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Genetics of *Drosophila nasuta*: developmental and population studies at esterase loci

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Gel electrophoresis technique is employed to detect allelic variants at the α-esterase loci in four natural populations of *Drosophila nasuta*. Four zones of activity were detected for α-esterases. The *Est-2* has four, the *Est-3* has two, and the *Est-4* has eight allelic variants, whereas allelic variants could not be detected at the *Est-1* because it was not satisfactorily resolved on the polyacrylamide gels. The degree of heterozygosity varies from locus to locus and also in different populations. Nearly 13 % individuals were heterozygous at the *Est-2*, 27 % at the *Est-3*, and 22 % at the *Est-4* locus. The zymogram pattern exhibits significant variation during the development. The most significant observation is the specificity of the Est-$4^{1.03}$ to the larval, and Est-$4^{0.95}$ and Est-$4^{1.02}$ to the pupal stages. The significance of differential gene activity is discussed.

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A basic approach to the study of evolution is to find out how much genetic variation exists in natural populations of various organisms. That is, the genetic variation is one of the fundamental parameters of the evolutionary process. Thus the amount of genetic variation in a population measures its evolutionary potential. But the difficulty is that with the application of Mendelian principles one can only detect variable genetic variations while invariant ones go undetected. This problem was solved by the development of gel electrophoresis, a technique that permits the detection of variable and invariant genetic variations at the same time (see AYALA 1978).

The *nasuta* subgroup of the *immigrans* species group of the genus *Drosophila* represents a very interesting group of species. Among them, *D. nasuta* is very common and widely distributed in the Indian subcontinent. A considerable lot of data on naturally occurring chromosomal polymorphisms have been accumulated in this species (see KUMAR and GUPTA 1986a, b, 1988). However, the knowledge regarding genic polymorphism in its natural populations is completely lacking. The present article provides a preliminary account of genetic variation at the α-esterase loci in four natural populations and also during development in *Drosophila nasuta*.

Materials and methods

Flies used in this study were collected from four different localities: Patna, Allahabad, Jaunpur, and the Banaras Hindu University campus. The majority of chemicals were purchased from Sigma Chemical Co., USA, and BDH Chemical Ltd., Poole, England.

Individual flies, larvae or pupae were homogenized in 20 µl of 0.1M Tris-HCl-EDTA buffer, pH 8.9 and centrifuged at 9,000 g at 4°C for 5 min. The supernatant was loaded in each gel pocket. The esterase enzymes were separated on polyacrylamide gels. Slab gels, 2 mm in thickness, were composed of a 5 % stacking gel and a 8 % running gel. The gels were run for 16 h at 5°C in 0.08M Tris-Borate-EDTA buffer, pH 7.1. After running, the gels were incubated in 0.05M Boric Acid room temperature for 30 min. The gels were thoroughly washed in distilled water and incubated in 50 ml phosphate buffer (pH 6.4) containing 1 ml each of 0.1M $MnCl_2$, 0.1M $MgCl_2$, and 0.1M NaCl, 25 mg

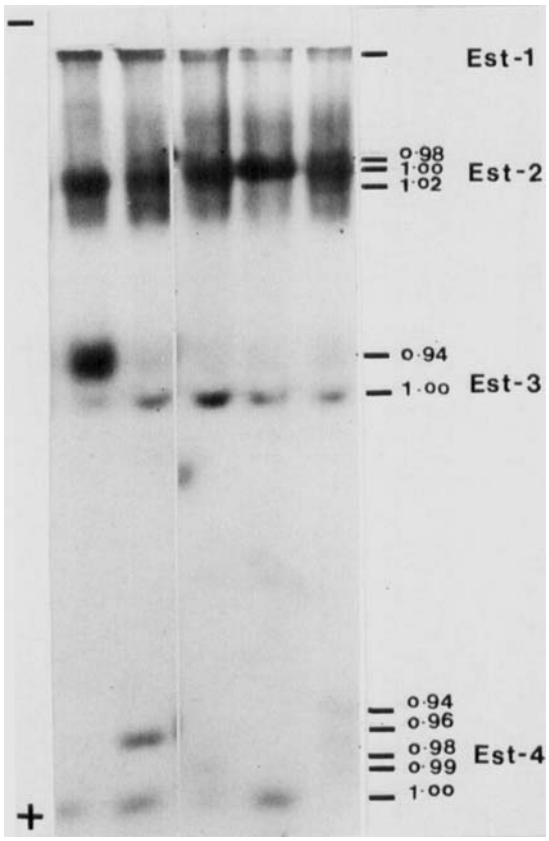

Fig. 1. Electrophoretic patterns of α-esterases from single flies homogenates of *Drosophila nasuta*

