

## Enzyme variability in local populations of *Drosophila* species

R. Parkash and K.S. Gill

Department of Genetics, Punjab Agricultural University, Ludhiana 141004 (India), 27 April 1979

**Summary.** Dimeric acid phosphatases are coded by allelic variants of a single autosomal gene. Esterases have revealed allelic as well as non-allelic polymorphism. Allelic frequencies and proportion of heterozygosity at the polymorphic loci have been described.

Isozymes have been used as markers to analyze genetic architecture and differentiation of natural populations<sup>1-3</sup>. However, information on enzyme polymorphism in local populations of *Drosophila* species is not available. The present studies were undertaken to investigate degree and pattern of acid phosphatase and esterase variability in local populations of 6 *Drosophila* species.

Enzyme variability was analyzed by starch gel electrophoresis<sup>4</sup> in *D. malerkotliana* (n=582), *D. takahashii* (n=264); *D. nepalensis* (n=769); *D. jambulina* (n=393); *D. punjabiensis* (n=173) and *D. immigrans* (n=72), where n represents the number of wild-caught individuals analyzed electrophoretically. The enzyme banding patterns in different *Drosophila* species (figure 1) have been treated as phenotypes and investigated by genetic tests that determined which of the bands are coded by allelic variants at a locus

and which are under the control of separate loci<sup>5,6</sup>. The resulting species specific banding patterns for acid phosphatases and esterases have been represented in figure 2 which includes only the homozygous variants. Acid phosphatases are coded by single autosomal gene *Acph-1* in six *Drosophila* species analyzed. This locus is monoallelic in *D. jambulina* and *D. immigrans*; triallelic in *D. punjabiensis*; and tetra-allelic in *D. malerkotliana*, *D. takahashii* and *D. nepalensis*; and the alleles are expressed codominantly. In the polymorphic species, a heterozygote reveals a hybrid band (figure 1) in addition to 2 parental bands, suggesting that acid phosphatase is a dimer molecule. Furthermore, the neighbouring acid phosphatase allozymes are separated by same mobility difference (figure 2), indicating that any 2 neighbouring allozymes differ by 1 amino acid substitution or by 1 mutation. Thus, it is suggested that the origin of

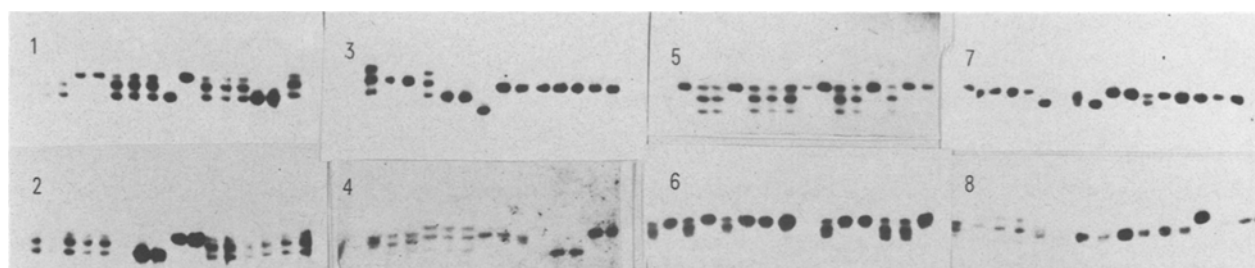


Fig. 1. Acid phosphatase variability in wild-caught individuals of different *Drosophila* species: 1-2 *D. malerkotliana*; 3-4 *D. takahashii*; 5-6 *D. nepalensis*; 7-8 *D. punjabiensis*.

