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STUDIES ON MIGRATION PATHWAYS OF *DROSOPHILA ALBOMICANS* DUDA IN TAIWAN AS ELUCIDATED BY ESTERASE ISOZYME PATTERNS*

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ABSTRACT

C. T. Peng, Y. L. Hsu, and J. C. Su (1972) *Studies on Migration Pathways of* Drosophila Albomicans *Duda in Taiwan as Elucidated by Esterase Isozyme Patterns.* Bull. Inst. Zool., Academia **11**(2): 21-27. Polyacrylamide gel electrophoretic technique was used to evaluate esterase isozymes of *Drosophila albomicans* Duda obtained from six localities in Taiwan. Zymogram variations were observed in both interstrain and intrastrain comparisons. By analyzing the frequency of appearance of some esterase isozymes, subunits of the population and migration pathways of this species in Taiwan are discussed.

Analyses of the developmental progressions of isoenzymes have been proved of considerable value in the studies of tissue differentiation[5,6], the control of enzyme synthesis[1], ecology and evolutionary and phylogenic relationships of organisms[7].

Recent studies[2,3,4] on the esterases of a number of species groups of the genus *Drosophila* indicated a close agreement between the isozyme differences and taxonomic categories. Stone *et al.*[11,12] have investigated electrophoretic variation of several enzymes in *Drosophila* species of the *nasuta* subgroup from two South Pacific island—Samoa and Fiji.

Through painstaking efforts, Mr. F. J. Lin of this Institute established the laboratory cultures of several geographic races of *Drosophila albomicans* Duda. In this study, we are most interested in the problem of migrntion pathways of these drosophilid flies in Taiwan because of the very special geographic features of Taiwan (Fig. 5). A glance on the map of Taiwan, the migration of lowflying drosophilid flies may be limited to the narrow coastal corridors. The steep central mountain range will not permit the east-west cross migration. Results fully substanting this view are reported in this paper.

MATERIALS AND METHODS

Materials: The *Drosophila albomicans* flies were collected from six different localities as indicated in Table I and Fig. 5.

These were raised from isofemale lines. The flies were reared on banana-agar medium. The medium has the following constituents: water 850 ml, agar 16.8 gm, dry yeast 35 gm, banana

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TABLE I
Geographic sources of *Drosophila albomicans* used in this study

Localities	Stock number	Collector(s)	Date
1. Taroko	0055.1	J. I. Ting	I 16, 1970
2. I-Lan	0059.1	J. I. Ting	IV 1970
3. Nan-kang	0003.4	F. J. Lin	XI 28, 1966
4. Mei-shan	0088.12	J. I. Ting	VII 12, 1971
5. Ken-ting	0057.1	J. I. Ting	IV 1970
6. Chi-beng	0075.1	F. J. Lin & A. H. Wang	VII 9, 1971

fruits 550 gm, with propionic acid 7 ml added for mold prevention.

Electrophoresis: Adult male flies were squashed singly on a microslide and ground in about 30 μl of 0.1 M Tris-borate buffer at pH 8.9 containing 1.5 mM EDTA, 5% sucrose and a little bromophenol blue as the electrophoretic marker. The slurry without centrifugation was electrophoresed in polyacrylamide gel according to the method of Nerenberg[9]. About 10 μl of the homogenized slurry was layered into the gel pocket and the electrophoretic run was conducted at a potential difference of 250–300 v (25 v/cm) for 150 minutes. All the experiments were performed at 4°C. After electrophoresis, the gels were incubated in 0.5 M boric acid for 1.5–2 hours to lower the pH of the gel to approximately 6.5. The gel were then incubated in 100 ml of 0.1 M phosphate buffer pH 6.5 containing 25 mg α-naphthyl acetate in acetone-water 1:1 (v/v) and 50 mg fast red TR (both from Sigma Chemical Company).