α-Naphthyl acetate (predissolved in 50 % acetone) and 30 mg fast blue BB salt. The nomenclature used here is that used by LAKOVAARA and SAURA (1971). We have used gels with 12 wells. The outer two wells were used for control samples. The inner ten wells were used for unknown samples. The mobility of allozymes in the inner wells was recorded with respect to the control samples.

Results and discussion

Four zones of activity were observed in zymograms of single fly homogenates when gels were assayed for α-esterases (Fig. 1). These zones were designated as Est-1 to Est-4 in the order of increasing mobility towards anode. For instance, the Est-1 is most cathodal and the Est-4 is most anodal. Among four zones detected, the Est-4 is most polymorphic and exhibits eight allelic variants, namely, Est-$4^{0.94}$, Est-$4^{0.95}$, Est-$4^{0.96}$, Est-$4^{0.98}$, Est-$4^{0.99}$ Est-$4^{1.00}$, Est-$4^{1.02}$, and Est-$4^{1.03}$. The Est-3 is less polymorphic and exhibits only two allelic variants, Est-$3^{0.94}$ and Est-$3^{1.00}$, whereas the Est-2 shows four variants, Est-$2^{0.96}$, Est-$2^{0.98}$, Est-$2^{1.00}$, and Est-$2^{1.02}$. However, no variants were detected at the Est-1 because it was not satisfactorily resolved on the polyacrylamide gels in spite of several changes made in the gel concentration, buffer pH, and time of running the gels. Among these variants, the Est-$2^{0.98}$, Est-$2^{1.02}$, Est-$4^{0.94}$ and Est-$4^{0.99}$ are rare variants, which were detected in a single and same individual, collected on the Banaras Hindu University campus. Based on the banding pattern in hybrid individuals, it is inferred that the allozymes coded by the *Est-*$2^{0.96}$, *Est-*$2^{1.00}$, *Est-*$3^{0.94}$, *Est-*$3^{1.00}$, *Est-*$4^{0.96}$, *Est-*$4^{0.98}$, *Est-*$4^{1.00}$ are monomeric, and allozymes coded by the *Est-*$2^{0.98}$, *Est-*$2^{1.02}$, *Est-*$4^{0.94}$, *Est-*$4^{0.95}$, *Est-*$4^{0.99}$, *and Est-*$4^{1.02}$ are dimeric. The most puzzling is the behaviour of the *Est-*$4^{1.03}$, which exhibits four-banded pattern in heterozygous condition with the *Est-*$4^{0.96}$. Since the Est-$4^{0.96}$ is monomeric, it may be possible that the *Est-*$4^{1.03}$ is an exceptional allele showing a three-band homozygous condition as has been earlier observed for the Est-$\beta^{1.28}$ allozyme in *D. virilis* by TSUNO et al. (1984). Alternatively, it is also possible that the Est-$4^{1.03}$ allozyme is the product of three similar closely linked alleles. However, unless genetic crosses (recombination tests) using the three-band homozygotes are made, it is difficult to interpret the unusual three-band pattern of the *Est-*$4^{1.03}$ allele. Moreover, similar situations are not uncommon in *Drosophila*. There are instances where multi-band homozygotes have been detected. Notable instances are at the *Est-6* locus of *D. melanogaster* (COSTA et al. 1977), the *6PGD* locus of the Japanese Quail (OHNO et al. 1969), and the α- and β-esterase loci of *D. virilis* (TSUNO et al. 1984).

