Variation and heritability of aristal morphology in a natural population of *Drosophila mediopunctata*

BLANCHE CHRISTINE BITNER-MATHÉ[1,2] and LOUIS BERNARD KLACZKO[3]

[1] *Departamento de Biologia, Instituto de Biociências, USP, São Paulo, Brazil*
[2] *Departamento de Genética, Instituto de Biologia, UFRJ, Rio de Janeiro, Brazil*
[3] *Departamento de Genética e Evolução, Instituto de Biologia, UNICAMP, Campinas, Brazil*

Bitner-Mathé, B. C. and Klaczko, L. B. 1998. Variation and heritability of aristal morphology in a natural population of *Drosophila mediopunctata*. — *Hereditas 128*: 67–71. Lund, Sweden. ISSN 0018-0661. Received March 27, 1997. Accepted December 5, 1997

We studied the major sources influencing the variation of the number of aristal branches in a natural population of *Drosophila mediopunctata*. Flies were collected on six occasions at different altitudes in *Parque Nacional do Itatiaia* (Brazil). The progenies of these flies were reared in the laboratory at 16.5°C. The number of aristal branches ranges from 11 to 15 and is influenced by sex. Estimates of the natural heritability showed that at least 20 % of the total phenotypic variation is due to additive genetic variation. Although the heritability of this trait estimate in the laboratory was larger (42 %), the difference between the two estimates is not statistically significant. Thus, for the number of aristal branches, laboratory estimates of heritability provide reasonable estimations of both the magnitude and significance of heritabilities in nature. The mean numbers of aristal branches in the wild-caught flies from different altitudes or months are homogeneous. The same was observed for the means of its progeny kept in the laboratory under controlled conditions. On the other hand, wild-caught females have significantly fewer aristal branches than their laboratory-raised daughters, which suggests that an environmental factor or factors may have an important influence on this trait.

Blanche Christine Bitner-Mathé, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Caixa Postal 68011, 21944-970, Rio de Janeiro — RJ, Brazil

The *Drosophila* arista is a multiply branched structure composed of the three terminal antennal segments (KELLOGG et al. 1962). In many *Drosophila* species the aristae are involved in the reception of courtship sounds (MANNING 1967; BENNET-CLARK and EWING 1970; SPIETH 1974). The number of aristal branches varies in several species, and it might be expected that this variation has some fitness effect, particularly on mating behavior. However, the experimental evidence is rather controversial. PASTEUR (1969) and PYLE (1978) observed that strains of *D. pseudoobscura* and *D. melanogaster*, successfully selected for divergent geotactic behavior, had altered aristal morphology so that geopositive flies had more and geonegative flies had fewer branches than control flies. In another laboratory study, selection for increased and decreased number of aristal branches in *Drosophila melanogaster* did not have a consistent influence, neither on geotaxis nor on mating speed or ethological isolation between the divergent strains (PYLE and RICHMOND 1979). Furthermore, the relaxation of selection pressures did not cause reversion to the preselection value, and flies function normally regardless of the number of branches. These results led PYLE and RICHMOND (1979) to conclude that the number of aristal branches in *Drosophila* is a neutral trait under laboratory conditions and that the correlations between aristal morphology and behavior found in other investigations were probably due to linkage disequilibria. The number of aristal branches appears to be under polygenic control, the genes being located on both the X chromosome and the autosomes (PYLE and RICHMOND 1979).

We do not know anything about aristal morphology variation in nature, nor about its "natural" heritability — a critical parameter in the capacity to respond to selection in nature. Does the number of aristal branches have additive genetic variation in nature? Is the laboratory estimate of heritability useful indices of field value? Is the number of aristal branches a neutral trait or it is under selection in nature? In this work, we started to try to answer these questions. We describe the phenotypic and genetic variation in the number of aristal branches along an altitudinal gradient and among collections in a natural population of *Drosophila mediopunctata* — a Neotropical species belonging to the *tripunctata* group in Brazil (VAL et al. 1981). We estimate the heritability of his trait in the laboratory and in the natural populations on several occasions during the year. And we also investigate the effect of the different gene arrangements of X chromosome and chromosome II inversion on the aristal morphology.

MATERIAL AND METHODS

Altitudinal and temporal variation

Flies were collected at *Parque Nacional do Itatiaia*

(22°25′S, 44°50′W — state of Rio de Janeiro, Brazil) in September, 1986; in June, August, and November, 1987, and in March, May, and November, 1988. In August 1987, we took samples from five different altitudes: 700 m, 850 m, 970 m, 1020 m, and 1300 m.

