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Comparison of Recessive Lethal Accumulation in Inversion-bearing and Inversion-free Chromosomes in *Drosophila*

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Yung-Yu Yang, Fei-Jann Lin and Hwei-yu Chang (2002) Comparison of recessive lethal accumulation in inversion-bearing and inversion-free chromosomes in *Drosophila*. *Zoological Studies* **41**(3):271-282. Frequencies of recessive lethal alleles in 3 *Drosophila* species were investigated to test the "trapping hypothesis" (i.e., the accumulation of deleterious alleles by large inversions in a population with seasonal subdivision into small units). The low recessive lethal frequencies of the 3rd chromosome of *D. albomicans* and the 2nd chromosome of *D. formosana* are consistent with their low inversion heterozygosities as predicted by the hypothesis. In addition, the frequencies of recessive lethal alleles on the 2nd and the 3rd chromosomes of *D. melanogaster* are also correlated with those of inversions. However, the correlation is not as obvious as in the *D. albomicans* and *D. formosana* cases, and this is probably due either to interactions between inversions or to the population structure. Locations of those lethal genes may be correlated with inversion breakpoints in *D. melanogaster*. The correlation between recessive lethals and inversions suggests that genetic load instead of heterozygous superiority is a major reason for maintaining inversion polymorphism in natural populations of *Drosophila* with a large simple inversion, and seasonal subdivision into small, inbreeding subpopulations. http://www.sinica.edu.tw/zool/zoolstud/41.3/271.pdf

Key words: Chromosomal inversions, *Drosophila*, Recessive lethal alleles.

High frequencies of chromosomal inversions have been observed in natural populations of many species of *Drosophila*. The superior fitness of heterokaryotypes was usually explained by either dominance or overdominance (Sperlich and Pfriem 1986). The dominance hypothesis supposes that the heterosis of inversions is due to the lack of recombination and hence the holding together of a set of genes that reduces the appearance of homozygotes of deleterious alleles (da Cunha 1955, Ohta 1971). Alternately, the overdominance hypothesis assumes that individual loci are overdominant for fitness (i.e., the fitness of heterozygotes is higher than that of homozygotes). Since the effects of recessive deleterious alleles on fitness are rather complex and heterogeneous, experimental models for analyzing these genes remain inadequate.

According to Chang and Lin (1995) the heterozygosity of an inversion, $In(2L)B_1D_5$, on the 2nd chromosome in natural populations of *D. albomicans* often exceeded 50%, and the frequency of recessive lethal alleles associated with it was unexpectedly higher than that of many other species. The coincidence of having high inversion heterozygosity and high recessive lethal frequency together implies that the dominance hypothesis may be true.

In previous studies, a "trapping hypothesis" was proposed to explain the maintenance of an inversion polymorphism in *D. albomicans* (Chang and Lin 1995, Chang et al. 1996). In brief, when an inversion appears in a population, lethal alleles accumulate on this inverted chromosome due to lack of recombination. If the population remains large, the inverted chromosome will reach a mutation-selection balance. On the contrary, if the population breaks into many small populations during

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the winter, bottleneck effects occur, and the frequency of heterokaryotypes increases (Chang et al. 1987) due to inbreeding depression. During the summer, all subpopulations expand and merge into a large population, and the previously increased heterokaryotypic frequency influences the inverted chromosome frequency relatively more than the non-inverted chromosome because the former is much lower than the latter. Therefore, the inverted chromosome frequency increases. The cycle of winter contraction and summer expansion is repeated year after year. The frequency change continues until the frequencies of the non-inverted and inverted chromosomes become balanced, while the inversion heterozygosity continues increasing during this process. Inverted chromosomes and non-inverted chromosomes form 2 distinct subpopulations, and coevolve in the same chromosome population. Natural selection can no longer keep the total lethality of the inverted region on both chromosome arrangements at low frequencies by the protection of the inversion heterozygotes (Chang and Lin 1995). This hypothesis can explain what has been observed in *D. albomicans*, such as the high lethal frequency, the equal amount of inverted and non-inverted chromosomes, the Hardy-Weinberg karyotypic frequencies in summer, and the greater than 50% heterokaryotypic frequency in winter.

The lethal frequency in *D. albomicans* was compared with that of other *Drosophila* species such as *D. melanogaster*, *D. willistoni*, and *D. pseudoobscura* (Chang et al. 1996). If *D. albomicans* or even the entire *D. immigrans* lineage had unusually high average lethal frequency, the above explanation would be false. Therefore, in this article we determine whether or not the frequency of recessive lethal alleles on the 3rd chromosome, which does not contain a high-frequency inversion as does the 2nd chromosome (Lin and Chang 1986), is indeed lower. The 2nd chromosome of *D. formosana* (which belongs to the *D. immigrans* species group as does *D. albomicans*) in Taiwan has little inversion heterozygosity (Chang et al. 1994), and is also compared.

In *D. melanogaster*, a species distantly related to *D. albomicans*, chromosomal inversion polymorphism and lethality have been frequently and separately studied (Watanabe and Watanabe 1973, Watanabe et al. 1976, Watanabe and Yamazaki 1976), but the interaction between inversions and recessive lethal alleles remains unclear. Using this model species, we have additional genetic tools with which to explore whether the "trapping hypothesis" is plausible.

To sum up, these 3 species, *D. albomicans*, *D. formosana*, and *D. melanogaster*, are valuable materials to test the "trapping hypothesis", which explains why inversion heterozygosity and accumulation of recessive lethal alleles can be closely associated in natural populations.

