Zoological Studies **36**(3): 251-259 (1997)

Zoological Studies

Karyotype Polymorphism in Hybrid Populations of *Drosophila nasuta* and *D. albomicans*

Yuh-Chyn Yu[1], Fei-Jann Lin[2] and Hwei-yu Chang[1,2,*]

[1]*Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan 106, R.O.C.*
[2]*Institute of Zoology, Academia Sinica, Taipei, Taiwan 115, R.O.C.*

(Accepted May 2, 1997)

Yuh-Chyn Yu, Fei-Jann Lin and Hwei-yu Chang (1997) Karyotype polymorphism in hybrid populations of *Drosophila nasuta* and *D. albomicans*. *Zoological Studies* **36**(3): 251-259. *Drosophila albomicans* (2*n* = 6) and *D. nasuta* (2*n* = 8) are 2 sibling species with indistinguishable morphology; but distinct karyotypes. The former is distributed from Japan through Taiwan to Thailand, and the latter from India to the east coast of Africa. Although they are completely crossable in the laboratory, no polymorphic karyotype has ever been found in any one population of these two species in nature. In this report, 2 questions are addressed. First, does karyotype polymorphism exist in hybrid populations of these 2 species after long-term cultivation? Second, do the chromosomes originating from the same species interact with each other during their transmission from generation to generation in hybrid offspring? To answer these questions, 22 interspecific hybrid strains were established. Each strain was initiated by crossing one *D. nasuta* male and one *D. albomicans* female. Their hybrid offsprings were maintained with the non-overlapping generation method for 2.5 y (i.e., 45 generations) and then subjected to karyotype analysis. The origins of the 3rd, 4th, and sex chromosomes in the hybrid offspring were identified by larval karyotyping, while an esterase electrophoretic technique was applied for distinguishing the origin of the 2nd chromosome. Among these hybrid offsprings, 92% of the 4th chromosomes and 38% of the 2nd chromosomes are derived from *D. albomicans*. The frequencies of the 4th and the 2nd chromosomes are significantly different from their initial values (50%), therefore, we may conclude that chromosome 4 is leaning to *D. albomicans*, and chromosome 2 to *D. nasuta* in hybrid strains. On the contrary, 72.2% of the 3rd and X chromosomes (70.3% in male and 72.8% in female) are derived from *D. albomicans*, which is close to the initial value (66.7%). Our data clearly reveal that karyotype polymorphism exists in hybrid offspring after long-term cultivation, and the evolution of the 3rd and X chromosomes is apparently not associated with other chromosomes.

Key words: Hybridization, Karyotype selection.

Drosophila albomicans and *D. nasuta* belong to the *D. nasuta* subgroup of the *D. immigrans* species group. Some species of the *D. nasuta* subgroup can produce viable or fertile hybrids and hence this species subgroup is used for many aspects of population genetic studies. All taxa belonging to this subgroup are almost identical morphologically but have various degrees of reproductive isolation. Among them, *D. albomicans* and *D. nasuta* are not only morphologically indistinguishable, but also they show little reproductive isolation under laboratory manipulation. Thus, it is disputable whether these 2 taxa should be classified as 2 species (Duda 1940), or 2 subspecies, *D. nasuta nasuta* and *D. n. albomicana* (Nirmala and Krishnamurthy 1972). Based on extensive morphometric, reproductive, and allozyme studies, Kitagawa et al. (1982) finally concluded that *D. nasuta* and *D. albomicans* are 2 biologically different species, and they considered them as semispecies of the *D. nasuta* complex.

During the past years, numerous studies have shown that *D. nasuta* and *D. albomicans* have different chromosomal configurations (Wilson et al. 1969, Wakahama et al. 1971, Nirmala and Krishnamurthy 1972). The karyotype of *D. nasuta* (2*n* =

*To whom correspondence and reprint requests should be addressed. E-mail: hwei@gate.sinica.edu.tw

8) consists of an acrocentric X chromosome, a submetacentric (J-shaped) Y, a metacentric 2nd, a large acrocentric 3rd, and a small dot-like 4th chromosomes. In *D. albomicans* ($2n$ = 6), a large metacentric neo-X or neo-Y chromosome constitutes about 60% of its nuclear genome. The metacentric 2nd chromosome of *D. albomicans* is the same as that of *D. nasuta*. Although *D. albomicans* has only 3 pairs of chromosomes, the nomenclature used follows that of *D. nasuta* (i.e., the 3rd pair of chromosomes is also called the 4th in *D. albomicans*). As compared to *D. nasuta*, the dot-like 4th chromosome of *D. albomicans* is larger because of heterochromatin accumulation (Wang et al. 1988). In addition, the karyotypes are different in many other aspects, such as heterochromatin distribution and differentiation, positions of nucleolar organizer regions, patterns of C-bands and Z-bands (Ranganath et al. 1982 1983 1986).

