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The Fate of Neo-Sex Chromosomes in *Drosophila albomicans-nasuta* Hybrid Populations

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Yung-Yu Yang, Chun-Yen Lee, Yi-Hua Yang, Shu-Ping Huang, Te-Pin Chang, and Hwei-yu Chang (2008) The fate of neo-sex chromosomes in *Drosophila albomicans-nasuta* hybrid populations. *Zoological Studies* **47**(1): 84-95. *Drosophila albomicans* (2*n* = 6), bearing a pair of neo-sex chromosomes, can be crossed to an allopatric sibling species *D. nasuta* (2*n* = 8). We previously proposed a "stepwise chromosome evolution" hypothesis to elaborate 2 stages in the karyotype evolution of *D. albomicans* derived from the ancestral *nasuta*-like state. The 1st stage included the formation and maintenance of the fused 3-X chromosome, and the 2nd stage included the fusion of 3-Y and the increased frequency of both fused chromosomes. The 2-stage model is supported by a previous study using experimental populations with either the X- or Y-type sex chromosome fixed. In this study, we attempted to explore the fates of sex chromosomes in populations initially consisting of all 4 types of sex chromosomes (i.e., the derived 3-X and 3-Y, and the ancestral X and Y). Our present results revealed that the 3-X chromosome is capable of reaching a high frequency as 3-Y reaches fixation, and the frequency of 1 neo-sex chromosome depends on that of the other in the same population. In another set of experimental populations, the existence of a meiotic driver located on the neo-X chromosome showed no significant effect on the fate of this chromosome. The efficacy of extrapolating these results obtained from hybrid populations to the initial chromosomal dynamics of *D. albomicans* is discussed.
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Key words: Evolution, Fused chromosome, Karyotype, Meiotic driver.

Cryptic species are not distinguishable by morphology but significantly differ at the genetic level. They exist in a variety of taxonomic groups (Walker 1964, Gupta and Sundaran 1994, Baimai 1998, He et al. 2003). Among genetic variations, karyotype differentiation is frequently found among the most closely related species (Miller and Stone 1962, Wilson et al. 1969, Baimai and Chumchong 1980, He et al. 2003). Within the *Drosophila immigrans* species group, *D. albomicans* and *D. nasuta* are a pair of such species. *Drosophila albomicans* has neo-sex chromosomes (3-X and 3-Y), and *D. nasuta* contains the ancestral chromosome set (3/3, X, and Y) (Fig. 1). Interspecific mating success between these 2 species reaches 93% in the laboratory (Chang and Ayala 1989), and their fertile hybrids can be maintained for over 300 generations (Tanuja et al. 1999). However, no hybrids have ever been found in natural populations.

The hypothesis that a centric fusion between the X chromosomes and the ancestral 3rd chromosome occurs, followed by another centric fusion between the Y chromosome and the 3rd chromosome producing the karyotype of *D. albomicans* (2*n* = 6), has been proposed (Ranganath and Hägele 1981, Yu et al. 1997). Such multiple fusion events are rare, but what possibly occurred in *D. albomicans* is not unique. For example, fusions between autosomes and sex chromosomes are likely the origin of neo-sex chromosome systems

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in 5 Heteroptera species (Jacobs 2004). Comparing the examples given above, *D. albomicans* is a very young system which came into existence less than 0.5 mya (Chang et al. 1989, Bachtrog 2006). Transcription profiling (Mahesh et al. 2001) showed little degeneration in the 3-Y chromosome of *D. albomicans*.

We previously proposed a "stepwise chromosome evolution" model to explain the evolutionary processes from 2*n* = 8 to 2*n* = 6 (Yu et al. 1999). In this model, when the 3-X fusion first occurred, certain advantages must have existed to overcome structural incompatibilities in the 1st stage. Otherwise selective forces would have excluded this newly arranged chromosome. The formation of a 3-Y chromosome would subsequently have relieved the population from sex chromosome incompatibility during meiosis and led to the fixation of these 2 neo-sex chromosomes in the population. Our previous study (Yu et al. 1999) showed that the 3-X chromosome in a hybrid population together with the ancestral X and Y remained polymorphic over 10 generations, whereas the 3-Y one was soon expelled under the same conditions. This is supporting evidence for stage 1, i.e., 3-X was formed prior to 3-Y and was sustained in the population. Furthermore, the frequency of 3-Y was then fixed within a few generations under a fixed 3-X condition (Yu et al. 1999). The results of this simple experimental design support the proposed stage 2.

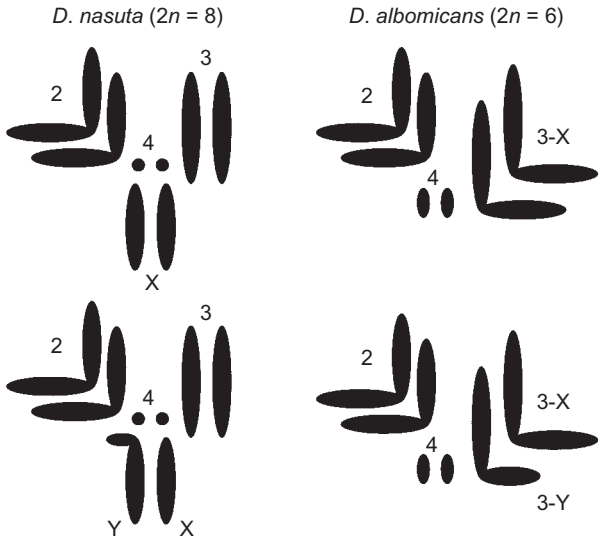

Fig. 1. Diagram of the metaphase chromosomes of *Drosophila nasuta* and *D. albomicans*: female above; male below.

The selective forces favoring a *D. albomicans*-like karyotype (2*n* = 6) are still unknown. Our previous hybridization study showed the presence of a sex-ratio distorter on the 3-X chromosome in certain populations of *D. albomicans* (Yang et al. 2004). In over 20 species of Diptera, males carrying a driver on the X chromosome have been reported to predominantly produce X-bearing sperm and hence more daughters (Wilkinson and Sanchez 2001). Theoretically, the meiotic driver should increase the frequency of the chromosome on which it is located. The role of a meiotic driver in increasing the frequency of the 3-X chromosome warrants further investigation.

