* the two pairs have different sizes. Surprisingly, the two Oriental *Zaprionus* species of the subgenus *Anaprionus* had different phenotypes. One, *Z. bogoriensis*, harbored the ancestral state of a single pair, while the other, *Z. obscuricornis*, had the derived phenotype of four bristles shown in *Z. indianus*. All species belonging to the Afrotropical subgenus *Zaprionus* had four bristles with slight variability. The precise phylogenetic affinity among the two *Zaprionus* subgenera and the genus *Phorticella* is still ambiguous (M. Toda, personal communication.) and the four bristle phenotype cannot therefore be considered as a shared derived character (synapomorphy).

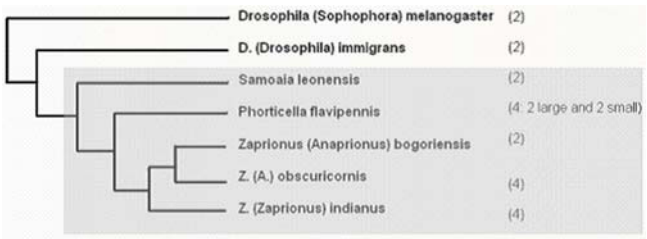

Figure 1. Schematic diagram showing the evolutionary relationships between two *Drosophila* species and five species belonging to the *Zaprionus* species group (shaded). For each species, mesosternal bristle number is given in parentheses.

Thermal plasticity among isofemale lines

The overall distributions of MS bristles at different temperatures are given in table 1. Six different phenotypes, ranging from one to six bristles, were observed (but no 0–0 phenotype) (figure 2). There was no significant difference between

the two sexes (ANOVA not shown) and, thus, the observations were pooled over sexes. The total distribution is clearly biased toward lower values with respect to the wild-type phenotype (2-2): 159 flies had less than four bristles and only 29 had more than four (table 1).

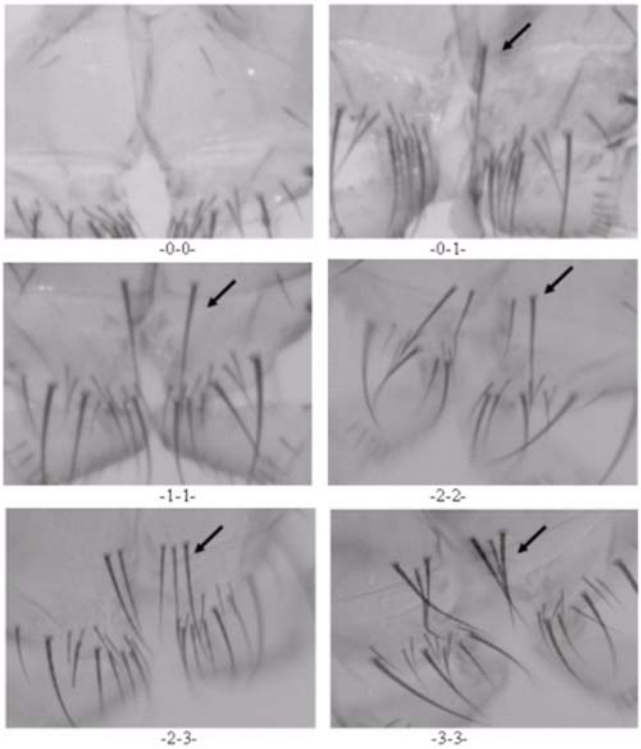

Figure 2. Illustration of six mesosternal bristle number phenotypic classes observed in different lines of *Zaprionus indianus*. Arrows indicate the location of bristles, and phenotype annotations are given below the photographs.

Table 1. Number of flies with different msosternal bristle numbers at different growth temperatures. The results are the sum of 10 isofemale lines of *Z. indianus* from a Rio de Janeiro population. For each temperature, 200 flies (100 females and 100 males) were investigated.