It has been suggested that the large neo-X or neo-Y chromosome of *D. albomicans* possibly evolved by fusion of the ancestral acrocentric 3rd and sex chromosomes (Wilson et al. 1969, Wakahama et al. 1971, Nirmala and Krishnamurthy 1972). The fused chromosome is regarded as a derived state in the phylogeny of the *D. nasuta* subgroup. The fixation of a new type is usually explained by either genetic drift or natural selection. Since these 2 sibling species have a broad geographic distribution and share several chromosome inversions, such as the one named $In(2L)B_1D_5$ in *D. albomicans* (Lin et al. 1974) and IIL-2 or 2L-B in *D. nasuta* (Kumar and Gupta 1986, Ranganath and Krishnamurthy 1975, Singh and Kalisch 1991), the speciation of *D. albomicans* was unlikely initiated by a unique small population with severe genetic drift. Whether *D. albomicans* evolved through karyotype selection is an interesting question that remains to be answered. Furthermore, whether the evolution of the neo-X, neo-Y chromosome was independent from other chromosomes during transmission also remains to be investigated.

According to their collection sites, Kitagawa et al. (1982) considered them as allopatrically distributed in nature. However, they did not collect specimens from the distribution rims of these 2 species. Thus, it remains to be clarified whether these 2 species are parapatric in certain areas. Since these 2 species produce fertile hybrid offspring in the laboratory, these hybrid progenies could serve as useful material to examine their karyotype change after long-term cultivation. The purpose of this report is to examine the karyotype polymorphism in hybrid offspring of *D. nasuta* and *D. albomicans*, in order to answer the following 2 questions. Does karyotype polymorphism exist in hybrid populations of these 2 species after long term cultivation? Do chromosomes originating from the same species interact with each other in hybrid offspring?

MATERIALS AND METHODS

Drosophila strains

A *Drosophila albomicans* isofemale strain from Okinawa, Japan (strain #0163.05), and a *D. nasuta* from Mysore, India (strain #0193.7) were used in this study. Twenty-two hybrid strains were established, and each was from 1 pair of 3-d-old virgin *D. albomicans* female and *D. nasuta* male. The virgin flies were sexed within 8 h after emergence. All hybrid strains were raised by a non-overlapping generation method in a 23 °C incubator for 2.5 y. Each generation, about 100 pairs of flies for each strain were collected from 6 culture tubes, mixed and transferred to 6 new tubes with fresh medium. These flies were discarded before the adult flies of the next generation emerged. The hybrid offsprings of the 45th generation were used throughout the experiments.

Karyotype assay

The 3rd-instar larvae of hybrid strains were fed with 0.02% colchicine in yeast paste for 1.5 to 2 h. The brain ganglia were removed and soaked in a hypotonic solution (1% sodium citrate aqueous solution) for 6 min. Afterward, they were fixed in a methanol-acetic acid (3:1) solution for 1 h, and then transferred to 50 μl of 60% acetic acid. Cells were dispersed by pipetting the brain tissue up and down several times and dropping on a warm slide (50 °C) for approximately 15 s. The solution was aspirated off. The air-dried slides were stained with 5% Giemsa in phosphate buffer, pH 6.8, for 1 h.

Sex ratio determination

For each hybrid strain, at least 100 adult flies were sampled and sexed morphologically to determine their sex ratio.

Esterase analysis

Electrophoretic analysis of esterase was per-

formed as described previously (Chang and Lin 1990). In brief, each individual fly was homogenized with 20 μl distilled water in an Eppendorf tube. After centrifugation for 5 min, 10 μl supernatant was mixed with 2 μl bromophenol blue-glycerol solution, and loaded on a well of a 10% polyacrylamide slab gel. Gel was run with Tris-glycine buffer (pH 8.3) at 4 °C until the dye front reached the gel bottom. Esterase patterns were then visualized by the specific staining method described by Ayala et al. (1972).