A possible genetic heterogeneity in the number of aristal branches among populations was investigated. For this analysis, wild-caught *Drosophila mediopunctata* females were brought to laboratory, set individually in shell vials with fresh culture medium, and kept in a constant temperature room (16.5°C). Each day the flies were transferred to a new vial. These procedures ensured homogeneous and near optimal conditions for the F_1 larval development and avoided overcrowding as well. Up to three F_1 females were taken from each isofemale strain. To prevent common environment, each fly came from a different vial.

Natural and laboratory heritabilities

Wild-caught females and their daughters were used to estimate the heritabilities of the number of aristal branches in the field and in the laboratory. The additive genetic variance and the heritability in the laboratory (V_{Al} and h^2_L, respectively) were estimated by full-sib covariance (e.g., BECKER 1992), using the F_1 females reared in the laboratory.

Heritability estimates in nature could be done by correlating phenotypic characters of wild-caught flies with those of their laboratory-reared offspring (PROUT 1958; FALCONER 1989), as in *Drosophila* it is impossible to obtain family groups in the wild. We estimated the natural heritability by doubling the regression coefficient of laboratory-reared females on their wild-caught mothers ($h^2_N = 2b_{(Ol.Pn)}$) and by the ratio of the additive genetic variance in the laboratory to the phenotypic variance in nature ($h^2_V = V_{Al}/V_{Pn}$).

Lande (in appendix to COYNE and BEECHAM 1987) and RISKA et al. (1989) showed that it is possible to estimate a lower bound heritability in nature ($\gamma^2 h^2_N$) as: $\beta^2_{(Ol.Pn)}(\sigma^2_{Pn}/\sigma^2_{Al}) = \gamma^2 h^2_N \leq h^2_N$, where γ denotes the additive genetic correlation of the character between individuals that develop in nature and individuals that develop in a particular laboratory environment. In our case the lower bound heritability in nature ($\gamma^2 h^2_N$) was estimated by the formula $4b^2_{(Ol.Pn)}(V_{Pn}/V_{Al})$ — the squared regression coefficient should be multiplied by 4 because the regression on one parent is half that on midparent.

The effect of chromosome inversions

To investigate the effect of different gene arrangements in the number of aristal branches, wild-caught males were brought to the laboratory and individuallly crossed to three virgin females of a known homokaryotypic strain. Male gene arrangements in X chromosome and chromosome II were determined by the analysis of up to eight F_1 larvae, ensuring that the probability of incorrect assignments was smaller than 1 per cent (ARNOLD 1981). For chromosome II, since there were many possible karyotypes and some of them were very rare, they were pooled before carrying out the analysis. We formed five classes: (1) the commonest homokaryotype (DA-PAO/DA-PAO); (2) the commonest heterokaryotype (DA-PAO/DI-PBO); (3) the heterokaryotypes between DA-PAO and reasonably frequent haplotypes; (4) homokaryotype and heterokaryotypes formed by the reasonably frequent haplotypes; and (5) karyotype that had one rare arrangement. Detailed descriptions of the gene arrangements as well as their frequencies and the classes formed for the analyses can be seen in BITNER-MATHÉ et al. (1995).

For all flies analyzed, the number of branches of the right aristae was counted, including the major branches and the small terminal ones. Statistical analyses were performed using SYSTAT 5 (WILKINSON 1992).

RESULTS

Altitudinal variation

Altitude did not affect significantly the number of aristal branches, neither in wild-caught flies nor in the laboratory ones. The linear regression coefficient obtained in the two cases were near zero. We also performed a two-way analysis of variance considering as sources of variation the altitude and the fly type: wild-caught male, wild-caught female, and laboratory female. The ANOVA did not detect, neither the effect of altitude ($F_{1, 628} = 0.652$; $P = 0.42$) nor the interaction between altitude and fly type ($F_{2, 628} = 0.748$; $P = 0.47$). However the effect of the fly type is very near the significance ($F_{2, 628} = 2.945$; $P = 0.05$).

