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On the Low Genetic Variability in *Drosophila immigrans* and *D. formosana**

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Hwei-yu Chang, Chau-Ti Ting and Fei-Jann Lin (1994) On the low genetic variability in *Drosophila immigrans* and *D. formosana*. *Zoological Studies* **33**(4): 287-295. Restriction patterns of mitochondrial DNA, esterase isozyme electromorphs, and chromosome inversions were chosen as indicators to investigate the genetic variability of natural populations of a sibling species pair, *Drosophila immigrans* and *D. formosana*, in Taiwan. In addition to local populations, we also surveyed sixteen laboratory-maintained stocks of the cosmopolitan species *D. immigrans* originating from different countries. All three indicators consistently showed that the local populations of both species in Taiwan have a very low genetic variation. No trace of introgression was found although they can produce fertile hybrids. The low genetic variability of these two species may be due to both the founder effect and their recent establishment in Taiwan.

Key words: Chromosome inversions, Esterase isozymes, Founder effect, Genetic variation, MtDNA restriction patterns.

Drosophila immigrans and *D. formosana* are a pair of sibling species. Although the male flies can be identified by the different hair patterns on the first tarsal segment of their forelegs (Duda 1926), females are morphological indistinguishable. Despite a morphological similarity, they have different spatial and temporal distributions and probably different niches. The former is a cosmopolitan species but the latter has a restricted distribution in Asia (Wheeler 1981). In addition to their different geographical distributions, they have different altitudinal distribution as well. The population of *D. formosana* is limited to elevations below 1,500 m, but that of *D. immigrans* extends up to 2,000 m. They also have different annual maxima in abundance in Taiwan: *D. immigrans* peaks in the spring, while *D. formosana*, the summer. The seasonal difference indicates that they occupy different niches.

The genetic variability of a population, sometimes reflecting the environmental complexity of its niche, is influenced by its phylogenetic background and the duration of its existence. Through the detailed study of polymorphic chromosome inversions, mtDNA restriction patterns, and esterase electrophoretic patterns of a local species *D. albomicans* (Lin and Chang 1986, Chang et al. 1987 1988a 1988b, Chang and Lin 1990), we have discussed the meaning of genetic variability of populations in evolutionary study. For instance, we suggested that the polymorphic pattern is more important than the polymorphic frequency in revealing an interpopulation relationship, because the latter may be influenced by too many factors, especially selection. Considering the specific properties of different indicators, we should be aware that a common chromosome inversion type is a better indicator of common ancestry than a common isozyme allele, since the formation of an inverted chromosome in a natural population is a rare event. But isozyme analysis can be a good indicator for revealing population structure. A good indicator of the intraspecific relationships among populations is not necessarily a good one for evaluating the

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phylogenetic relationship among species. The matrilineal inherited mitochondrial DNA is a more sensitive indicator for introgression than nuclear genes which are under homeostatic selection (Moritz et al. 1987), but the detective ability is only one way rather than reciprocal. We report here the low intraspecific variation in *D. immigrans* and *D. formosana* by using these three indicators. Since both species have a similar degree of genetic variability, environmental constraint does not seem to be an important factor in this case. The two remaining possibilities are phylogenetic constraint and the short time period since their introduction and establishment on this island.

MATERIALS AND METHODS

Flies

Drosophila immigrans and *D. formosana* flies were collected from natural populations with banana baits sprayed with beer. The flies were either directly sacrificed for survey or used for the establishment of isofemale stocks. In order to avoid the negative aspect of the founder effect, during the first few generations of the establishment of isofemale stocks, the maximum number of offspring was reared by transferring the flies to new medium every other day. All stocks were reared with a standard corn meal medium and an environment of 22°C and 75% relative humidity was maintained throughout the experiment. The numbers of flies or stocks used in this study are summarized in Table 1. The majority of the stocks were isofemale ones. We are uncertain whether the three *D. immigrans* stocks from Venezuela, Colombia, and Nepal were isofemale or mass cultures.