In the present study, we attempted to further explore our "stepwise chromosome evolution" model by asking 2 questions: first, what is the fate of the 2 neo-sex chromosomes coexisting with the 2 ancestral sex chromosomes? And second, does a meiotic driver facilitate fixation of 3-X?

MATERIALS AND METHODS

All flies were maintained on standard cornmeal medium under conditions of a photoperiod of 12 h dark and 12 h light at 20°C and 75% relative humidity. Experimental populations were reared as non-overlapping generations. Newly emerged flies were sexed within 8 h and kept in separate vials for 5 d before crossing.

Drosophila strains

Seven *Drosophila albomicans* and 2 *D. nasuta* isofemale strains and a hybrid strain were employed in the experiments (Table 1).

Table 1. Localities and numbers of the 7 *Drosophila albomicans* and 2 *D. nasuta* strains

Species	Locality	Strain no.
D. albomicans	Ishigaki Is., Okinawa, Japan	162.4
	Nago, Okinawa, Japan	163.4
	Okayama, Japan	231.3
	Ilan, Taiwan	282.3
	Nantou, Taiwan	283.1
	Chiang Mai, Thailand	161.3
	Mae Sa, Thailand	254.29
D. nasuta	Mysore, India	193.7
	Mombassa, Kenya	252.21

Construction of experimental hybrid populations

For studying fates of each chromosome of *D. albomicans* in hybrids, we established hybrid populations through reciprocal crosses between *D. albomicans* (# 161.3) and *D. nasuta* (# 252.21). The 2 strains were chosen based on the results of Yu et al. (1999) because this combination showed the lowest frequency of the 3-X chromosome among the 3 sets in the 1st stage of the stepwise model (Yu et al. 1999). The origins of these strains are listed in table 1, and the procedure is illustrated in figure 2. In brief, reciprocal crosses between *D. albomicans* and *D. nasuta* were made, and each cross consisted of 10 vials (with 3 pairs of virgin flies in each vial). These 20 vials represented the founding population. Fifteen pairs of F_1 flies from each vial were evenly distributed into 15 new vials, i.e., each new vial contained 20 pairs of flies with 1 pair from each of the 20 initial vials. The 15 vials were randomly divided into 3 populations (E_1, E_2, and E_3) as 3 replicates. After 2 d, the flies were transferred to new vials. Therefore each population consisted of 10 vials. The collection of these 10 vials represented the G_0 generation. Afterward, each generation contained 10 vials, and each vial contained 20 pairs of flies from the previous generation (2 pairs from each vial). Each vial in the next generation contained equal numbers of flies from every vial in the previous generation. Genetic drift was reduced by evenly mixing and transferring the same number of flies from old to new vials.

Frequency of individual chromosomes in flies of hybrid populations

Karyotypes as percentages of the 3-X chromosome, the 3-Y chromosome, and the 4th chromosome in the 3 E hybrid populations were calculated at generations G_1, G_5, G_{10}, G_{20}, and G_{49} (Figs. 3-5). Three females and 3 males were sampled from each of the 10 vials, i.e., we examined the karyotypes of a total of 60 larvae for every tested generation. Except for the 2nd chromosome, other chromosomes of *D. albomicans* and *D. nasuta* are morphologically distinguishable. Three esterase loci related to a large inversion, $In(2L)B_1D_5$ (Yu et al. 1997), were adopted as a rough indicator of this chromosome. Electrophoretic patterns indicating these allozyme genotypes were used to distinguish the origin of a 2nd chromosome in hybrid offspring. Percentages of the *D. albomicans* 2nd chromosome in the 3 E hybrid populations were calculated at generations G_1, G_5, G_{10}, G_{20}, G_{43}, and G_{49} (Fig. 6). Since the karyotype was checked in G_1, the virginity of G_0 females was confirmed. Experimental populations were designed to make the initial frequency of the *D. albomicans* chromosome type 50% not only for autosomes but also for the 3-X and 3-Y chromosomes.

Effects of the meiotic driver on the rise of the 3-X chromosome

This experiment was designed to determine whether frequency changes in the 3-X chromosome differed with or without a meiotic driver on it. In this set of experiments, we established 6 hybrid populations: 3 (D populations) with and 3 (N populations) without the meiotic driver.

Identification of strains with and without the meiotic driver

Over 10 *D. albomicans* strains were checked for the existence of a meiotic driver. In brief, a *D. albomicans* virgin female and a *D. nasuta* male were crossed (the genome of *D. nasuta* lacks meiotic driver suppressors). After sib-mating of the F_1 generation, the sex ratio of the F_2 generation was checked. For each strain surveyed, we started with at least 12 original pairs to avoid artifacts due to individual variations. From each pair, 3 F_1 males and 3 F_1 females were randomly chosen and crossed, and the F_2 sex ratio was determined by counting at least 100 flies. As summarized in table 2, three *D. albomicans* strains containing the meiotic driver were chosen to establish the D populations and 3 without the meiotic driver for establishing the N populations.

Establishment of the D and N populations

We crossed the 6 *D. albomicans* strains (Table 2) with *D. nasuta* (#193.7) to establish 3 D and 3 N populations. The scheme for establishing these populations is illustrated in figure 7. For each population, 3 pairs of *D. albomicans* female and *D. nasuta* male flies were transferred to a vial, and 10 such vials constituted the initial population of 60 flies. After the emergence of F_1, 2 males from each vial were pooled (i.e., 20 males in total) and transferred to a new vial with 20 *D. nasuta* virgin females. From each cross, 10 F_1 replicates with a total of 400 flies constituted the G_0 generation. G_1 also contained 10 vials, and each vial

contained 20 pairs of flies from the G_0 generation (2 pairs from each vial). Five (D1, D2, and 3 Ns) of the 6 populations were maintained until G_{15} except for D3 which was maintained to G_{22}.

