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## 【Research report】

### 三個果蠅近緣種 (*Drosophila nasuta* Complex)之異構[西每]變異【研究報告】

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## Abstract

### 摘要

用膠體電泳法調查大學蠅種群 (*Drosophila immigrans* species group) 的輝顏亞種群 (*D. nasuta* species subgroup) 中三個近緣種的六種異構[西每]變異。比較的結果顯示種間及單雌品系間皆有變異。對於多態性的基因座，異結合型比率 (heterozygosity) 相當高。品系內並沒有低頻率的變異存在。這筆初步資料顯示在異構[西每]的系統看來，即使自然族群為多態性，單雌品系有趨於單態性傾向。因此個別的單雌品系不能代表自然族群中異構[西每]的分佈情形。必須作進一步的研究，才能用異構[西每]的調查結果來推斷族群結構。

### Key words:

#### 關鍵詞:

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## ISOZYME VARIATION IN THREE SPECIES OF THE *DROSOPHILA NASUTA* COMPLEX

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### Abstract

Polyacrylamide gel electrophoresis was applied for the analysis of the genetic variation of 6 enzyme systems in 3 species of the *Drosophila nasuta* species subgroup of the *D. immigrans* species group. Isozyme variations are shown both among strains and among species. The heterozygosity values for those polymorphic loci are pretty high. There has no minor variation within strain been detected. The preliminary data shows that *D. albomicans* and *D. nasuta* are more similar to each other than to *D. kepulauanana*, and isofemale strains tend to be monomorphic while the natural population is polymorphic for certain enzyme system. Thus, single isofemale strain can not represent a population for such isozyme system. Given a number of isofemale strains, it is possible to estimate the genetic variation in the natural population. Furthermore, heterosis seems to be one of the main mechanisms to maintain the polymorphism within isofemale strains.

**Key words:** *Drosophila*/Isozyme Variation.

### Introduction

The genetic variation is not only the fundamental material for natural selection to operate, or the opportunity for organisms to evolve, but also a clue to understand the population structure as well as the relationships among populations. There exists a large quantity of genetic variation in natural populations. Among a variety of different kinds of genetic variation, isozyme variation is frequently adopted for investigation.

*Drosophila albomicans*, *D. nasuta*, and *D. kepulauanana* are three species in the *D. nasuta* subgroup of the *D. immigrans* group. The close relationships among them were already noted by Wilson *et al.* (1969), Kitagawa *et al.* (1982), and others. In our laboratory, we have acquired and established nine isofemale strains of three species. Thus, in the present study, we compare the enzyme variation among those isofemale strains in order to get more information about the relationship among these species.

### Materials and Methods

Nine isofemale strains of 3 species of the *nasuta* complex of the *D. immigrans* group

Table 1. Species and strains used in this study

Species and strains	Locality	Collector(s)
<i>D. albomicans</i>		
0073.2	Wulai, Taiwan, R. O. C.	Lin, Ting and Wang
0161.2	Chiang Mai, Thailand	Kitagawa
0163.1	Okinawa, Japan	Lin, and Yamamoto
<i>D. nasuta</i>		
0181.8	Seycelles, Sri Lanka	#SEZ-4 from University of Texas
0193.6	Kandy, Sri Lanka	Kitagawa
0193.7	Mysore, India	Kitagawa
0193.8	Mombasa, Kenya	Kitagawa
<i>D. kepulauanana</i>		
0181.7	Brunei	#3056.8 from University of Texas
0193.4	Palawan, Philippines	Kitagawa

—*D. albomicans*, *D. kepulauanana*, and *D. nasuta*—were used in this study. The locality and collector were listed in Table 1.

An isofemale strain is the one established from the offspring of a single female fly captured from its natural habitat. Those isofemale strains were reared in our laboratory by standard corn meal medium under the condition of 22°C and 75% relative humidity.

Six isozyme systems such as alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), tetrazolium oxidase (TO),  $\alpha$ -glucose phosphate dehydrogenase ( $\alpha$ GPDH), malate dehydrogenase (MDH), and esterase (EST) were analyzed by vertical polyacrylamide gel electrophoresis. Briefly, each individual fly was homogenized with 20  $\mu$ l distilled water in an Eppendorf tube. After centrifugation for 10 min, 10  $\mu$ l of supernatant was mixed with 2  $\mu$ l Bromophenol blue-glycerol solution, and loaded on a well 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 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

Five of those isofemale strains were assayed for 6 enzyme systems and the other four were used for just EST assay only. The zymogram patterns observed in this study are shown in Fig. 1. Three enzyme systems, including ADH, TO and MDH, show only one zymogram pattern. Therefore, they do not provide useful information in this study. On the other hand, there are 2, 3 and 6 zymogram patterns for  $\alpha$ GPDH, ODH and EST, respectively. However, most of the isofemale strains are fixed on those isozyme gene loci (Table 2), except Okinawa *D. albomicans* (stock number 0.163.01) on ODH locus, and EST locus, and *D. kepulauanana* (0181.7) on  $\alpha$ GPDH locus.