Extraction of mitochondrial DNA

Mitochondrial DNA was extracted from adult flies according to a protocol modified from that of Chang et al. (1988). Briefly, 16 flies were gently homogenized in 320 μl of solution I (10 mM Tris, pH 7.8, 60 mM NaCl, 5% sucrose, and 10 mM EDTA), and lysed by the addition of 400 μl of freshly mixed solution II (300 mM Tris, pH 9.0, 1.25% SDS, 5% sucrose, 100 mM EDTA, and 8 μl/ml diethyl pyrocarbonate). After the reaction mixture was incubated at 65°C for 30 min, 120 μl of 3 M sodium acetate was added. The mixture was allowed to stand on ice for 45 min after which it was centrifuged on an Eppendorf for 10 min at 4°C. DNA was isopropanol precipitated at room temperature and resuspended in 500 μl double distilled water, thereafter phenol and chloroform extractions were made (Maniatis et al. 1982). Mitochondrial DNA was ethanol precipitated and dried in a vacuum desiccator.

Digestion of restriction endonucleases and agarose gel electrophoresis

Partially purified mitochondrial DNA was dissolved in 20 μl of double distilled water. The digestion of restriction endonucleases was performed according to the procedure described by Maniatis et al. (1982). The reaction was stopped by the addition of 1/10 volume STOP solution (0.5% bromophenol blue, 50 mM EDTA, 0.5% SDS, 50% glycerol, and 0.2% boiled RNase). The restriction pattern was analyzed on a 0.8% agarose gel in Tris-acetate buffer (40 mM Tris, pH 8.1, 20 mM acetic acid, and 2 mM EDTA). After staining with ethidium bromide, DNA bands were viewed under UV light. The size of fragments was estimated by a graphical method using *Hin*d III digested lambda phage DNA as a standard.

Esterase isozyme electrophoresis

Each individual fly was homogenized with 20 μl of double distilled water in an Eppendorf tube. After centrifugation for 10 min, 10 μl of the supernatant was mixed with 2 μl of bromophenol blue-glycerol solution, and loaded on a well of a 7.5%, pH 8.8 (with a 5% pH 6.8 stacking) polyacrylamide slab gel. The gel was run with Tris-glycine buffer (pH 8.3) at 4°C until the dye front reached the end of the gel. The isozyme patterns were then examined by the specific staining method as described by Ayala et al. (1972).

Chromosome inversion analysis

The chromosome preparation is the same as that used in our previous studies of *D. albomicans* (Lin and Chang 1986). The salivary glands dissected out from third instar female larvae were subsequently submerged in 45% acetic acid for 45 seconds, 1N HCl for 15 to 30 seconds and then kept in lacto-aceto-orcein for one hour. The stained salivary glands were squashed on a slide in 75% lacto-acetic acid (1:1.5). The inversion loops were identified microscopically and photographed for detailed comparisons.

RESULTS

The genetic variation of mitochondrial DNA (mtDNA) in these two species was examined by digestion using four restriction enzymes (*Eco*R I, *Hin*d III, *Pst* I, and *Hpa* II). All of the stocks indicated in the 'S' columns of Table 1 were checked by the restriction digestion of *Eco*R I. One hundred and sixteen *Drosophila immigrans* stocks and 53 *D. formosana* stocks were checked by *Hin*d III digestion. One hundred and fourteen *D. immigrans* stocks and 34 *D. formosana* stocks were checked by *Pst* I and *Hpa* II.

The representative restriction patterns of *D. immigrans* and *D. formosana* stocks are shown in Fig. 1. Among the *D. immigrans* stocks, including the foreign ones, 19 restriction sites on the mtDNA were recognized by these four enzymes. Three stocks with a 0.1 kilo-bases(Kb) smaller mtDNA (Fig. 2) among 326 *D. immigrans* were collected from Alishan, Tongpu, and Shihcho in Taiwan. Out of the 114 stocks only one has a second *Hpa* II restriction pattern (Fig. 1).

