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ISOZYME VARIATION IN THREE SPEICES OF THE *DROSOPHILA HYPOCAUSTA* COMPLEX[1]

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Fei-Jann Lin and Hwei-Yu Chang (1987) Isozyme variation in three species of the *Drosophila hypocausta* complex. *Bull. Inst. Zool., Academia Sinica* **26**(4): 311-315. Polyacrylamide gel electrophoresis was adopted to analyze 7 enzyme systems and their variations in three species of the *Drosophila hypocausta* species subgroup of the *D. immigrans* species group. Comparative isozyme patterns show variations both among strains and among species. Phenogram shows that differences among species agreed with the presumed degrees of relationship. Thus, the information obtained from isofemale strains may be used as a reference to the natural population. Under the condition of 100 individuals per sample, there has no minor variation within strain been detected, indicating that isofemale strains tend to be monomorphic. The heterozygosity for the only polymorphic locus is 0.52, which is pretty high. The polymorphism maintained in the isofemale strain could be mainly due to heterosis.

Genetic variation, the fundamental material for natural selection to operate, in population reveals the potential for the population to evolve. Genetic variation among populations is also a clue to understand the relationships among them. Isozyme variation is frequently adopted for these kinds of investigation.

Drosophila hypocausta, *D. siamana*, and *D. neohypocausta* are closely related species in the *D. hypocausta* subgroup of the *D. immigrans* group (Ikeda, *et al.*, 1983). The insemination tests showed that *D. siamana* and *D. hypocausta* are closer to each other than to *D. neohypocausta* (Hihara and Lin, 1984). The present study compares the enzyme variation among these species and describes their relationships by using isofemale strains.

MATERIALS AND METHODS

Seven isofemale strains of 3 species of the *hypocausta* complex of *D. immigrans* group —*D. hypocausta*, *D. siamana*, and *D. neohypocausta*—were used in this study. The locality, year of collection, and collector were listed in Table 1.

Seven isozyme systems including alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), tetrazolium oxidase (TO), acid phosphatase (ACPH), alkaline phosphatase (APH), esterase (EST) and leucine amino peptidase (LAP) were analyzed on vertical polyacrylamide gel electrophoresis. Each individual fly was homogenized with 20 μl distilled water in an Eppendorf tube. After centrifugation for 10 min, 10 μl supernatant was mixed with 2 μl Bromophenol Blue-glycerol solution, and loaded on a well of of a 7.5% polyacrylamide slab gel. Gel was run with Tris-glycine buffer (pH 8.3) at 4°C until the dye front reaching the end of the gel. The isozyme patterns were then visualized by the specific staining methods described by

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TABLE 1
Species and strains used in this study

Species and Strains	Locality	Year and Collector (s)
D. hypocausta		
0181.11	Palawan, Philippines	Hihara & Watanabe, 1979
0181.12	Rabaul, New Guinea	Masuzawa, 1979
D. siamana		
0181.13	Penang, Malaysia	Hihara & Fuyama, 1979
0181.14	Penang, Malaysia	Hihara & Fuyama, 1979
D. neohypocausta		
0005.1	Jiuntou, Yilan	Lin & Tseng, 1968
0154.1	Wulai, Taibei	Lin, Hsu & Chang, 1977
0173.2	Funglin, Hualian	Lin & Tung, 1980

Ayala, *et al.* (1972). In order to detect minor variation within an isofemale strain, 100 female flies from each strain were used for analysis.

RESULTS AND DISCUSSION

The various zymogram patterns of each isozyme observed in this study are shown in Fig. 1. These seven enzyme systems show 15–19 gene loci if we consider one band as the product of one gene locus and take into account the situation that monomers and polymers appear on the same gel. Except in one *D. siamana* strain (0181.13) on one esterase locus (the triple bands on esterase