MATERIALS AND METHODS

Flies

All strains were reared with 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 8 h and kept in separate vials for 3 d before crossing.

Drosophila albomicans

Flies of *D. albomicans*, examined for their 3rd chromosome lethality, were collected from Kenting (墾丁) in March 1994, from Hualien City (花蓮市) in June 1995, and from Litao (利稻) and Chihpen (知本) in July 1997. A strain homozygous for mutant *brick* eye allele (*br*) was constructed. This strain was used to extract 3rd chromosomes from the wild population.

Drosophila formosana

Flies of *D. formosana* used for the survey of recessive lethal allele frequency were collected from Chitou (溪頭) in September 1995. One isofemale line containing an *Est-F* rare allele was obtained from Kuantzuling (關仔嶺) during the survey in our previous study (Chang et al. 1994).

Drosophila melanogaster

Wild males of *D. melanogaster* were taken from Taipei City (台北市) in April and in July 1995 and from Hualien City and Tienhsiang (天祥) in August 1995. The *Cy/Sp;Ser/Sb* strain was provided by Dr. Chung-I Wu at the Univ. of Chicago. Two strains (*rucuca* and *ruPrca*) for mapping recessive lethal alleles on chromosomes were provided by Dr. Henry Sun at Academia Sinica, Taipei, and Dr. Ronny C. Woodruff at Bowling Green State Univ., Ohio, respectively.

Chromosome extraction

Drosophila albomicans

This species has a special genetic structure due to the male *D. albomicans* (*2n* = 6) having a pair of large metacentric sex chromosomes, which were formed by a fusion between the ancestral acrocentric chromosome 3 and the X chromosome (i.e., neo-X), and a fusion between chromosome 3 and the Y chromosome (i.e. neo-Y). A fly heterozygous for the mutant allele, *brick*, was originally collected from a Wulai (烏來) population in October 1993, and a pure line was constructed thereafter. The strain consists of brick-eye (homozygous for *brick* alleles) females, and wild-type-eye (heterozygous for *brick* and wild-type alleles) males due to the wild type allele on the neo-Y chromosome.

Method 1: Father-daughter inbreeding

One daughter, produced by the cross of a wild-caught male and a *br/br* virgin female, was crossed with her father. The procedure is summarized in a flow chart (Fig. 1). If the neo-X chromosome of the wild male carries a recessive lethal allele, this inbreeding will generate a 1:2 (♀:♂) instead of a 1:1 sex ratio. Only data with a sample size larger than or equal to 68 individuals were included in this study in order to significantly distinguish between the 1:2 and 1:1 ratios (for $n = 68$, $\chi^2 = 3.886$, d.f. = 1, $p < 0.05$). This sample size criterion was also adopted for the control groups. The sex ratio of the offspring produced by the initial cross was used as 1 control (control 1). The same wild-caught male was crossed with a 2nd virgin female from another pure line immediately after the 1st cross, and the sex ratio of the offspring produced by this cross was used as a 2nd control (control 2).

Method 2: Brick-eyed cross

A wild-caught male was crossed with a *br/br* virgin female and a sib-mating pair was formed with the F_1 offspring. Several F_2 sib-mating pairs from the wild-type offspring of this F_1 pair were formed, and one of them was chosen which did not produce brick-eyed F_3 females. From the offspring of this F_2 pair, 7 F_3 females were crossed with a male from the *br* strain to determine whether they were homozygous or heterozygous. If all 7 of them were heterozygous, then the 3rd chromosome from the wild-caught male was said to carry a recessive lethal allele. The procedure is summarized in a flow chart (Fig. 2).

Esterase haplotype method

Drosophila formosana

After a survey of flies collected from natural populations, we found no morphological markers. The only genetic marker available is a rare *Est-F*^{R} (located on the 2nd chromosome) allele found in the Kuantzuling population. After construction of a pure line with this rare allele *F*^{R} at *Est-F* (a dimer) and a common allele *C*^{S} at another esterase locus *Est-C* (a monomer), we used this marker strain for the chromosome extraction and to check the recessive lethal frequency on the 2nd chromosome of the flies collected from Chitou. The genetic distance between *Est-F* and *Est-C* is smaller than 0.43 cM (*n* = 928). The procedure, which is similar to that in Chang and Lin (1995), is summarized in a flow chart (Fig. 3).

Each male fly collected from a natural population was crossed with a virgin female from the *F*^{R}*C*^{S}/*F*^{R}*C*^{S} pure line. After F_1 larvae appeared in

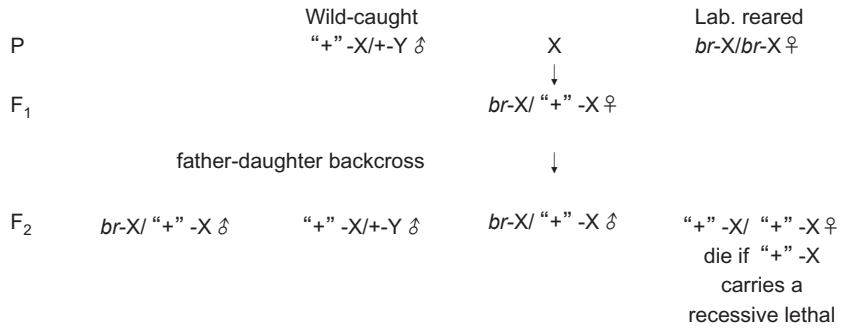

Fig. 1. Wild-caught males individually mated with brick-eyed females of *Drosophila albomicans*. An F_1 female was backcrossed with her father. A distorted sex ratio can be observed in F_2 progenies if the neo-X chromosome of the wild-caught male carried a recessive lethal allele.

the medium, the wild-caught male was sacrificed to determine its esterase pattern by electrophoresis. If this male was heterozygous at the *Est-C* locus, regardless of whether or not it contained the same allele as the pure line, it went into Program I; if homozygous, it went into Program II.