Sex ratios and sex chromosomes

The effects of the meiotic driver on the sex ratio were confirmed by examining the offspring sex ratios every generation from G_1 to G_7. At least three of 10 vials and over 100 adult flies from each vial were sampled and sexed morphologically. The sex ratio is represented as the percentage of male flies. Karyotypes of the D and N populations were checked at the G_5 and G_{12} generations. The frequencies of the 3-X, 3-Y, and 4th chromosome were determined by microscopic examination. Concomitantly, we also determined the esterase allozyme patterns of the 2nd chromosome composition in the D and N populations at the G_5 and G_{12} generations. Four females and 3 males were sampled from 5 of 10 vials of each tested experimental population, and 3 females and 4 males from the other 5 vials. The sample size for each tested generation was about 70, with 1/2 females and 1/2 males. At G_{15} and G_{22}, instead of checking the sex ratio of the population, males from the population were sampled. Each male was crossed with 5 *D. nasuta* (#193.7) females, and the sex ratio of their offspring was checked. At G_{22}, the chromosome type of a male was also determined by sacrificing one of his daughters in the larval stage. Therefore, both the offspring sex ratio and the sex chromosome type (3-X/3,Y or 3/3;X/Y) of a male were obtained.

Table 2. Hybrid F_2 sex ratios of the 6 *Drosophila albomicans* strains crossed with *D. nasuta* to establish the 3 D and 3 N populations

Population	Strain no.	F_2 sex-ratio (%) (males/total ± S.E.)	Total sample size
D1	162.4	18.3 ± 4.9	11,286
D2	163.4	18.4 ± 8.3	1686
D3	231.3	34.2 ± 6.3	5462
N1	254.29	51.5 ± 3.7	7012
N2	282.3	42.5 ± 5.4	11,585
N3	283.1	50.1 ± 7.4	1350

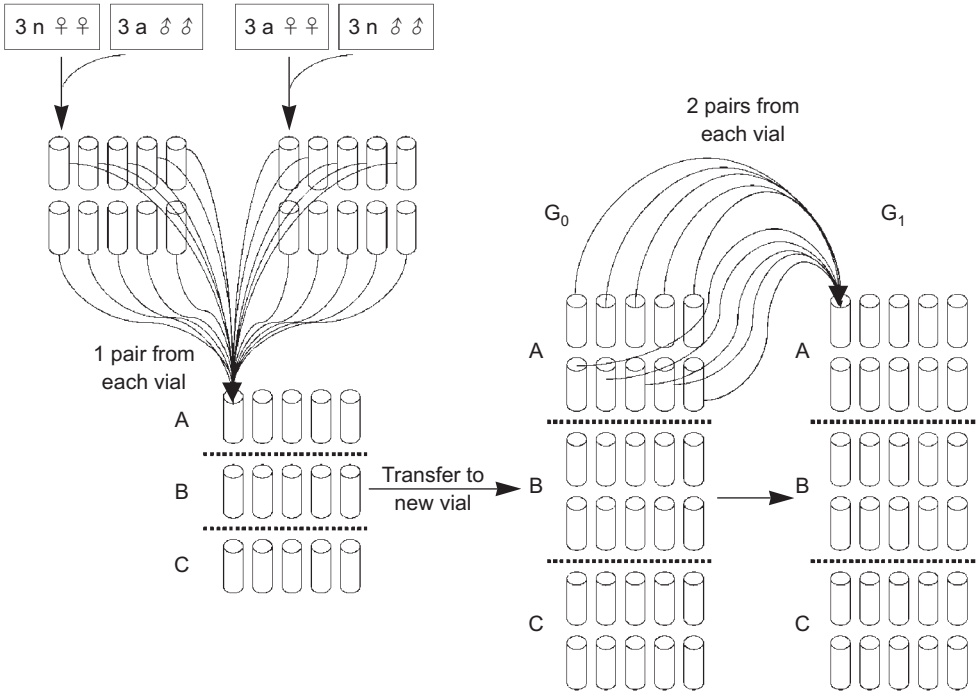

Fig. 2. Reciprocal crosses, each with 10 vials, between *Drosophila albomicans* and *D. nasuta*. Three pairs of virgin flies were put into 1 vial, and these 20 vials comprised the founding generation. After the emergence of F_1, 15 pairs from each vial in the founding generation were evenly distributed into 15 new vials, and therefore each new vial contained 20 pairs of flies from all 20 vials of the previous generation. The 15 vials were randomly assigned to 3 groups (A, B, and C). Each replicate contained 5 vials, and after 2 d, the flies were transferred to new vials. These 10 vials comprised the G_0 generation.

Karyotype and esterase analysis

Karyotyping and electrophoretic analysis of the esterase allozyme patterns were carried out as previously described (Yu et al. 1997). For each karyotype analysis of the 3 E populations, 60 chromosome slides (including 3 males and 3 females from each of the 10 vials of 1 generation) were examined, i.e., approximately 90 3-X and 30 3-Y sex chromosomes, and 120 autosomes were checked. Esterase electrophoresis was performed 6 times for each population, at generations G_1, G_5, G_{10}, G_{20}, G_{43}, and G_{49}. Each survey consisted of 35 individuals (3 males and 4 females from five of 10 vials for 1 generation). Analyses of the D and N populations were described in the previous paragraph.

Statistical analysis

Percentage data were arcsine-transformed before the statistical analyses. Frequency changes of the 3-X and 3-Y chromosomes in all hybrid populations were compared by *t*-test. Correlation coefficients of the 3-Y frequencies versus the 3-X, 2nd, and 4th chromosome frequencies in the E populations were determined using Pearson's correlation test. Analysis of variance (ANOVA) was used to test the effects of the population (D and N populations) and generation on frequency changes of the 3-X chromosome. In addition, the Kruskal-Wallis test was used to examine whether the sex ratio differed among replicates in the D and N populations. The *t*-test was used to examine the sex ratio difference between the D and N populations.

