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Evolutionary Changes in a Y-Like Chromosome in Hybrids of *Drosophila albomicans* and *D. nasuta*

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Hwei-yu Chang and Ting-Yi Kung (2008) Evolutionary changes in a Y-like chromosome in hybrids of *Drosophila albomicans* and *D. nasuta*. *Zoological Studies* **47**(4): 455-465. The initial steps of Y or even neo-Y chromosome evolution remain obscure, because it is difficult to study using contemporary species. In this report, we established 3 hybrid strains with Y-like chromosomes to mimic the neo-Y chromosome of *Drosophila albomicans* by crossing a *D. albomicans* female to a *D. nasuta* male. In hybrid strains, a specific 3rd chromosome from *D. nasuta* became a non-recombining, paternally inherited Y-like chromosome associated but not fused with the Y chromosome. Through backcrossing, we extracted Y-like chromosomes from hybrid-strain males, and then successfully established F_3 offspring with a pair of homozygous Y-like chromosomes. The presence of paired homozygous Y-like chromosomes in an organism is essential to demonstrate the level of recessive degeneration of Y-like chromosomes. Our results showed that higher recessive degeneration was observed in old Y-like chromosomes (~280 generations) compared to young ones (~20 generations). The small number of F_1 offspring during chromosome extraction indicated incompatibility between hybrid-strain males and *D. nasuta* females. For F_2 flies carrying a Y-like chromosome, two of the 8 extractions from the old hybrid strain showed fewer males than females. This observation supports the hypothetical dependence of this Y-like chromosome in males on its intra-strain homologue. Furthermore, one of 3 extractions from the young hybrid strains weakly supported the presence of a sexually antagonistic effect of a Y-like chromosome favoring males. Our study revealed that heterogeneous incompatibility with parental species emerged in the hybrid strains. Although the supporting data were not very strong, they suggested the appearance of sexual antagonism at an early stage (around the 20th generation) and the formation of inter-chromosome dependence at a later stage (around the 280th generation) of neo-Y chromosome evolution. Recessive degeneration on a Y-like chromosome could also be observed within 280 generations, instead of tens of thousands of years. This study indicates that Y-like chromosomes in hybrid strains are a unique experimental model to investigate the evolution of paternally inherited chromosomes without recombination. http://zoolstud.sinica.edu.tw/Journals/47.4/455.pdf

Key words: Degeneration, Hybridization, Sex chromosome interaction, Sexual antagonism.

A chromosome, usually carrying hundreds or thousands of genes, does not function alone. It cooperates with other chromosomes to manifest integrated systems of life. The value of a gene or chromosome should be judged by considering its interaction with others, as selection works on the overall expression of an organism. Although it is difficult to predict which specific gene will evolve or degenerate under a particular situation, general trends can be observed. Since Morgan (1912) first reported no crossing over during meiosis in males of *Drosophila melanogaster*, the maxim "no male recombination occurs in *Drosophila*" is generally accepted. Due to paternal transmission of the hemizygous Y chromosome, a *Drosophila* Y chromosome has little chance of recombination. The presence in males only and no recombination are 2 crucial aspects of the unique pattern of *Drosophila* Y chromosome evolution compared to that of autosomes or the X chromosome.

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Theories on the early stages of sex chromosome evolution proposed by Charlesworth and Charlesworth (1978) showed how the lack of crossing over between sex chromosomes evolves, and how this cessation of recombination can result in the genetic degeneration of this specific chromosome which exists only in heterogametic sex (Charlesworth and Charlesworth 2000). Crossing over should initially be suppressed only in the sex-determining region (Charlesworth et al. 2005). Sexual antagonism is a hypothetical cause for the inhibition of crossing over; meanwhile, a non-recombining Y chromosome may result in gradual accumulation of disadvantageous alleles that consequently lead to degeneration of the Y chromosome (Muller 1964). Since a Y chromosome always coexists as well as coevolves with an X chromosome in male organisms, there is no chance for an individual to possess a pair of homozygous Y chromosomes or to eliminate these detrimental alleles (Muller 1918). Recessive deleterious alleles on the Y chromosome can thus be maintained. On the other hand, alleles advantageous to males but harmful to females can be retained because they have no chance to enter a female and be selected out. However, evidence supporting the existence of cooperation between X and Y chromosomes or the accumulation of either deleterious alleles or sexually antagonistic alleles during the process of evolution in the initial stage of sex chromosome evolution remains limited.