Program I: Separate 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 were analyzed. Only $C^S/C^S \times C^S/C^S$ and $C^F/C^S \times C^F/C^S$ pairs were saved; others were discarded. Regardless of whether they were homozygous or heterozygous at the *Est-C* locus, those pairs were all $F^C/F^R \times F^C/F^R$ at the *Est-F* locus. With an ordinary chromosome extraction method, we could only extract 1 chromosome from each wild-caught fly, because there is no way to distinguish the homologous pair. With the help of the closely linked *Est-F*^{R} and *Est-C* haplotype, both chromosomes can be identified if heterozygous. This method is better than the ordinary chromosome extraction meth-

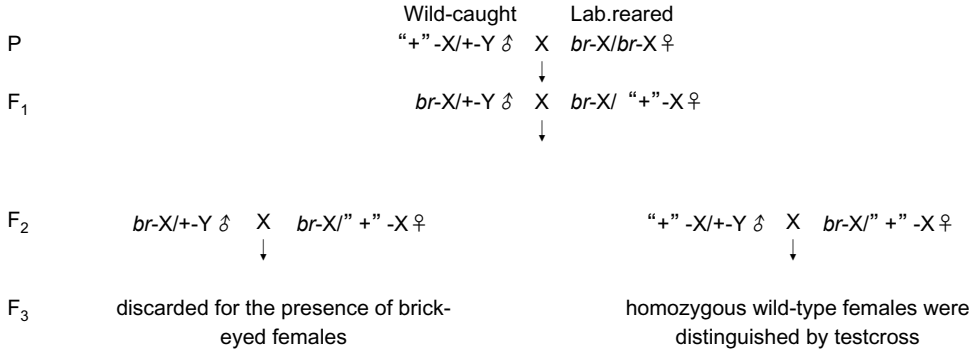

Fig. 2. Wild-caught males individually crossed with brick-eyed females of *Drosophila albomicans*. F_2 pairs were made with wild type F_1 males and females. Incorrect pairs were discarded based on the presence of brick-eyed female progenies, and 7 of the F_3 females from the correct pairs were checked by testcrosses.

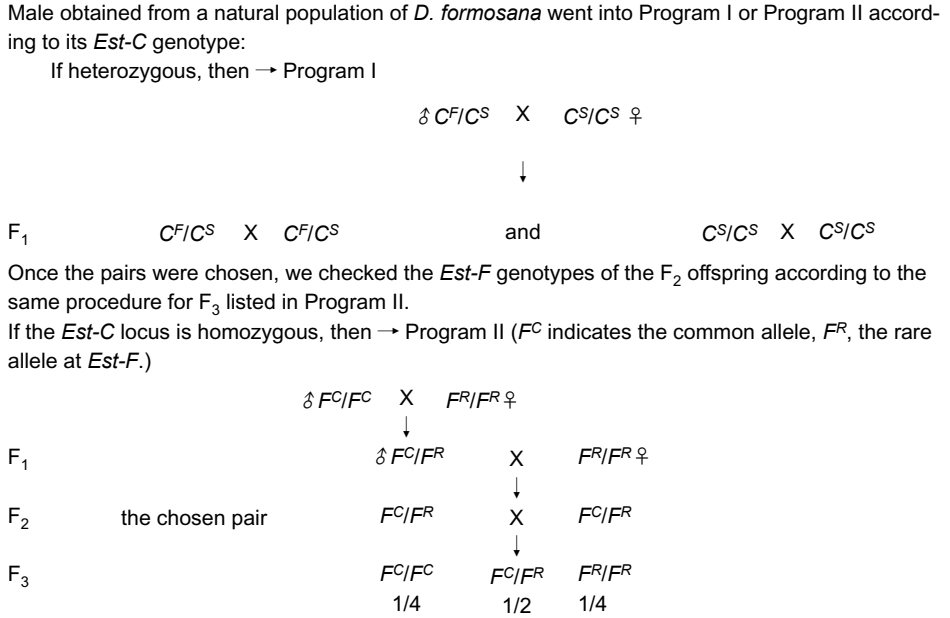

Fig. 3. Males obtained from a natural population of *Drosophila formosana* crossed with females of the extraction tool. The experiment proceeded into Program I or Program II according to the esterase genotype of these males. The expected possibility of the appearance of a homozygous offspring from a heterozygous pair was 1/4 if the chromosome did not contain a recessive lethal allele.

ods such as the brick-eyed cross of *D. albomicans*, program II for *D. formosana*, and the *Cy/Sp*; *Ser/Sb* method for *D. melanogaster*, because both chromosomes of the wild-caught fly can be identified.

Program II: One F_1 male produced from each wild-caught male, which was homozygous at the *Est-C* locus, was crossed to a virgin F^RC^S/F^RC^S female again; separate pairs were made from virgin F_2 flies, and a heterozygous pair (i.e., $F^C/F^R \times F^C/F^R$) was chosen.