RESULTS

The fate of *albomicans*-type chromosomes in hybrids

Three hybrid populations were successively maintained by a non-overlapping generation scheme for over 3 yr. Under our experimental conditions, the frequency of the 3-X chromosome of *D. albomicans* varied from 30% to 98% (Fig. 3). The frequency of the 3-Y chromosome of the 3 hybrid populations had reached 87% in one and about 10% in the others at G_{49} (Fig. 4). Among these 3 hybrid populations, neo-sex chromosomes 3-X and 3-Y were approaching fixation in E_1. The frequency of the *D. albomicans* 4th chromosome remained at an average of 73% (Fig. 5). The 2nd chromosome was less variable than the other chromosomes, and its frequency remained at an average of 56% (Fig. 6). The 3-Y chromosome frequency showed a significantly positive correlation with the 3-X chromosome frequency (r = 0.69, p = 0.001) and with the 4th chromosome frequency (r = 0.48, p = 0.04), but it did not have a significant correla-

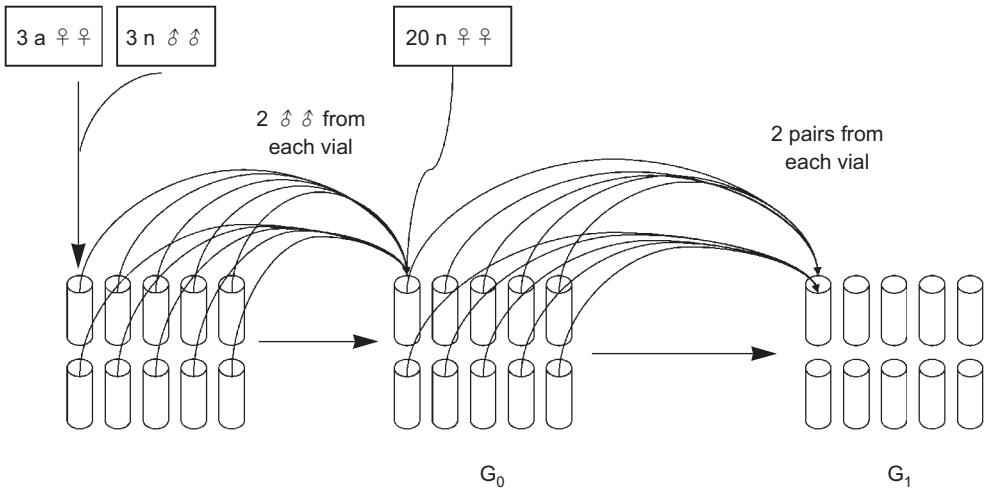

Fig. 3. Three pairs of *Drosophila albomicans* female and *D. nasuta* male flies placed into a vial, and 10 such vials constituted the initial population. After the emergence of F_1, 20 males from each vial were evenly distributed into 10 new vials. In addition to the 20 F_1 males from all 10 old vials, 20 *D. nasuta* virgin females were added to each of the 10 vials. These 10 new vials constituted the G_0 generation. G_1 also contained 10 vials, and each vial contained 20 pairs of flies (2 pairs from each vial of the G_0 generation).

tion with the 2nd chromosome frequency (r = 0.34, p = 0.15) (Pearson's correlation test).

Frequency change of neo-sex chromosomes in the D and N populations

Expected sex ratio changes were calculated based on an average of distorted values of offspring produced by hybrid 3-X/3,Y males when choosing strains for establishing the D populations (Table 2). According to the experimental design, there was 1 mating type (i.e., 3/3;X/X x 3-X/3,Y) at the founding generation, 1 mating type (3-X/3,X x 3/3;X/Y) in the 1st generation, 4 mating types in the 2nd generation, and 6 mating types in the 3rd and subsequent generations. According to the crossing scheme, females consisted of 3 genotypes (3-X/3-X, 3-X/3,X, and 3/3;X/X), while males consisted of 2 (3-X/3,Y and 3/3;X/Y) from the 3rd generation on. Under a random mating assumption, the frequency of each genotype was respectively standardized for females and males before calculating the probabilities for all types in a mating table. Since the meiotic driver is only active in males, the expected sex ratio in the 2nd generation was 50%. The frequency of each genotype was calculated from data from the previous generation with the same distortion value. The sex ratios of the D (with the meiotic driver) and N (without the meiotic driver) populations as well as expected values from G_1 to G_7 are summarized in figure 8. As expected, the N populations showed no bias (Fig. 8), whereas the D populations showed a significant female bias in the 1st generation, but the distortion diminished beyond the 3rd generation.

Compared to the initial frequency of 3-X (33%), the average frequencies of 3-X at G_5 and G_{12} increased in both the D (57.0%) and N (61.8%) populations (Table 3). Similarly, the frequencies of both of the *D. albomicans*-type autosomes began at 25% and increased in both the D and N populations to averages of 53.5% for the 2nd chromosome and 36.2% for the 4th chromo-

Table 3. Percentages (average ± S.E.) of the neo-X (3-X), 2nd (A-II), and 4th (A-IV) chromosomes of *Drosophila albomicans* in the D and N populations in the G_5 and G_{12} generations

		Percentage of the chromosome		
		3-X	A-II	A-IV
Initial frequency		33.3	25.0	25.0
G_5	D	54.3 ± 3.1	60.7 ± 5.3	37.0 ± 3.7
	N	61.7 ± 5.0	55.0 ± 4.9	24.3 ± 3.9
G_{12}	D	59.7 ± 12.8	53.0 ± 1.2	50.7 ± 14.1
	N	62.0 ± 5.1	45.3 ± 10.2	33.0 ± 11.1
Average		59.4 ± 8.1	53.5 ± 8.1	36.2 ± 14.5