In nature, Y chromosomes, usually carrying only a few functional genes because of cytological condensation or heterochromatinization (Charelesworth 1996, Rice 1987 1994), are too ancient to explore the issue of how deleterious variations accumulated in the non-recombining Y chromosome (Lucchesi 1978). A newly evolved Y chromosome, however, can show how genetic changes accumulate on a Y chromosome. A pair of sibling species may be good candidates for this kind of study. A *D. nasuta* male with 8 chromosomes (2*n* = 8) (Fig. 1a) has a non-recombining and heterochromatinized Y as do other *Drosophila* males. Males of its sibling species, *D. albomicans*, have 6 chromosomes (2*n* = 6) (Fig. 1b), with fusions of the ancestral 3rd chromosome to the X and Y chromosomes, respectively. We named these newly derived chromosomes neo-X and neo-Y (Yu et al. 1999). The 3rd chromosomal arm of the neo-Y was inferred to be non-recombining due to its fusion with a Y chromosome, but it has not yet been cytologically heterochromatinized. Since the generation of fused neo-Y chromosomes arose < 0.5 million yr (Ma) ago (Chang et al. 1989,

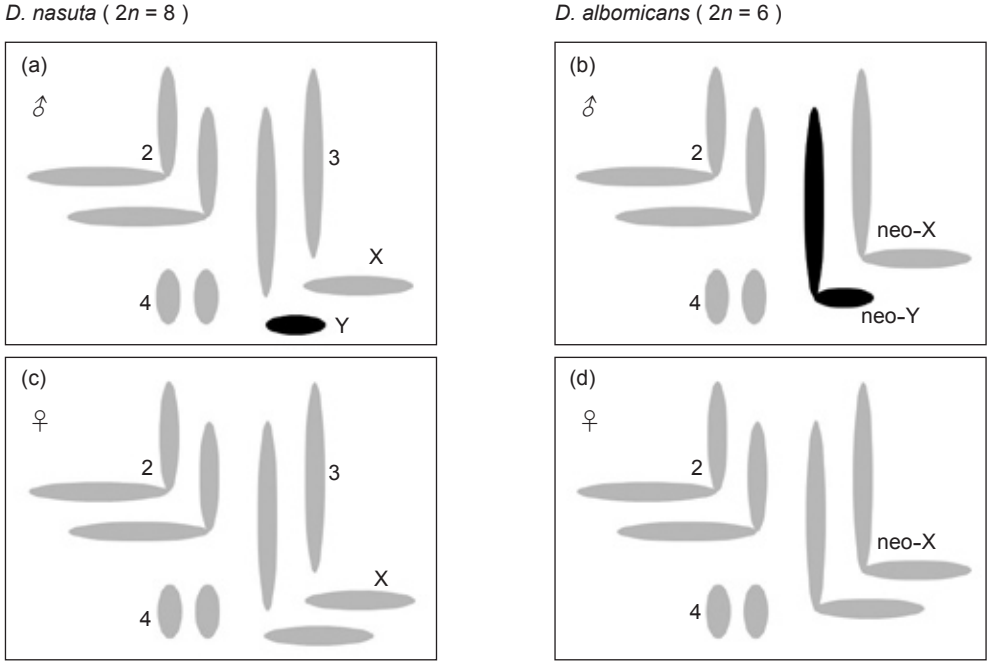

Fig. 1. Karyotypes of (a) *Drosophila nasuta* and (b) *D. albomicans*. *Drosophila nasuta* has 8 chromosomes with separate 3rd chromosomes and sex chromosomes, while *D. albomicans* has 6 chromosomes with neo-X and neo-Y chromosomes generated from fusions between the 3rd and sex chromosomes.

Bachtrog 2006), one can consider the 3rd arm of a neo-Y as a newly evolved Y chromosome. Similarly, the absence of homozygous individuals with 2 neo-Y chromosomes and the lack of females with a neo-Y chromosome have hindered investigators from examining recessive degeneration and sexual antagonism.