The following procedures are the same for both Programs I and II, except that there was a 1-generation delay, and only 1 type of heterozygous pair was saved for each wild-caught male in the latter. The esterase patterns of offspring from the chosen pairs were checked. If more than 16 flies were checked and no common allele homozygotes (i.e., F^C/F^C) were found, this wild chromosome was said to contain a recessive lethal allele.

Cy/Sp; *Ser/Sb* method

Drosophila melanogaster

The procedure is similar to that of Kosuda (1971) except that we used the *Cy/Sp; Ser/Sb* strain instead to do the extraction (Fig. 4). The tested chromosome was determined to be carrying a recessive lethal allele if the ratio of the wild type to *Cy* (or *Ser*) in the progeny was smaller than 0.05, a criterion adopted from Haldane (1956)

Chromosome preparation and detection of inversions in *D. melanogaster*

Wild-caught males, or a single male from each isofemale line, were individually crossed with virgin females of *Oregon R*, which has the standard chromosome arrangement. From each cross, 8 hybrid larvae were examined cytologically for inversions by the method used in Lin and Chang (1986). *D. melanogaster* has 5 cosmopolitan inversions: *2L(t), 2R(NS), 3L(P), 3R(P)*, and *3R(C)* (Sperlich and Pfriem 1986).

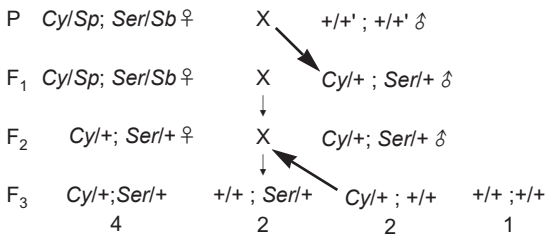

Fig. 4. Males obtained from a natural population of *Drosophila melanogaster* individually crossed with females of the *Cy*/*Sp*; *Ser*/*Sb* strain. An F_1 male from each cross was backcrossed with females from the same marker strain. F_2 pairs were made, and recessive lethal alleles on the 2nd and the 3rd chromosomes were checked in F_3.

Allelism test in *D. melanogaster*

In order to estimate the allelic rate, allelism tests were performed both within and between populations. All combinations of inter-strain crosses were made. Those cultures that yielded no wild-type flies were identified as containing allelic lethal alleles.

Mapping of recessive lethal alleles in *D. melanogaster*

Lethal alleles were maintained in balancer strains, and mapped by using *rucuca* (*ru, h, th, st, cu, sr, e^s, ca*) and *ruPrca* (*ru, h, th, st, cu, sr, e^s, Pr, ca*) strains. A single male from each balanced-lethal strain was crossed with 3 females of the *rucuca* strain, and their female progeny

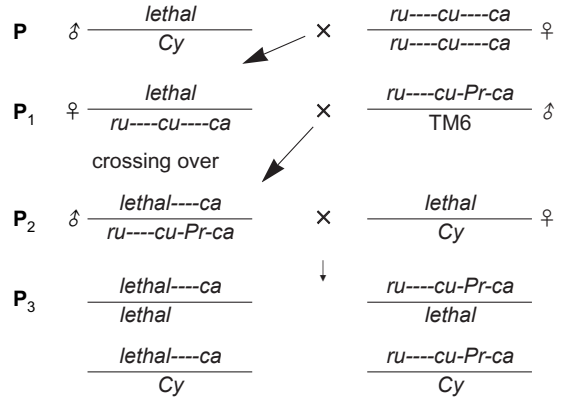

Fig. 5. A single male *Drosophila melanogaster* from each balanced-lethal strain crossed with females of the *rucuca* strain for mapping recessive lethal alleles on chromosome 3. Their female progeny (lethal/*rucuca*) were crossed to *ruPrca* males. F_2 recombinants between the lethal and the *rucuca* chromosomes were classified according to 8 recessive markers and F_2 males containing a dominant *Pr* marker singly backcrossed with females from the original balanced-lethal strain. An example of the chosen F_2 male here indicates a recombination between markers *e^s* and *ca*, and the order of markers can be seen in figure 7. The absence of F_3 wild type offspring was used to judge the existence of a lethal allele on this recombinant chromosome of the F_2 male. These lethal loci were mapped by analyzing the recombinant classes and the incidence of lethality.

(lethal/*rucuca*) were crossed to the *ruPrca* males. F_2 recombinants between the lethal and the *rucuca* chromosomes were classified according to 8 recessive markers, and F_2 males containing a dominant *Pr* marker were singly backcrossed with females from the original balanced-lethal strain. The absence of F_3 wild-type offspring was used to judge the existence of lethal alleles on this recombinant chromosome of the F_2 male. These lethal loci were mapped by analyzing the recombinant classes and the incidence of lethality. The procedure, which was modified from Spiess et al. (1963), is summarized in a flow chart (Fig. 5).

RESULTS

Third chromosome lethality in natural populations of *D. albomicans*

Two methods were used to estimate the frequency of recessive lethal alleles of the 3rd (neo-X) chromosome. According to method 1 (i.e., "father-daughter inbreeding"), the frequencies of recessive lethal alleles are 37%, 32%, and 29% in 3 natural populations, and method 2 (i.e., the "brick-eyed method") showed 15%, 11%, 7%, and 25% in 4 natural populations (Table 1). Method 1 is based on the Chi-square analysis of sex ratios, and the comparison for the Hualien City survey using controls 1 and 2 is listed in table 2. Data of method 1 shown in this study (Table 1) were based solely on a comparison with control 1, for reasons elaborated below.