RESULTS

Karyotype polymorphism

The karyotypes of a *D. nasuta* male, a *D.*

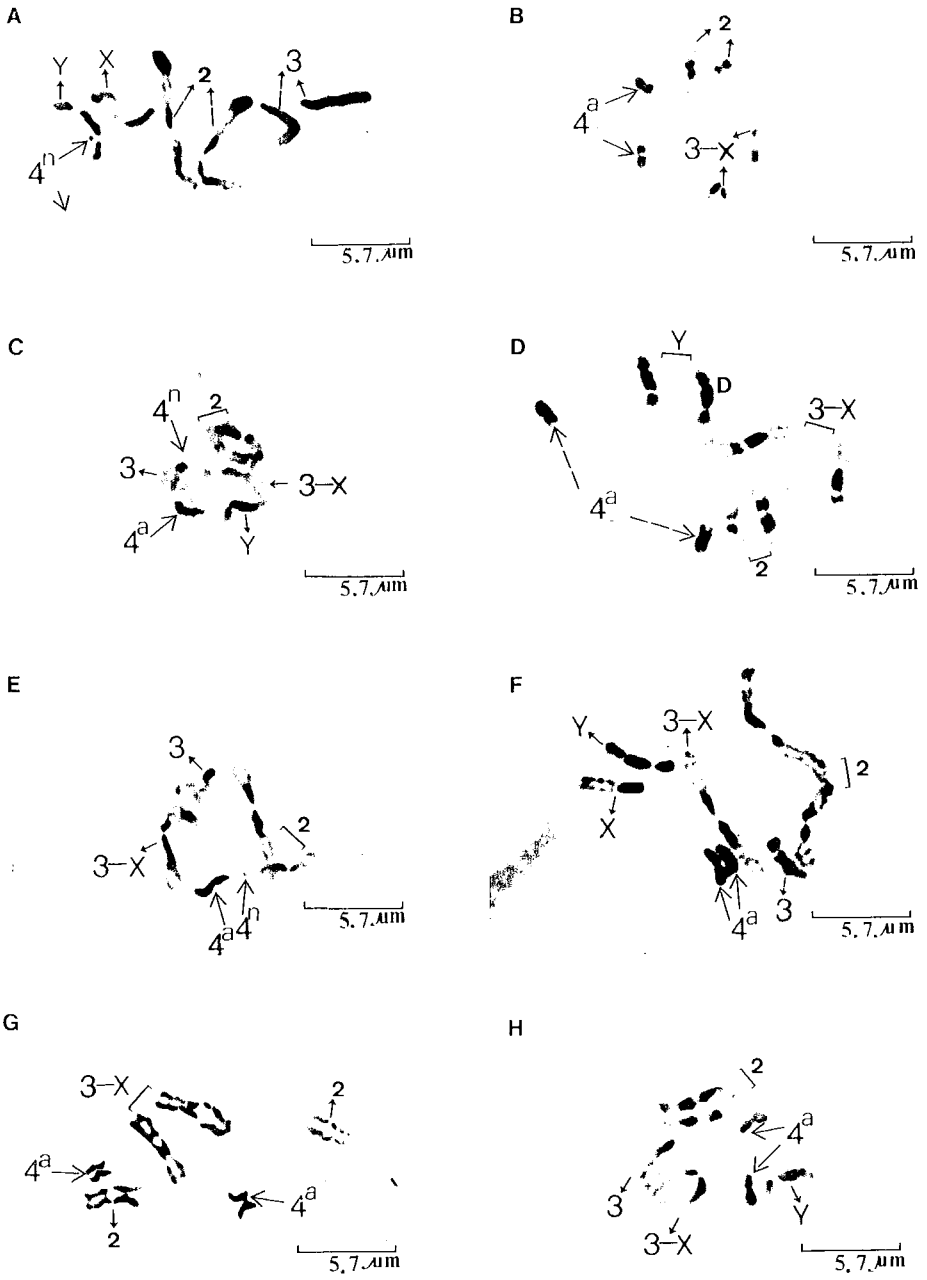

Fig. 1. Karyotypes of *Drosophila nasuta*, *D. albomicans*, and their hybrids. A: Indian *D. nasuta* ♂; B: *D. albomicans* ♀; C: hybrid F_1 ♂; D~F: aneuploids; G, H: ♀ and ♂ in a fixed strain.

Table 1. Observed and computer-simulated frequency distribution of the neo-X chromosome in 22 strains at the 45th non-overlapping generation

Observed frequency[a]	No. of strain	Frequency for different population size (pairs)					
		10	20	30	40	50	100
0	0	4.32	1.37	0.40	0.12	0.04	0
0 – 0.1	1	0.48	0.75	0.60	0.42	0.24	0.24
0.1 – 0.2	2	0.62	1.03	0.91	0.73	0.58	0.68
0.2 – 0.3	1	0.63	1.14	1.21	1.13	0.99	1.34
0.3 – 0.4	1	0.65	1.24	1.48	1.59	1.56	2.23
0.4 – 0.5	0	0.72	1.43	1.79	2.00	2.14	3.13
0.5 – 0.6	2	0.64	1.47	1.94	2.27	2.65	3.86
0.6 – 0.7	3	0.68	1.59	2.19	2.69	3.05	4.09
0.7 – 0.8	3	0.69	1.68	2.42	2.92	3.30	3.65
0.8 – 0.9	2	0.68	1.72	2.48	2.92	3.19	2.31
0.9 – 1	1	0.49	1.52	2.28	2.62	2.69	0.42
1	6	11.42	7.05	4.30	2.59	1.56	0.05
χ^2		33.29*	6.72	5.88	11.22	22.59*	719.06**

[a]from Fig. 2.
*$p < 0.05$, **$p < 0.01$, $df = 11$.

albomicans female, and their hybrid F_1 male are shown in Fig. 1 (A, B, C, respectively). From each of the 22 hybrid strains, at least 10 larvae (a total of 234 larvae) were subjected to karyotype analysis. According to the karyotype differences between *D. albomicans* and *D. nasuta*, the origins of the 3rd, 4th, and X chromosomes in hybrid offspring could be easily identified. Thirty-five out of 234 hybrid offspring (15%) were aneuploids, for instance, with 1 or 2 extra Y chromosomes in females, or no Y chromosome in males (Fig. 1D, E, F), and were excluded from the karyotype analysis. The remaining individuals with a diploid karyotype were used to estimate the frequencies of chromosomes originating from *D. albomicans*.