In our laboratory, we have established a hybrid strain, H10, derived from a cross between a *D. albomicans* female and a *D. nasuta* male (Yu et al. 1997). The H10 hybrid strain, kept in our laboratory for 280 generations, has a unique and fixed karyotype in males. H10 males have 7 chromosomes including a neo-X, a Y, and a separate 3rd chromosome; while H10 females, just like *D. albomicans* females, have 6 chromosomes. Although this 3rd chromosome is not physically attached to the Y chromosome, it goes to the same pole with the Y during meiosis, while the neo-X chromosome segregates to the opposite pole. Since this 3rd chromosome only exists in males and presents a sex linkage relationship, we refer it as a Y-like chromosome and consider it to be the prime neo-Y in *D. albomicans*. However, the Y-like chromosome does not represent the ancient *Drosophila* Y chromosome because the cessation of recombination along the entire chromosome is instantaneous. However, it mimics the evolution of a neo-Y chromosome because it is uniparentally inherited without recombination. Furthermore, the Y-like chromosome contains a large number of functional genes. Therefore, it has great potential to coevolve with neo-X chromosomes and could serve as a unique experimental model to explore how deleterious alleles accumulate on neo-Y chromosome via inhibition of recombination.

On the other hand, we established 2 new hybrid strains, Hn_1 and Hn_2, with karyotypes exactly the same as H10 (i.e., $2n = 7$ in males and $2n = 6$ in females) for comparative investigations of the evolution of a Y-like chromosome extracted from young hybrid-strain males (Hn_1 and Hn_2 at the 20th generation) and old hybrid-strain males (H10 at the 280th generation). A series of crossing experiments was conducted to extract specific Y-like chromosomes from a number of hybrid strains (H10, Hn_1, and Hn_2) and pass them on to female offspring, hence allowing us to examine how genetic variations gradually accumulate on a neo-Y chromosome, homologous to the 3rd chromosome of *D. nasuta*.

MATERIALS AND METHODS

Drosophila strains

All flies were reared in glass vials (3 cm in

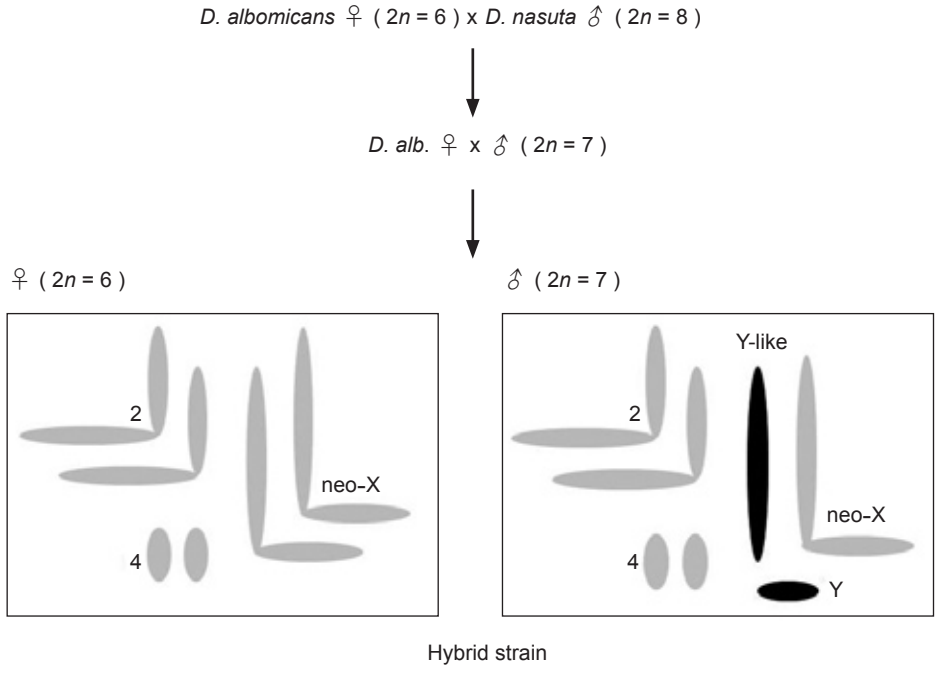

Fig. 2. Scheme for establishment of hybrid strains. A hybrid strain was generated by crossing a *Drosophila nasuta* male to a *D. albomicans* female, and then backcrossing a hybrid male to a *D. albomicans* female.

diameter and 10 cm high) containing 2.5 cm (depth) of standard corn meal *Drosophila* medium; these were placed in an incubator maintained at 23 ± 1°C, and 75% relative humidity with a 12: 12 h light: dark photoperiod. A *D. albomicans* strain (#163.5 from Okinawa, Japan) and a *D. nasuta* strain (#193.7 from India) were used to establish all 3 hybrid strains (H10, Hn_1, and Hn_2) carrying the Y-like chromosomes. Each hybrid strain originated from 1 pair of flies. The establishment of H10 was previously described (Yu et al. 1997), and the protocol for generating Hn_1 and Hn_2 is illustrated in figure 2. H10 has been maintained in this laboratory for 280 generations, and Hn_1 and Hn_2 for 20 generations, hence they are called "old" and "young" hybrid strains, respectively. In addition to hybrid strains H10, Hn_1, and Hn_2, the *D. nasuta* strain (#252.11, also from India), carrying the same genetic markers as those on the neo-X from *D. albomicans* strain #163.5, was chosen to extract the Y-like chromosome from hybrid strains. Experimental populations were reared with non-overlapping generations. Newly emerged virgin flies were sexed within 8 h and were kept in separate vials for 4 d before crossing.