Temporal changes

Fig. 1 shows the mean number of aristal branches and one standard error for wild-caught flies and laboratory females among collections. First, we consider the collections from August/87, March/88, and November/88, for which we have samples from the two sexes in nature. A two-way analysis of variance shows that the number of aristal branches is influenced by the sex ($F_{1, 502} = 16.891$; $P < 0.001$), being greater in males than in females. We did not detect

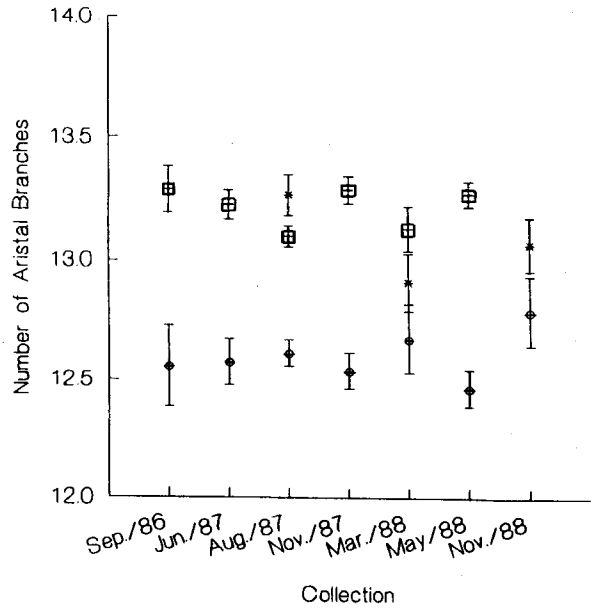

Fig. 1. Mean number of aristal branches per individual per collection for wild-caught females (⊖), their daughters reared in laboratory (⊟) and males from the wild (*). Each bar represents 1 standard-error.

neither any significant difference between collections ($F_{2,\ 502} = 0.880$; $P = 0.42$) nor a sex × collection interaction ($F_{2,\ 502} = 2.869$; $P = 0.06$). Next, we compared the wild-caught females with their daughters reared at 16.5°C. The two-way analysis of variance showed a very significant environment influence on the number of aristal branches (the field *versus* the laboratory: $F_{1,\ 1715} = 154.447$; $P < 0.001$). However, as in the previous analysis, temporal changes seem to have no influence on it ($F_{5,\ 1715} = 0.602$; $P = 0.70$) and no developmental environment × collection interaction was detected ($F_{5,\ 1715} = 1.549$; $P = 0.17$).

Heritability

Estimates of laboratory and natural heritabilities are shown in Table 1. Although the values obtained among collections vary widely, this may be due to sampling error. When we submitted the laboratory females to a hierarchical analysis of variance where family was nested within collection (Table 2), we observed that the collection effect was not significant. We used the amount of variance related to the family source to estimate the pooled full sib covariance. We also tested the homogeneity of the slopes in the regression analyses (laboratory reared females on their mothers from the field) among collections. The analysis of covariance performed indicates that the slopes are homogeneous; a non-significant F value was obtained for the interaction term ($F_{5,\ 322} = 0.52$; $P = 0.76$). With the pooled regression coefficient of females on their mothers, we were able to estimate the h^2_L, h^2_N, h^2_V, and $\gamma^2 h^2_N$ for the data from all collections (Table 1).

Our pooled value of natural heritability from offspring-parent regression across environments (h^2_N) was 27 % ± 7 %, for h^2_V was 38 %. and for $\gamma^2\ h^2_N$ was 20 %. According to RISKA et al. (1989), considering the ratio $k = h^2_N/\gamma^2\ h^2_N = h^2_V/h^2_N$, the relationship between these estimates can be understood. If $|k| < 1$, then $\gamma < 1$, $\sigma^2_{Al} < \sigma^2_{An}$ — the lower bound is larger than the other two approximations and these are therefore too small. If $|k| > 1$, as in our case (Table 1), nothing is known about the relative magnitudes of genetic variances in the two environments. The lower bound $\gamma^2\ h^2_N$ is the smallest of the three approximations. One of the other approximations could be the closest to the real value of heritability in nature, but

Table 1. *The heritability of the number of aristal branches estimated by analyses of females obtained in different collections at Parque Nacional do Itatiaia and in the pooled data. The heritability estimates in nature are:* $h^2_N = 2b_{(Ol.Pn)}$; $h^2_V = V_{Al}/V_{Pn}$ *and the lower bound heritability,* $\gamma^2 h^2_N = 4b^2_{(Ol.Pn)}\ (V_{Pn}/V_{Al})$ *where* $b_{(Ol.Pn)}$ *is the regression coefficient of laboratory-reared females on their wild-caught mothers,* V_{Pn} *is the phenotypic variance of the mothers from nature and* V_{Al} *is the additive genetic variance in the laboratory.* $k = \beta_{(Ol.Pn)}/\gamma^2 h^2_N = h^2_V/\beta_{(Ol.Pn)}$ *and* h^2_L *is the laboratory heritability. n is the number of families analyzed*