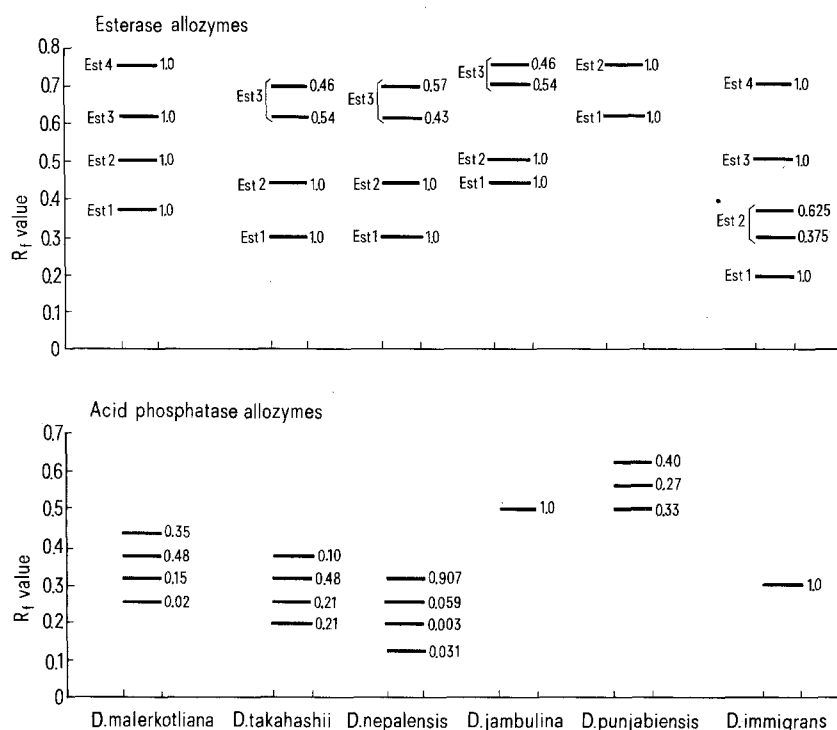


Fig. 2. Diagrammatic representation of esterase and acid phosphatase allozymes and their frequencies in different *Drosophila* species.

9 acid phosphatase allozymes (allelic isozymes) in these species might have involved 8 mutational events.

There are 2 esterase zones in *D. punjabiensis*; 3 in *D. takahashii*, *D. nepalensis* and *D. jambulina*; 4 in *D. malerkotliana* and *D. immigrans*. The segregating esterase phenotypes at any esterase zone do appear in the 1:2:1 proportions, suggesting monogenic control of that zone. Heterozygotes at variable esterase zone reveal only the parental bands, suggesting that esterases under their control are monomers. The enzyme phenotypes being direct representatives of genotypes, the frequencies of different *Acph/Est* alleles have been determined from the zymograms of the wild-caught individuals (figure 2). The observed heterozygosity at *Acph-1* locus is 0.17, 0.61, 0.62 and 0.63 in *D. nepalensis*, *D. malerkotliana*, *D. punjabiensis* and *D. takahashii* respectively. The 4 esterase loci showing diallelism have heterozygotic frequencies varying from 0.42 to 0.49 and thus contribute to genetic polymorphism. The local populations of 6 *Drosophila* species show a good fit to the Hardy-Weinberg equilibrium with respect to *Acph-1* and *Est* loci, indicating that selection is not operating. Ohno<sup>7</sup> hypothesised that functional differences might exist between allelic variants, and such differences could be selectively important. In the present studies, 3 allelic acid phosphatases of *D. malerkotliana* have been characterized on the basis of their specific

activities,  $V_{max}$ - and  $K_m$ -values, effect of metallic ions, and inhibitor sensitivity. The specific activities of 3 allelic acid phosphatases ( $A_1$ ,  $A_2$  and  $A_3$ )<sup>5</sup> are 60, 89 and 100  $\mu\text{M}$   $\alpha$ -naphthol released/min/mg protein respectively. The differences in catalytic efficiency of allelic acid phosphatases (allozymes) can be argued on the basis of marked differences observed in  $V_{max}$ -values at different temperatures<sup>6</sup>. However,  $K_m$ -differences between allozymes are not significant<sup>6</sup>. One of the allelic acid phosphatase ( $A_3$ ) has revealed characteristically least affinity for an inhibitor (sodium fluoride) while  $\text{Mg}^{++}$  or  $\text{Mn}^{++}$  have shown a promotory effect. It is suggested that such biochemical differences might be physiologically relevant and subject to selection.

