ON THE POLYMORPHIC *ESTERASE-F* LOCUS IN *DROSOPHILA ALBOMICANS*[1]

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Hwei-Yu Chang and Fei-Jann Lin (1990) On the polymorphic *Esterase-F* locus in *Drosophila albomicans. Bull. Inst. Zool., Academia Sinica* 29(2): 81-88. The EST-F esterase in *D. albomicans* is a dimer enzyme. Electromorphs have shown that the locus of this enzyme contains 5 alleles, F^a, F^b, F^c, F^d and a null form, F^o. High polymorphism was conspicuous not only in isofemale stocks but also in natural populations in Taiwan. Since the allelic frequencies of the *Est-F* locus did not show differentiation among natural populations, a balancing selection force may exist in natural populations in Taiwan to maintain the allelic frequencies. Since heterozygous males have higher opportunity in mating success, heterosis expressed at male mating competition may play an important role on maintaining polymorphic *Est-F* in natural populations. Alternatively, heterosis expressed at the survival rate is the main mechanism of maintaining the polymorphism in laboratory isofemale stocks.

Key words: Allozyme variation, *Drosophila*, Fitness, Polymorphism.

Polymorphism usually shows a strong correlation with fitness. Chromosomal inversions and mitochondrial DNA restriction patterns are highly polymorphic in natural populations of *Drosophila albomicans* (Lin and Chang, 1986; Chang *et al.,* 1988). Certain inversion patterns and isozyme alleles have been shown to have higher fitness value under specific environmental conditions (Santos *et al.,* 1989; Serradilla and Ayala, 1983). Kanapi and Wheeler (1970) have reported that *Esterase-F* (*Est-F*) locus in a strain of *Drosophila albomicans* collected from Wulai, Taiwan contains three alleles, indicating that this locus is polymorphic. The purpose of this communication aims to investigate the allelic frequencies of *Est-F* locus in natural populations of *D. albomicans* all over this island in order to provide a better understanding of the relationship between polymorphism and fitness.

MATERIALS AND METHODS

Flies

One hundred and fourteen isofemale

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stocks of *D. albomicans* from 16 different geographical locations (Table 3) have been established in the laboratory for more than ten years. Flies recently collected from Fenglin, Jiuntou (宜蘭、圳頭), Shitou and Wulai were sacrificed to check their *Est-F* genotypes. The genotypes of the mates of some wild-caught females from Wulai were also determined by the examination of their F_1 offepring. Both laboratory maintained and recently collected isofemale stocks were reared by standard corn meal medium under the condition of 22°C and 75% relative humidity.

Electrophoretic analysis of esterase

Each individual fly was homogenized in 20 μl distilled water in an Eppendorf tube. After centrifugation for 5 min, 10 μl supernatant was mixed with 2 μl 0.25% Bromophenol Blue - 50% glycerol aqueous solution, and loaded in a well of a polyacrylamide slab gel with 5% stacking gel (pH 6.8) and 7.5% separating gel (pH 8.8). Gel was run with Tris-glycine buffer (pH 8.3) at 4°C until the dye front reaching the end of the gel. The EST-F (according to Kanapi and Wheeler, 1970) patterns were then visulized by the specific staining method described by Ayala *et al.* (1972).

The frequencies of F^a and F^d were calculated from genotypic frequencies. As for the old 45 Wulai isofemale stocks, each stock represents one genotype (i. e. monomorphic and polymorphic stocks were treated as homozygotes and heterozygotes, respectively). Part of the frequencies of F^b and F^c is estimated by other determinable genotypes.

Analysis of allelic frequencies

Flies for fitness study were collected from the same valley at Wulai as mentioned in a previous study (Chang *et al.*, 1987). Fifty seven isofemale stocks were established for the fitness study. From their F_1 generation, 515 single pairs

were cultured separately. The electrophoretic patterns of *Est-F* locus of these F_1 were examined when their F_2 generations were established. Fifty six out of 515 single pairs were found to be heterozygous pairs. From the offsprings of these 56 heterozygous pair, 1,189 F_2 single pairs were established. Again, the EST-F electrophoretic patterns of these F_2 single pairs were examined when F_3 generations were established. The frequencies of each *Est-F* allele of F_1, F_2 and F_3 generations were then determined.

