Zoological Studies

The Interaction between Chromosomal Inversion and Recessive Lethals in *Drosophila albomicans*

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Hwei-yu Chang and Fei-Jann Lin (1995) The interaction between chromosomal inversion and recessive lethals in *Drosophila albomicans*. *Zoological Studies* **34**(1): 47-54. In order to reveal the mechanism for the high heterozygosity of an inversion on the left arm of the second chromosome, $In(2L)B_1D_5$, several pure lines were established of *Drosophila albomicans* for an esterase dimer locus (*Est-F*). This locus was shown to be either inside the inversion region, or outside, but closely linked with it. By a chromosome extraction procedure, we have found that the Wulai *D. albomicans* population exhibits high heterozygosity (0.54) of the $In(2L)B_1D_5$ and that it also has a high frequency of recessive lethals (0.42), but low allelism. These genetic marker strains not only made the chromosome extraction possible, but were used to reveal that the Wulai population is at linkage equilibrium, whereas the Chitou population is at linkage disequilibrium. A possible mechanism, accumulation of recessive lethals in the presence of a chromosomal inversion in a seasonally fluctuating population, is proposed to explain the phenomena of high inversion heterozygosity, high lethality, and low allelism in the Wulai population.

Key words: Chromosomal inversion, *Drosophila albomicans*, Esterase, Lethality, Population genetics.

The fly *Drosophila albomicans*, one of the most abundant species in Taiwan, has high genetic variation, indicated by inversion polymorphism (Lin and Chang 1986, Chang et al. 1987). We previously proposed that the extraordinarily high heterozygosity (i.e. an average of 66.1%) of $In(2L)B_1D_5$ in winter at Wulai is maintained by some overdominant selective force (Chang et al. 1988). Our recent studies also show heterosis by esterase polymorphism in this species (Chang and Lin 1990). These two indicators, $In(2L)B_1D_5$ and the *Est-F* locus, being closely linked would indicate that the heterosis is possibly caused by a genetic element located near or within the $(2L)B_1D_5$ region.

There are five alleles at the esterase dimer locus (*Est-F*) in the natural populations (Chang and Lin 1990) of this species. Without any suitable morphological markers, esterases seem to be the only suitable tool for identification of the genetic element involved in the heterosis.

This study confirmed that the two genetic markers, *Est-F* and $In(2L)B_1D_5$, are closely linked. An isofemale stock #0131.5 which originated from Wulai in March 1975 was found to be a balancer stock with F^a and a recessive lethal allele at one locus on the standard $(2L)B_1D_5$ chromosome, as well as F^c and a recessive lethal allele at another locus on the inverted chromosome. Linkage disequilibrium and lethality were investigated by use of a chromosome extraction procedure similar to the "Curly-Plum" technique (Dubinin 1946, Wallace 1956), except that the genetic markers we used are enzymatic instead of morphological. A hypothesis integrating the interactions among chromosomal inversion frequency, recessive lethals and population structure was proposed to explain the heterosis. The high lethality revealed in the Wulai population supports this hypothesis.

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MATERIALS AND METHODS

Flies

Drosophila albomicans flies were collected from natural populations using banana bait sprayed with beer. Each male was crossed to a virgin female from a laboratory-maintained stock. The esterase genotype of these flies was determined by electrophoresis after a sufficient number of progeny had been produced.

There are five Est-F alleles including four visible variants and a null form (Chang and Lin 1990), and pure lines for the five alleles have been established. Their chromosome arrangements on the left arm of the second chromosome were determined according to the standard map of the giant chromosomes of D. albomicans (Lin et al. 1974). Therefore, the F^aF^a, F^cF^c, and F^dF^d pure lines were arbitrarily called "normal" (NN) configurations, whereas the F^bF^b and the F^oF^o pure lines were called "inverted" (II).

The balancer stock, #0131.5, with 100% esterase heterozygosity (i.e., F^aF^c) and 100% inversion heterozygosity (i.e., $In(2L)B_1D_5$) used in this study is an isofemale stock which originated from Wulai in March 1975.

All stocks were reared with a standard corn meal medium and maintained in an environment of 22°C and 75% relative humidity throughout the experiment. Newly emerged flies were sexed within eight hours and were kept in separate vials for three days before crossing.