Molecular markers of Y-like chromosomes

Two PCR-RFLP markers, c29-*Rsa*I and a52-*Hae*III, were used to trace the Y-like chromosomes in this study. Primers for these 2 markers were previously designed in our laboratory. The PCR products of c29 and a52 are about 530 and 800 base pairs (bp) in length, respectively (Chang et al. 2008). Amplified c29 products from *D. albomicans* strain #163.5 can be digested by the *Rsa*I restriction enzyme to form 2 fragments (of around 250 and 280 bp), whereas that from *D. nasuta* #193.7 is resistant to *Rsa*I digestion. They were designated alleles c^F and c^S, respectively. After *Hae*III digestion, the major band of a52 amplified from *D. albomicans* strain #163.5 on an electrophoresis gel was 740 bp, whereas that from *D. nasuta* #193.7 was 600 bp. They were designated alleles a^S and a^F, respectively. Therefore, the Y-like chromosome derived from *D. nasuta* #193.7 carries the markers of a^S and c^F, while the neo-X chromosome derived from *D. albomicans* strain #163.5 carries the markers of a^F and c^S. Since *D. nasuta* strain #252.11 carries the same c29-*Rsa*I and a52-*Hae*III alleles as those on the neo-X from *D. albomicans* strain #163.5, it was chosen to extract the Y-like chromosome containing the a^S and c^F alleles through designated crossing experiments described below. DNA preparation from a single fly and PCR amplification were carried out according to methods described by Gloor et al. (1993 from http://engels.genetics.wisc.edu/).

Y-like chromosome extraction and generation of homozygous Y-like offspring

To reveal recessive degeneration of the neo-Y chromosome, we needed to extract the Y-like chromosome from young (Hn_1 and Hn_2) and old hybrid (H10) strains and transmit them into both male and female offspring to examine the presence of recessive deleterious alleles on the Y-like chromosome. The scheme for the crossing experiments is illustrated in figure 3. Accordingly, a specific Y-like chromosome from a hybrid-strain male could be extracted by crossing it with a *D. nasuta* (#252.11) female. The extracted Y-like chromosome only appeared in F_1 males. In order to pass the Y-like chromosome to both male and female flies, we backcrossed the F_1 male to a *D. nasuta* female. Only 1 F_1 male from each extraction cross was backcrossed to a *D. nasuta* female to obtain both F_2 males and females carrying the Y-like chromosome. To acquire flies with homozygous Y-like chromosomes, a number of virgin F_2 flies were then individually mated. After producing offspring, F_2 flies were subjected to a chromosome marker analysis to check whether they carried a Y-like chromosome derived from the hybrid strain. Only the F_3 offspring obtained

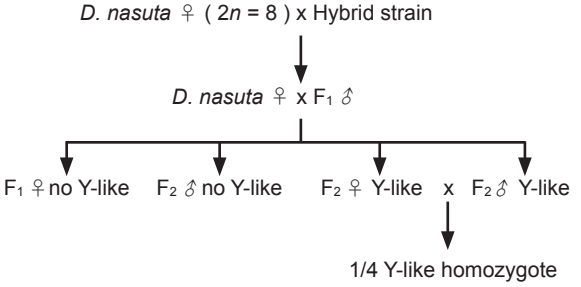

Fig. 3. Extraction of Y-like chromosomes from a hybrid-strain male. Y-like chromosomes could be extracted from a hybrid strain by crossing it with a *Drosophila nasuta* female as described in "Materials and Methods". The extracted Y-like chromosome could be passed to a female by backcrossing the F_1 male to a *D. nasuta* female. There were 2 types of males and 2 types of females in the F_2 generation. Heterozygous pairs with the Y-like chromosome were chosen, and the expected proportion of homozygous Y-like individuals in F_3 was 1/4.

from heterozygous F_2 parents (both carrying the Y-like chromosome) were subjected to genotyping. Approximately 16 males and 16 females of F_3 from each heterozygous F_2 pair were genotyped. The presence of recessive deleterious alleles on the Y-like chromosome was supported by the decreased number of F_3 individuals with homozygous Y-like chromosomes produced from the heterozygous F_2 pair. Genotypes of the Y-like chromosome and 3rd chromosome of *D. nasuta* were $a^S c^F$ and $a^F c^S$, respectively. The number and sex ratio of the F_1 and F_2 generations were also recorded.