(1) The 3rd and X chromosomes

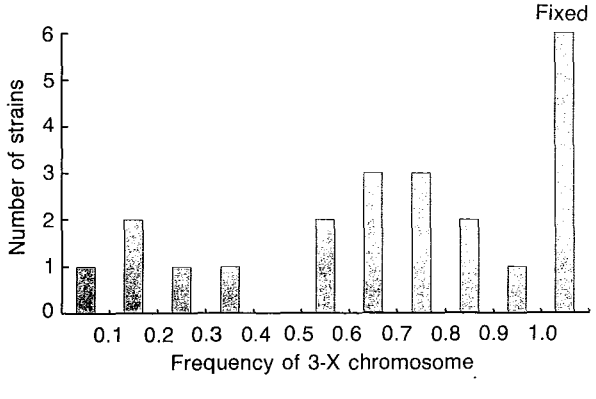

Fig. 2. Frequency distribution of the neo-X chromosome in 22 hybrid strains.

Fig. 2 shows the frequency distribution of the *albomicans* 3-X (neo-X) chromosome in 22 hybrid strains. The neo-X chromosome of 6 strains (#4, #5, #10, #12, #13, and #16) had apparently reached fixation, whereas the others remained polymorphic. The female and male karyotypes of the fixed strain are shown in Fig. 6G, H. However, the frequency of the neo-X chromosome varied from 5.5% to 90.0% among these polymorphic hybrid strains. The mean frequency of these 22 strains, including those reaching fixation, was 72.2%, which is not statistically different from the initial value (66.7%). None of the 22 strains had lost the neo-X chromosome after 2.5 y (at least 45 generations).

Several neo-X frequency distributions at the 45th generation based on different population sizes were generated by computer simulation through sampling for 100 000 times, assuming random mating and no selection. In the 1st generation, there were only 1 type of male and 1 type of female; in the 2nd generation, there were 2 male and 2 female genotypes; in the following generations, there were 2 male and 3 female genotypes. There are a total of 6 possible mating types, each of which generates offspring according to the Mendelian ratio. The frequency of a mating type depends on the frequencies of male and female genotypic frequencies. For comparison, expected neo-X frequency distributions of 22 stocks were calculated from the result of computer simulation (Table 1).

(2) The 4th chromosome

The frequency of the *albomicans* 4th chromosome is shown in Fig. 3. Eighteen strains had reached fixation to the *albomicans* type. The mean frequency (92%) was significantly different from the initial value (50%). Thus, the 4th chromosome in hybrid offspring trended forward *D. albomicans*.

Esterase electrophoresis

From each of the 22 hybrid strains, 24 adults were subjected to esterase electrophoretic analysis. *D. albomicans* and *D. nasuta* have 3 esterase loci, *Est-F*, *Est-C*, and *Est-A* (nomenclature follows Kanapi and Wheeler 1970) on their 2nd chromosome, and the esterase patterns of 2 parental strains and their hybrids are illustrated in Fig. 4. The electrophoretic patterns of EST-C and EST-A of these 2 species were easily distinguished on 10% polyacrylamide gel (Fig. 4, left), while the EST-F patterns could be differentiated on 7.5% gel (data not shown). Fortunately the homogenate from a single fly is enough for loading on 2 separate gels. Since it is impossible to show all 3 isozyme zones clearly on one gel, 8 different patterns (including 1 *albomicans*, 3 *nasuta*, 2 hybrids, and 2 recombinant patterns) observed in hybrid offspring are illustrated in the right panel of Fig. 4. The *Est-F* and *Est-C* loci are polymorphic in the *D. nasuta* stock, therefore there are 3 *nasuta* patterns.

Two hybrid strains, #9 and #13, showing recombinant types of esterase pattern, were excluded for the determination of frequency of *albomicans* 2nd chromosome (Fig. 4, right). As shown in Fig. 5, the frequency of the *albomicans* 2nd chromosome varied from 17% to 57%. The mean frequency of 37% was significantly different from the initial value of 50%. Thus, the trend of the 2nd chromosomes existing in the hybrid offspring was apparently forward *D. nasuta*.

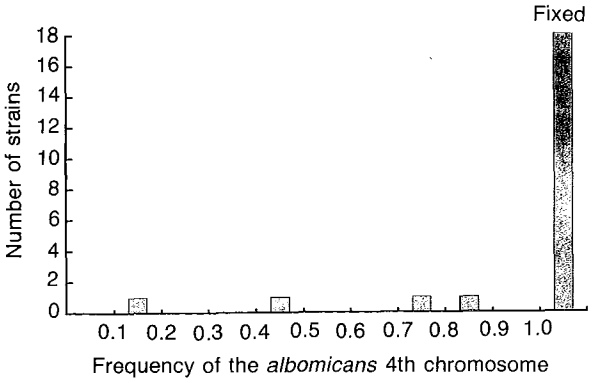

Fig. 3. Frequency distribution of the *albomicans* 4th chromosome in 22 hybrid strains.