RESULTS

As shown in Fig. 1, *Est-F* was present as 5 alleles including 4 visible variants and a null form. They are named F^a, F^b, F^c, F^d and F^o. The dimer enzyme a_2 has the fastest locomotion on the gel, and d_2 the slowest. F^o indicates the null form. The band of the homodimer d_2 is hardly visible in the heterozygous forms of F^b/F^d, F^c/F^d and F^a/F^d. Therefore double bands, instead of triple bands, are demonstrated in lane 4, lane 6 and lane 9 from the left of Fig. 1. This result was confirmed by the analysis of zymogram patterns in natural populations as well as in isofemale stocks and by crosses of homozygous forms.
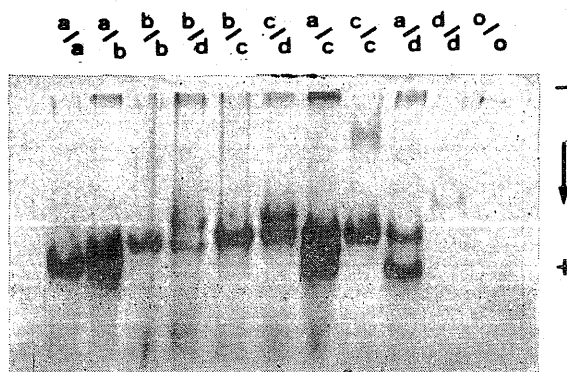

Fig. 1. Electrophoretic variants of EST-F including all five homozygous types and six heterozygous types of the four visible different alleles.

Table 1
The survey of EST-F patterns of flies from four different
geographic localities

Localities	Sample size	Sex	a/a	a/b	a/c	a/d	U[1]	b/b	b/c	c/c	b/d	c/d	d/d	a/o
Fenglin	172	F	52	25	43	10	7	5	2	13	4	8	3	0
	171	M	46	21	41	15	13	8	5	9	5	5	3	0
Jiuntou	124	F	35	17	30	9	0	12	4	15	2	0	0	0
	175	M	55	22	50	9	0	14	1	17	0	6	1	0
Shitou	10	F	3	1	2	1	3	0	0	0	0	0	0	0
	12	M	7	4	1	0	0	0	0	0	0	0	0	0
Wulai	99	F	32	17	26	7	1	3	3	5	1	2	2	0
	107	M	34	16	31	5	15	1	0	1	3	0	1	0
	57	M[2]	5	14	23	2	—	0	6	1	4	2	0	0
	45	iso-stocks[3]	8	8	14	1	—	3	2	6	0	2	0	1

1. U indicates the indistinguishable b/b, b/c or c/c.
2. The pattern of each male in this category was determined by the mate and the F_1 offspring. And this category is not included in the category of the 107 males from Wulai.
3. Each isofemale stock is treated as one genotype: monomorphic one as a homozygote and polymorphic one as a heterozygote.

The genotypes of *D. albomicans* collected recently from four localities in Taiwan (Fenglin, Jiuntou, Shitou and Wulai) are summarized in Table 1. According to χ^2 analysis, there is no significant difference in genotypic frequencies between males and females. The data were, therefore, pooled together to calculate the allelic frequencies for the four localities. As shown in Table 2, there was no apparent difference in the frequency of each allele in these four localities. F^a is the most common allele of the *Est-F* locus. EST-F patterns of flies from 114 laboratory maintained old iso-female stocks are shown in Table 3. Only the Wulai stocks show all 5 alleles. Three alleles (F^a, F^c and F^o) at most are observed in iso-stocks collected from Thailand, Okinawa and localities other than Wulai in Taiwan. Although individual iso-stock could not represent the variation in the population, the allelic frequencies of the *Est-F* gene in 45 Wulai iso-stocks were similar to those obtained from natural populations (Table 2).

The heterozygosity of natural populations calculated from genotypic frequencies was statistically lower than the expected one (Table 4). However, the

Table 2
Allelic frequencies of the *Est-F* locus

Localities	Sample size	f(F^a)	f(F^b)	f(F^c)	f(F^d)	f(F^o)
Fenglin	343	.51	.14	.26	.09	0
Jiuntou	299	.53	.16	.26	.05	0
Shitou	22	.66	.20	.12	.02	0
Wulai	206	.57	.15	.22	.06	0
males*	57	.43	.21	.29	.07	0
iso-stocks	45	.44	.18	.33	.03	.01

* The mates of the 57 isofemales.