Frequencies of different alleles at esterase loci in natural populations of *D. nasuta* are given in Table 1. The degree of heterozygosity ranges from 7.5 % to 20 % at the *Est-2* locus, 5 % to 75 % at the *Est-3* locus, and 15 % to 27.5 % at the *Est-4* locus. Moreover, the weighted heterozygosity (average heterozygosity) at the *Est-2*, *Est-3*, and *Est-4* are 13 %, 27 % and 22 %, respectively. The occurrence of a high degree of allelic variation at esterase loci in natural populations of *D. nasuta* is similar to that of the other species of *Drosophila*, such as *D. melanogaster* (WRIGHT 1963), *D. pseudoobscura* (HUBBY and LEWONTIN 1966; COYNE et al. 1978; SINGH 1979), *D. aldrichi* and *D. mulleri* (JOHNSON et al. 1968), *D.*

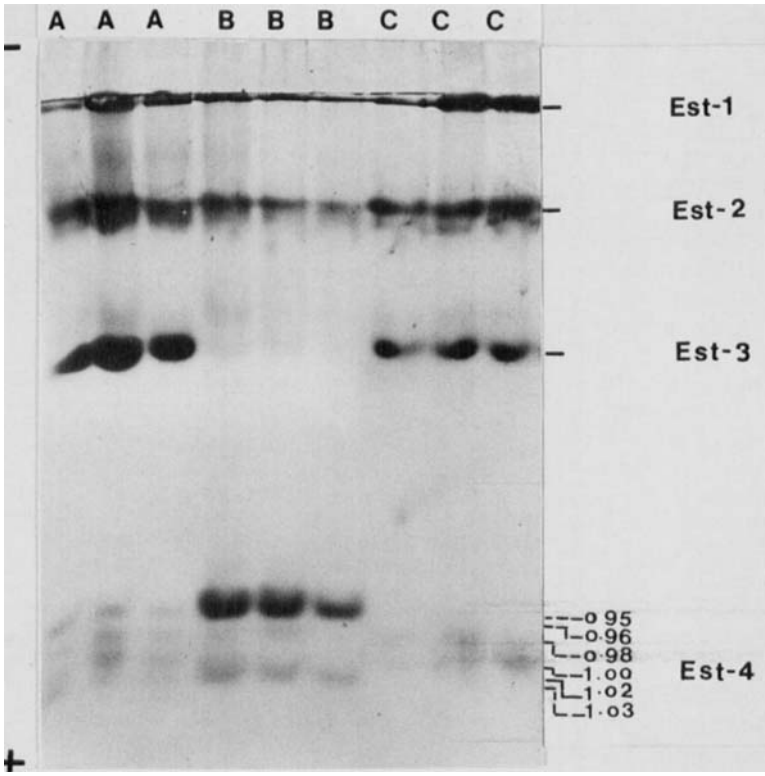

Fig. 2. Developmental zymogram patterns of α-esterases of *Drosophila nasuta*
Abbreviations: A = Larva; B = Pupa; C = Adult

subobscura (LAKOVAARA and SAURA 1971), *D. equinoxialis* (AYALA et al. 1972), and *D. persimilis* (PRAKASH 1977). According to GILLESPIE and LANGLEY (1974), the amount of genetic variation exhibited by an enzyme is determined by its substrate specificity. Enzymes, such as alcohol dehydrogenase, esterases and others, capable of utilizing a family of substrates are more variable than enzymes, such as malate dehydrogenase, pyruvate dehydrogenase and others, utilizing a unique substrate. Thus, the large amount of genetic variation at esterase loci is not unusual. JOHNSON (1974), extending this notion further, has shown that, in addition to substrate specificity, enzymes involved in regulatory metabolic pathways are generally more polymorphic than enzymes whose functions are primarily non-regula-

Table 1. Allelic variation at esterase loci in four natural populations of *Drosophila nasuta*

Gene loci	Alleles	Localities*			
		Patna	Allahabad	Jaunpur	BHU campus
Est-2	0.96	0.25	0.125	0.20	0.40
	1.00	0.75	0.875	0.80	0.60
Heterozygosity		0.10	0.075	0.125	0.20
Est-3	0.94	0.10	0.15	0.15	0.225
	1.00	0.90	0.85	0.85	0.775
Heterozygosity		0.05	0.75	0.10	0.175
Est-4	0.96	0.20	0.05	0.025	0.125
	0.98	0 15	0.15	0.125	0.275
	1.00	0.65	0.80	0.85	0.60
Heterozygosity		0.25	0.20	0.15	0.275