Sex-ratio distortion

A total of 3626 adult flies from these 22 strains were sexed and their ratio of males was 0.42. The sex ratio distribution among these 22 strains is shown in Fig. 6A. Although the average sex ratio is significantly different from the expected 0.5 sex ratio ($\chi^2 = 104.44$, $df = 1$), only 12 out of the 22 strains significantly deviated from it ($\chi^2 > 3.84$, $df = 1$, $p < 0.05$) if each strain was checked separately. These 12 hybrid strains include all 6 strains with the neo-X chromosome fixation and were all

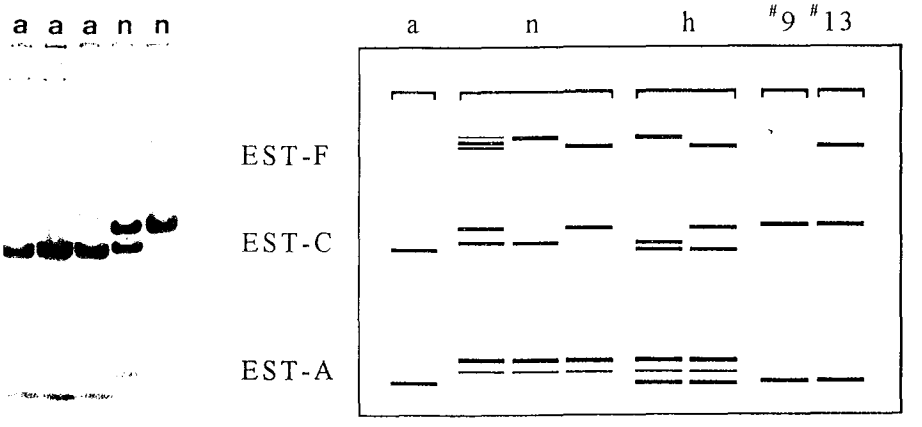

Fig. 4. Esterase patterns of *Drosophila albomicans*, *D. nasuta*, and their hybrids. Left, the esterase pattern of *D. albomicans* (a; 3 columns) and *D. nasuta* patterns (n; 2 columns) on a 10% acrylamide gel; Right, a drawing of different patterns including 1 *D. albomicans*, 3 *D. nasuta*, 2 hybrids and 2 recombinants. a: Okinawa *D. albomicans*; n: Indian *D. nasuta*; h: hybrid strains; #9, #13: hybrid strains with recombinant types.

female biased. It is important to note that the other 10 strains statistically fit the 0.5 sex ratio. In other words, 62% (10/16) of the neo-X polymorphic strains had a normal sex ratio (Fig. 6B), but 100% (6/6) of the neo-X fixed strains showed a female bias (Fig. 6C).

DISCUSSION

Drosophila nasuta and *D. albomicans* are 2 sibling species with distinct karyotypes. No karyotype polymorphism has ever been found in these 2 species in nature. Therefore, it is important to know whether chromosomal cohesion plays a role in keeping their karyotypes homogeneous within a species and hence reducing the mixing of gene pools in the natural environment. The chromosomal cohesion concept was derived from Templeton's (1989) cohesion species concept. Templeton suggested that individuals of 1 species have the potential for acquiring phenotypic cohesion through intrinsic cohesion mechanisms, including genetic and environmental factors. It is very difficult to demonstrate what the intrinsic forces are for maintaining phenotypic cohesion.

If chromosomal cohesion is responsible for preventing karyotype mixing of these 2 species, the karyotype of hybrids will soon be fixed to 1 type. We would expect to see karyotype fixation in hybrid offspring of *D. nasuta* and *D. albomicans* under experimental manipulation. The chromosomes X, Y, 3, and 4 are easily identified by their morphology in these 2 species. However, chromosome 2 was morphologically indistinguishable. Fortunately, there are 3 esterase loci located on the 2nd chromosome of these 2 species. We have demonstrated previously that the genetic distance between *Est-F* and *Est-C* was smaller than 0.13% (n = 767), and that between *Est-C* and *Est-A* was 17.3% (n = 505) in *D. albomicans* (unpubl. data). However, we also found that the recombination rate between *Est-C* and *Est-A* was 2.69% (n = 223) in hybrids of *D. albomicans* and *D. nasuta* (unpubl. data). The very low recombination rate between *Est-C* and *Est-A* suggests that crossing over of the whole chromosome in hybrids was probably inhibited, but the reason is unknown. Due to the low recombination rate, these 3 loci could serve as good indicators to trace the source of 2nd chromosomes in these hybrid strains. All 3 esterase loci are monomorphic in the Okinawa *D. albomicans* strain #0163.5, but *Est-F* contains a null allele. Although *Est-A* in the Indian *D. nasuta* strain #0193.7 is monomorphic, *Est-F* and *Est-C* are polymorphic: each contains 2 alleles. Because of this complicated situation, all 3 esterase loci have to be used as genetic markers to indicate the origin of the 2nd chromosome. Although they cannot represent the whole 2nd chromosome, they appear to be adequate indicators for a major portion of it.

Ramachandra and Ranganath (1986 1988 1990)

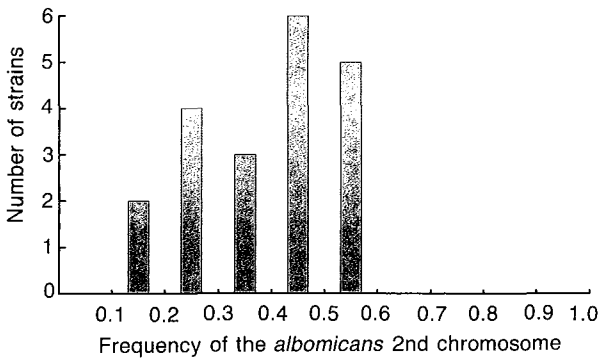

Fig. 5. Frequency distribution of the *albomicans* 2nd chromosome in 22 hybrid strains.