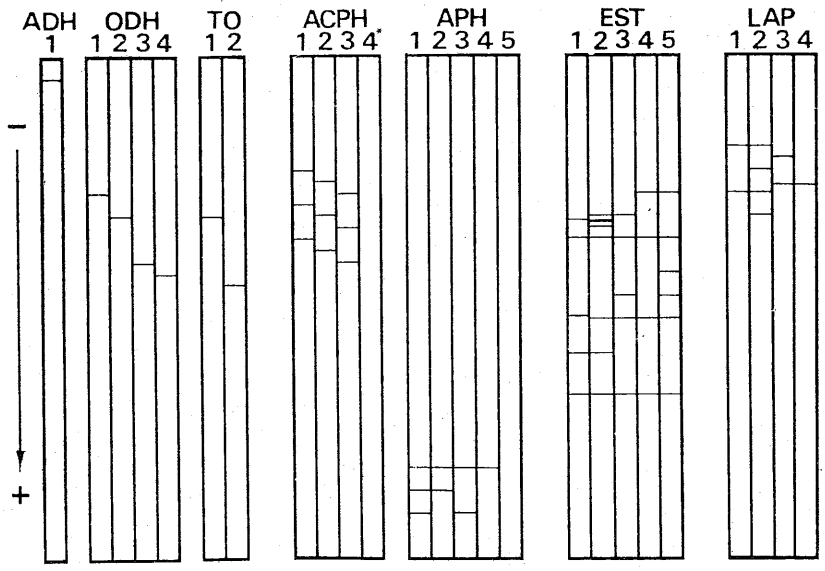

Fig. 1. Diagrams showing the zymogram patterns resulting from single adult flies of the *D. hypocausta* subgroup species after alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), tetrazolium oxidase (TO), acid phosphatase (ACPH), alkaline phosphatase (APH), esterase (EST) and leucine amino-peptidase (LAP) staining of the 0.75% polyacrylamide gels.

* the fourth column under ACPH indicates that there is no distinguishable band but patches of smear.

TABLE 2
Zymogram patterns of seven strains of *D. hypocausta* subgroup species

Strain number	ADH	ODH	TO	ACPH	APH	EST	LAP
0181.11	1	2	2	1	1	1	1
0181.12	1	1	2	3	1	1	1
0181.13	1	4	2	2	2	2	2
0181.14	1	4	2	2	2	3	2
0005.1	1	3	1	4	5	4	3
0154.1	1	3	1	4	4	5	4
0173.2	1	3	1	4	3	4	4

* 1 to 5 refer to the patterns shown in Fig. 1 for each isozyme.

pattern 2), there is no detectable variation within every isofemale strain. The zymogram pattern of each isofemale strain is listed in Table 2. Since 100 flies are used for each analysis, it is obvious that all isozyme gene loci examined except one of esterase locus are fixed in all of the seven isofemale strains. These isofemale strains could be either monomorphic at beginning or fixed through genetic drift. All of these isofemale strains have been maintained in the laboratory for a long time (Table 1). If we take into account the average 10 years per stock, about 20 generations per year, more than 200 flies transfered per generation, and 15 gene loci plus 100 individuals per sample the result in this study is accumulated at 6×10^7 level. The high degree of monomorphism in isofemale strains compairing to natural populations indicates that the probability of generating a neutral allele is very low. In other words, the isozyme polymorphism in natural population is unlikely just due to mutation and drift.

According to these isozyme data, we may construct the phenogram of these seven isofemale strains. Briefly, if each band on zymogram is treated as a character, the character states are coded as presence/absence of the band, the zymogram results in a data set of 36 characters. The middle band, which represents a heterozygous diameter form, of the esterase triple bands (pattern 2) is omitted in order to reduce redundancy. The Sorensen resemblance coefficient is then calculated according to the binary data set recoded from the original zymogram patterns of Table 2. Furthermore, the unweighted pair group method using arithmetic average (UPGMA) is adopted for cluster analysis. Phenogram of these seven isofemale strains is shown in Fig. 2.

The relationship among these three species shown in Fig. 2 is consistent to the result of insemination tests in the report of Ikeda, *et al.* (1983). *D. hypocausta* and *D. siamana* are thought to be the closely related species on the basis of morphology and the interspecific hybridization test. Therefore, this analysis, which is based on the information obtained from isofemale strains, may be used as a reference to the natural populations. The variation between the strains of *D. hypocausta* is higher than the intraspecific variation of the other two species. It may be due to that these two strains were collected from different geographic environment, i.e. one from Philippines and the other from New Guinea.