RESULTS

According to the crossing scheme illustrated in figure 3, the Y-like chromosome was first individually extracted from F_1 males. A small number of offspring and significant sex ratio distortion were observed in F_1 (Table 1). All sex ratios of F_1 from hybrid-strain males and *D. nasuta* females were significantly female biased, while the sex ratio of progeny produced by *D. nasuta* pairs did not deviate from 1: 1 (Table 1). A 2 × 2 contingency table analysis ($\chi^2 = 4.29$, $p = 0.038$) showed that the sex ratio distortion was more serious with the young Y-like strains (Hn_1 and Hn_2) than with the old Y-like strain (H10). This sex ratio distortion might have been due to a meiotic driver hidden in the hybrid strain, and this possibility is discussed below. By the Tukey-Kramer honest significant difference (HSD) test, the reduction in F_1 was significant, and the young strains were more seriously affected than the old one. But the number of offspring recovered in F_2 (Table 2). Y-like chromosomes were transmitted to males in the F_1 generation and were further transmitted to both males and females in the F_2 generation through backcrossing these F_1 males to *D. nasuta* females. Chi-square (χ^2) test of the sex ratio at F_2 showed that the male: female ratio did not deviate from 1: 1 for 10 of the 11 extracted Y-like chromosomes, except one from Hn_1 (no. 1) which produced significantly male-biased offspring (Table 2). This young Y-like chromosome showed significant differences from the other 2 young Y-like chromosomes in a 2 × 2 contingency table analysis ($\chi^2 = 5.09$, $p = 0.024$). Nevertheless, this young hybrid strain was the same as the other 2 young strains showing a female bias in F_1 (Table 1). The possible genetic disturbance in a hybrid strain causing reduction of offspring when crossed to *D. nasuta* is explored in the "Discussion" section.

The presence of a Y-like chromosome was determined with the aid of PCR-RFLP markers. Approximately equal numbers of F_2 males and females were randomly selected and subjected to genotyping analysis. The electrophoretic patterns of the 2 PCR-RFLP markers, c29-*Rsa*I and a52-*Hae*III, are illustrated for H10 males and females in figure 4. After 280 generations, no recombinants were found in this hybrid strain. Table 3 summarizes the F_2 genotyping results. Among F_2 flies heterozygous for Y-like chromosomes (c^F/c^S), there were more females than males if they carried nos. 4 and 11, but there were more males than females if they carried the no. 1 chromosome. The former two are old Y-like, but the latter one was a young Y-like chromosome. However, the statistical support was weak. In particular, the equal numbers of males and females sampled from the no. 1 extraction were inappropriate because the

Table 1. Sex ratio (male/total) and number of F_1 progeny produced from a cross between a *Drosophila nasuta* #252.11 (Dnas) female and a hybrid-strain male with 2 intra-strain crosses from Dnas as controls

Chromosome extracted	Source	F_1 sex ratio	No. of F_1 progeny	χ^2
1	Hn_1	0.375	72	4.50*
2	Hn_1	0.132	38	20.63***
3	Hn_2	0.111	18	10.89***
4	H10	0.410	195	6.28*
5	H10	0.417	175	4.81*
6	H10	0.246	167	43.26***
control 1	Dnas	0.509	287	0.09
control 2	Dnas	0.522	320	0.62

* $p < 0.05$; *** $p < 0.001$.

result showed a sex ratio distortion (Table 2), and this may have been the reason why it was not statistically significant (X^2 = 3.14, p = 0.076).