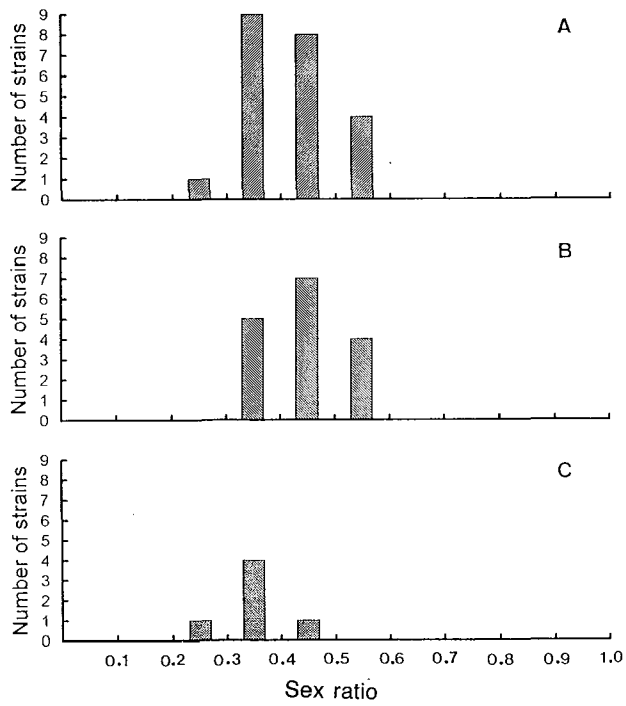

Fig. 6. Sex ratio (male/total) distribution in hybrid strains. A: distribution of the total 22 strains; B: distribution of the 16 neo-X polymorphic strains; C: distribution of the 6 neo-X fixed strains.

reported that mating between Indian *D. nasuta* and Okinawa *D. albomicans* could establish 4 stable cytoraces (Cytoraces I, II, III, and IV) in a short period. The stable cytoraces from the cross of *D. nasuta* male and *D. albomicans* female were cytorace I (male: $2n = 7$; female: $2n = 6$) and cytorace IV (male: $2n = 8$; female: $2n = 8$). Cytorace I contains the neo-X chromosome, while cytorace IV the *nasuta* 3rd and X chromosomes. They also noticed that chromosomes 2 of these cytoraces consisted of one from *D. nasuta* and the other from *D. albomicans*. According to Ramachandra and Ranganath's expectation, half of the 22 strains in our experiment should become cytorace I and the other half cytorace IV. However, our data revealed that the karyotypes of hybrid strains remained highly variable after 45 generations, 6 among the 22 became cytorace I, but the remaining 16 were polymorphic. To avoid severe genetic drift in a hybrid strain, we mixed about 200 flies from all 6 tubes of that strain and redistributed them into 6 new tubes for the next generation. A week later, the flies were discarded; therefore the population was constituted by discrete generations. Due to the prevention of severe genetic drift, no cytorace IV was formed in our experiments.

In our hybridization experiments, the hybrid progeny of each strain were established from a single pair of flies. If hybrid progeny can last for generations, the Y chromosome that came from the paternal species has to be retained in the population. In the case of the *D. nasuta* male and *D. albomicans* female cross, selection favors chromosomes which cooperate well with *nasuta* 3, Y. When the neo-X chromosome is paired with 3, Y in a hybrid individual, this individual has a higher risk of producing abnormal gametes during meiosis and aneuploids in the next generation, and hence results in genetic load in hybrid strains. The chromosomal cohesion selection should drive the neo-X chromosome away from the hybrid populations. If the cohesion selection were the only factor controlling the evolution of neo-X chromosome, we would expect to see a decreased frequency of neo-X chromosome, and the sex ratio distortion returning to normal. After long-time cultivation, 6 strains were fixed to the neo-X chromosome with a female-biased sex ratio, but none had lost the neo-X chromosome. This implies the existence of an opposite selection force favoring neo-X.

The average population size in our non-overlapping-generation rearing system was 200 flies. The computer simulation showed that the effective population size may be much smaller. The distribution is still bell shaped at the 45th generation if the population size is 100 pairs. The result shows that if genetic drift is the only influencing force, the population size should be about 30 pairs (Table 1). There are 2 reasons for this lower effective population size: the limited cultural capacity, and the sex ratio distortion. Since the χ^2 test shows that the distribution fits an expected one with a lower population size, it is probable that genetic drift is more influential than selection for the frequency change of the neo-X chromosome.