Esterase isozyme electrophoresis and chromosome inversion analysis

The esterase isozyme patterns were analyzed according to the electrophoretic method described in Chang and Lin (1990). The salivary gland chromosome preparation was performed as described in Lin and Chang (1986). In order to simultaneously study the chromosomal arrangement and esterase polymorphism, we dissected salivary glands from a third instar female larva in 20 μl of autoclaved TE buffer (10 mM Tris and 1 mM EDTA) for chromosome preparation; and the remaining parts of this larva together with the buffer were transferred into and homogenized in an Eppendorf tube for esterase electrophoresis.

The determination of hatchability

Eggs were collected within five hours of being laid and placed on autoclaved sheets of moist, black paper in petri dishes. Hatchability of the eggs was determined forty hours later.

The determination of the genotype of captured flies

Male flies of the first collection from Wulai (November 5, 1990) were mated with F^dF^d(NN) virgin females. With this method, no F^o alleles can be detected. Thereafter, males collected from Wulai (April 16, May 17, May 27, and July 13, 1991) and Chitou (June 12, 1991) were mated with F^oF^o(II) virgin females. Chromosomal analysis and esterase electrophoresis of these F_1 larvae were simultaneously performed to reveal the exact genotypes of the two chromosomes. The esterase data of the F_1 larvae were double checked against the paternal record.

Chromosome extraction

Male flies collected from Wulai (November 6, 1991) were crossed with F^dF^d(NN) females. Synchronized analysis of the esterase patterns and chromosomal arrangements of these F_1 larvae should reveal the genotypes of the two chromosomes. Using EST-F isozymes as markers, if the male is a heterozygote with alleles F^a, F^b or F^c, both chromosomes can be extracted (Program I). But if the male is a homozygote or if it contains an F^d or an F^o, only one chromosome can be extracted (Program II).

Program I: Separate single pairs (ca. 30 – 40 pairs/captured male) were made from virgin F_1 flies. After F_2 larvae appeared in the medium, the esterase patterns of these F_1 pairs of flies were checked, and only the heterozygote pairs with the same wild chromosome were saved; the others were discarded. These F_2 flies from the two kinds of heterozygous pairs were checked for their esterase patterns. If more than sixteen flies were checked and no homozygotes with the wild chromosome marker were observed, then this wild chromosome was said to contain a recessive lethal.

Program II: One F_1 male from each wild-caught male was crossed to an F^dF^d(NN) virgin female, and separate single pairs were made from the virgin F_2 flies just as in the procedure performed for the F_1 generation in Program I. The remaining procedures followed the same protocol as in as Program I except there was one generation delay, and only one kind of heterozygous pair was saved for each captured male.

The procedure is summarized in a flow chart (Fig. 1).

Placing lethal alleles into balancer stocks

Flies with chromosomes judged to contain a recessive lethal were crossed with flies from #0131.5, and the lethal alleles were kept in a new balancer stock with one chromosome with the opposite arrangement at the $(2L)B_1D_5$ from the #0131.5. About thirty to forty pairs of F_1 were made. After the F_2 larvae appeared in the medium, the esterase genotypes of those F_1 flies were checked, and only the desired heterozygous pairs were chosen; the others were discarded. The procedure is summarized in a flow chart (Fig. 2). Allelism of the lethal alleles was investigated by

complementation testing of these newly established balancer stocks.

RESULTS

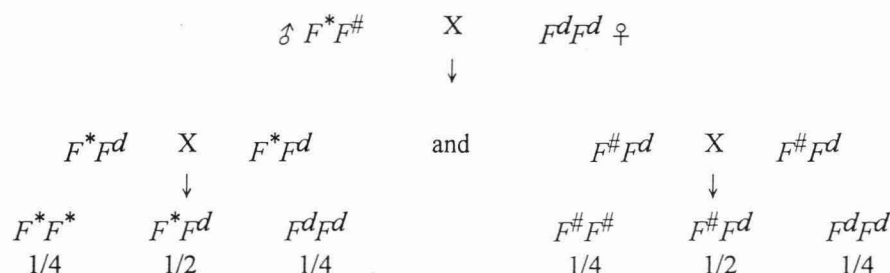### The linkage relationship between *Est-F* and *In(2L)B₁D₅*