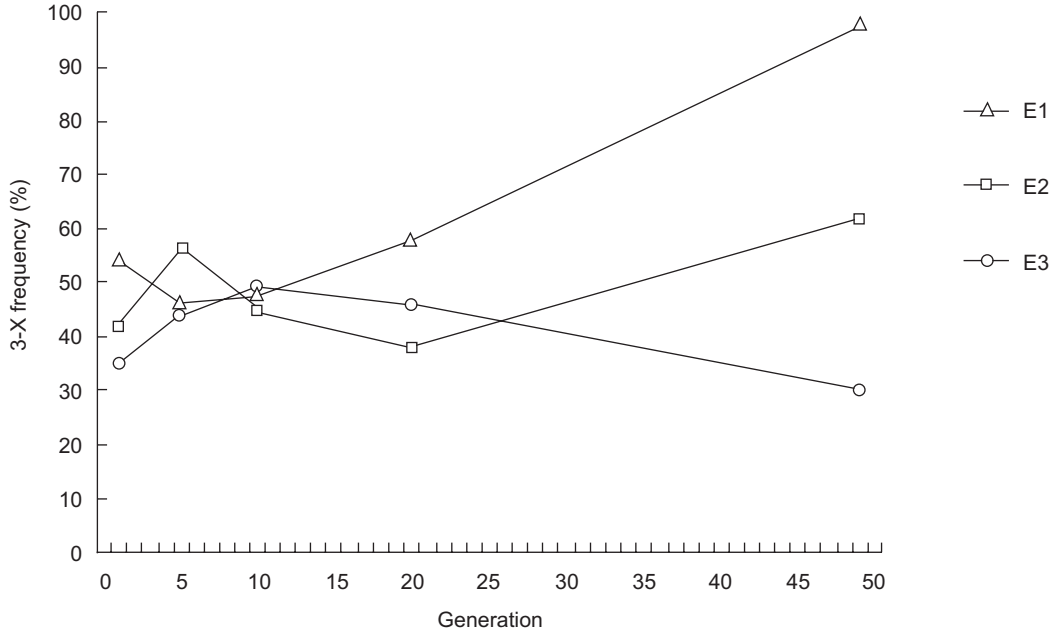

Fig. 4. Percentages of the 3-X chromosome in the 3 E hybrid populations calculated at generations G_1, G_5, G_{10}, G_{20}, and G_{49}.

some, respectively. However, the frequencies of these 3 chromosomes showed no statistically significant difference between the D and N populations, or between generations G_5 and G_{12} (two-way ANOVA, the 3rd chromosome: $p = 0.59$ for the generation effect, $p = 0.40$ for the population effect, and $p = 0.61$ for the interaction effect; the 2nd chromosome: $p = 0.06$ for the generation effect, $p = 0.13$ for the population effect, and $p = 0.21$ for the interaction effect, and the 4th chromosome: $p = 0.18$ for the generation effect, $p = 0.08$ for the population effect, and $p = 0.14$ for the interaction effect).

No significant role of the meiotic driver in determining the 3-X chromosome frequency

In order to test whether the meiotic driver still existed in the D populations, we examined the sex ratio of the offspring by crossing males from 3 D and 3 N populations at G_{15} with *D. nasuta* (#193.7) females, respectively. Since there was no significant difference in the sex ratio among the 3 replicates (Kruskal-Wallis test, $p = 0.25$ for Ds and $p = 0.63$ for Ns, respectively), the data were separately pooled to represent the D and N populations. The distributions of the offspring sex ratio of these tested males are shown in figure 9. There was no statistically significant difference in the sex ratio between the D and N populations (43.0% and 44.8% males in the D and N populations, respectively, *t*-test, $p = 0.11$). Based on an assumption of random mating and calculated with an average value for the meiotic driver, the expected frequency of 3-X was 63% and that of 3,X was 37% at G_{15} in the D populations. To further explore the existence of the meiotic driver, we performed a follow-up experiment by sampling 59 males from the D3 populations at G_{22}. These males were crossed with *D. nasuta* (#193.7) females, and then we examined the sex ratios of their offspring as well as the karyotypes of their daughters. As shown in figure 10, the sex ratio of offspring produced by 33 3-X/3,Y males (mean = 41.7%) was significantly female-biased compared with that by 26 3/3;X/Y males (mean = 49.4%) (*t*-test, $p < 0.001$). These results indicated that the meiotic driver was still present in the D population on the 3-X, but the effect of the driver was weaker than in the initial generations (Table 2).

DISCUSSION

What is the fate of the 2 neo-sex chromosomes coexisting with 2 ancestral sex chromosomes?

We previously proposed a "stepwise chromosome evolution" model to explain how the derived karyotype in *D. albomicans* was established from the ancestral karyotype (Yu et al.1999). In this model, a selective advantage was hypothesized to

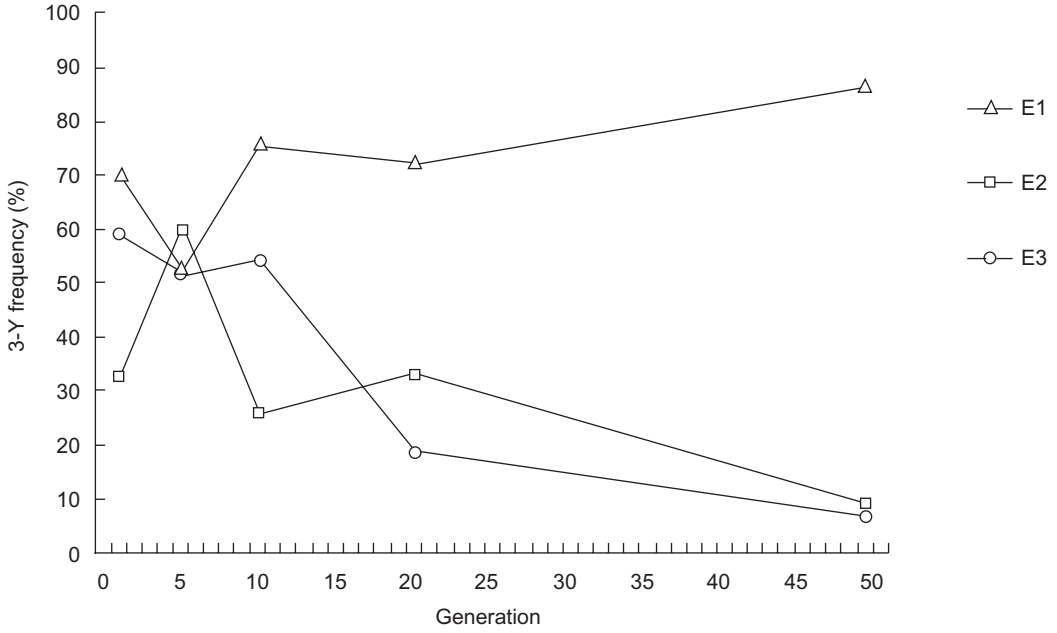

Fig. 5. Percentages of the 3-Y chromosome in the 3 E hybrid populations calculated at generations G_1, G_5, G_{10}, G_{20}, and G_{49}.