As for *D. formosana*, a total of 17 different restriction sites were detected by using these four restriction endonucleases. Eight out of the 103 stocks contained a 0.3 Kb larger mtDNA (Fig. 2). Two of them were from Hoshe, two Wulai, one Tongpu, one Fenchihu, one Chitou, and one Shihcho. There is a second *Pst* I restriction pattern,

Table 1. Number of captured flies or isofemale stocks whose esterase or mtDNA restriction patterns were checked

Locality	*Drosophila immigrans*			*D. formosana*		
	M[1]	F[2]	S[3]	M	F	S
TAIWAN						
Alishan, Chiayi (Alishan, Jiayi)[4]	0	0	27	–[5]	–	–
Chiayi City (Jiayi City)	0	0	0	0	2	2
Chitou, Nantou (Shitou, Nantou)	30	71 + 4*	9	46	114	12
Chungtou, Ilan (Jiuntou, Ilan)	0	0	0	0	1	1
Fenchihu, Chiayi (Fenchihu, Jiayi)	0	109	109	0	8	8
Hoshe, Nantou (Hoshe, Nantou)	0	32	32	0	29	29
Kuantzuling, Tainan (Guantzling, Tainan)	0	32	31	0	16	14
Paileng, Taichung (Baileng, Taijung)	0	1*	1	0	0	0
Shihcho, Chiayi (Shrjuo, Jiayi)	0	25	25	0	3	3
Taipingshan, Ilan (Taipingshan, Ilan)	12	15	6	0	1	0
Tongpu, Nantou (Dungpu, Nantou)	0	323 + 2*	20	0	11	10
Wulai, Taipei (Wulai, Taibei)	224	187 + 4*	64	76	95	22
Wuling, Taichung (Wuling, Taijung)	0	2	2	0	2	2
USA						
Winters, California[6]	19	11*	11	–	–	–
Vietnam						
Hanoi[7]	0	2*	2	–	–	–
Venezuela						
Caripe[8]	0	1*	1	–	–	–
Colombia						
Palmira[8]	0	1*	1	–	–	–
Nepal						
Patan[8]	0	1*	1	–	–	–
Total	285	823	342	122	282	103

[1]M = number of male flies used for the esterase survey.
[2]F = number of female flies or stocks (marked with *) used for the esterase survey.
[3]S = number of stocks used for the mtDNA survey.
[4]Spelling in parentheses is officially used by the Education Administration of R.O.C. since 1984, in contrast to the romanization. Chinese names of these localities are attached as appendix.
[5]"–" indicates no *D. formosana* in these localities.
[6]Ten isofemale stocks from Dr. M. Turelli, Section of Evolution and Ecology, University of California, Davis, were established in 1993; one stock was established from an isofemale collected by Dr. W. Anderson in 1983.
[7]Two isofemale stocks from the Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.
[8]Three stocks from the National Drosophila Species Resource Center, Bowling Green, Ohio, U.S.A.

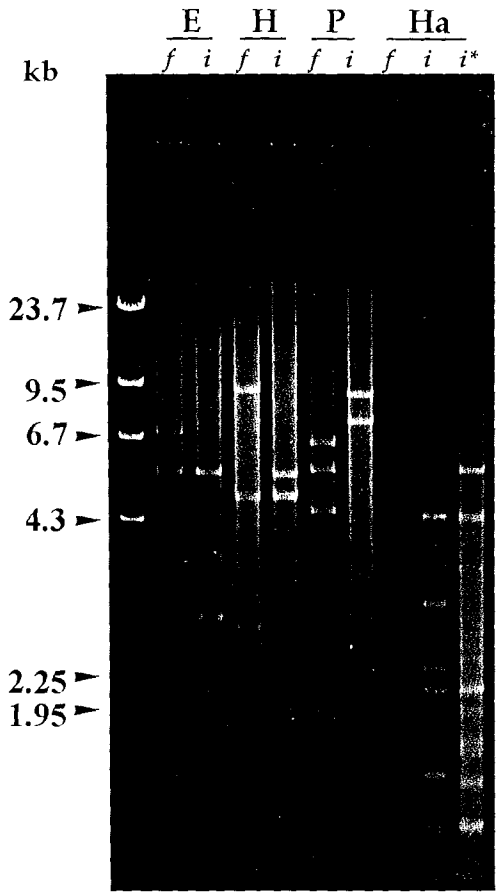

Fig. 1. The *Eco*R I (E), *Hind* III (H), *Pst* I (P), and *Hpa* II (Ha) restriction patterns of the mitochondrial DNA of *Drosophila formosana* (*f*) and *D. immigrans* (*i*). The right-most lane is the varient *Hpa* II pattern of *D. immigrans* (*i**). The left-most lane is the lambda DNA digested with *Hind* III as size markers.

different from the standard by the loss of one restriction site (Fig. 2), presented by eight among 34 stocks. Five among the 34 stocks has another *Hpa* II pattern which has one less restriction site compared to the standard. Only one among the 34 stocks had both the rare *Pst* I and the rare *Hpa* II patterns; thus it had 2 less restriction sites in total, compared to the standard pattern.