The first step in locating the *Est-F* locus was to determine whether it is sex-linked. The sex chromosomes of *Drosophila albomicans* are "neo-X" and "neo-Y" formed by the fusion of an X and an ancestral 3rd chromosome, and another fusion of a Y and a 3rd chromosome during speciation. The F_1 males from a cross between F^cF^c females and F^dF^d males were test crossed. Since both

$\male$ obtained from a natural population $F^*F^\#$

$$\text{if } * \neq \# \quad \text{and} \quad * \text{ or } \# \neq d \text{ or } null \quad \rightarrow \text{Program I}$$
$$\text{if } * = \# \quad \text{or} \quad * \text{ or } \# = d \text{ or } null \quad \rightarrow \text{Program II}$$

Program I:

$$\male\ F^*F^\# \quad \times \quad F^dF^d\ \female$$
$$\downarrow$$

F^*F^d	$\times$	F^*F^d	and	$F^\#F^d$	$\times$	$F^\#F^d$

$$\downarrow \qquad\qquad\qquad\qquad\qquad\qquad\qquad \downarrow$$

F^*F^*	F^*F^d	F^dF^d		$F^\#F^\#$	$F^\#F^d$	F^dF^d
1/4	1/2	1/4		1/4	1/2	1/4

Program II:

$$\male\ F^*F^\# \quad \times \quad F^dF^d\ \female$$
$$\downarrow$$
$$\male\ F^*F^d \quad \times \quad F^dF^d\ \female$$
$$\downarrow$$
$$F^*F^d \quad \times \quad F^*F^d$$
$$\downarrow$$

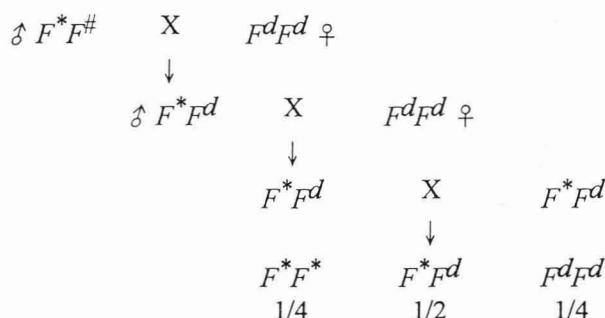| F^*F^*	F^*F^d	F^dF^d
1/4	1/2	1/4

Fig. 1. Males obtained from a natural population were crossed with F^dF^d females; the experiment should proceed into Program I or Program II according to the esterase genotype of these males.

A lethal allele is located on a *(2L)B₁D₅* arrangement A with a *Est-F* marker *

if A = N

$$F^*F^d \qquad X \qquad F^aF^c$$
$$\downarrow$$

choose only: F^*F^c X F^*F^c

discard other F_1 pairs

if A = I

$$F^*F^d \qquad X \qquad F^aF^c$$
$$\downarrow$$

choose only: F^*F^a X F^*F^a

discard other F_1 pairs

Fig. 2. Establishment of new balancer stocks to maintain recessive lethals extracted from the natural population.

males and females from the test cross of the F^cF^d males with the F^dF^d females have both F^cF^d and F^dF^d genotypes, the *Est-F* locus is not sex linked. This result is reproducible by using other combinations of pure lines, and it confirmed that the *Est-F* locus is neither located on the XY portion nor on the homologous arm of the sex chromosome.

By using dihybrid crosses between the pure lines of *Est-F* and $(2L)B_1D_5$ (e.g., F^aF^a(NN) and F^bF^b(II)) and then test crossing the F_1 females to the null(II) males, the linkage relationship between them was revealed. The synchronized analysis of 125 F_2 larvae from the test cross showed no recombinants. Therefore, the recombination rate was lower than 0.8%. This low recombination rate indicates that the *Est-F* locus must reside within the $(2L)B_1D_5$ region or else be closely linked with it.

The balancer stock found in the laboratory

From the survey of the 63 isofemales kept in the laboratory, four stocks showed no homozygotes in small samples. As the sample size increased, homozygotes were observed in three of them. Since no homozygote had ever been found in the isofemale stock #0131.5 even after the sample size reached 128, we continued investigating this stock.