Since we successfully extracted and transmitted Y-like chromosomes from young and old hybrid strains to F_2 offspring, we were able to further demonstrate the presence of recessive deleterious alleles on Y-like chromosomes by crossing heterozygous F_2, the third step in figure 3. Although no recombination existed in F_2 males during meiosis, F2 females with a Y-like chromosome (a^Fc^S/a^Sc^F) could generate 4 kinds of gametes, 2 without recombination (a^Fc^S and a^Sc^F, designated **Y** and **3** for short) and 2 recombinants (a^Fc^F and a^Sc^S, designated $\mathbf{Y^a}$ and $\mathbf{Y^c}$, respectively). Therefore, 7 possible genotypes could be observed in F_3 from the cross between a heterozygous male and heterozygous female of F_2 (Fig. 5). For

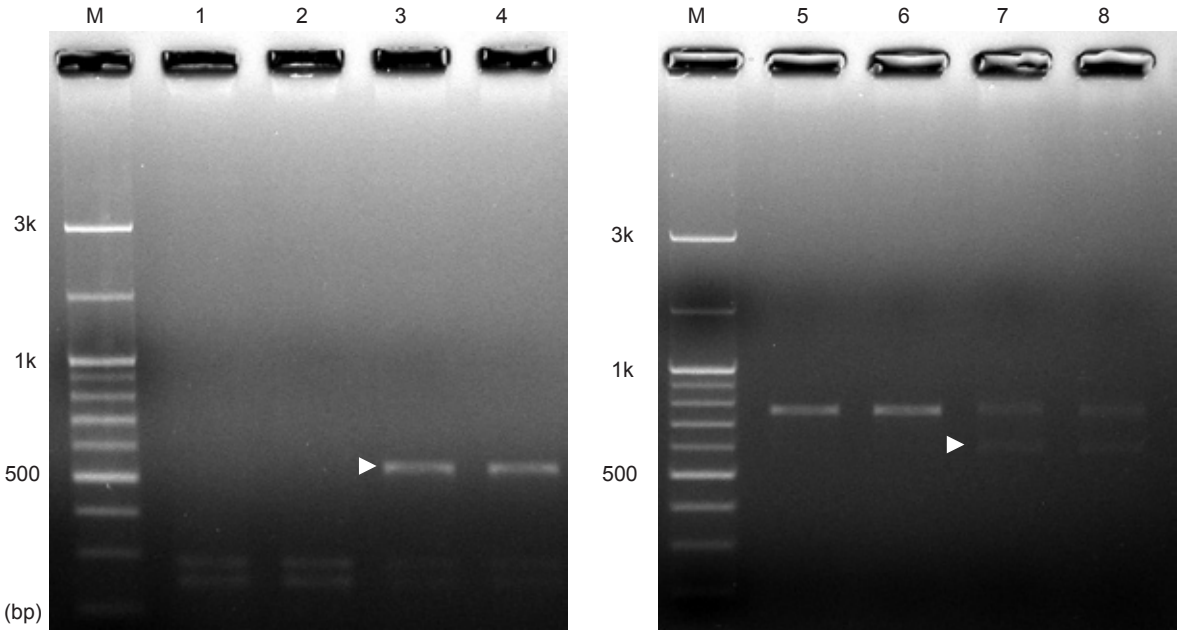

Fig. 4. PCR-RFLP patterns of hybrid strain H10. Left, c29-*Rsa*I; right, a52-*Hae*III. The 530-bp band of c29-*Rsa*I and the 600-bp band of a52-*Hae*III, marked by arrows, indicate the Y-like chromosome. Well numbers 1, 2, 5, and 6 are females; and 3, 4, 7, and 8 are males; M, markers.

Table 2. Sex ratio (male/total) of F_2 progeny produced from the chromosome extraction scheme and χ^2 test for deviation from a 1: 1 ratio

Y-like chromosome[a]	Source	F_2 sex ratio	Total number of F_2 progeny	χ^2
1	Hn_1	0.576	243	5.63*
2	Hn_1	0.466	279	1.29
3	Hn_2	0.508	372	0.10
4	H10	0.484	364	0.40
5	H10	0.480	298	0.48
6	H10	0.522	276	0.52
7	H10	0.519	268	0.37
8	H10	0.514	282	0.23
9	H10	0.484	353	0.34
10	H10	0.470	281	1.03
11	H10	0.472	352	1.14

[a] Eleven Y-like chromosomes were extracted by the crossing scheme shown in figure 3.
* $p < 0.05$.

comparison, the genotyping data of F_3 were pooled into 2 groups, H-old (i.e., H10) vs. H-young (i.e., Hn_1 plus Hn_2), to represent the situation of old and young Y-like chromosomes, respectively (Table 4). The distribution of these 7 genotypes with old Y-like chromosomes significantly differed from that with young ones (p = 0.02). By comparing non-recombinant chromosomes, **Y/Y** to **3/3** was 12 vs. 33 and 13 vs. 10 for the old and young Y-like chromosomes, respectively (x^2 = 5.84, p = 0.01). There were significantly fewer homozygous Y-like individuals (**Y/Y**) if the chromosome was extracted from the old hybrid strain than from the young hybrid strains. A similar situation was observed