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## Chromosome complement of two species of Amelinae (Dictyoptera: Mantodea)

J. C. Orozco, M. Espejo and A. Pretel

Departamento de Genética, Facultad de Ciencias, University of Granada, Granada (Spain), 3 April 1979

**Summary.** The karyotypes of *Apteromantis aptera* (Fuente) and *Ameles decolor* (Charpentier) are described. Chromosomes numbers in females are  $2n = 30$  and  $2n = 28$  respectively. Sex determination mechanism in both is XO-XX.

Matthey<sup>1</sup>, White<sup>2,3</sup> and Gupta<sup>4</sup> described in detail the karyotypes of some species from the Amelinae subfamily. At least 7 species have been studied. In *Ameles heldreichi* Br.<sup>5</sup> and *Ameles abjecta* Cyr.<sup>6</sup> intraspecific polymorphism has been found. Matthey<sup>1</sup> was the first to study the karyotype of *Apteromantis bolivari* Wern. This species has  $2n = 29$  in males: 28 acrocentric autosomes and 1 metacentric X. The author quotes as an interesting point in the analysis of this species the presence of an unusual diakinetik stage. Wahrman<sup>5</sup> reported an intraspecific chromosomal polymorphism in Amelinae. He found 3 karyotypic variants in *Ameles heldreichi*, which he calls type A ( $2n = 27$  in males, having 24 acrocentric and 2 metacentric autosomes and 1 metacentric X); type B ( $2n = 28$  in males, with 26 acrocentrics, 1 unpaired metacentric and 1 metacentric X) and type C ( $2n = 29$  in males, all 28 autosomes being acrocentrics and 1 metacentric X). These data were confirmed in the meiotic analysis. Type A, in fact, showed in M-I 1 ring-shaped bivalent, formed by the only pair of metacentric autosomes. Type B showed, instead of it, a trivalent composed of the only unpaired metacentric autosome and 2 acrocentric homologues; and type C rod-shaped bivalents only. The 3 types had the same fundamental number. A mechanism of the Robertsonian type was proposed to explain intraspecific variation in this species. Besides, Wahrman quotes the presence, in some individuals, of a small metacentric autosome, originated by a pericentric inversion. The author did not find homozygous individuals for this structural rearrangement.

**Material and methods.** 6 individuals (4 males and 2 females) of *Apteromantis aptera* Fuente, an endemic species from the

South of the Iberian Peninsula, were analysed. Individuals were collected in La Mala (Granada, Spain) during April and May 1977. Also 10 individuals (2 males and 8 females) of *Ameles decolor* (Charpentier) were studied. They were collected from Sierra de Cazorla (Jaen, Spain) in 1977. For classification of specimens the code of Morales Agacino<sup>7</sup> was used. For male meiosis analysis, testes, fixed in acetic acid-ethanol (1:3) mixture, were squashed in 1% acetic orcein. For somatic chromosome analysis ovarioles were fixed in the same fixative. Females were previously injected with 0.05% colcemid solution in isotonic saline, and material was squashed in 1% acetic orcein.

**Results.** The karyotype of *Apteromantis aptera* is described here for the first time (figure 1). This species displays 14 pairs of acrocentric autosomes and a pair of submetacentric X ( $C.I. = 2.27$ ) in females, with  $FN = 30$  in males. In male meiosis, 14 bivalents and 1 X univalent were observed. Meiotic analysis showed the existence of a diakinetik stage in this species. Diakinesis in another species of *Apteromantis*, *A. bolivari*, was previously reported by Matthey<sup>1</sup>. Another point to be noted is the side-arm bridge seen in approximately half of the A-II meiotic cells analysed of this species. These bridges are due to an adherence between the long arms of the sister chromatids of an X chromosome presumably by specific regions situated in them. The existence of such bridges has been reported in grasshoppers<sup>8</sup>.

The karyotype of *Ameles decolor*, also described here for the first time (Figure 2), shows 2 pairs of metacentric chromosomes of different size (Nos 1 and 2), a pair of subacrocentric ones (No. 13) and 10 pairs of acrocentrics (Nos 4-13): It also has a pair of metacentric X-chromo-