The mtDNA restriction variation in Taiwanese *D. immigrans* and *D. formosana* populations is summarized in Table 2. The sample size, indicated in parentheses, is the number of haplotypes checked, equal to the number of isofemale stocks checked. Since there was one *D. formosana* stock with both variant *Pst* I pattern and *Hpa* II pattern, the frequency was not additive in this case.

The esterase zymogram (Fig. 3) showed two zones representing enzymes encoded from different loci. The upper bands are dimer proteins and the lower bands are monomer proteins, according to the triple and double bands of heterozygotes, respectively. The standard dimer and monomer gene product from *D. immigrans* moves faster on an electrophoretic gel than those from *D. formosana*.

In order to understand the genetic variability of these esterases, the isozyme patterns of stocks established long ago, and of flies collected recently, were examined (Table 1). All stocks including the foreign ones of *D. immigrans* are homogeneous and have the common esterase type. Genetic variation, at a low level, does exist in the natural Taiwan populations. The monomer esterase of *D. immigrans* is monomorphic, whereas that of *D. formosana* is polymorphic with 3 alleles, *F*, *S*, and *S′*. The *F* and *S* alleles are common alleles in every population. The frequency of the *F* allele is 0.43 (N = 171) of the Wulai population, 0.45 (N = 160) of the Chitou population, 0.45 (N = 352) on average. The *S′* allele is a rare one which has been found in Chitou, Wulai, and Kuantzuling (0.006, N = 352). The difference of allelic frequency between Wulai and Chitou populations is statistically insignificant. In contrast, the dimer esterases of both species are nearly monomorphic. *D. im-*

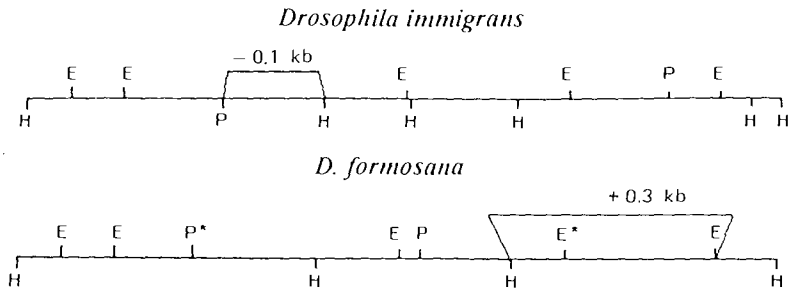

Fig. 2. Diagram of the restriction patterns and the size differences obtained with endonucleases *Eco*R I (E), *Hind* III (H), and *Pst* I (P) in *Drosophila immigrans* and *D. formosana* mtDNA. These three enzymes generated a total of 9 and 10 restriction sites in *D. immigrans* and *D. formosana*. Only a 0.1 Kb smaller mtDNA have been detected in *D. immigrans*, and a 0.3 Kb larger mtDNA and 2 restriction differences (marked with *) have been detected in *D. formosana*.

migrans possesses a rare allele found under a heterozygous condition in one out of 323 Tongpu females. A pure line established from that isofemale stock, and the homo-dimer encoded by this rare allele, runs faster than the common one on an electrophoretic gel (Fig. 3). In addition, a dimer null allele (*n*) was found in Chitou and Tongpu. The dimer esterase of *D. formosana* also has a faster allele and a null one with low frequencies. The only heterozygotous female with the faster dimer allele was found in the Kuantzuling population. A pure line with the rare alleles was established from this isofemale stock. The *n* alleles was found in one isofemale stock from the Wulai population.

The esterase variation in the populations of both species is summarized in Table 2. The sample size, indicated in parentheses, is the number of alleles checked, which is twice the number of individuals checked. The frequencies of these dimer rare alleles were very low and were therefore indicated in the form of common fractions.