explain the maintenance of the 3-X chromosome despite structural disadvantages in the 1st stage. In order to explore the results with those of Yu et al. (1999), 2 strains were chosen to construct the hybrid populations. The hybrid E populations began with an average of 50% 3-X which was the same as in the previous study, while in the previous study the 3-Y chromosome was absent. In the 10th generation, the average of 47.3% for 3-X (Fig. 3) was already higher than the 32.3% shown in Yu et al. (1999), and this is consistent with our prediction that the presence of 3-Y may facilitate an increase in 3-X. In one of the 3 E populations, 3-Y reached a high frequency by G_{49} (Fig. 4) together

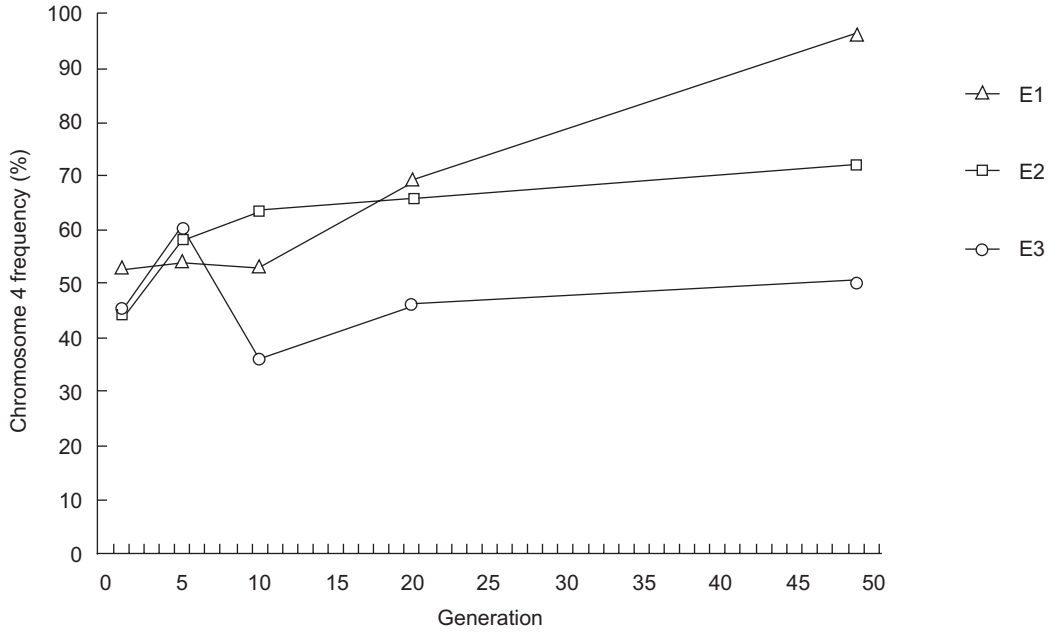

Fig. 6. Percentages of the *Drosophila albomicans* 4th chromosome in the 3 E hybrid populations calculated at generations G_1, G_5, G_{10}, G_{20}, and G_{49}.

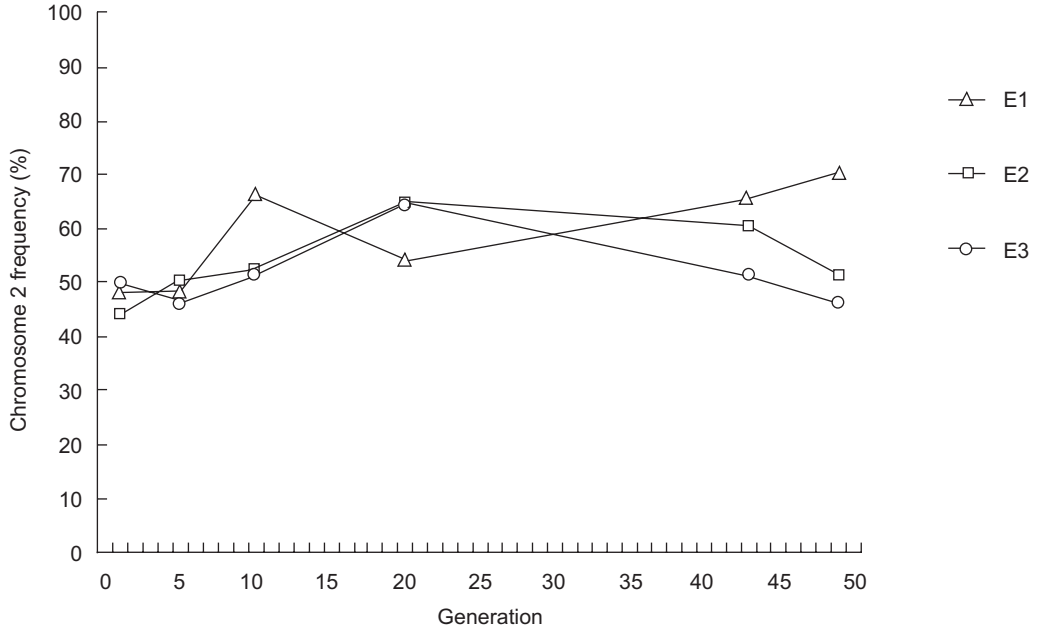

Fig. 7. Percentages of the *Drosophila albomicans* 2nd chromosome in the 3 E hybrid populations calculated at generations G_1, G_5, G_{10}, G_{20}, G_{43}, and G_{49}.

with the nearly fixed 3-X (Fig. 3). These results indicate that it is possible for 3-X to reach fixation in the presence of 3-Y even when using hybrids with the lowest 3-X frequency at the 1st stage of the stepwise model.