Table 3
The survey of *Est-F* variation of old isofemale stocks

Locality	No. of stocks	No. of polymorphic stocks	No. of alleles	Types of alleles
Thailand	2	1	3	F^a, F^c, F^o
Okinawa	13	5	3	F^a, F^c, F^o
Taiwan				
Baileng	3	2	2	F^a, F^c
Dawu	5	3	2	F^a, F^c
Fenglin	5	1	3	F^a, F^c, F^o
Guguan	2	2	2	F^a, F^c
Kending	5	2	2	F^a, F^c
Lanyu	2	0	2	F^a, F^c
Puli	6	3	2	F^a, F^c
Rueifang	4	3	2	F^a, F^c
Shanhutan	5	3	3	F^a, F^c, F^o
Shitou	5	4	3	F^a, F^c, F^o
Shrtoushan	4	3	2	F^a, F^c
Tailuge	3	1	2	F^a, F^c
Wulai	45	28	5	F^a, F^b, F^c, F^d, F^o
Yuenshuei	5	2	2	F^a, F^c
Total	114	63		

Table 4
Observed and expected heterozygosity of the *Est-F* locus

Categories	Sample size	H_{obs}	H_{exp}
Natural populations	206	.53	.63
Successful males	57	.89	.68
Isofemale stocks	45	.62	.66

heterozygosity of the mates of 57 iso-females is significantly higher than the expected value, i.e. 0.89 vs. 0.68. *Est-F* genotypes of these 57 males were determined by examining their F_1 offspring (data not shown).

Among 99 old Taiwan stocks, 57 of them are still polymorphic. Only one is polymorphic for F^a-F^c-F^o three alleles, are the other 56 stocks have two-allele polymorphism. When 3 stocks containing null allele are excluded, 75% (40/53) of polymorphic stocks have heterozygosity values higher than those estimated by Hardy-Weinberg proportion (data not
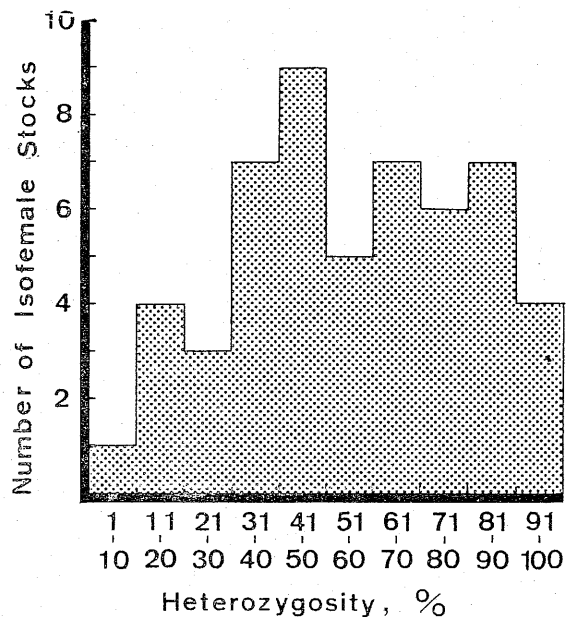

Fig. 2. The distribution of 53 polymorphic isofemale stocks on the heterozygosity of the *Est-F* locus.

shown). The frequencies with heterozygosity higher than the expected in stocks with alleles F^a-F^c, F^a-F^b, F^b-F^c,

Table 5
The abundance of the 28 observed mating types

No. of pairs	Mating types (F×M)			Frequency
6	$F^a/F^a \times F^a/F^c$;	$F^a/F^c \times F^a/F^c$;	$F^a/F^b \times F^a/F^c$	.11
5	$F^a/F^a \times F^a/F^b$			.03
4	$F^a/F^c \times F^a/F^b$			.03
3	$F^a/F^b \times F^a/F^b$;	$F^a/F^c \times F^b/F^c$		.07
2	$F^a/F^b \times F^a/F^a$;	$F^b/F^c \times F^a/F^c$;	$F^a/F^a \times F^b/F^d$	.11
1	$F^a/F^c \times F^a/F^a$;	$F^a/F^d \times F^a/F^a$;	$F^a/F^a \times F^b/F^c$;	.64
	$F^a/F^a \times F^c/F^d$;	$F^b/F^d \times F^a/F^a$;	$F^c/F^c \times F^c/F^d$;	
	$F^b/F^b \times F^a/F^b$;	$F^b/F^b \times F^b/F^d$;	$F^d/F^d \times F^b/F^c$;	
	$F^d/F^d \times F^a/F^d$;	$F^a/F^b \times F^b/F^c$;	$F^a/F^c \times F^a/F^d$;	
	$F^a/F^d \times F^a/F^c$;	$F^c/F^d \times F^a/F^c$;	$F^c/F^d \times F^a/F^b$;	
	$F^a/F^b \times F^b/F^d$;	$F^a/F^d \times F^a/F^b$;	$F^a/F^a \times F^c/F^c$	

F^a-F^d or F^c-F^d are 73% (30/41), 75% (6/8), 100% (2/2), 100% (1/1) and 100% (1/1), respectively. These results suggest that the high heterozygosity is in common to all allelic pairs. Furthermore, 29 out of these 53 polymorphic stocks have heterozygosity values even higher than 0.5 (Fig. 2).