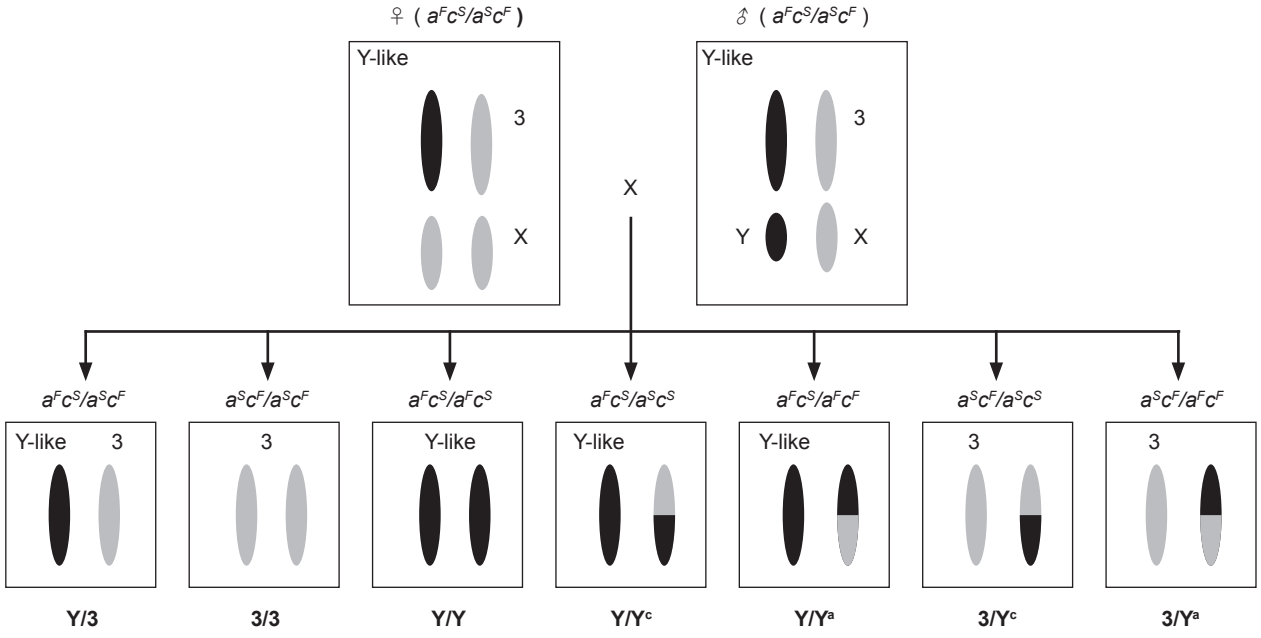

Fig. 5. Seven genotypes produced by a couple of heterozygtes. The male produces 2 kinds of gametes but the female produces 2 non-recombined and 2 recombinant gametes. The non-recombined Y-like chromosome indicated by a^Fc^S was designated **Y**; the 3rd chromosome of *D. nasuta* indicated by a^Sc^F was designated **3**. Two recombinants, a^Fc^F and a^Sc^S, were designated **Y^a** (i.e., a partial Y-like indicated by the a locus) and **Y^c**, respectively. The 7 genotypes were **Y/3**, **3/3**, **Y/Y**, **Y/Y^c**, **Y/Y^a**, **3/Y^c**, and **3/Y^a**.

Table 3. Number of F_2 progeny with and without a Y-like chromosome[a] as well as 2 × 2 cnotingency χ^2 values

Y-like chromosome[b]	Source	F^2 ♀		F^2 ♂		χ^2
		c^F/c^F	c^F/c^S	c^F/c^F	c^F/c^S	
1	Hn_1	48	29	37	40	3.18
2	Hn_1	35	34	36	33	0.03
3	Hn_2	39	32	30	42	2.52
4	H10	30	47	42	34	4.08*
5	H10	49	29	42	35	1.10
6	H10	43	33	44	33	0.01
7	H10	29	42	39	30	3.44
8	H10	32	39	36	35	0.45
9	H10	27	49	36	40	2.20
10	H10	36	33	37	33	0.01
11	H10	31	45	45	28	6.48*

[a]Numbers of F_2 progeny with and without a Y-like chromosome were determined according to the c29-*Rsa*I patterns: c^F/c^F and c^F/c^S represent those without and with a Y-like chromosome, respectively. [b]Eleven Y-like chromosomes were extracted by the crossing scheme shown in figure 3. * $p < 0.05$.

if the individual was partially Y-like homozygous. The ratios of partial Y-like homozygotes (i.e., **Y/Y^c** plus **Y/Y^a**) to partial 3 homozygotes (**3/Y^c** plus **3/Y^a**) were 31 vs. 47 and 25 vs. 18 for old and young Y-like chromosomes, respectively (x^2 = 43.43, p < 0.001). By comparing the old vs. young Y-like chromosomes, the fewer Y-like homozygotes may reflect recessive degeneration levels of this chromosome. The present results indicate that old Y-like chromosomes carried higher levels of recessive degeneration than did young ones.