Our present results show that 15% of samples were aneuploids and 12 out of 22 strains had a female-biased sex ratio distortion. All 5 strains with neo-X frequencies lower than the initial value (Fig. 2) showed a normal sex ratio, whereas all 6 strains with fixed neo-X (Fig. 2) showed a female-biased sex ratio (Fig. 6C). This obviously indicates the relationship between high neo-X frequency and sex ratio distortion. The present data are slightly different from those of a previous hybridization experiment (Chang and Ayala 1989) which showed that: all strains remained substantially polymorphic after 20 generations; and the F_2 sex ratio distortion was restored at the F_3 generation. These discrepancies are probably due to different methods for initiating hybrid strains. In the previous study (Chang and Ayala 1989), the hybrid strains were initiated by 5 pairs of flies, but 1 pair was used in this study. If a strain were started with 1 pair of flies, there might not be enough variation for selection to work with. When the strain were started with 5 pairs of flies, selection might decrease the sex ratio distortion by decreasing neo-X frequency. The relationship of high neo-X frequency and a female-biased sex ratio distortion is obvious and can explain both the polymorphism without fixation and the sex ratio restoration in that experiment.

Furthermore, *albomicans* 2nd, neo-X, and 4th chromosomes have completely different fates in hybrid populations (Figs. 5, 2, 3). The distribution of *albomicans* 2nd chromosome indicates that the 2nd chromosome evolves under a balancing selection (Fig. 5). In fact, there are 4 strains showing a significant excess of heterozygotes. Since the mean frequency of *albomicans* 2nd chromosome is smaller than 0.5, the homozygotes of *albomicans* 2nd chromosome are inferior to those of *nasuta* 2nd chromosome. As for the trend of the neo-X chromosomes, it is highly variable mainly due to genetic drift (Fig. 2). In contrast, 18 out of 22 strains were fixed to the larger *albomicans* 4th chromosome, indicating a directional selection. The remaining 4 polymorphic strains with scattered

frequency distribution could be due to the effect of genetic drift which is weaker than the directional selection force in this case.

If we further examine the *albomicans* 2nd and neo-X chromosome frequencies, 3 strains have higher frequencies than the initial values on both *albomicans* 2nd and neo-X chromosomes, 7 strains have lower frequencies on both, and 10 strains have 1 frequency higher but the other lower. These data indicate that the increase or decrease in frequency of non-homologous chromosomes is apparently not in parallel. In other words, the cohesion selection, even if it existed, cannot be an important factor affecting karyotype evolution in hybrid populations.

From the observation of hybrid strains the following conclusions can be made: chromosomal cohesion in either *D. nasuta* or *D. albomicans* is unlikely to be important in maintaining karyotype homogeneity, and each pair of chromosomes has its own fate in hybrid populations. Therefore, we can take advantage of the property that each pair of chromosomes does not influence other non-homologous chromosomes, we can then focus our attention on the changes of the 3rd and sex chromosomes to investigate the mechanism of the fixation of neo-X and neo-Y chromosomes during the speciation of *D. albomicans*. This study is presently underway.

Acknowledgements: We thank Professor Fushing Hsieh and Mr. Ting-Li Chen of the Department of Mathematics, National Taiwan University, for computer simulation. This work was supported by the National Science Council of the Republic of China (Grant No. NSC-85-2321-B002-024).

REFERENCES

Ayala FJ, JR Powell, ML Tracey, CA Mourao, S Perez-Salas. 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. Genetics **70:** 113-139.

Chang H, FJ Ayala. 1989. On the origin of incipient reproductive isolation: the case of *Drosophila albomicans* and *D. nasuta*. Evolution **43:** 1614-1624.

Chang H, FJ Lin. 1990. On the polymorphic *Esterase-F* locus in *Drosophila albomicans*. Bull. Inst. Zool., Acad. Sinica **29:** 81-88.

Duda O. 1940. Revision der afrikanischen Drosophiliden (Diptera), II. Ann. Mus. Nat. Hung. **33:** 19-53.

Giles RE, H Blance, HM Cann, GC Wallace. 1980. Maternal inheritance of human mitochondrial DNA. Proc. Natl. Acad. Sci. USA **77:** 6715-6719.

Hagele K, HA Ranganath. 1982. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. nasuta albomicana*. II. Differences between their microchromosomes. Chromosoma **85:** 215-220.

Kanapi CB, MR Wheeler. 1970. Comparative isozyme patterns in three species of the *D. nasuta* complex. Texas Rep. Biol. Med. **28:** 261-278.

Kitagawa O, KI Wakahama, Y Fuyama, Y Shimada, E Takanashi, M Hatsumi, M Uwabo, Y Mita. 1982. Genetic studies of the *Drosophila nasuta* subgroup, with notes on distribution and morphology. Jpn. J. Genet. **57:** 113-141.

Kumar A, JP Gupta. 1986. Inversion polymorphism in *Drosophila nasuta*. Drosophila Information Service **63:** 78-80.

Lin FJ, HC Tseng, TC Wang. 1974. Standard map of the salivary gland chromosomes of *Drosophila albomicans* Duda. Drosophila Information Service **51:** 42-43.

Nirmala SS, NB Krishnamurthy. 1972. *Drosophila albomicana* — a race of *Drosophila nasuta*. Drosophila Information Service **49:** 60.

Rajasekarasetty MR, L Siddaveere Gowda, NB Krishnamurthy, HA Ranganath. 1979. Population genetics of *Drosophila nasuta nasuta*, *Drosophlila nasuta albomicana* and their hybrids. I. Karyotypic mosaicism in the hybrid populations. Genetics **93:** 211-215.