Temperature	Bristle number						Total number of bristles
	1	2	3	4	5	6	
15°C	0	22	37	134	5	2	728
17°C	0	6	34	151	6	3	766
21°C	0	8	15	168	7	2	780
25°C	0	5	33	162	0	0	757
28°C	1	12	25	162	0	0	748
31°C	0	15	46	135	3	1	729

The effect of temperature is illustrated by the reaction norms of the wild-type, four bristle phenotype (figure 3). The average norm shows that the wildtype is canalized at benign temperatures between 21 and 28°C. On the other hand, the wild-type decanalization (i.e. the appearance of abnormal bristle numbers) was observed at extreme temperatures, reaching maxima at 15°C (33%) and at 31°C (33.5%). Figure 3 also illustrates the variability among lines. The shapes of the norms are quite variable, some being more sensitive to cold, others more sensitive to heat. A precise comparison of the shapes would, however, require a much greater number of flies in each line.

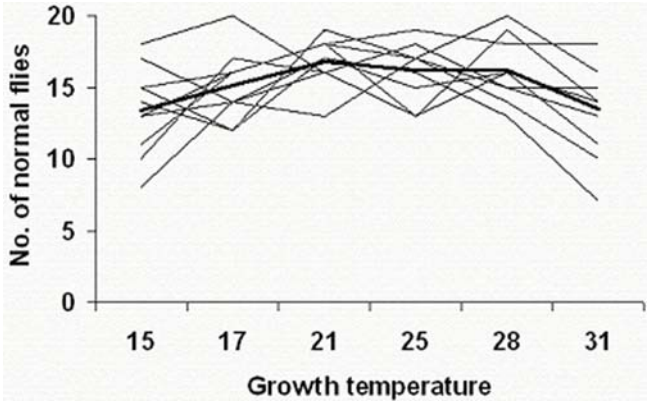

Figure 3. Depiction of reaction norms of mesosternal bristle number according to growth temperature in 10 isofemale lines of *Zaprionus indianus*. The graph shows the number of normal flies (phenotype 2-2) among 20 flies (10 females and 10 males) for each line, investigated at each temperature. The thick line shows the average curve.

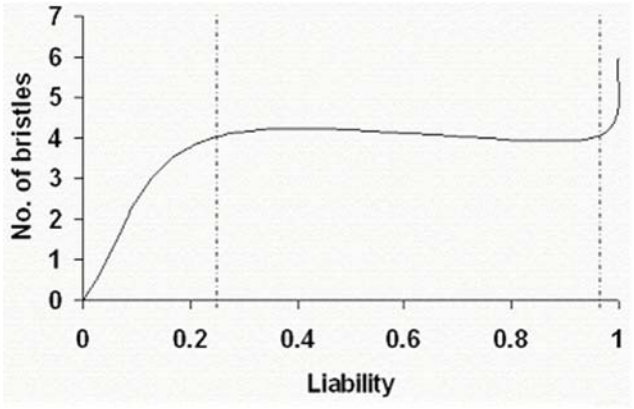

Figure 4. Developmental map of mesosternal bristle number estimated from probit analysis (see **Materials and methods** for details). Dashed lines determine the threshold of the canalization zone of the wild phenotype (2-2). Thresholds were determined according to average value for all isofemale lines. Note the bias towards bristle loss (≤3 bristle phenotypes).

As stated earlier, we also analysed the developmental maps. The mean MS bristle developmental map of all isofemale lines summed over all thermal regimes was $2.76\,\sigma \pm 0.15\,\sigma$. Moreover, considerable variation was found in the strength of canalization across isofemale lines, ranging from $2.39\sigma \pm 4.51\sigma$ to 4.45σ (with no variance, as no bristle gain had been observed in this line). We also estimated the developmental map for each phenotype and results are given in figure 4. It is clear that the decanalization distribution across