Genetic drift is usually unavoidable during such long-term cultivation. However, this effect was reduced by evenly mixing flies from all vials and transferring the same number of flies from old vials to new ones through each discrete generation. The coordinate frequency changes of 3-X and 3-Y in these populations indicated selection for an interaction of the neo-sex chromosomes. Frequencies of the 4th chromosomes of the *D. albomicans* type were variable after long-term cultivation (Fig. 5). The 4th chromosome of *D.*

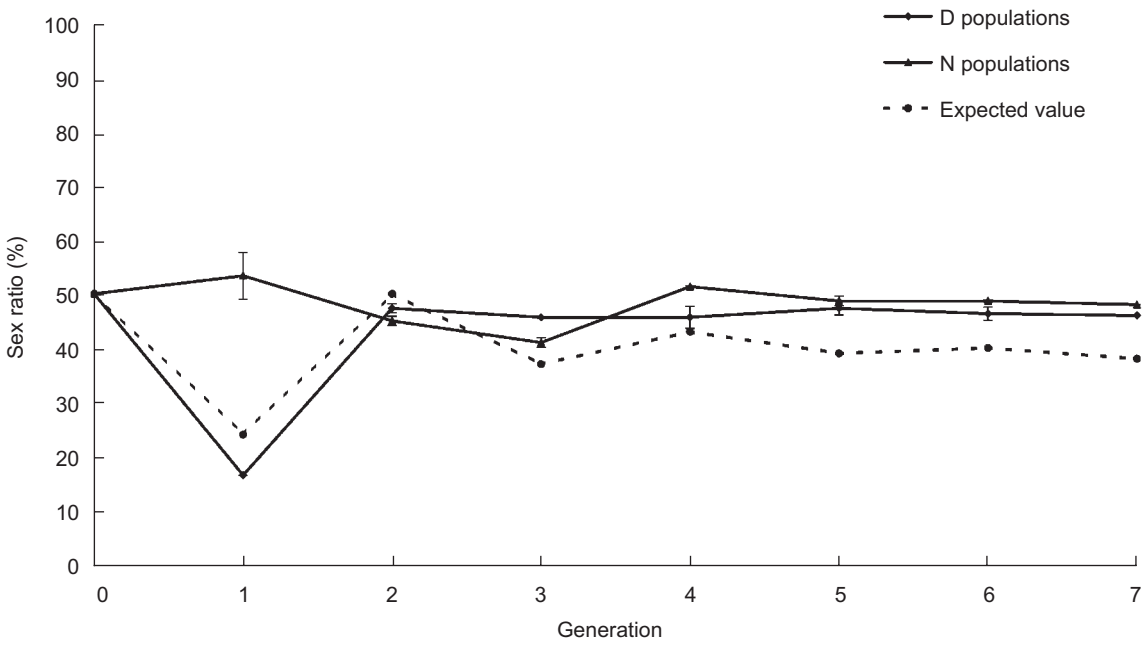

Fig. 8. Average sex ratios of the 3 D and 3 N populations as well as expected values from G_1 to G_7.

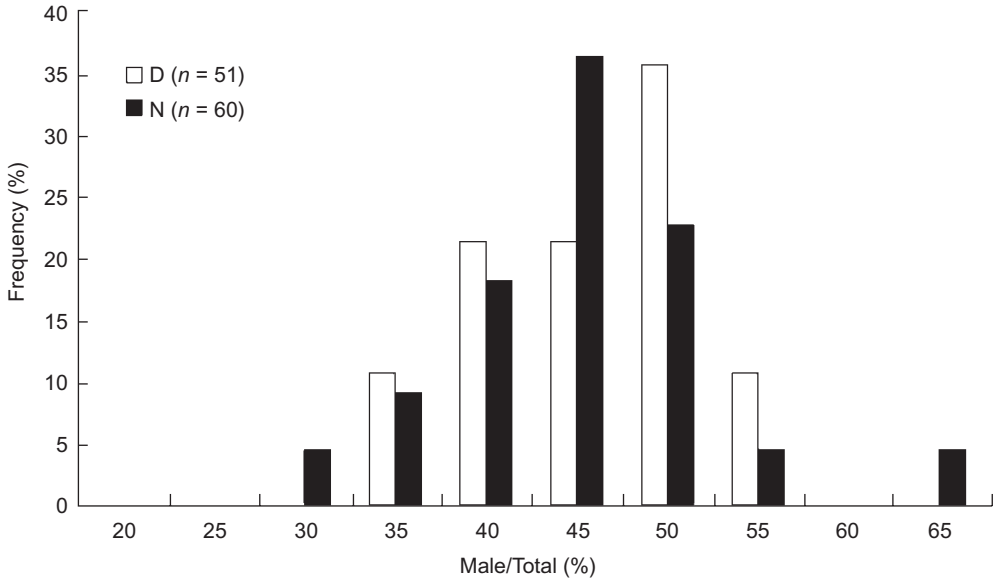

Fig. 9. Males at generation G_{15} sampled from 3 D and 3 N populations, and crossed to *Drosophila nasuta* females. The sample size is indicated in parentheses. The black and white bars respectively indicate the sex ratio distributions of the N and D populations.

albomicans is several times larger than that of *D. nasuta* (Hatsumi 1987, Wang et al. 1988). Wang et al. (1988) discovered that the length of the 4th chromosome was inversely correlated to that of the Y chromosome among different *D. albomicans* populations, implying the occurrence of translocation between the 4th and Y chromosomes. The frequency of the Y arm of *D. albomicans* might have been part of the reason for the frequency changes in the 4th chromosome; for instance, they were both highest in the E_1 population.