Ramachandra NB, HA Ranganath. 1986. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. nasuta albomicana*. IV. Hybridization and karyotype repatterning. Chromosoma **93:** 243-248.

Ramachandra NB, HA Ranganath. 1988. Estimation of population fitness of parental races *Drosophila nasuta nasuta* and *Drosophila nasuta albomicana* and of the newly evolved Cytorace I and Cytorace II. Genome **30:** 58-62.

Ramachandra NB, HA Ranganath. 1990. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. nasuta albomicana*. V. Introgression and the evolution of new karyotypes. Zeitschrift für zoologishe Systematik und Evolutionsforschung **28:** 62-68.

Ranganath HA, K Hagele. 1982. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. nasuta albomicana*. I. Differentiation and differentiation of heterochromatin. Chromosoma **85:** 83-92.

Ranganath HA, K Hagele. 1983. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. nasuta albomicana*. III. Localization of nucleolar organizer regions. Genetica **60:** 123-128.

Ranganath HA, NB Krishnamurthy. 1975. Chromosomal polymorphism in *Drosophila nasuta*. III. Inverted gene arrangements in South Indian populations. J. Heredity **66:** 90-96.

Ranganath HA, NB Krishnamurthy. 1981. Population genetics of *Drosophila nasuta nasuta*, *Drosophila nasuta albomicana* and their hybrids. IV. Pattern of meiotic F_1 chromosome segregation. Heredity **72:** 19-21.

Ranganath HA, MR Rajasekarasetty, NB Krishnamurthy. 1974. Evolutionary status of Indian *Drosophila nasuta*. Ind. J. Hered. **6:** 19-25.

Singh OP, WE Kalisch. 1991. SSP technique applied to EM genome analysis and photo mapping in *Drosophila nasuta*. Drosophila Information Service **70:** 257-259.

Templeton AR. 1989. The meaning of species and speciation: a genetic perspective. *In* P Otte, JA Endler, eds. Speciation and its consequences. Sunderland, Massachusetts: Sinauer Association Inc., pp. 3-27.

Wakahama K, O Kitagawa, O Yamaguchi. 1971. Evolutionary and genetical studies of the *nasuta* subgroup. I. Chro-

mosomal polymorphism found in the natural populations of *D. albomicans*. Drosophila Information Service **46:** 144.
Wang TC, CC Chen, FJ Lin. 1988. Intraspecific polymorphism of karyotype in *Drosophila albomicans*. Bull. Inst. Zool., Acad. Sinica **27:** 127-131.
Wilson FD, MR Wheeler, M Harget, M Kambysellis. 1969. Cytogenetic relations in the *Drosophila nasuta* subgroup of the *immigrans* group of species. Univ. Texas Publ. **6918:** 207-254.

紅果蠅和輝顏果蠅雜交族群之染色體核型多態性

余玉琴[1] 林飛棧[2] 張慧羽[1,2]

同胞種 (sibling species) 紅果蠅(*Drosophila albomicans, 2n*=6) 與輝顏果蠅 (*D. nasuta, 2n*=8) 具有不同的染色體核型 (karyotype)，卻有相同的外型。前者的分布從日本、臺灣到泰國，後者從印度到非洲東岸。牠們在實驗室中可以很容易的雜交而且後代可繼續繁殖，但在野外卻看不到任何混雜的核型。我們試圖探討兩個問題：一、核型的多態性 (polymorphism) 是否可長久維持？二、是否可能在雜交後代中，一條染色體的頻率變化影響到來自同種的非同源 (non homologous) 染色體的頻率變化？我們以世代不重疊的累代培養方法，建立了 22 個雜交品系。每個品系最初是由一對印度的輝顏果蠅雄蟲和琉球的紅果蠅處女雌蟲在實驗室中雜交產生。將第 45 代 (兩年半之後) 幼蟲及成蟲分別作核型分析及酯酶電泳，可區辨其染色體分別來自親本的那一方。由核型的分析可顯示第三、第四及性染色體X來自紅果蠅 (癒合型的 3-X 及較大的 4) 或是來自輝顏果蠅 (原始的分離型式 3 與 X)，而酯酶電泳可供辨認第二對染色體。雜交後代的第四對和第二對染色體分別有 92% 及 38% 來自紅果蠅，這兩對染色體的組成比例與族群起始值 (50%) 作卡方分析，均有極顯著之差異。由頻率分布顯示第四對染色體受方向性選汰 (directional selection) 趨向紅果蠅的染色體，而第二對則受平衡性選汰 (balancing selection) 其平均值偏向輝顏果蠅的染色體。另外，3-X 的比例佔 72.2% (雌、雄分別爲 72.8% 及 70.3%)，此觀測值與 66.7% 的族群起始值之間，利用卡方檢測發現並無顯著性差異。這些數據顯示核型的多態性可以長久維持在雜交族群中，同時各個染色體有自己的特性，非同源染色體各受自己的選汰作用，因此我們可以設計實驗來研究紅果蠅性染色體的進化而不必顧慮其非同源染色體頻率變化的干擾，這個部分的研究正在進行中。

關鍵詞：雜交試驗，核型選汰。

[1] 國立臺灣大學植物病蟲害學系
[2] 中央研究院動物研究所