Second chromosome lethality in *D. formosana* natural populations

Fifty-eight males and 19 females were collected from Chitou in 1995. At the dimer locus (*Est-F*), none of these flies contained the rare dimer allele (F^R) used as the genetic marker in the chromosome extraction; all of them were homozygous for the common allele (i.e., F^C/F^C). The allele frequencies of the monomer locus (*Est-C*) of these 77 flies were: *f* (C^F) = 46%, *f* (C^S) = 53%, and *f* ($C^{S'}$) = 1%.

Twenty-nine (29) crosses of wild-caught males with pure-line females went into Program I, and 58 second chromosomes were extracted. Fifteen crosses went into Program II, and 15 second chromosomes were extracted. Nineteen crosses of F_1 males, each from 1 isofemale, went into Program I, and 38 second chromosomes were extracted. Eighteen of the 111 extracted 2nd chromosomes contained recessive lethal alleles. The lethality of the 2nd chromosome was 16%.

Chromosomal inversion heterozygosity, lethality, and lethal allelism in *D. melanogaster*

Three populations (Taipei City, Hualien City,

Table 1. Third chromosome lethality in *Drosophila albomicans* natural populations (method 1 = father-daughter inbreeding; method 2 = brick-eyed cross)

Locality	Date	Lethality with sample size in parentheses	
		Method 1	Method 2
Hualien City	June 1995	0.367 (30)	0.154 (52)
Litao	July 1997	0.320 (25)	0.111 (27)
Chihpen	July 1997	0.288 (52)	0.068 (44)
Kenting	Mar. 1994	–	0.250 (12)

Table 2. Chi-square analysis of the Hualien City *Drosophila albomicans* lethality survey by the father-daughter inbreeding method using 2 different controls

	Male biased			Female biased			Sample size (*n*)
	***	**	*	***	**	*	
Control 1	11	0	0	0	0	2	30
Control 2	5	1	3	0	0	0	25

***$p < 0.005$, highly significant; **$p < 0.01$, intermediately significant, *$p < 0.05$, mildly significant.

and Tienhsiang) of *D. melanogaster* were analyzed. Five types of cosmopolitan inversions were observed. The chromosomal inversion heterozygosity and lethality of the 2nd and 3rd chromosomes are presented in table 3. The heterozygosity of *In*(*2R*)*NS* and *In*(*3L*)*P* did not significantly differ in the 3 local populations, whereas that of the *In*(*3R*)*P* significantly differed in different seasons (April and July). The heterozygosities of *In*(*2L*)*t* and *In*(*3R*)*C* showed weak positive correlations with lethality of the 2nd and 3rd chromosomes individually (Table *3*). However, 3 inversions, *In*(*2R*)*NS*, *In*(*3L*)*P*, and *In*(*3R*)*P*, showed no correlation at all with lethality. The lethality of the 3rd chromosome was higher than that of the 2nd one (Table 3), but there was no difference in total inversion heterozygosities between these 2 chromosomes. The only difference found was that the inversions of the 3rd chromosome covered a larger proportion than did those on the 2nd chromosome (Fig. 6). The physical map is based on Lindsley and Zimm (1992).

Intra-population and inter-population allelism is very low (Table 4). We could not calculate the size of each population based on allelism because most of them were 0. Allelic lethal alleles were found only in the Taipei population.

Location of recessive lethal alleles on the 3rd chromosome

Figure 7 shows the mapping result of recessive lethal alleles on the 3rd chromosome. The physical and genetic map locations are from Lindsley and Zimm (1992). The distribution differed from randomness, because 13 (approximate 44%) of the recessive lethal alleles were located close to the breakpoints of inversions (2 recessive lethal alleles near the proximal breakpoint of *In*(*3L*)*P*, and 11 recessive lethal alleles near the proximal breakpoint of *In*(*3R*)*P*). Dividing the chromosome into 7 regions, the observed frequencies differed significantly from the expected ones (based on genetic distance, not physical distance), and this analysis confirmed the non-random distribution (Table 5).

DISCUSSION

Methods of analyzing recessive lethal frequencies in *Drosophila albomicans*

Different methods were used to obtain recessive lethal frequencies of different chromosomes as well as of different species. For example, it was possible to detect the lethality of the 3rd chro-

Table 3. Chromosomal inversion heterozygosity and lethality (with sample size in parentheses) of the 2nd and 3rd chromosomes in Drosophila melanogaster natural populations

Locality	Time	Second chromosome			Third chromosome			
		Inversion heterozygosity (%)		Lethality (%)	Inversion heterozygosity (%)			Lethality (%)
		(*2L*)*t*	(*2R*)*NS*		(*3L*)*P*	(*3R*)*P*	(*3R*)*C*	
Taipei	Apr. 1995	8.3	22.2 (36)	0 (57)	13.9	0	27.8 (36)	32.8 (57)
	July 1995	18.9	20.8 (53)	1.9 (48)	13.2	15.1	11.3 (53)	17.0 (48)
Hualien City	Aug. 1995	31.4	20.0 (35)	10.5 (29)	17.1	20.0	17.1 (35)	26.3 (29)
Tienhsiang	Aug. 1995	25.6	23.2 (43)	8.6 (27)	14.0	23.2	11.6 (43)	25.7 (27)