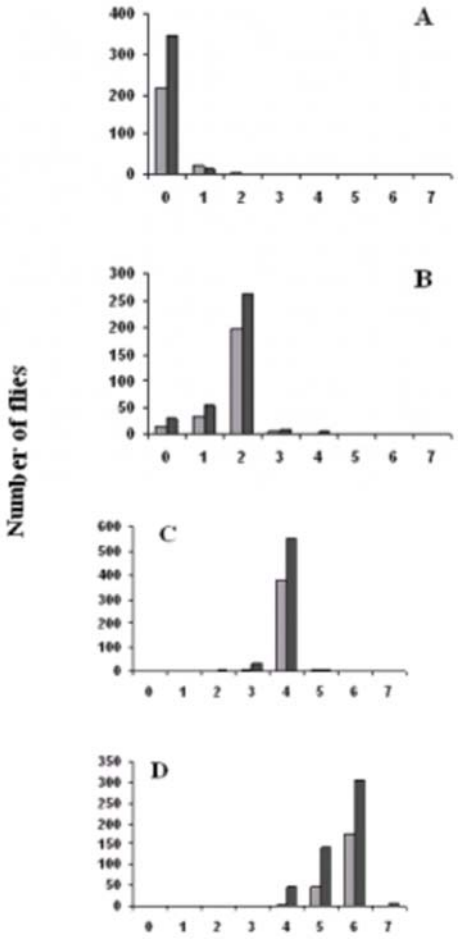

Figure 5. Frequency distributions of different mesosternal bristle number phenotypes in males (black) and females (gray) of four selected lines: (a) 0-0, (b) 1-1, (c) 2-2 and (d) 3-3. Frequencies are pooled from generations 10 to 20. Note that selection for (0-0) and (2-2) phenotypes was the most successful (see text for details).

the two phenotypic classes (bristle loss *vs*. bristle gain) is not symmetrical (Kolmogorov–Smirnov statistic = 0.2212; $P < 0.001$), and that variability at extreme environments is strongly biased towards MS bristle loss.

Directional selection on mesosternal bristles

As stated earlier, a selection experiment was undertaken to decrease the number of MS bristles, starting from an isofemale line from Antananarivo. Flies with two or three bristles, instead of four, were pooled to produce progeny, and the operation was repeated in each generation. The number of reproductive adults were not precisely noted, but was never less than 15. A fast response to selection was observed with the appearance of (0-1) and (0-0) phenotypes. After about 10 generations of selection, an almost complete response, that is an almost total suppression of the bristles, was observed, and the phenotypic distribution is given in figure 5a. Some rare individuals with one or sometimes two bristles were still observed, and regularly appeared in successive generations, in spite of continuing selection.

In parallel, normalizing selection for the normal phenotype of four bristles on the same line was also carried out. Approximately 15–20% of abnormal phenotypes, harboring two or three bristles, were eliminated at each generation, and the number of reproductive flies were around 50. This normalizing selection resulted in a small but significant decrease of the abnormal flies, the frequency of which fell to less than 10%, as illustrated in figure 5c.

Interestingly, the number of STP bristles was not modified during the selection process. After 20 generations of selection, the number of STP bristles was 19.6 ± 0.26 in the wild-type (2-2) line, and 21.0 ± 0.25 in the (0-0) line (25 males and 25 females were measured for each line). However, when the sexes were considered separately for STP bristle number analysis, the STP sexual dimorphism normally encountered in *Z. indianus* was less pronounced in the (0-0) line. These numbers lie within the range of STP bristle number variability in *Z. indianus* (David *et al*. 2006b). The same phenomenon, i.e. a normal appearance of STP bristles, was observed in all other lines and in crosses, although these bristles were not precisely counted.

In the line selected for a low MS bristle number, phenotype (1-1) was the most frequent at generation 5. It was decided to start a new selection for this phenotype, and the average results, for generation 10 to 20 are shown (figure 5b). We see that the selection was successful, and that the frequency of flies with two bristles rose to 80%. However, the other phenotypes with less or more than two bristles could not be completely eliminated.

Phenotypes with more than four bristles are also encountered in natural populations. In a sample from Rio de Janeiro, a line grown at 25°C was found with a large number of such flies, and they were selected over successive generations. At the beginning, the proportion of these flies was less than 10%, but the number of reproductive individuals was then extended to 30 or more. After 20 generations, the phenotype distribution (figure 5d) exhibited a mean number of bristles much higher than four, but up to now it has not been possible to obtain a pure line with six (3-3) bristles.

In conclusion, it was possible through selection to generate all possible phenotypes, odd or even, ranging between (0-0) and (3-4), of which six are illustrated in figure 2.

Crosses between lines (0-0) and (2-2)

We chose the two lines (0-0) and (2-2) for genetic analysis for three reasons. First, they were obtained from the same isofemale line and are, therefore, likely to share the same genetic background; second, the difference between them is high, almost four bristles; third, both lines are almost monomorphic and exhibit a low proportion of individuals not corresponding to the selected phenotype. Results of the various crosses are shown in table 2.