Salivary gland chromosomes of the F_1 offspring from the captured flies were examined. Eight to ten chromosome sets from each isofemale stock collected from Chitou (31 *D. immigrans* and 30 *D. formosana* isofemales) and Wulai (30 *D. immigrans* and 20 *D. formosana*) were checked for chromosome inversion loops. The initial two sets of chromosomes in each isofemale line could be determined from their F_1 offspring. Due to the low frequency of these chromosome inversions, the existence of an inversion loop in the F_1 offspring indicates a cross between a homozygote with the standard chromosome type and a heterozygote.

The pure line with the standard chromosome type of *D. formosana* and that of *D. immigrans* have been established in the laboratory. Males (19 *D. immigrans* and 12 *D. formosana*) collected from Wulai were crossed with virgin flies from the pure line of the same species with the standard chromo-

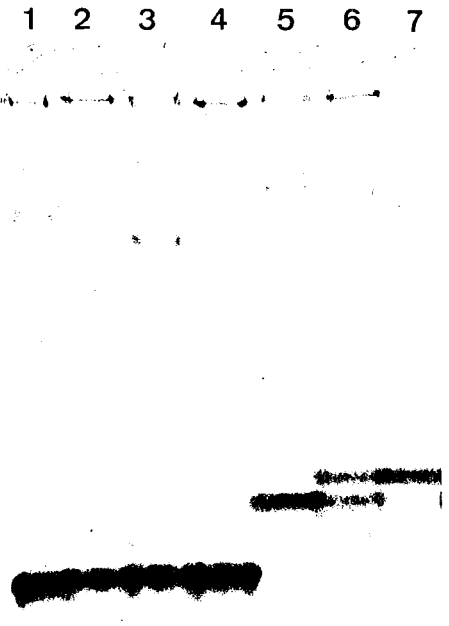

Fig. 3. The representive esterase patterns of *Drosophila immigrans* (lane 1, D^{SS}; lane 2, D^{FS}; lane 3, D^{FF}; lane 4, D^{nn}) and *D. formosana* (lane 5, $D^{SS}M^{FF}$; lane 6, $D^{FS}M^{FS}$; lane 7, $D^{FF}M^{SS}$). *D* and *M* indicate the dimer and monomer loci, respectively.

Table 2. A summary of genetic variation in Taiwanese populations of *Drosophila immigrans* and *D. fomosana*, sample sizes in parentheses

		Drosophila immigrans	*D. formosana*
MtDNA restriction patterns			
	Common type	0.982 (326)	0.639 (103)
	Size variant	−0.1 kb: 0.009 (326)	0.3 kb: 0.078 (103)
	Size variant	−*Hpa* II site: 0.009 (114)	−*Pst* I site: 0.235 (34)
			−*Hpa* II site: 0.147 (34)
Esterase loci			
	Monomer	common: 1 (2126)	*F*: 0.450 (808)
			S: 0.540 (808)
			S': 0.006 (808)
	Dimer	*S*: ~1 (2126)	*S*: ~1 (808)
		F: 1/2126	*F*: 1/808
		n: 2/2126	*n*: 1/808
Chromosome arrangements			
		standard chr. II: 0.975 (282)	standard chr. III: 0.986 (224)
		inverted chr. II: 0.025 (282)	inverted chr. III: 0.004 (224)

some type. Eight to ten chromosome sets from each cross had been analyzed; therefore, the two chromosomes from the initial male can be determined.

Only one kind of inversion loop on the second chromosome was detected in *D. immigrans*, and also only one kind of inversion loop on the third chromosome was found in *D. formosana* (Fig. 4). In the *D. immigrans* populations, 6 among the 31 isofemale stocks from Chitou, and one among the 30 isofemale stocks from Wulai, had the inversion. The inversion heterozygosity was estimated as 6 among 62 and 1 among 60, respectively. The fact that no offspring of the males from Wulai contained any inversion indicated that none of the 19 males was a heterozygote. Since the difference among these three samples are not statistically significant ($\chi^2 = 5.29$, $df = 2$, $p > 0.05$), the observed heterozygosity can be indirectly estimated as 5% (7/141). In the *D. formosana* populations, one among the 30 isofemale stocks from Chitou had the inversion. Neither the 20 *D. formosana* stocks nor the 12 males from Wulai contained any inversion. Heterozygosity is estimated to be 0.9% (1/112). The sample sizes indicated in Table 2 are the estimated numbers of chromosome surveyed. Since both the inversion types are rare, the frequencies are half of the heterozygosity.