	Sep/86	Jun/87	Aug/87	Nov/87	Mar/88	May/88	Total
$h^2_N \pm SE$	0.04 ± 0.26	0.28 ± 0.16	0.34 ± 0.12	0.46 ± 0.22	0.04 ± 0.42	0.28 ± 0.14	0.27 ± 0.07
n	24	50	117	45	18	80	334
V_{Pn}	0.781	0.649	0.708	0.583	0.458	0.607	0.650
V_{Al}	0.708	0.170	0.308	0.424	0.072	0.100	0.24
h^2_V	0.91	0.26	0.44	0.73	0.16	0.17	0.38
$\gamma^2 h^2_N$	0.00	0.30	0.27	0.29	0.01	0.48	0.20
$\|k\|$	22.7	0.94	1.28	1.58	4	0.59	1.39
$h^2_L \pm SE$	1.07 ± 0.27	0.32 ± 0.19	0.58 ± 0.14	0.34 ± 0.15	0.18 ± 0.34	0.21 ± 0.20	0.42 ± 0.08
n	20	49	107	81	19	72	348

Table 2. *Hierarchical analysis of variance on the number of aristal branches of the* F_1 *daughters reared in the laboratory from wild-caught females collected on different occasions at the Parque Nacional do Itatiaia. Family was nested within collection*

Source	df	SS	MS	*F*	*P*
Collection	5	7.27	1.45	1.88	0.10
Family	342	261.84	0.77	1.72	0.001
Error	590	263	0.45		

the data provide no way of deciding whether this is so or not. Anyway, all the pooled estimates for heritability in nature are not very different and $|k|$ is close to unity.

According to ROFF and SIMON (1997), an approximate test of the difference between the heritability estimates in the field and in the laboratory can be obtained using the *t*-test, which shows no significant difference between laboratory and field estimates: $t = (0.42 - 0.27)/\sqrt{(0.08^2 + 0.07^2)} = 1.42$; $df = 680$; $0.10 < P < 0.20$.

The effects of chromosome inversions

To test the influence of different chromosome inversions on aristal morphology, we performed a two-way analysis of variance considering the karyotypes and collections as factors of variation. Neither for chromosome II (sources of variation: chromosome II — $F_{4, 239} = 2.10$; $P = 0.08$; collection — $F_{2, 239} = 0.57$; $P = 0.56$; interaction — $F_{8, 239} = 0.54$; $P = 0.83$) nor for X chromosome (sources of variation; X chromosome — $F_{2, 231} = 0.72$; $P = 0.40$; collection — $F_{2, 231} = 4.02$; $P = 0.02$; interaction — $F_{4, 231} = 1.17$; $P = 0.32$) did we observe a significant difference between different inversion karyotypes.

DISCUSSION

Our results show that the variation in the number of aristal branches is heritable in nature. The estimate of the lower bound heritability shows that at least 20% of the total variation in the field is caused by the additive genetic variation.

Under the laboratory condition we obtained, for the heritability estimate, a value of approximately two times the value obtained under field conditions. This can have two explanations: (1) full-sib estimates are potentially biased by dominance effects; and (2) the environmental variance is considerably larger in the field than in the laboratory, thereby increasing the phenotypic variation. In this study, we observed that the phenotypic variances were similar between wild-caught flies and the laboratory ones (0.6416 and 0.5592, respectively, $\chi^2 = 3.32$; $df = 1$; $P = 0.07$ — Bartlett test). Moreover, the difference between the natural and laboratory estimates of heritability is not statistically significant. In *Drosophila melanogaster*, PYLE and RICHMOND (1979) obtained, in the laboratory, a realized heritability of 0.11 ± 0.03 and 0.20 ± 0.03 from females subjected to high and low selection, respectively.

WEIGENSBERG and ROFF (1996), in a review of the literature, and ROFF and SIMON (1997), using wing dimorphism in the *Gryllus pennsylvanicus*, observed that laboratory heritabilities tended to be higher than field estimates, but the difference was not significant. According to the authors, the major implications of this study are that laboratory estimates of heritability should generally provide reasonable estimations of both the magnitude and the significance of heritabilities in nature. The results we had for the number of aristal branches, support this conclusion.