A balanced inversion polymorphism on the 2nd chromosome of natural populations of *D. albomicans* was reported (Chang et al. 1988), and the possible mechanism for this inversion heterosis was discussed in Chang et al. (1996). A similar phenomenon was observed in a study by Tanuja et al. (2003). The inversion, $(2L)B_1D_5$, is common in *D. albomicans*, and it also exists in *D. nasuta* where it is called IIL-2 or 2L-B (Ranganath and Krishnamurthy 1975, Kumar and Gupta 1986, Singh and Kalisch 1991). The frequencies of the 2nd chromosome in all 3 E populations remained at around 60% from the 10th to the 49th generations, which indicated that the chromosomes might be balanced (Fig. 6). The correlated frequency changes among the 3-X, 3-Y, and 4th chromosomes revealed a possibility of selective forces. These 2 cases showed that genetic drift was not significant compared to selective forces on the 2nd chromosome and on interactions among the other 3 chromosome types. Nevertheless, the effective transmission number of 3-Y was only 1/4 that of an autosome. The frequency of 3-Y might influence those of the 3-X and 4th chromosomes. The reason why we did not observe the same high frequencies in the other E populations might have been due to insufficient reduction of genetic drift on the 3-Y chromosome.

Does the meiotic driver facilitate fixation of 3-X?

Although Yang et al. (2004) reported that a meiotic driver, located on the 3-X chromosome, exists in many natural populations of *D. albomicans*, our present results showed no significant effect of this driver on 3-X frequencies (Table 3). The D populations were established by backcrossing F_1 males to *D. nasuta* females, which contained no suppressors. Since these F_1 males were produced by crosses between *D. albomicans* females and *D. nasuta* males, the suppressors on the 3-Y chromosomes of *D. albomicans* did not enter the hybrid D populations. The G_1 generation showed a sex ratio distortion as expected. Then the meiotic driver moved into females, and the sex ratio of the G_2 generation returned to normal. Considering the meiotic drive only, changes in the sex ratio in an ideal population were theoretically calculated, and our experimental data fit the expectation only for the 1st few generations (Fig. 8). The sex ratio in the D populations was restored and was indistinguishable from that of the

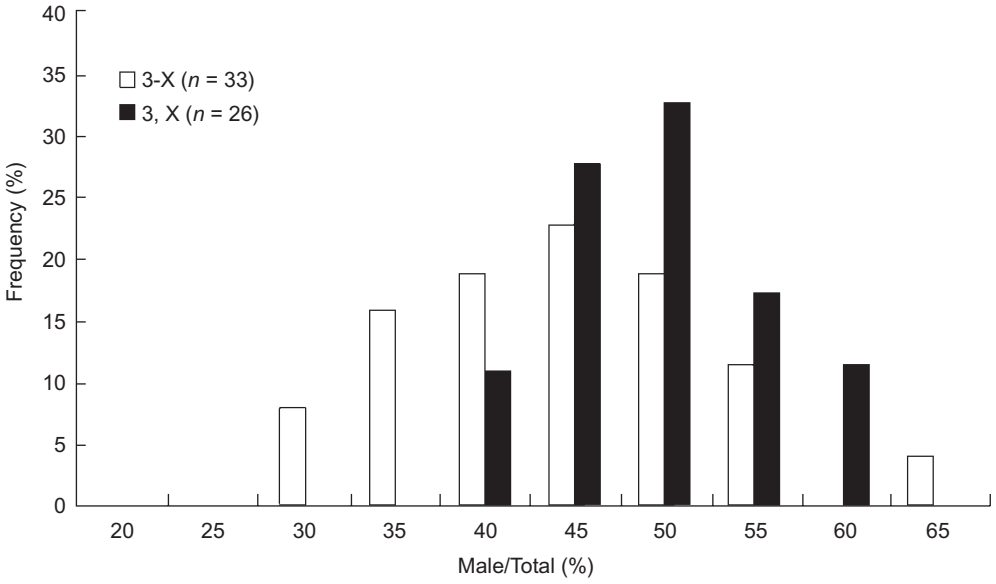

Fig. 10. At G_{22}, males were sampled from the D3 population and crossed with *Drosophila nasuta* (#193.7) females to check the sex ratio of their offspring. The sex ratio distributions of the offspring of 3, X/3, Y males and 3-X/3, Y males are shown separately.

N populations from the 4th generation onward. These results suggest that the meiotic driver is unlikely to be a major factor in the increase or maintenance of 3-X chromosome frequency. However, this suggestion was made according to the results under our experimental conditions, and the possibility of violating the proxy assumption should be considered. Yang et al. (2004) previously reported that more than 1 suppressor exists in the *D. albomicans* genome and functions in hybrid populations. The suppressors might be efficiently reactivated soon after a disturbance by hybridization.

G_{15} males in the D populations no longer showed a detectable sex ratio bias in producing offspring (Fig. 9), but there still may be a mixture of 3-X/3, Y and 3/3; X/Y males as observed at G_5 and G_{12} (Table 3). We examined males in the D3 population at G_{22} in detail (Fig. 10). 3-X/3, Y males showed a significant female bias in their offspring compared to 3/3; X/Y. Obviously the meiotic driver was still being segregated in the population, but the sex ratio distortion was weaker than that in the initial state because the sex ratio increased from 34.2% to 41.7% after 22 generations. This suggests that the effect of the sex ratio distorter might have been inadequate to facilitate fixation of the 3-X chromosome under our experimental design. Since contemporary *D. albomicans* and *D. nasuta* chromosomes are only proxies for the ancient sex chromosomes, we cannot rule out the possibility of its role in the initial increase in the frequency of 3-X.

CONCLUSIONS

The chromosome evolution of *D. albomicans* probably consisted of 2 fusion events: fusion of a 3rd chromosome with the X, and subsequently another 3rd chromosome with the Y. The 1st fusion of the 3rd chromosome and the X would be expected to cause structural incompatibilities. Before the 2nd fusion occurred, selective advantages as well as a meiotic drive may have counteracted the structural incompatibilities in the population. In the present study, we began hybrid matings with the same set of strains which showed the lowest 3-X frequencies in our previous study (Yu et al. 1999). The experimental design was intended to reduce genetic drift, but the drift of 3-Y might still have been significant due to the small effective transmission number. In one of the 3 replicates, the 3-X and 3-Y chromosomes increased to a combined high frequency as predicted in the 2nd stage of the "stepwise chromosome evolution" model. Although a meiotic driver has been found in many natural *D. albomicans* populations (Yang 2001), it was indicated under our experimental condition that the meiotic driver was unlikely to have been a major driving force in the increase of the neo-X chromosome (Table 3).

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