The females of the reciprocal F_1 are very similar and the mean bristle number is very close to the mid-parent value

Table 2. Frequency distribution of mesosternal bristle phenotypes in parental (P_1 and P_2) selected lines, reciprocal F_1 and backcross generations.

Generation	Sex	*n*	Bristle number 0	1	2	3	4	5	Mean ± SE
Parents									
P_1 (2-2)	F	595	0	0	8	35	550	2	3.92 ± 0.01
[summed from G9 to G20]	M	390	0	0	0	4	379	7	4.07 ± 0.01
P_2 (0-0)	F	362	346	15	1	0	0	0	0.05 ± 0.01
[summed from G9 to G20]	M	238	216	20	2	0	0	0	0.10 ± 0.02
$\underline{F_1}$									
$P_2 \times P_1$	F	250	4	16	159	54	17	0	2.26 ± 0.05
	M	250	109	72	68	0	0	0	0.84 ± 0.05
$P_1 \times P_2$	F	250	4	26	172	38	10	0	2.10 ± 0.04
	M	250	0	0	3	29	218	0	3.86 ± 0.02
RBC									
$F_{1(1\text{-}1)} \times P_1$	F	150	3	7	59	29	52	0	2.80 ± 0.08
	M	150	38	27	7	7	71	0	2.31 ± 0.14
$F_{1(1\text{-}1)} \times P_2$	F	150	47	24	66	11	2	0	1.31 ± 0.09
	M	150	37	22	21	7	63	0	2.25 ± 0.14
CBC									
$F_{1(0\text{-}0)} \times P_2$	F	100	56	20	23	0	1	0	0.70 ± 0.09
	M	100	39	7	6	14	34	0	1.97 ± 0.18
$F_{1(0\text{-}1)} \times P_2$	F	100	50	20	26	4	0	0	0.84 ± 0.10
	M	100	47	4	7	11	31	0	1.75 ± 0.18
$F_{1(1\text{-}1)} \times P_2$	F	100	47	18	34	1	0	0	0.89 ± 0.09
	M	100	35	11	5	6	43	0	2.11 ± 0.18
$F_{1(1\text{-}2)} \times P_2$	F	100	34	22	37	6	1	0	1.18 ± 0.10
	M	100	36	10	9	5	40	0	2.03 ± 0.18
$F_{1(2\text{-}2)} \times P_2$	F	100	37	18	33	10	2	0	1.22 ± 0.11
	M	100	28	17	9	2	44	0	2.17 ± 0.18

RBC: reciprocal backcrosses between selected $F_{1(1-1)}$ females and males of the parental lines. CBC: categorical backcrosses between F_1 females with different phenotypes and P_2 males.

(2.26 *vs*. 2.10). We notice, as illustrated in figure 6a, a broadening of the distributions and an increase of the variance. By contrast, a major and highly significant difference discriminates the reciprocal F_1 males, with average values of 0.84 and 3.86 ($\chi^2 = 487.5, P < 0.001$). There is an obvious trend of the males to be much more similar to their mother than to their father (figure 6b), demonstrating a major genetic effect of the X chromosome. The genetic basis was further investigated by studying the offspring of two types of backcrosses, that we denote reciprocal (RBC) and categorical (CBC) backcrosses, respectively. Results of both backcross analyses are also presented in table 2.

RBCs were done by mass-mating the median class F_1 females (1-1) with either male parent in order to confirm the X chromosome effect hypothesis. For the RBC females, as heterozygosity breaks down, the phenotypic distributions are very different and each appears more or less bipartite (figure 6c). On the other hand, the bimodal, U-shaped distribution of RBC males becomes more apparent (figure 6d). This can be regarded as a strong evidence for the X-linked hypothesis; F_1 female heterozygosity was reflected in the high phenotypic variance of their hemizygous RBC sons.