Among 57 isofemale stocks newly established for further study of fitness of *Est-F* alleles, 28 mating types were identified (Table 5). All of these isofemale stocks were originated with more than two alleles (29 with 2 alleles, 27 with 3 alleles, and 1 with 4 alleles). The abundance of different types of dihybrid crosses found in parental, F_1 and F_2

Table 6
Established heterozygous parental,
F_1 and F_2 single pairs

Types of heterozygous pair	Number of dihybrid pairs in		
	P	F_1	F_2
$F^a/F^c \times F^a/F^c$	6	22	22
$F^a/F^b \times F^a/F^b$	3	20	18
$F^a/F^d \times F^a/F^d$		7	19
$F^c/F^d \times F^c/F^d$		3	
$F^b/F^d \times F^b/F^d$		2	
$F^b/F^c \times F^b/F^c$		2	
Total	9	56	59

generations was shown in Table 6. EST-F patterns in the F_1, F_2 and F_3 generations from these dihybrid crosses were sampled and surveyed. The genotypic frequencies in the offspring from those parental, F_1 and F_2 pairs are summarized in Table 7. Heterosis was obviously demonstrated in the second and the third generations from these dihybrid crosses.

DISCUSSION

From the survey of 870 flies collected from natural populations and 114 laboratory maintained isofemale stocks, 5 alleles of *Est-F* locus were identified in *D. albomicans*. *Est-F* locus shows high polymorphism in natural populations and isofemale stocks. To investigate the mechanisms for the maintenance of polymorphism is fundamentally important to understand the evolution of living organisms.

That both natural populations and the F_1 of dihybrid crosses sampled directly from natural populations show lower heterozygosity than expected (Tables 4 and 7) would suggest that the *Est-F* locus may be under the control of underdominance or directional selection on survival. Since this locus shows high polymorphism in natural populations, the

Table 7

The genotypic frequencies in the offspring from dihybrid crosses
in parental, F_1 and F_2 pairs in the iso-stock

Mating type	Offspring genotype	Frequencies in generation		
		1	2	3
$F^a/F^b \times F^a/F^b$	F^a/F^a	.28	.24	.26
	F^a/F^b	.37	.62	.70
	F^b/F^b	.35	.14	.04
		($n=178$)	($n=807$)	($n=768$)
$F^a/F^c \times F^a/F^c$	F^a/F^a	.36	.20	.26
	F^a/F^c	.43	.59	.55
	F^c/F^c	.21	.21	.19
		($n=191$)	($n=1,179$)	($n=1,157$)
$F^a/F^d \times F^a/F^d$	F^a/F^a		.21	.28
	F^a/F^d		.67	.56
	F^d/F^d		.12	.16
			($n=134$)	($n=953$)
$F^b/F^d \times F^b/F^d$	F^b/F^b		.09	
	F^b/F^d		.91	
	F^d/F^d		0	
			($n=34$)	
$F^c/F^d \times F^c/F^d$	F^c/F^c		.39	
	F^c/F^d		.60	
	F^d/F^d		.01	
			($n=170$)	

maintenance of polymorphism is possibly due to certain forces of balancing selection. Whatever the mechanism is, it cannot be simply explained by heterosis model acting at survival rate.

Alternatively, the maintenance of high polymorphism of *Est-F* locus in natural populations could be due to the heterosis expressed at male mating competition, because the heterozygosity of the 57 males, which were the successful mates of the 57 isofemales from Wulai, was significantly higher than the expected value. This result suggests that the heterozygous males have higher opportunity to compete for females.

However, heterosis at survival rate may be the main mechanism for the maintenance of polymorphism at this locus in isofemale stocks, because 75% of the polymorphic stocks had the observed

heterozygosity higher than the expected one. Our previous study (Chang *et al.*, 1988) also has suggested that the major mechanism of maintaining inversion polymorphism within isofemale stocks is heterosis too.