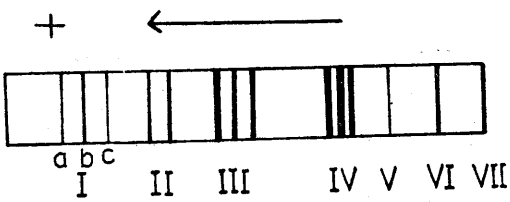

Fig. 1. Composite diagram of the relative positions of the 7 primary zones of esterase activity observed in strains of D. albomicans obtained from 6 localities. Right margin represents the sample slots; arrow indicates the direction of migration. a, b, and c are subbands of esterase I.

As described in a previous report[8], each of the seven esterse zones after electrophoresis was identified by their electrophoretic mobilities. Esterase bands were numbered Est I to Est VII in decreasing order of mobilities from the anode. Bands with only slight difference in mobility were designated as sub-bands (Fig. 1, Ia, Ib, and Ic).

RESULTS

Both inter- and intrastrain variations of esterase zymogram within each of six geographic strains of this species were observed.

Esterase I is present in zymograms of all strains. It consists of one or more bands of Est Ia, Est Ib and Est Ic. The zymograms are shown in Fig. 2. Est Ia is the band with a relative mobility of 88% (the mobility of the marker bromophenol blue is taken as 100%) and is present in the strains of Taroko and I-Lan, but not in other localities. Est Ib is the band with 83% relative mobility and is present in all strains. Est Ic moving slower than Est Ib is sometimes present in the strains of I-Lan, Nankang, Mei-shan, Ken-ting and Chi-beng.

Esterase II occures rarely in the strains of Mei-shan (3 out of 41) and Chi-beng (1 out of 30) and is absent in other localities.