F^aF^c females and males obtained from the cross between a $F^aF^c \times F^aF^c$ pair from the #0131.5 stock were test crossed with F^cF^c flies. Both F^aF^c,

and F^cF^c genotypes were observed in the F_1 generation. Sib-matings of $F^aF^c \times F^aF^c$ were made from these F_1 flies, and 29 F^aF^c and 16 F^cF^c were produced, but no F^aF^a were observed in the F_2 generation. This differs significantly from the Mendelian 1:2:1 ratio ($\chi^2 = 15.13$, $df = 1$, $p < 0.001$). When the cross was repeated with F^aF^a instead of F^cF^c, the results of the test cross showed both F^aF^a and F^aF^c genotypes in the F_1 generation, and the lack of F^cF^c in the F_2 generation was confirmed. These results indicate that there are two recessive lethal alleles at different loci: one linked with F^a(N), and another with F^c(I) in the #0131.5 stock (Fig. 3). These two loci must reside within the inversion loop or be very closely linked with it.

The hatchability of the #0131.5 stock was 35/41 and that of a normal isofemale stock #0909.1 was 48/50. Statistically they were not significantly different ($\chi^2 = 3.19$, $df = 1$, $p > 0.05$). This result indicates that some homozygotes can complete their embryonic development.

Synchronized analysis of 30 third instar larvae, which matured at normal developmental rates, showed that they were heterozygotes for both $In(2L)B_1D_5$ and *Est-F*. Among 46 third instar larvae collected from a culture after most of the individuals in the same vial had pupated, 29 were heterozygotes, but 17 were homozygotic for both genetic markers. Among 43 second instar larvae collected under the same conditions, nine were
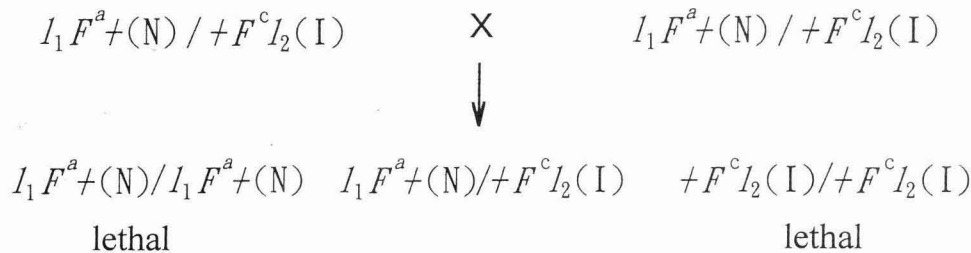
$$l_1 F^a + (N) / + F^c l_2 (I) \qquad \times \qquad l_1 F^a + (N) / + F^c l_2 (I)$$

$$\downarrow$$

$$l_1 F^a + (N) / l_1 F^a + (N) \qquad l_1 F^a + (N) / + F^c l_2 (I) \qquad + F^c l_2 (I) / + F^c l_2 (I)$$

$$\text{lethal} \qquad\qquad\qquad\qquad\qquad\qquad\qquad \text{lethal}$$

Fig. 3. The balancer stock #0131.5 contains two recessive lethals (l_1 and l_2). The arrangement of these two loci and the *Est-F* locus is arbitrary.

Table 1. Chromosome arrangements and esterase patterns of larvae of the laboratory stock #0131.5 under different conditions

Condition of larvae	Genotypes[a]			n[b]
	+ +	− −	+ −	
early 3rd instar	1.00	0	0	30
late[c] 3rd instar	0.63	0.37	0	46
late[c] 2nd instar	0.21	0.79	0	43
14-day-old	0.50	0.50	0	10
16-day-old	0.33	0.67	0	3

[a] + +: both inversion and esterase heterozygous;
− −: both inversion and esterase homozygous;
+ −: one heterozygous and the other homozygous.
[b] n: sample size.
[c] "late" means the larvae were collected after most of the individuals in the same vial had pupated.

heterozygotes but thirty four were homozygotic for both markers. Since the egg laying in these vials was not controlled, we can not determine how old these "late" larvae were. The result was similar when we repeated the synchronized analysis under controlled conditions. Flies were allowed to lay eggs in vials for five hours and then discarded. On average, *D. albomicans* larvae pupate on their seventh day. Of ten 14-day-old larvae, five were heterozygotes and five were homozygotes. Of three 16-day-old larvae, one was heterozygotic and two were homozygotes (Table 1). This indicates that the lethal effect occurs mainly during larval development and explains why developmentally retarded homozygous larvae were detected, but no homozygous adults have ever been observed in this stock.