* A total of 40 individual females were assayed from each locality

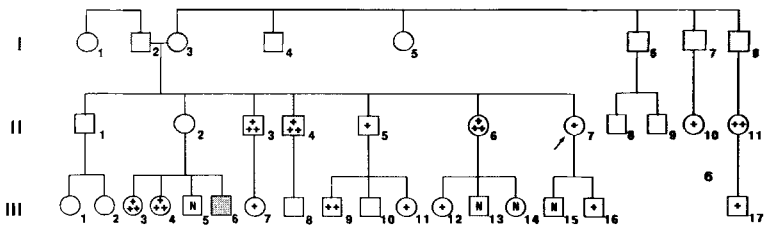

Fig. 1. Pedigree of 36 members of a family with high incidence of cancer. Shaded symbol indicates carcinoma (see Table 1). Square symbols = males; round symbols = females. The proband has been marked by an arrow. The subjects which could be studied cytogenetically have been listed in Table 2. For each of these result of the visual classification for chromosome 1 heterochromatin (1qh) heteromorphism (Table 2) has been indicated inside the symbol. N = no heteromorphism; 1–3 plus signs = increasing degree of heteromorphism.

bers with a history of malignant disease were collected. In addition, available members of the family were interviewed about the incidence of cancer in the family. On the basis of the data obtained, a pedigree including 36 family members was constructed (Fig. 1).

Cytogenetic analysis. – Peripheral blood samples were drawn from 19 members of the family. A few drops of blood were suspended in 10 ml of McCoy5A medium supplemented with 15 % fetal bovine serum and PHA. The cells were cultured for 72 hours. Colcemid was added during the last hour of culture at a final concentration of 0.005 μg/ml, and air dry chromosome spreads were prepared and stained for G-bands according to a standard method (MARTINSSON et al. 1982). For each individual several well-spread metaphases were photographed and 3 complete karyotypes were made from cut out chromosomes of enlarged prints. Some slides from each patient were stained for C-banding according to the BSG method of SUMNER (1972).

Estimation of constitutive heterochromatin polymorphism in chromosome 1. – Polymorphism of the proximal heterochromatic segment in chromosome 1 (1qh) was estimated independently by 2 methods. (1) Visual classification was made independently by 2 of us without knowledge of the clinical data. No appreciable difference between the homologues was scored with the designation N; increasing degrees of asymmetry were scored with 1, 2 or 3 plus signs. Only in one case was there a difference of opinion about the classification among the observers. This case was resolved by the detailed analysis of several mitoses in the microscope. (2) Quantitative analysis was performed by means of a computer-based interactive image-analysis system (IBAS I/II, Kontron Bildanalyse GmbH). The lengths of the heterochromatic segments of both homologues were approximated by a curve introduced via a cursor and the length of this curve was calculated by the computer. The length values obtained were used to calculate the ratio of the longer and the shorter heterochromatic segments of chromosome 1.

Results

Occurrence of malignancies

The occurrence of tumors in the family has been recorded in Table 1. A total of 9 family members have developed malignant tumors, mostly carcinomas of the colon and/or of the ovary.

The proband (II:7) was a member of the second generation in the pedigree of Fig. 1. Her father (I:2) was one of a sibship of two. Neither sib has shown any sign of tumors and the sister (I:1) is alive and well at the age of 93. The proband's mother (I:3) was one of a sibship of six. Three of the sibs (including the mother) are known to have developed and died from malignancies.

At the outset of the present investigation, tumors had been diagnosed in 4 members of the second generation, i.e., in 4 of the 7 children of I:2 and I:3. Since then, a fifth sib (II:4) developed a colon carcinoma.

The third generation consists exclusively of young persons. Only one member of this generation has developed a tumor (III:6).

Cytogenetic findings

The 19 individuals available for cytogenetic analysis have been listed in the figure legend of the pedigree (Fig. 1). Generally, normal karyotypes were

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