Some of the variations within and between

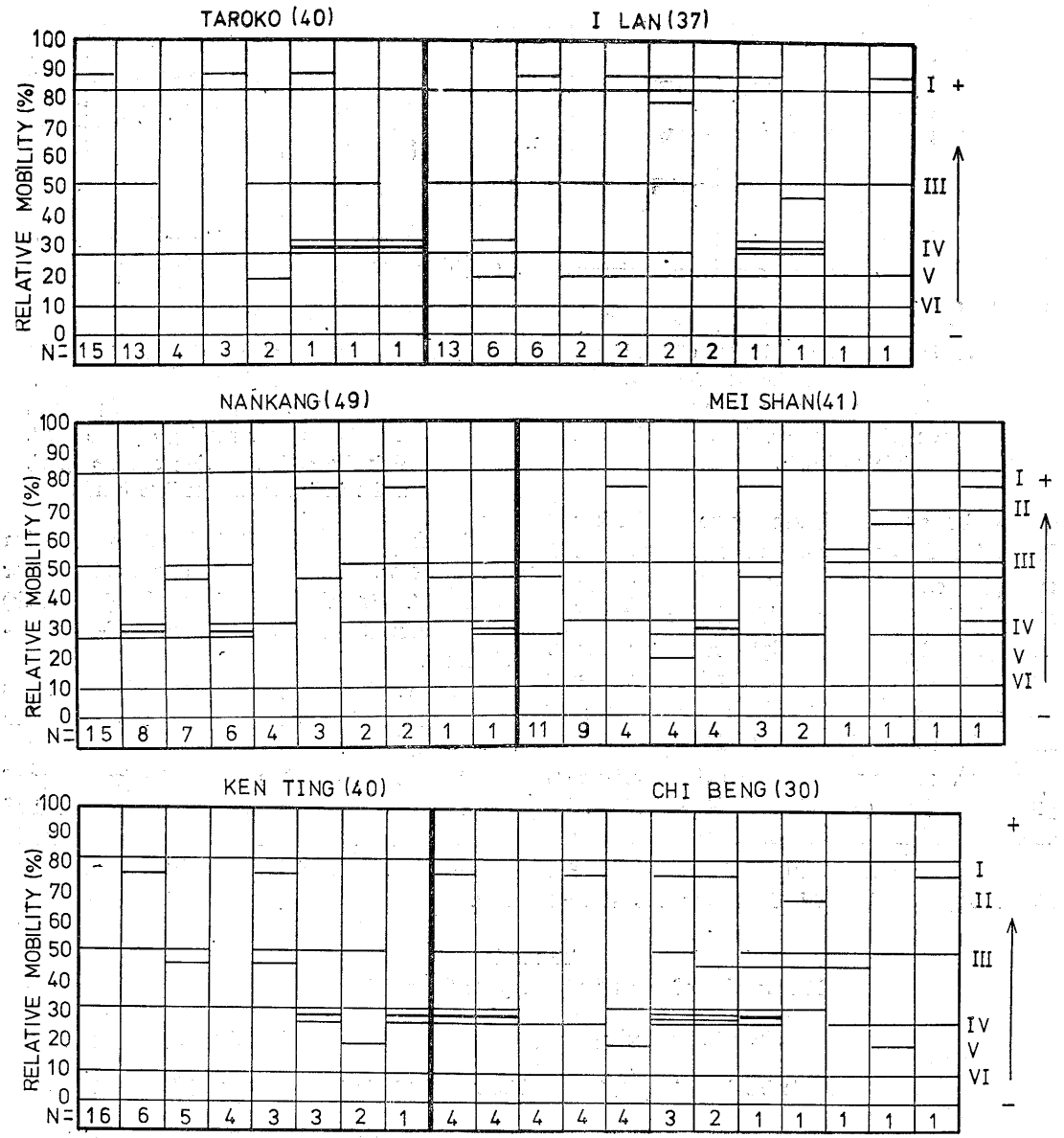

Fig. 2. Diagrams of esterase isozymes showing variations within and between strains. The numbers at the left correspond to the relative mobilities of the enzyme. N indicates the number of individuals showing the particular zymogram. The number in parenthesis at right of each locality is total samples for test.

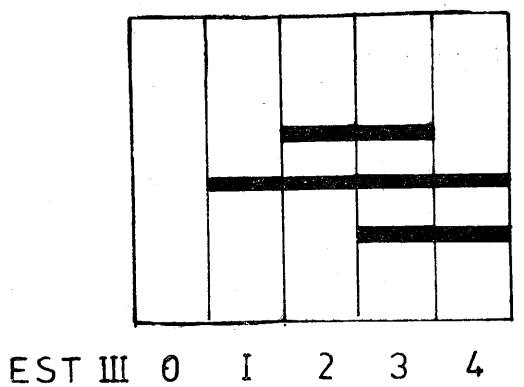

Fig. 3. Electrophoretic variations of Est III observed in the various strains. Number 0 indicates no band observed, 1 indicates single band and 2, 3, 4 indicate multiple subbands.

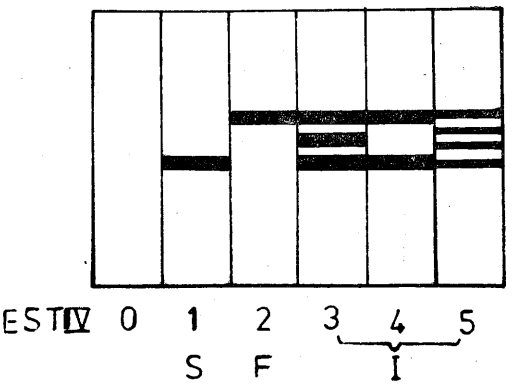

Fig. 4. Diagram showing variation of Est IV observed in various strains. The numbers indicate the phenotypes of patterns, S indicated slow moving esterase band, F indicates fast moving esterase band, and I indicates intermediate bands.

strains for estrase III are shown in Fig. 2 and Fig. 3 (Est III corresponds to bands with 50% relative mobility). Est III consisting up to 5 subbands is arbitrarily divided into 3 categories, namely, single band, multiple bands and none. Frequency of the multiple bands phenotype is higher in the strain of Mei-shan than that of strains from other localities (Table II).