The foreign *D. immigrans* could be crossed to the local flies; therefore, the salivary gland chromosomes of hybrid larvae were examined. The 16 foreign *D. immigrans* are all genetically homogeneous, and their esterase pattern, mtDNA restriction patterns, and salivary gland chromosome sequence proved to be the same as those of the local *D. immigrans*.

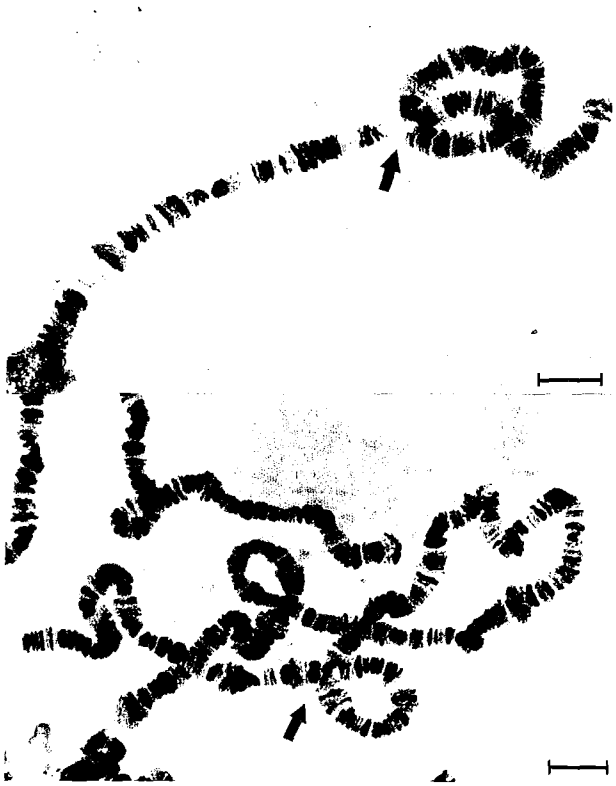

Fig. 4. Inversion loops on salivary gland chromosomes of *Drosophila immigrans* (upper) and *D. formosana* (lower). The inversion of *D. immigrans* is located on the left arm of the second chromosome with a frequency of 2.5%. That of *D. formosana* is located on the third chromosome with a frequency of 0.45%. (Scale bars indicate 10 μl)

DISCUSSION

The mitochondrial DNA (mtDNA) variation of the local populations of both species is revealed according to their restriction pattern analysis. The only restriction site variation (with a frequency of 0.9%) in *Drosophila immigrans* was the absence of one among 19 sites. The restriction site variation of *D. formosana* involved the loss of two among 17 sites (which could occur singly or simultaneously) (Fig. 2). The size difference variants also have been observed, i.e., *D. immigrans* contains a smaller sized mtDNA; *D. formosana* contains a larger sized mtDNA. Although the frequency of the size difference variants is very low, they are not detected in *D. albomicans* which has highly polymorphic mtDNA restriction sites (Chang et al. 1988). The mtDNA restriction patterns of *D. albomicans* are so highly polymorphic that flies collected from a single bait could have more than one mtDNA morph (Chang et al. 1988). Fourteen restriction mtDNA morphs had been found in *D. albomicans* by the use of only two restriction enzymes: *Eco*R I and *Hin*d III. The mtDNA restriction patterns showed that the genetic variation of *D. immigrans* and *D. formosana* is much lower than that of *D. albomicans* and it exhibits a different pattern (i.e., size difference vs. base substitution). From the mtDNA restriction patterns of the 326 *D. immigrans* isofemale stocks and the 103 *D. formosana* stocks we did not find any sign of introgression although these two species can be crossed in the laboratory (Lin and Yeh 1980).

The females of *D. immigrans* and *D. formosana* are morphologically indistinguishable; morphologically similar males could only be distinguished by the different hairs on their fore-tarsi. The degree of biochemical difference is similar. Although these two species have many similar isozyme patterns (unpublished data) due, probably, to their common

ancestry, the profound difference between their esterase electromorphs provides a useful and easy way to identify larvae or female flies of these two sibling species. Otherwise, identification of captured females of this species pair has to await the emergence of their male offspring. Using this esterase criterion, we have clearly identified over one thousand *D. immigrans* and over four hundred *D. formosana* flies. This also confirms that there is no introgression between this sibling pair although hybrids produced in the laboratory are partially fertile (Lin and Yeh 1980).