In the F_1 females (figure 6a), we observed an intermediate mean number, close to 2, but also a very significant increase of the variance, and the appearance of a few extreme phenotypes, 0-0 or 2-2. We asked the question: does this variability correspond to some underlying genetic variability (for instance autosomal), or is it a purely phenotypic phenomenon, revealing developmental instability and imperfect canalization? This was investigated in the second kind of backcross: the CBC. To answer this problem, we sorted out F_1 virgin females into five phenotypic classes ranging from 0-0 to 2-2. These females were backcrossed to males of the parental (0-0) strain. The results (table 2) were straightforward. Male distributions were clearly bimodal, while those of the females had less bristles and an asymmetrical distribution skewed to the right. In the females, a genetic effect was observed as mean MS bristle number in the CBC offspring increased as compared to the MS bristle number of the F_1 mother (Spearman's rank correlation = 1.0, $P < 0.001$). In the males, the correlation with the mother's phenotypes was lower and nonsignificant (Spearman's correlation = 0.8,

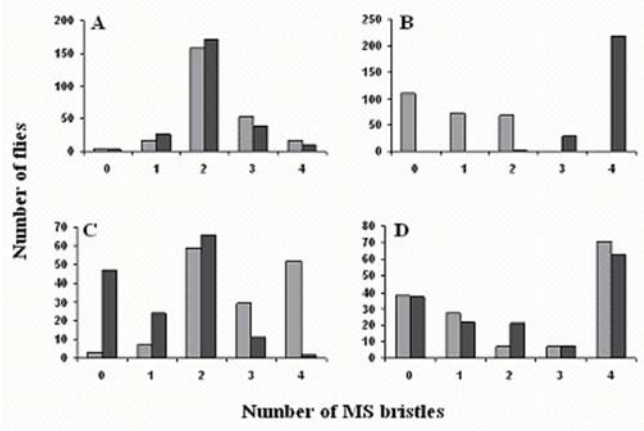

Figure 6. Frequency distributions of mesosternal bristle number in F_1 (a) females and (b) males of two reciprocal crosses between lines (0-0) and (2-2) (gray: $P_{(0-0)} \times P_{(2-2)}$; black: $P_{(2-2)} \times P_{(0-0)}$), and in offspring of reciprocal backcross (RBC's, see text for details) (c) females and (d) males (gray: $F_{1(1-1)} \times P_{(2-2)}$, black: $F_{1(1-1)} \times P_{(0-0)}$). Note that for F_1 females (a), the major class corresponds to the intermediate phenotype (1-1) indicating a codominant effect. F_1 males (b), on the other hand, exhibit a majority phenotype of their mother, indicating X-linked inheritance. In the RBC offspring, note that the shape of the distributions seems bipartite in females (c) and U-shaped in males (d), confirming the X-linked hypothesis.

$P = 0.10$), although it is similar in sign to that in the females. The most likely interpretation is that the phenotypic variance which is observed among F_1 females harbors a small genetic component, which might be an autosomal effect, interacting with the major X chromosome effect.

Geographical variation and founder effect

Working with a cosmopolitan species with a known invasion history like *Z. indianus* provides a unique opportunity to test for the cryptic genetic variation versus developmental noise hypotheses in natural populations. We examined six geographical populations, four of which (Belem, Rio de Janeiro, Montevideo, Alexandria) are known to be recently founded. The other two (Sao Tome and Brazzaville) belong to the Afrotropical region which is known to harbour the ancestral populations (Tsacas 1985). Genetic bottlenecks that accompany introduction into new environment are thought to be a major cause for the release of cryptic genetic variation via genetic disturbance (Waddington 1957; Eshel and Matessi 1998). We may assume, then, if the phenotypic decanalization of MS bristle number phenotypes is due to genetic factors, that ancestral populations should be more stable than recently introduced ones. An average phenotype (pooled over sexes) in each population was calculated from the mean MS bristle number of isofemale lines and then used in a cluster analysis. A UPGMA dendrogram shows clearly that the two Afrotropical populations were more stable, and clustered away from the other introduced populations (figure 7). This result was obtained regardless of the number of the isofemale lines examined (ranging from 6 to 15 in ancestral populations *vs* 6–20 in introduced ones), and after 1000 bootstrap iterations. The wild-type phenotype, thus, appears to be more strongly canalized in ancestral, large sized populations, possibley consequence of a stabilizing selection.