In the field as well as in the laboratory, estimates of heritability indicate that the number of aristal branches is potentially capable of responding to natural selection. However, the experiments of PYLE and RICHMOND (1979) lead them to conclude that number of aristal branches in *Drosophila* is not subject to natural selection under laboratory conditions. In our study, in the natural population of *Parque Nacional do Itatiaia*, we also did not detect any indication that altitudinal and temporal variation would exert selection on this trait. The offspring reared in the laboratory under uniform conditions, had the same means independently from where their mothers came. These results might be interpreted as an indication of neutrality. Alternatively, it is possible that the number of aristal branches is under some kind of natural selection that could not be detected by our experimental design; such as a selection that does not vary among altitudes and seasons.

Another interesting point is the homogeneity of means observed in wild females among collections when there is clearly temporal variation in nature. In these same natural populations a significant phenotypic variation in wing size was observed among collections (KLACZKO and BITNER-MATHÉ 1990; BITNER-MATHÉ et al. 1995). On the other hand, highly significant differences observed between females from nature and their daughters reared in the laboratory, suggest that an environmental factor or factors may have an important influence on this trait. Preliminary data suggest that the difference observed between wild-caught and laboratory-reared females is possibly related to a different intensity of food stress. The latter were maintained at low density with a

good food supply, the former, in nature, could be subject to more stressed food conditions. As we have no information about the micro-habitat where these flies develop in nature, no conclusion can be drawn at this moment. However, other studies are under way in view of determining the major factors that influence the morphology of aristal branches.

ACKNOWLEDGMENTS

We thank Dr. Antonio Bernardo de Carvalho for valuable suggestions in the manuscript. Dr. Sergio F. Reis helped us providing computer facilities. This work has been supported by Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos (FINEP), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Fundo de Apoio ao Ensino e Pesquisa da UNICAMP (FAEP-UNICAMP).

REFERENCES

Arnold J, (1981). Statistics of natural population. I: Estimating an allele probability in cryptic fathers with a fixed number of offspring. Biometrics 37: 495–504.

Becker WA, (1992). Manual of Quantitative Genetics (5th edn). Academic Enterprises, Pullman, Washington, USA.

Bennet-Clark HC and Ewing AW, (1970). The love song of the fruit fly. Sci. Am. 223: 85–92.

Bitner-Mathé BC, Peixoto AA and Klaczko LB, (1995). Morphological variation in a natural population of Drosophila mediopunctata: altitudinal cline, temporal changes and influence of chromosome inversions. Heredity 75: 54–61.

Coyne JA and Beecham E, (1987). Heritability of two morphological characters within and among natural populations of Drosophila melanogaster. Genetics 117: 727–737.

Falconer DS, (1989). Introduction to Quantitative Genetics. Longman Scientific and Technical.

Kellogg FE, Friezel DE and Wright RH, (1962). The olfactory guidance of flying insects. IV. Drosophila. Can. Entomol. 94: 884–888.

Klaczko LB and Bitner-Mathé BC, (1990). On the edge of a wing. Nature 346: 321.

Manning A, (1967). Antennae and sexual receptivity in Drosophila melanogaster. Science 158: 136–137.

Pasteur G, (1969). Morphological differences between Drosophila pseudoobscura populations selected for opposite geotaxes and phototaxes. Genetics 62: 837–847.

Prout T, (1958). A possible difference in genetic variance between wild and laboratory populations. Drosophila Inf. Serv. 32: 148–149.

Pyle DW, (1978). Correlated response to selection for a behavioral trait in Drosophila melanogaster Behav. Genet. 8: 333–340.

Pyle DW and Richmond RC, (1979). Genetic basis of aristal morphology in Drosophila melanogaster and its correlation with behavior: selection for increased and decreased aristal branching. Behav. Genet. 9: 297–308.

Riska B, Prout T and Turelli M, (1989). Laboratory estimates of heritabilities and genetic correlation in Nature. Genetics 123: 865–871.

Roff DA and Simon AM, (1997). The quantitative genetics of dimorphism under laboratory and 'field' conditions in the cricket Gryllus pennsylvanicus. Heredity 78: 235–240.

Spieth HT, (1974). Courtship behavior in Drosophila. Annu. Rev. Entomol. 19: 385–403.

Val FC, Vilela CR and Marques MD, (1981). Drosophilidae of the Neotropical Region. In: Genetics and Biology of Drosophila, vol. 3a (eds M Ashburner, HL Carson and JN Thompson) Academic Press, London p. 123–168.

Weigensberg I and Roff DA, (1996). Natural heritabilities: Can they be reliably estimated in the laboratory? Evolution 50: 2149–2157.

Wilkinson L, (1992). SYSTAT for Windows, version 5. SYSTAT Inc., Evanston, IL.