Esterase isozymes are highly polymorphic in some other species of the same species group, such as *D. albomicans* (Chang and Lin 1990) and *D. ruberrima* (unpublished data). The monomer esterase locus of *D. ruberrima* has 6 visible variant alleles plus a null one, but it has only three alleles in *D. formosana* and is monomorphic in *D. immigrans*. The dimer esterase locus is equivalent to the esterase locus *Est-F* in *D. albomicans*. The *Est-F* locus has 4 visible variant alleles plus a null one in *D. albomicans* and the observed heterozygosity is 0.53. The equivalent dimer locus in *D. ruberrima* is also variable with 4 visibly variant alleles with high heterozygosity. The dimer locus of *D. immigrans* contains a null allele and a rare visibly different allele at very low frequencies; the same holds true for *D. formosana*. One heterozygote with the visibly different allele has been found in the Tongpu population of *D. immigrans*; the observed heterozygosity was in this sample 0.003 (N = 325). Possibly the rare allele was not found in most other populations because of the smaller sample size. Still it was not found in the Wulai population which had an even larger sample size (N = 415). The same situation held for *D. formosana*. One heterozygote with the visibly different allele was found in a sample of 16 females from the Kuantzuling population. But it was not found in the Chitou and Wulai populations with sample sizes of 160 and 171 respectively. Since only one heterozygote was found in each case, and if the Tongpu *D. immigrans* population and the Kuantzuling *D. formosana* population are not special, the real heterozygosities of these two rare alleles in natural populations must be lower than that which we observed in these two local populations. Both the number of alleles and the heterozygosity of esterases showed that the genetic variability of *D. immigrans* and *D. formosana* is much lower than that of *D. albomicans* or *D. ruberrima*.

Taiwanese *D. immigrans* populations contain only one chromosome inversion type, with low frequency. This long median inversion on the left arm of the second chromosome is the "A" inversion type according to Brncic's nomenclature (1955). *D. formosana* has one in low frequency, whereas *D. albomicans* contains 91 inversions (Lin et al. 1977). Apparently, both the number of inversion types, and the inversion heterozygosity of *D. immigrans* and *D. formosana* are much lower than those of *D. albomicans* which is a member of the *D. nasuta* subgroup that displays a high degree of polymorphism for paracentric inversions in natural populations (Wilson et al. 1969, Ranganath and Krishnamurthy 1975, Balwin 1982, Clyde 1982, Lin and Chang 1986, Casu 1990).

All three indicators (mtDNAs restriction patterns, esterase isozyme electromorphs, and chromosome inversions) showed that *D. immigrans* and *D. formosana* are genetically much less variable compared to *D. albomicans*. If the discussion were to stop here, it might suggest that the low variability is due to phylogenetic constraint. In other words, the historical background may not have contained much polymorphism in the lineage of this species pair. However, the isozyme survey (Rao and Ranganath 1990) of the Indian *D. formosana* population falsified this hypothesis. According to their analysis of the variation of eight isozymes controlled by 10 loci, *D. formosana* has a much higher degree of polymorphism than does *D. nasuta* in India. Even if we only look at the esterases, *D. formosana* still is more variable than *D. nasuta*. Therefore, the low variation of *D. formosana* in Taiwan can not be attributed to phylogenetic constraint. The alternative possibility is that the Taiwanese *D. formosana* population is still young. Genetic drift and insufficient time for the accumulation of mutations may explain its low genetic variability.