Esterase IV is present as five visible variations plus a null form (Fig. 4). Variations of Est IV observed within and between strains are shown in Fig. 2 (relative mobility of ranging between 26 to 31%). Est IV^S designates the slowest moving from while Est IV^F the fastest moving one. Three kinds of bands are grouped together as Est IV^I. Est IV complex (consisting 4 subbands) was observed in the strain of Chi-beng exclusively.

The frequencies of occurence of phenotypes Est IV^F, Est IV^S and Est IV^I are shown in Table III.

In the strain of Taroko, the frequency of Est IV^S is the highest while that of Est IV^F extremely low. On the contrary, in the strain of Ken-ting, Est IV^F is the highest but Est IV^S low.

TABLE II

Frequencies of Est III phemotypes from different strains

Strains	No. of flies each phenotype			Frequencies of each phenotype		
	Single band	Multiple bands	None	Single band	Multiple bands	None
Taroko	32	0	0	1.000	0	0
I-Lan	34	1	2	0.919	0.027	0.054
Nan-kang	28	9	12	0.571	0.184	0.254
Mei-shan	22	17	2	0.537	0.415	0.488
Ken-ting	27	8	5	0.675	0.200	0.125
Chi-beng	19	3	8	0.633	0.100	0.267

TABLE III
Frequencies of Est IV phenotypes from different strains

Strains	No. of flies of each phenotype				Frequency of each phenotype			
	Est IV^F	Est IV^S	Est IV^I	None	Est IV^F	Est IV^S	Est IV^I	None
Taroko	0	37	3	0	0	0.925	0.075	0
I-Lan	0	25	8	4	0	0.676	0.216	0.108
Nan-kang	9	22	15	3	0.184	0.449	0.306	0.061
Mei-shan	13	18	9	1	0.317	0.439	0.220	0.024
Ken-ting	36	0	4	0	0.900	0	0.100	0
Chi-beng	5	11	14	0	0.166	0.367	0.470	0

TABLE IV
Analysis by Chi-square test of divergence between locality, based on the frequencies of occurence of all phenotypes of Est IV

Localities compared	Degree of freedom	x^2	p
Taroko vs. I-Lan	5	12.40	0.05-0.01*
Taroko vs. Chi-beng	5	26.19	<0.001*
Ken-ting vs. Mei-shan	5	34.80	<0.001*
Mei-shan vs. Nan-kang	5	13.23	0.05-0.01*
Ken-ting vs. Chi-beng	5	40.67	<0.001*
Nan-Kang vs. I-Lan	5	24.07	<0.001*

*: Significant difference

Esterase V has 18% relative mobility and is present in almost all individuals of the strain of I-Lan but occurs rarely in the strains of Taroko, Mei-shan, Ken-ting and Chi-beng.

Esterase VI zone has 10% relative mobility and occurs in zymograms of all strains.

Esterase VII represents the band remaining at origin slot and occurs in all strains. Whether it is an unsolubilized enzyme remains unknown.

Significance in frequencies difference of all phenotypes of Est IV between two neighborhoods were analyzed by means of ratio method of calculating x^2 in a $2 \times c$ table[10]. The results of these tests are preseted in Table IV.

DISCUSSION

This study has revealed intraspecies differences of zymograms of esterase isozymes among various strains of *D. albomicans* obtained from different localities. In spite of the presence of a high degree of polymorphism in esterase isozymes, the present investigation has yield meaningful information concering Est IV complex.

From the frequency of occurence of Est IV^F and Est IV^S, the subunits of population and migration pathways of *D. albomicans* in Taiwan could be elucidated from the following view points.