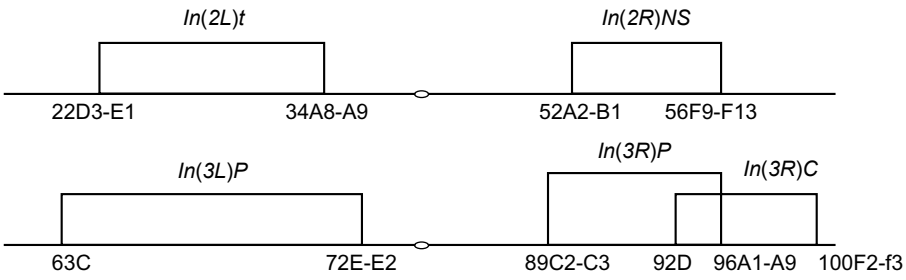

Fig. 6. Cytological size and location of 5 polymorphic chromosome inversions observed in 3 populations of *Drosophila melanogaster*.

mosome of *D. albomicans* by the deficiency of females in the father-daughter inbreeding experiment due to sex linkage (3-X, i.e., the neo-X). Fusions between ancestral 3rd chromosomes and the sex chromosome formed the neo-X and neo-Y chromosomes in this species. The rationale of the father-daughter inbreeding method is that recessive lethal alleles can be shown by a sex ratio change, since inbreeding should cause the death of 1/2 of female progeny due to the formation of homozygous females if the tested male contains a recessive lethal allele. Theoretically, the sex ratio of females to males should become 1:2, given the normal 1:1 Mendelian ratio. The other method of analyzing the recessive lethal frequency of the 3rd chromosome of *D. albomicans* is the construction of homozygotes by chromosome extraction. Chromosome extraction was the common method used in this study to analyze recessive lethal frequencies of all 3 species.

Chi-square analysis can not properly discriminate 1:2 from 1:1 if the sample size is smaller than 68; therefore we adopted a criterion of $n \geqq 68$. Because the sex ratio might be genetically influenced and variable in natural populations, 2 different controls were tested. From the Hualien City survey, only 30 of the 44 replicates had proper control 1 data, 25 had control 2 data, and 19 had both control 1 and control 2 data by this $n \geqq 68$ criterion (Table 2). If a high standard ($p < 0.005$) was adopted to judge the existence of recessive lethal alleles, the Chi-square analysis with control 1 showed that 36.7% (11/30) were significantly male biased, while control 2 showed 20% (5/25) only. Among the 19 replicates with both control 1 and control 2 data, the inconsistency was 26% (5/19). The high proportion of difference indicates

Table 4. Lethal allelism within and between 3 *Drosophila melanogaster* populations

Population	Crosses	Allelism (%)
Taipei	629	0.47
Hualien City	66	0
Tienhsiang	28	0
Taipei vs. Hualien City	432	0
Hualien City vs. Tienhsiang	108	0
Tienhsiang vs. Taipei	324	0

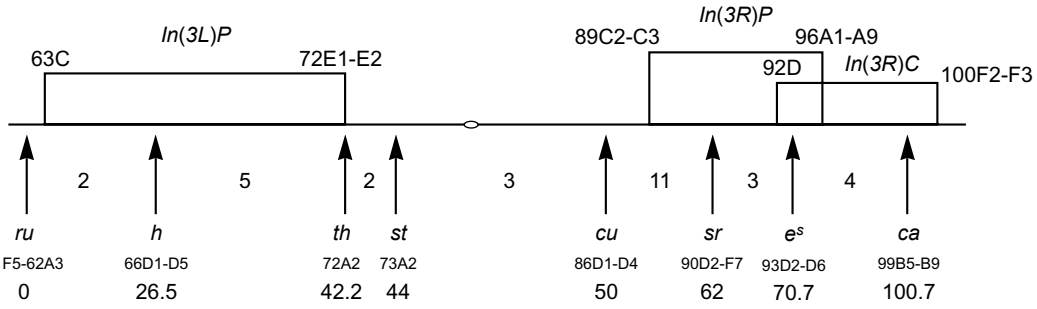

Fig. 7. Thirty recessive lethal alleles mapped on chromosome 3 of *Drosophila melanogaster*. The circle on the horizontal axis marks the centromere. The cytological locations of the 3 inversions are indicated above the horizontal axis. Arrows under the horizontal line indicate the locations of morphological markers, and the cytological and the genetic map locations are both written beneath each marker. The number between the 2 arrows indicates the number of recessive lethal alleles mapped in this region.

Table 5. Chi-square distribution of 30 recessive lethals on chromosome 3 of *Drosophila melanogaster*

Regions	*ru-h*	*h-th*	*th-st*	*st-cu*	*cu-sr*	*sr-e^s*	*e^s-ca*
Genetic distance	26.5	15.7	1.8	6.0	12.0	8.7	30.0
Expected number	7.89	4.68	0.54	1.79	3.57	2.59	8.94
Observed number	2	5	2	3	11	3	4

$\chi^2 = 27.455$ $df = 6$, $p < 0.1$.

a non-negligible maternal influence on variations in the sex ratio. Although control 1 and control 2 flies had the same father as the experimental group, these 2 control groups had different mothers. Since the control 1 flies were the full-sibs of the "daughter" in the "father-daughter inbreeding" experiment, biologically it is a better control than control 2. Statistical analysis revealed another advantage of choosing control 1: it is unambiguous. All 11 male biases according to control 1 were highly significant, whereas control 2 showed 1 intermediately ($p < 0.01$) and 3 mildly ($p < 0.05$) significant male biases in addition to the 5 highly ($p < 0.005$) significant cases. Therefore, the data of method 1 shown in this study were solely based on comparison with control 1.