Determination of the genotype of wild-caught flies

The genotypes determined are summarized

in Table 2. Six individual and combined data sets from Wulai were analyzed statistically, and none of them showed significant linkage disequilibrium. Therefore, the one-year survey showed that the natural population at Wulai was at linkage equilibrium. However, the June 1991 sample showed that the Chitou population was at significant linkage disequilibrium ($D = -0.074$ for F^a vs. non-F^a, $\chi^2 = 14.7$, $df = 3$). According to these data, the linkage disequilibrium possibly results from natural selection and/or genetic drift. The frequency of the inverted *(2L)$B_1 D_5$* arrangement (I) was 0.53 in the Wulai population and 0.69 in the Chitou population. The average inversion heterozygosity was 0.54 in the Wuali population and 0.56 in the Chitou population.

Recessive lethals in the Wulai population

Sixteen of thirty-eight (42%) chromosomes extracted from the natural population contained recessive lethals. The results of chromosome extraction are tabulated on Table 3. None of them is allelic to another.

DISCUSSION

The extremely low mutation rates of chromosomal inversions make them consistent genetic markers in natural populations. In *Drosophila albomicans*, the heterozygosity of *In(2L)$B_1 D_5$* in natural populations and some laboratory stocks is very high and fluctuates seasonally (Chang et al. 1987). This inversion type is maintained under balancing selective forces (Chang et al. 1988). What characteristic of the inversion loop makes the heterozygotes more fit during the cold and humid winter weather? We tried postulating that the cold resistant allele found in the Wulai population was mainly responsible, but this scenario

Table 2. Genotypes of second chromosomes from natural populations

Chromosome arrangement	Est-F	Wulai						Chitou
		110590	041691	051791	052791	071391	110691	061291
N	F^a	11	1	8	14	18	11	7
	F^b	1	1	2	1	3	2	3
	F^c	5	6	3	10	6	7	7
	F^d	5	1	0	2	1	1	3
	F^o	—	1	0	3	1	1	1
I	F^a	10	4	8	25	23	9	31
	F^b	2	2	2	2	5	3	10
	F^c	1	6	1	9	11	6	5
	F^d	0	0	0	2	1	0	0
	F^o	—	6	0	2	1	0	1

Samples are marked by their collection dates, eg., 110590 was collected on November 5, 1990 (see Materials and Methods).

Table 3. The list of extracted chromosomes with or without recessive lethals

	Program I		Program II	
	with	without	with	without
F^a(N)	1	3	2	5
F^a(I)	2	4	2	1
F^b(N)	0	1	0	1
F^b(I)	1	0	1	1
F^c(N)	1	2	3	1
F^c(I)	3	1	0	2
Total	8	11	8	11

was soon invalidated. This question remained difficult to resolve due to the lack of appropriate morphological genetic markers. Fortunately, use of the closely linked *Est-F* locus with multiple alleles plus a balancer stock (#0131.5) made it possible to answer the question.

There are four possible hypotheses explaining the maintenance of 100% heterozygosity of the stock #0131.5: 1) extreme meiotic drive (Sandler and Novitski 1957) produces sperm with one kind of allele and eggs with the other; 2) duplicated genes fix to different alleles; 3) F^a and F^c are both recessive lethal alleles; and 4) F^a and F^c are both closely linked to some recessive lethal alleles. The test cross showed that both male and female flies produce two kinds of gametes, thus invalidating the first two hypotheses. The results of sib-mating indicated that either the F^a has a pleiotropic recessive lethal effect, or it is closely linked with a recessive lethal allele. However, there are many F^aF^a and F^cF^c individuals both in natural popula-

tions and in laboratory-maintained stocks, which makes the third hypothesis unlikely. Therefore, the fourth hypothesis appears to be the most likely. The balancer stock #0131.5 with two *Est-F* alleles (F^a and F^c) is supposed to contain at least two recessive lethal alleles at different loci within or near the $In(2L)B_1D_5$ heterozygous region. The maintenance of this balancer stock is illustrated in Fig. 3. The hatchability of this stock, together with the synchronized esterase and chromosome data, show that the lethal effect does not occur mainly during egg developmental, but during the larval stage.