A. From the standpoint of Est IV

The frequency of the appearence of Est IV^S is the highest in the strain of Taroko. The Est IV^S frequency is lower in strains that are collected at places farther from Taroko in two

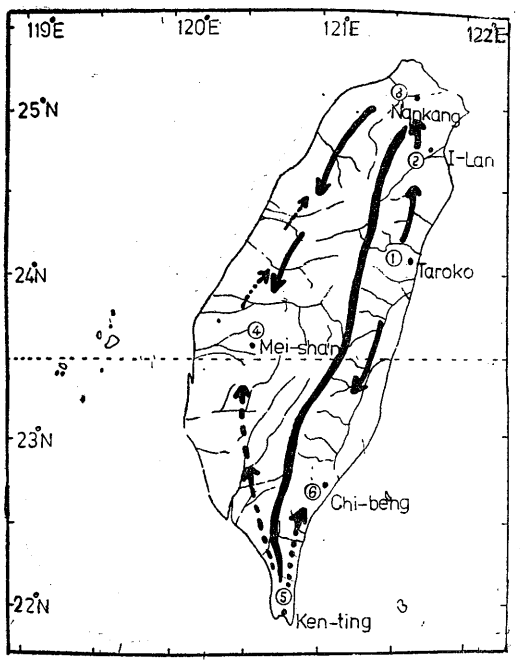

Fig. 5. The map of Taiwan showing geographic sources (•) of *D. albomicans* used in this study. Black line in center indicates the Central Mountain Range as a geographical barrier (altitude over 2,600 m), ➡ shows migration pathways of the population possessing Est IV^S ◂▪▪▸ shows migration pathways of the population possessing Est IV^F.

possible directions (Fig. 5). The frequency of Est IV^F is the highest in the strain of Ken-ting, and the frequency is lower in the strains that are obtained from places farther from Ken-ting in two possible directions (Fig. 5). High frequencies of Est IV^I are fonnd in the two possible crossing areas of the two subunits of population, namely Chi-beng and the west costal plain. The Est IV^I may be formed by the mixing of two branches of population possessing Est IV^F and Est IV^S, respectively.

B. From the standpoint of geographic situation

D. albomicans has never been collected at an altitude of 1,600 m or higher. The Central Mountain Range of Taiwan has so many higher mountains (over 2,600 m) streching from north to south that it is most probable that no *D. albomicans* can fly across the island. This leaves the costal zone the only possible route of migration (Fig. 5).

Based on the above considerations, we may conclude that *D. albomicans* in Taiwan has two subunits of population at the vicinities of Taroko and Ken-ting, respectively. They then migrated according to the pathways depicted in Fig. 5.

From the high frequencies of certain phenotypes such as Est IV^S and Est IV^F in the strains of Taroko and Ken-ting respectively, and the occurence of these two phenotypes in the strains of Chi-beng and west costal plain, it seems appropriate to say that the crossing areas of the two subunits of population would be on the west coastal plain and east coastal corrider of Taiwan respectively. At the same time, we have also tested the significance of the difference in frequencies of all phenotypes of Est IV between two neighbor localities. The results showed significant difference between them.

From the experience obtained in this study, we may conclude that the analysis of the frequency of some esterase iszymes is an applicable tool for detections the subunits of population centers and the migration pathways of some *Drosophila* species.

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根據異構酯水解酵素電泳圖解釋臺灣紅果蠅之移居路線

彭清次　徐亞莉　蘇仲卿

用多醯胺膠電泳法，將臺灣六地區（大魯閣、宜蘭、南港、梅山、墾丁、知本）採集之紅果蠅做異構酯水解酵素分析。發現各地之果蠅雖係同種，但因地區不同，其所含某種異構酯水解酵素顯然有所不同；而各地區內之果蠅，個體之間亦有少數差異。

藉着各地區某些異構酯水解酵素出現頻率之不同，可以解釋該果蠅在臺灣有兩小族羣 (Subunits of Population) 一在墾丁，另一在大魯閣，它們之移居路線分別由上述二地沿着海岸平原地帶向兩方向移居，而在臺灣西部平原及東部之知本附近滙合。