Within-species comparison agreement with the "trapping hypothesis"

In *D. albomicans*, method 1 showed a much higher lethality than method 2, because the former includes every lethal allele on the neo-X chromosome, whereas the latter includes only those near the marker, due to recombination. The "trapping hypothesis" (Chang and Lin 1995) predicts that the 3rd chromosomes of *D. albomicans* should contain a lower frequency of recessive lethal alleles due to the low inversion heterozygosity. The result is in accord with our expectation, and indeed the average frequency of recessive lethal alleles on the 3rd chromosome (method 1, 28%, $n = 122$; method 2, 15%, $n = 91$) was significantly lower than that on the 2nd chromosome (44%, $n = 213$) (Chang and Lin 1995). Method 2 is similar to the esterase method used for detecting the lethality of the 2nd chromosome (Chang and Lin 1995). The difference is even larger if we take into account that the 3rd chromosome arm of the neo-X consists of 40% of the genome, while the 2L has only 20%. Although the entire neo-X chromosome consists of 60% of the genome (i.e., the X chromosome portion is about 20%), we neglected the X portion because the heterochromatinized Y portion was unable to cover the recessive lethal alleles on the X portion. It is obvious that the lethality of the 2nd chromosome is much higher than that of the 3rd. Here we can remark that our hypothesis holds in *D. albomicans* due to the internal comparison within species and without the complication of species specificity.

Between-species comparison agreement with the hypothesis

Drosophila nasuta, the sibling species of *D. albomicans*, has 3 heterozygous inversions, *2L-2*, *3-2*, and *3-35*, which were very common and detected in high frequencies in Indian populations (Kumar and Gupta 1988). Kumar and Gupta found that heterozygotes for those inversions were always in excess of homozygotes, and the standard homozygotes were more frequent than inversion homozygotes. Kumar and Gupta (1989) suggested that the scarcity of inversion homozygotes might be due to reduced viability of their carriers, which probably have lethal or sub-lethal combinations of alleles in the inverted sections of their chromosomes. Our "trapping hypothesis" would predict that recessive lethal frequencies of 2nd and 3rd chromosomes were both high in *D. nasuta* due to the high inversion heterozygosity.

A rare allele for an esterase dimer in *D. formosana* was found in a heterozygous female among 404 flies (Chang et al. 1994). A pure line of this rare allele was established and proved to be a useful tool for chromosome extraction. None of the 58 males or 19 females plus their mates sampled from Chitou contained this rare allele.

The result of the allele frequencies of monomers in this study is also consistent with that of our previous study (Chang et al. 1994). In Program I, both chromosomes could be extracted, but in Program II, only 1 could. Since the data among different sample sets were statistically indistinguishable, they were pooled together and showed 16% lethality, which is significantly lower than the 44% ($n = 213$) of *D. albomicans* ($\chi^2 = 24.3$, $df = 1$). No inversion was found on the 2nd chromosome of *D. formosana* (Chang et al. 1994). Both *D. albomicans* and *D. formosana* belong to the same species group, so the high lethal frequency on the 2nd chromosome of *D. albomicans* cannot be due to an unusual nature of this lineage.

Does the "trapping hypothesis" work in *D. melanogaster?*

No strong correlation was found between heterozygosity of any particular inversion and lethality in *D. melanogaster* (Table 3), nor did total inversion heterozygosity show any correlation with lethality. The average lethality (25.4%) of the 3rd chromosome of *D. melanogaster* was higher than that (16%) of the homologous 2nd chromosome of *D. formosana* as predicted by the hypothesis. But, the average lethality (5.2%) of the 2nd chromosome of *D. melanogaster* was lower than that

(14.5%) of the homologous 3rd chromosome of *D. albomicans*. Schultz and Refield (1951) suggested inter-chromosomal effects of crossing over, and that may be why we found no obvious relationship between inversion and lethality in *D. melanogaster*. *D. albomicans* has only 1 large *In*(*2L*)B_1D_5 with high frequency and hence a more significant correlation.

Another explanation for the lower average lethality (5.2%) of the 2nd chromosome of *D. melanogaster* compared to that (14.5%) of its homologous chromosome (i.e., the 3rd) of *D. albomicans* could be that double crossovers take place in an inverted region (Navarro et al. 1997). Hasson and Eanes (1996) found that the *esterase-6* gene region, which is located in the center of *In*(*3L*)*P*, shared DNA sequence polymorphism between *St* chromosomes and inverted chromosomes. In contrast, the *heat shock 83* gene (*Hsp83*) locus, located close to the distal breakpoint, had no shared sequence polymorphism. Although it is widely accepted that inversions are recombination inhibitors, genetic exchange may still occur in the middle of long inversions, e.g. *In*(*3L*)*P* and *In*(*2L*)t in *D. melanogaster* (Depaulis et al. 1999). In addition, selection would act on recombination length, not physical length (Caceres et al. 1999). Therefore, recessive lethal alleles located in a large inversion would be eliminated by a double crossover. But this cannot explain the present case for 2 reasons: 1) the recessive lethals still accumulated in the large inversion, *In*(*2L*)B_1D_5, and 2) the lethality of the 2nd chromosome of *D. melanogaster* with 2 inversions is even lower than that on its homologous chromosome without inversions. There is an important component in the "trapping hypothesis" for explaining the accumulation of lethals: the cyclically changing population structure. *D. melanogaster* is a "domestic" species, and can still be collected easily during the winter although the population becomes smaller. The inter-chromosomal effects plus the lack of a dynamic population structure may restrict the use of the "trapping hypothesis". Data on *D. melanogaster* suggest that the hypothesis may be limited to species with large simple inversions and a cyclical population structure.