Finding linkage disequilibrium in the Chitou population, but not in the Wulai population suggests that the latter is an older and a more stable population. Although the Wulai population decreases in size dramatically and forms small patches during the winter, it expands and resumes the status of a single large population again after spring. Because linkage equilibrium is then restored, the number of population reservoirs must be large.

We propose a hypothesis to show that inversions are not necessarily good for populations. The number and frequencies of recessive lethals in natural populations may support the hypothesis that "Genetic load", instead of groups of cooperative genes, is the major reason for the maintenance of inversion polymorphism in natural populations. Genetic load implies that the optimal fitness for any genetic locus is reduced by mutations that are deleterious if heterozygous and/or homozygous. The concept of genetic load was developed originally by Muller (1950).

Hypothetical scenario

1. Inverted chromosome (I) occurs in a population as heterozygotes (NI).
2. Recessive lethals accumulate on the common N chromosomes.
3. The increasing recessive lethals decrease the fitness of NN individuals. By contrast, NI individuals have higher fitness value. This effect is more significant in the isolated winter population groups because of inbreeding due to small population size.
4. Natural selection can not maintain the recessive lethals at low frequencies because of the protection of the inversion heterozygotes. Therefore, the frequency of I chromosomes increases.
5. Recessive lethals accumulate on the I chromosomes too, as the frequency of I chromosome increases.
6. The frequency of inversion heterozygotes increases until it reaches 0.50 with an equal number of N and I chromosomes, due to the unavoidable increase of recessive lethals on both of them.

Although the maintenance of high variability as a whole usually has certain advantages under natural selection, the inversion loop of $In(2L)B_1D_5$ is not able to increase the fitness of the population. On the contrary, the maintenance of high inversion variability may be due to the genetic load because of the presence of recessive lethal alleles inside the inversion region.

Begon et al. (1985) have shown the accumulation of limited recessive lethals with high allelism in a population after a bottleneck. The difference between such a population and the Wulai population is that after the bottleneck they had a homogeneous population while the Wulai population contained a high inversion heterozygosity during the winter; also, they introduced limited amount of genetic variation into the post-bottleneck population, while the Wulai population probably was divided into many small population groups during the winter time and then merged into a single large population during summer. From the high frequencies of both chromosome arrangements, high lethality, and low allelism, it seems that the Wulai *D. albomicans* represents a large old population.

The 42% lethality on the left arm of the second chromosome of *D. albomicans* is relatively high compared with the average of 26.4% for the entire second chromosome of *D. melanogaster* (Yamazaki et al. 1986). According to the criteria we have adopted, the so-called lethal alleles include sublethals with fitness values less than 0.19. Therefore, for comparison, we must further investigate the lethality on the third chromosome, which does not contain a large inversion with significant frequency in the natural population.

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紅果蠅 (*Drosophila albomicans*) 的染色體逆位
與隱性致死因子間之交互作用

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爲了探討紅果蠅第二對染色體左臂逆位 "*In(2L)B₁D₅*" 的高異結合性而建立了幾個酯酶 "*Est-F*" 的純系，此酯酶基因座和這個染色體逆位有連鎖關係。利用染色體粹取交配法發現烏來的紅果繩族群具有染色體逆位的高異結合性(0.54)及隱性致死因子的高頻率(0.42)，但所粹取出的隱性致死因子各不相同。此外並發現烏來的族群處於連鎖平衡狀態而溪頭的族群卻處於連鎖不平衡的狀態。根據這些資料，我們提出一個在染色體逆位存在的前題下季節性變動的族群累積隱性致死因子的假說，來解釋烏來族群所呈現的逆位高異結合性、致死因子的高頻率及低等位性。

關鍵詞：染色體逆位，紅果蠅，酯酶，隱性致死因子，族群遺傳。

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