Although heterosis was profoundly demonstrated in all samples of the second and the third generations within isofemale stocks from dihybrid crosses, it is still unclear whether the overdominance in the offspring of dihybrid crosses is due to heterozygous advantage of this particular *Est-F* locus influenced by the genetic background within isofemale stock or other closely linked genes. Since *D. albomicans* at Wulai is a large outbreeding population, it may contain a lot of recessive deleterious alleles in the gene pool. Thus, the frequencies of the homozygous *Est-F* genotypes may decrease in

the first few generations of dihybrid crosses because of inbreeding of the flies.

Due to the high polymorphism in natural populations, genetic variation within single isofemale strain is not substantial to represent the natural population. However, our result showing that allelic frequencies of *Est-F* locus estimated from 45 Wulai isofemale stocks are similar to those of natural populations indicates that a large number of isofemale stocks may overcome this problem (Lin and Chang, 1987). This also shows that the most common allele, F^a, does not have higher fitness to get better chance to be preserved in isofemale stocks. The change from the average 2.5 alleles per stock at beginning to the average 1.6 alleles per stock shows that genetic drift is conspicuous in isofemale sotcks. But it still is possible to preserve the allelic frequencies in a collection of isofemale stocks.

There are several other interesting findings in this study. The flies freshly collected from the natural populations of four different localities contain four visible *Est-F* variants, but only the Wulai isofemale stocks contain all of them. This result may be due to the sample size and the way of establishing isofemale stocks. There are 45 isofemale stocks from Wulai but only 6 stocks at most from other localities. Furthermore, 39 of them were sampled from 308 isofemale stocks established with special care during 1977-1978, *i.e.* almost all the offspring were collected during the first few generations. According to the founder effect, Carson (1983) has suggested that the early generations are a crucial stage for maintaining genetic information successfully. The six routinely established stocks from Wulai contain the same two alleles as those from other localities.

Why are the alleles F^b and F^d more frequently lost as compared to F^a and F^c? Our results seem to indicate that different *Est-F* alleles have different levels of fitness, particularly under laboratory cultivation during the first few generations of establishing isofemale stocks. The flies with allele F^b or F^d seem to have the least fitness value. However, these two alleles are apparantly not deleterious since they are present in natural populations as well as in the 39 Wulai isofemale stocks. Whether some detrimental effects associated with these alleles in the genetic background of the natural population are remained for further investigation. With special care during the crucial period, these alleles may be maintained in the isofemale stocks by breaking up the associated detrimental genes through recombination. Thus, the establishment of isofemale stocks without special care will result in losing of a portion of genetic information carrying in natural populations.

The null allele is more frequently observed in iso-stocks collected from Thailand (1 out of 2) and Okinawa (5 out of 13). Among the 99 Taiwan stocks, there were only one monomorphic null stock and four polymorphic null stocks (including one F^c-F^o, two F^a-F^o and one F^a-F^c-F^o). These results indicate that the null allele may have better fitness in Thailand and Okinawa than in Taiwan. However, we did not find the null allele in the samples directly taken from natural populations in Taiwan. It is possible that the heterozygous null form cannot be distinguished from other homozygous forms, and that the frequency of null homozygotes is too low to be sampled.

According to χ^2 analysis, there is no significant difference in allelic frequencies among the flies collected from four localities in Taiwan. These results are consistent with our previous finding that

the frequency distribution of major restriction patterns of mitochondrial DNA is quite similar among different populations of *D. albomicans* (Chang *et al.*, 1988). Since inversion data showed that there exists certain degree of isolation among populations, allelic frequencies of *Est-F* as well as major morphs of mtDNA seem to be maintained by balancing natural selection in stead of high migration rate.

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紅果蠅 (*Drosophila albomicans*) 的多態性酯酶
Est-F 基因座

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紅果蠅的酯酶 EST-F 是一具兩個亞單位的蛋白質。電泳顯示該酵素的基因座 *Est-F* 包括五個對偶因子 (alleles)。不論是單雌品系或是臺灣的自然族羣均表現出明顯的多態性。由於各因子頻率在自然族羣間並無顯著的差異，加之以往由染色體逆位的調查發現族羣間有某些程度的隔離，因此推測有平衡性選汰壓在維持因子的頻率。在自然族羣當中異結合型的 (heterozygous) 雄蟲有較多的機會獲得配偶，所以表現在雄性競爭配偶上的雜種優勢 (heterosis) 似乎是維持多態性的重要機制 (mechanism)。而表現在存活率上的雜種優勢却是單雌品系維持多態性的主要機制。