The triple bands on esterase pattern 2 shows the heterozygous form of this esterase locus in strain 0181.13, which is the only strain with a polymorphic esterase locus. If the two alleles are named F for fast, and S for slow locomotion on the gel, the frequencies of FF, FS, and SS individuals are 0.21, 0.52, and 0.27, respectively. This result indicates that heterosis could be a possible mechanism for maintaining polymorphism in isofemale strains. This possibility is supported

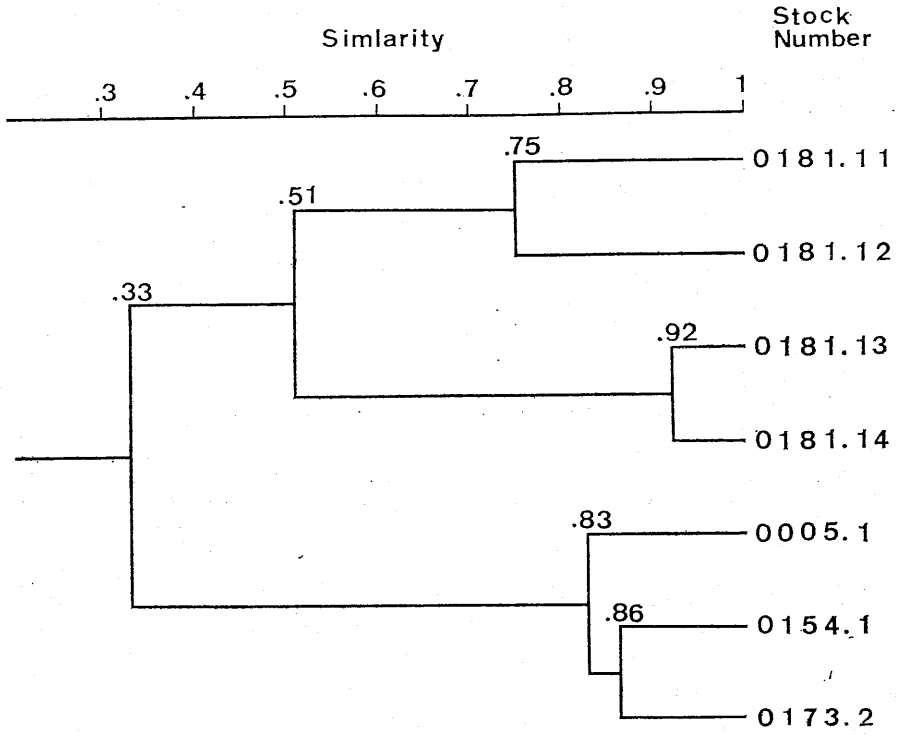

Fig. 2. The phenogram of seven isofemale stocks of the *Drosophila hypocausta* complex constructed by the UPGMA method.

by our provious finding that heterozygous inversion In(2L)B_1D_5 of *D. albomicans* is also preserved at high frequencies in isofemale strains (Chang, *et al.*, 1987). These two alleles were first recognized in 1983 and again checked in 1986. Since there is no significant difference between the two samples, we suppose this strain is at equilibrium, and the equilibrium frequency of F allele is 0.47. If the polymorphism existed at the beginning, the initial frequency of the F allele would be 0.25, 0.50, or 0.75. If the polymorphism is due to mutation, the initial frequency is around 0.0025. Although we can not rule out the possibility that the F allele is generated through mutation during the period of laboratory maintenance, the possibility for the allele frequency to reach 0.47 from 0.0025 within four years is pretty low. However, more study is needed to clarify how polymorphism in natural populations is maintained.

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三個果蠅近緣種 (*Drosophila hypocausta* complex) 之異構酶變異

林 飛 棧　　張 慧 羽

用膠體電泳法調查大果蠅種羣 (*Drosophila immigrans* species group) 三個近緣種 *D. hypocausta*, *D. siamana* 和 *D. neohypocausta* (*D. hypocausta* species subgroup) 七種異構酶的變異。比較結果顯示種間和單雌品系間皆有變異。依據異構酶數據所作出之表型圖 (phenogram) 與其他研究結果符合。用 100 個體作一樣本，發現單雌品系內沒有低頻率的變異。唯一的那個多態性基因座之異結合型比率 (heterozygosity) 偏高 ($H=0.52$)。初步資料顯示單雌品系有趨於單態性的傾向。異結合型優勢 (heterosis) 可能是維持單雌品系多態性的主要機制。