Four paracentric inversions have been recorded in *D. immigrans*. One of them is probably endemic to the Hawaiian islands (Paik and Sung 1974). Three of them, Inversions "A", "B", and "C", named by Brncic (1955), are widely distributed throughout the world. All three "cosmopolitan" inversions have been found in Chile (Brncic 1955), the Hawaiian islands (Richmond and Dobzhansky 1968, Paik and Sung 1974), Japan (Hirumi 1961), and Korea (Paik 1973). Two inversions "A" and "B" have been found in the populations near the Strait of Magellan (Brncic 1991). Only inversion "A" has been found in Brazil (with heterozygosity 19.6%) (Freire-Maia et al. 1953), India (Singh and Gupta 1979), Israel (33.9%) (Gruber 1958), and Taiwan (5%). Although the frequency of inversion

"A" in Taiwan is closer to that in Japan and Korea (7.2% and 7.7% respectively), the population here does not contain inversion "B", which has higher heterozygosity values (11.8% and 10.4%, respectively) in those two areas. Chromosome polymorphism data showed that the population in Japan is similar to that in Korea, but that the Taiwanese population remains different from these two. The difference may be due to either a founder effect during the establishment of this population in Taiwan, or to the dissimilarities in environmental conditions between Taiwan, Korea, and Japan.

According to the variation of eight isozymes controlled by 14 loci, the Indian *D. immigrans* populations showed polymorphism at six loci with an average heterozytosity of 15%, and no genetic differentiation among the four populations (Parkash and Yadav 1989). *D. immigrans* is a rare species in Hungary. The alcohol dehydrogenase, octanol dehydrogenase, and malate dehydrogenase-M of the Hungarian population are monomorphic; but malate dehydrogenase-S is polymorphic with a 6.7% heterozygosity (Pecsenye 1987). The Indian population seems to be more polymorphic than the Hungarian one. According to our study of esterases, the monomer esterase locus is monomorphic in Taiwan with the total sample size of 1,063, while it is polymorphic in the Indian populations with an average heterozygosity of 36%. Since the Californian stocks we sampled showed the same esterase pattern, mtDNA restriction patterns, and salivary gland chromosome sequence, it is likely that the spread of this cosmopolitan species may be a recent event and the Indian populations are probably older.

In conclusion, the low genetic variation of the Taiwanese *D. immigrans* and *D. formosana* revealed by the three different indicators may be due both to the founder effect and to their recent establishment in Taiwan. Australian *D. buzzatii* was reported to have no mtDNA variation (neither size nor restriction-site) (Halliburton and Barker 1993), a low level of allozyme variation (Barker and Mulley 1976), and low chromosome inversion frequency (Carson and Wasserman 1965, Knibb et al. 1987). It was suggested that a founder effect with only a few individuals colonizing Australia between 1931 and 1936 has caused the low genetic variation. But, for the time being no information is available to indicate the time of the establishment of the sibling species pair in Taiwan.

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Appendix:

The Chinese collected localities used in the context in contrast to their Romanizations are listed as follows:

Romanization	Chinese	Romanization	Chinese	Romanization	Chinese
Alishan, Chiayi	阿里山	Hoshe, Nantou	和社	Tongpu, Nantou	東埔
Chiayi City	嘉義市	Kuantzuling, Tainan	關仔嶺	Wulai, Taipei	烏來
Chitou, Nantou	溪頭	Paileng, Taichung	白冷	Wuling, Taichung	武陵
Chungtou, Ilan	圳頭	Shihcho, Chiayi	石卓		
Fenchihu, Chiayi	奮起湖	Taipingshan, Ilan	太平山		

大果蠅(*Drosophila immigrans*)與臺灣大果蠅(*D. formosana*)之低遺傳變異性

張 慧 羽[1,2] 丁 照 棣[1] 林 飛 棧[2]

不論用粒腺體DNA、染色體逆位或酯酶的異構酶來當指標，同胞種臺灣大果蠅(*Drosophila formosana*)和大果蠅(*D. immigrans*)均呈現極低的遺傳變異性。這個結果和紅果蠅(*D. albomicans*)相比，可以得到下面的推論：(1)這三個指標在顯示遺傳變異性上是一致的；(2)這一對同胞種的低遺傳變異性是由於這二種在臺灣立足時爲小族群及在臺灣的歷史不長所致，而不是受環境限制所塑造的。此外，由調查中顯示，此二同胞種雖然在實驗室可雜交且雜交後代部份可孕，但由粒腺體DNA及酯酶電泳二項指標都顯示自然族群無遺傳漸滲(Introgression)的現象。

關鍵字：染色體逆位，酯酶，拓荒者效應，遺傳變異，粒腺體DNA限制酶切型。

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