Discussion

Mesosternal bristle number is a variable trait in drosophilids

We have demonstrated in *Zaprionus* that MS bristle number, a trait considered as strongly canalized and invariant in *D. melanogaster*, can vary genetically, responds rapidly to selection, and is mostly determined by genetic factors on the X chromosome. The genus *Zaprionus* is close to the subgenus *Drosophila*, but distant from the subgenus *Sophophora* to which *D. melanogaster* belongs (Throckmorton 1975; Grimaldi 1990). In the latter species, the wild-type phenotype is one pair of bristles while in *Z. indianus*,

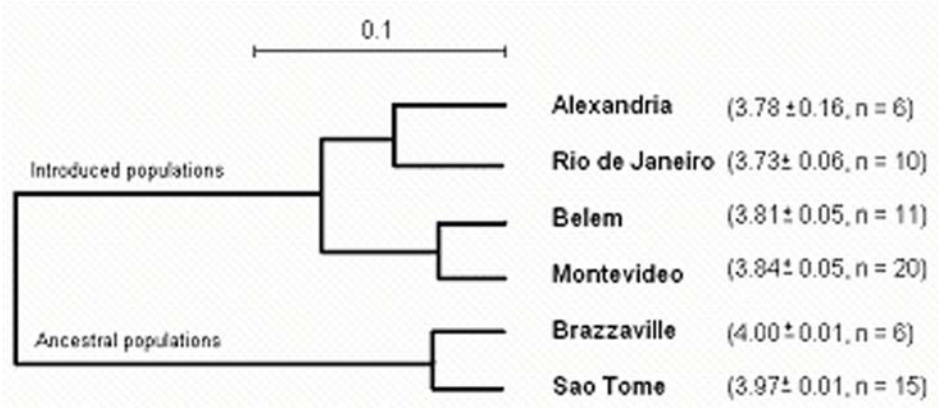

Figure 7. Relationship between six natural populations of *Z. indianus* with respect to their mesosternal bristle numbers (n = number of isofemale lines). The dendrogram was calculated with UPGMA after 1000 bootstrap iterations. The graph sharply contrasts the four recently introduced populations from the two Afrotropical ancestral ones that show stronger canalization of the normal (2-2) phenotype.

it is two pairs. We have revised other *Zaprionus* species, as well as a number of species belonging to *Drosophila* or to other genera in a phylogenetic hierarchy. In all *Zaprionus* species belonging, like *Z. indianus*, to the Afrotropical radiation (subgenus *Zaprionus*), the wild-type is always two pairs. Moreover, in all species, phenotypic variants with three, or sometimes two or five bristles, are encountered. However, when species belonging to the Oriental radiation (subgenus *Anaprionus*) were considered, the four bristle phenotype was not fixed (four in *Z. obscuricornis*, but two in *Z. bogoriensis*). We have also considered other species in the *Zaprionus* genus complex including genera *Phorticella* and *Samoaia*. The first bears two pairs of unequal size, while the second has a single pair. In *D. melanogaster*, we found only one strain where phenotypes (0-0) and (0-1) could be observed. Selection, however, failed to increase the proportion of these deviant phenotypes. In natural populations of *D. simulans*, by contrast, phenotypes with an extra bristle (1-2) are not rare. Directional selection has been successful, but after 30 generations, the selection line is still a mixture of 1-2, 2-2 and 2-3 phenotypes (J. R. David, unpublished data). Another problem is that, in many species belonging to the subgenus *Drosophila s. str*., there is no clear gap between the sternopleural (STP) and the MS bristles, but a continuity between the two types. Such seems to be the case in all species belonging to the *repleta* group. In these species, it is very difficult to discriminate both types of bristles, although this has been done in some cases (Polak 1997).