The triple bands of ODH pattern 1 (Fig. 1) indicate that a dimer protein form is present in the heterozygous individual in the polymorphic Okinawa *D. albomicans* strain (0163.01). 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 0.38, 0.49, and 0.13, respectively. The ODH allele of *D. nasuta* is indistinguishable from the F allele of *D. albomicans*, but *D. kepulauanana* has a different ODH allele (Fig. 1).

The polymorphic locus of  $\alpha$ GPDH also corresponds to a dimer protein enzyme. The

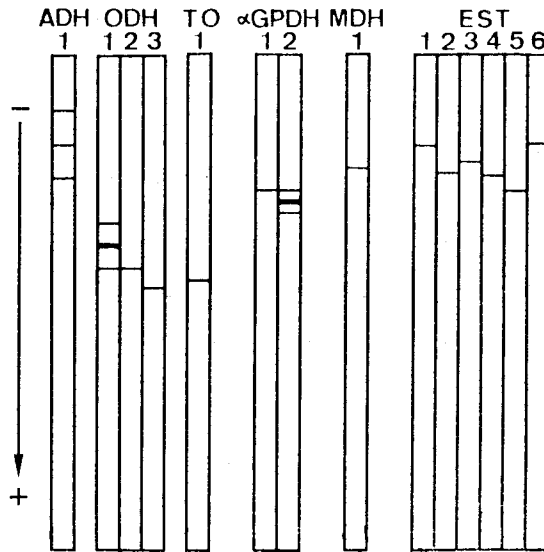


Fig. 1. Zymogram patterns observed in flies of the *D. nasuta* subgroup species. See Materials and Methods for detail. ADH, alcohol dehydrogenase; ODH, octanol dehydrogenase; TO, tetrazolium oxidase;  $\alpha$ GPDH,  $\alpha$ -glycerophosphate dehydrogenase; MDH, malate dehydrogenase; and EST, esterase.

frequencies of FF, FS and SS individuals are 0.42, 0.58, and 0 respectively in the *D. kepulauanana* strain (0181.7). *D. albomicans* and *D. nasuta* are both fixed at an allele which is indistinguishable from the S allele of *D. kepulauanana*.

The esterase variation shown in Fig. 1 is controlled by alleles at the EST-F locus (Kanapi and Wheeler, 1970). The Okinawa *D. albomicans* is polymorphic on EST locus for Fast and null alleles (F-: null=64:36). Although eight out of nine strains are fixed, the result shows that they are all polymorphic within species. *D. albomicans* has three alleles—F, S and null. *D. nasuta* and *D. kepulauanana* have their own F and S alleles.

In general, our data provide three implications. (1) these preliminary data support the previous reports that *D. albomicans* and *D. nasuta* are more similar to each other than to *D. kepulauanana* (Kitagawa, *et al.*, 1982); (2) The three polymorphic loci in isofemale strains are all showed high heterozygosity. Our previous finding that heterozygous

Table 2. Zymogram patterns of five strains of *D. nasuta* subgroup species

Strain number	ODH	$\alpha$ GPDH	EST
0073.2	2	1	1
0161.2	2	1	2
0163.1	1	1	2*
0181.8	2	1	3
0193.6	ND	ND	4
0193.7	ND	ND	4
0183.8	ND	ND	3
0181.7	3	2	5
0193.4	ND	ND	6

\* This strain contains a null allele and is polymorphic at this EST locus.

ND—none done

inversions, such as In(2L)B<sub>1</sub>D<sub>5</sub>, are preserved in isofemale strains at high frequencies too (Chang, *et al.*, 1986). These results indicate that heterosis could be the main mechanism for maintaining the polymorphism in isofemale strains. Therefore, it is obvious that the maintenance of polymorphism in isofemale strain might be different from that in natural population. (3) Genetic variation within single isofemale strain can not represent the whole natural population. However, it is possible to estimate the genetic variation in a natural population through the analysis of a large number of isofemale strains.

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## 三個果蠅近緣種 (*Drosophila nasuta* Complex) 之異構酶變異

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用膠體電泳法調查大果蠅種羣 (*Drosophila immigrans* species group) 的輝顏亞種羣 (*D. nasuta* species subgroup) 中三個近緣種的六種異構酶變異。比較的結果顯示種間及單雌品系間皆有變異。對於多態性的基因座，異結合型比率 (heterozygosity) 相當高。品系內並沒有低頻率的變異存在。這筆初步資料顯示在異構酶的系統看來，即使自然族羣為多態性，單雌品系有趨於單態性的傾向。因此個別的單雌品系不能代表自然族羣中異構酶的分佈情形。必須作進一步的研究，才能用異構酶的調查結果來推斷族羣結構。