D. melanogaster was chosen for this study because its many genetic markers make mapping possible. The distribution of recessive lethal alleles deviates from a random distribution on chromosome 3 of *D. melanogaster* (χ^2 = 27.455, $p <$ 0.1, Table 5), with 13 (approximately 44%) recessive lethal alleles located close to 2 breakpoints of inversions. Comparing the region with and without inversion breakpoints, we found 2 lethal loci located within *th-st* (0.8 cM) which contains a breakpoint of *In*(*3L*)*P*, and 11 within *cu-sr* (12.0 cM) which contains a breakpoint of *In*(*3R*) *P*, but only 3 within *st-cu* (6.0 cM) which contains no inversion (Table 5). The relative density of lethals (13 loci/12.8 cM) near breakpoints is about twice that (3 loci/6 cM) of a region with no inversion. Therefore, the effect of inversion breakpoints on the accumulation of recessive lethals may be important on the 3rd chromosome of *D. melanogaster.*

More on the "trapping hypothesis"

In this article, we confirm the high lethalilty of the 2nd chromosome of *D. albomicans* by a within-species comparison with its own 3rd chromosome and by a between-species comparison with its homologous chromosome of *D. formosana*. This evidence indicates that the high lethality is neither a species-specific phenomenon nor a historical event in this lineage. In fact, a nonrandom association between recessive lethal alleles and chromosomal inversions has been described in *D. pseudoobscura* (Epling et al. 1961, Mayhew et al. 1966). Dobzhansky et al. (1963) and Crumpacker and Salceda (1969) proposed that rare gene arrangements in a population would tend to have a greater number of recessive lethal alleles than would common ones. This can been seen as the effect of "Muller's ratchet" on the small chromosomal population of the rare type in the absence of recombination. A similar hypothesis was proposed by Eanes et al. (1992). Their observations showed significantly increased numbers of *P* elements within regions associated with minority inversions in natural populations. Because the opportunity for recombination is suppressed in the heterozygotes, the rarer the inversion in a population, the greater the frequency of the inversion present in the heterozygous state and the lower the potential recombination rate in homozygous inversions. Since transposable element insertions often produce deleterious effects on fitness (Charlesworth and Langley 1989), it is believed that the results of Eanes et al. have the same meaning and indicate that selection may participate in the process of the initial stage in the "trapping hypothesis". When an inverted chromosome occurs in a population as heterozygotes at low frequency (Chang et al. 1996), recessive lethals will accumulate in the inverted region.

However, in this stage, the recessive lethal frequency will not be too high due to mutation selection balance. The "trapping hypothesis" introduces population dynamics to explain the forces which result in the high recessive lethal frequency, and then, high inversion heterozygosity will further be attained by this genetic load.

The "trapping hypothesis" stresses small scattered winter subpopulations. Since inversions suppress recombination, they can function as a trap for detrimental alleles (Muller 1964, Felsenstein 1974, James 1992). Chance fixation of detrimental or even lethal alleles may result in an increased superiority of heterokaryotypes (Albornoz and Dominguez 1994). That is why bottleneck effects cause the frequency of heterokaryotypes to increase due to inbreeding depression. On the contrary, during summer, the Hardy-Weinberg expectation is observed when sub-populations merge into a large population. Inoue and Watanabe (1992) showed that natural populations must be considered as sets of divided or semi-divided small populations. In such subpopulations, inversion polymorphism is maintained by heterokaryotypic advantage; they found that the advantage was destroyed when inversions from different subpopulations are mixed. Although they interpreted their results on the basis of coadaptation, the same would be expected if chromosomal heterosis were due to recessive deleterious or lethal alleles. We suggest that genetic load (recessive lethals) is better than "coadaptation" for explaining inversion heterozygosity. The concept of genetic load is an important issue in population genetics of sexually reproducing diploid organism. It also significantly contributes to the modern synthesis theory of evolution.

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果蠅的染色體逆位和隱性致死因子的累積

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在我們先前的研究中指出，紅果蠅的第二對染色體具有很高的染色體逆位 *In*(2*L*)B_1D_5 異結合型頻率以及隱性致死因子的比率。因而提出一個假說：大區段的逆位在特定族群結構影響之下會像陷阱一樣造成隱性致死因子的堆積。在本文中，我們調查在自然族群中幾乎不含染色體逆位的紅果蠅第三對染色體 (亦即neo-X染色體)和臺灣大果蠅第二對染色體的隱性致死因子的頻率，以及具有逆位多態性的黃果蠅第二和第三對染色體。前兩者的隱性致死因子頻率均低於紅果蠅的第二對染色體，這個結果與我們的預期相符。而黃果蠅隱性致死因子的頻率與染色體逆位的相關並沒有那麼顯著，可能是由於逆位間的交互作用所致。此外，我們訂出黃果蠅隱性致死因子在染色體上的位置。這些隱性致死因子在染色體上的分布與逆位的斷接點可能有關。本研究著眼於隱性致死因子，由遺傳負荷的觀點去探討自然族群中維持染色體逆位多態型的機制。

關鍵詞：染色體逆位，果蠅，隱性致死因子。

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