Mesosternal bristle number variability is a complex X-linked trait, independent of sternopleural bristle number

To explore the genetic basis of MS bristle number variability in *Zaprionus*, we conducted a selection experiment in which two pure lines were obtained, one carrying no bristles and the other with the wild-type phenotype, denoted (0-0) and (2-2), respectively. These two lines, however, did not show any significant difference in the number of STP bristles, indicating an independent genetic basis of MS and STP bristle number. Recent studies have shown that *Drosophila* mesonotal macrochaetae and microchaetae may perform different functions, and that they evolve independently (Usui-Ishihara and Simpson 2005). When the two *Zaprionus* lines, (0-0) and (2-2), were reciprocally crossed, two important results were observed. First, the phenotype of the F_1 males always followed that of their mothers, denoting a major effect of the X chromosome. Second, this effect was codominant, as the majority of F_1 females, heterozygous for the X, harbored an intermediate phenotype (1-1). A simple search on FLYBASE reveals that eight out of 26 major genes responsible for bristle morphogenesis are found on the X chromosome in *D. melanogaster*. Among them, the genes of the *achaete-scute* complex (AS-C) are of major importance (reviewed in Calleja *et al*. 2002; Skaer *et al*. 2002). Using *in situ* cytogenetic hybridization, Su *et al*. (1992) showed that an X chromosome synteny existed between *D. melanogaster* and *Z. tuberculatus*, another Afrotropical species. Hence, a member of the AS-C complex remains a major candidate for determining MS bristle number and a comparative analysis at the molecular level is strongly needed.

In *Z. indianus*, the penetrance of the character was incomplete, as both hemizygous males and heterozygous females showed a certain increase of phenotypic variance (with individuals bearing different phenotypes other than the 0-0 or 2-2 phenotypes in males or the 1-1 in females). The question thus, was whether this variance was due to other minor genetic effects, or simply the result of developmental instability. Backcross analyses have shown that MS variability was partly genetic, most probably due to some autosomal modifiers.

Mesosternal bristle number as a model for studying canalization and its role in enriching preadaptation

MS bristle number in *Zaprionus* is an interesting model for studying genetic canalization. Canalization was defined by Waddington (1949) as the insensitivity of a phenotype to variability in the underlying environmental and/or genetic factors. This mechanism will lead to the buildup of a hidden variability (liability), and aberrant phenotypes will appear only when the liability exceeds a threshold value. A regulatory system is thus needed to keep the phenotype within the canalization zone (estimated as the probit value of the canalized phenotype on a developmental map, D_m) (Lynch and Walsh 1998). Many studies, however, have demonstrated a failure of these regulatory systems under extreme environmental conditions, or after major genetic disturbances (reviewed in Gibson and Dworkin 2004). In order to estimate the amplitude of the canalization zone for the wild MS bristle number phenotype (2-2), we tested the effect of six different growth temperature regimes on the frequency of normal individuals. The developmental maps obtained clearly showed that liability could change among different lines; line means crossed among different thermal regimes and the among-line variance increased under extreme temperatures resulting in a nonlinear reaction norm. Moreover, MS bristle number decanalization was always biased towards bristle loss, i.e. very few individuals with phenotypes of more than four bristles were encountered, although they could be selected for.

How such cryptic genetic variation is maintained, and what its role is, compared to new mutations, in driving morphological evolution have long been and continue to be debated in the literature. Schmalhausen (1949) proposed the stabilizing selection model in which natural selection tends to repress any deviation from the optimal phenotype in the most common environment. Hence, under drastic environmental changes such as colonization of a new habitat, a number of phenotypic changes are likely produced as a result of decanalization (Waddington 1957; Eldredge and Gould 1972). This was taken as a strong evidence for the role of canalization in enriching cryptic genetic variation for potential adaptation (Rendel 1967, Rutherford and Lindquist

1998, Eshel and Matessi 1998, Queitsch *et al*. 2002, Masel 2005, 2006, but see also Siegal and Bergmann 2002, and Hermisson and Wagner 2004, for neutral models of evolution of canalization systems). Indeed, Eshel and Matessi (1998) showed that a canalization system that tends to be inactivated in extreme environments is more advantageous than a rigid one. Here again, *Z. indianus* appears to be a very interesting species for studying decanalization in natural populations. A comparison between ancestral and introduced populations revealed clearly how the MS bristle number wild-type in ancestral Afrotropical populations was more canalized than in introduced (Brazilian and Egyptian) populations, that harbored more individuals with aberrant phenotypes. Our results offer a new perspective on bristle number variability in drosophilids and the MS bristle system now deserves more attention at both the phenotypic and the molecular level.

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