DISCUSSION

Although a Y-like chromosome and an original Y chromosome are not physically attached in the hybrid strain, the sex linkage between the Y-like and Y chromosomes is similar to the fused neo-Y chromosome of *Drosophila albomicans*. Through crossing a hybrid strain male (2n = 7) with a *D. nasuta* female (2n = 8), the association between the Y and Y-like chromosomes could be broken in F_1 male progeny, because they separately paired with X and 3rd chromosomes that were derived from *D. nasuta* (Fig. 6). The Y-like chromosome was transmitted to F_2 females via a backcross and subsequently appeared in F_3 flies with homologous Y-like chromosomes via crosses of heterozygous F_2 males and females. This crossing scheme allowed us to establish flies with a pair of homologous Y-like chromosomes. This permitted us to examine whether the deleterious alleles gradually accumulated on Y-like chromosomes. From the results shown in table 4, the viability of flies with homozygous Y-like chromosomes derived from the old hybrid strain (H10) was apparently lower than that with homozygous Y-like chromosomes derived from the young hybrid strains (Hn_1 and Hn_2). Comparing either whole-arm homozygotes or partial homozygotes showed the same χ^2 significant results indicating that old Y-like chromosomes accumulated more recessive deleterious alleles than did young Y-like chromosomes. A giant synthetic neo-Y chromosome was established in a *D. melanogaster* model system (Rice 1994) to test Muller's ratchet (Muller 1964). As reported by Rice, harmful genetic variations accumulated on non-recombining chromosomes faster than on control chromosomes with normal recombination efficiencies. In our previous study, no recessive deleterious allele accumulation was detected when the H10 strain was in its 70th generation (Cheng 1999). If the lethal mutation rate of this Y-like chromosome is also 0.0063 per chromosome per generation (Mukai 1965) the chance of seeing a chromosome without recessive lethal mutations is 0.64 in the 70th generation, but 0.17 in the 280 generation. Due to a lack of sufficient genetic markers and inversions which inhibit recombination, the comparison merely provides a rough picture. Our results are in agreement on a theoretical speculation, i.e., a lack of recombination could accelerate the accumulation of small-effect deleterious alleles on a primitive Y sex chromosome. Therefore, deleterious alleles become notable at a later stage.

During the crossing experiment, several interesting phenomena associated with Y-like

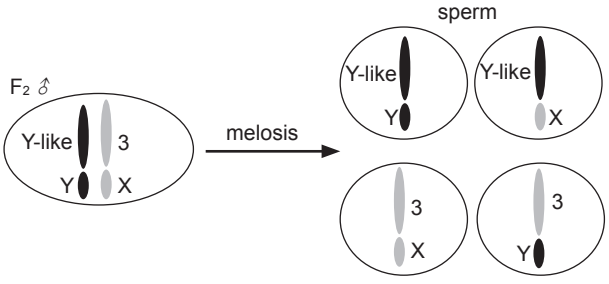

Fig. 6. Meiosis of an F_1 male illustrated showing the 3rd, Y, Y-like, X, and neo-X chromosomes only. In an F_1 male, the Y-like and Y chromosomes could independently segregate because of the absence of a neo-X.

Table 4. Numbers of 7 genotypes (indicated by a52-*Hae*III allele followed by the c29-*Rsa*I allele; a slash separates homologous chromosomes) in F_3 shown in a 2 × 7 contingency table

	Y/3	3/3	Y/Y	Y/Y^a	Y/Y^c	3/Y^a	3/Y^c	χ^2
H-young	21	10	13	7	18	6	12	14.45*
H-old	63	33	12	13	18	20	27	

* p < 0.05.

chromosomes were also observed. The sex ratios of F_1 from both the young and old strains were significantly female biased (Table 1). We previously demonstrated the existence of a meiotic driver on the neo-X chromosome of *D. albomicans* strain #163.5 (Yang et al. 2004), which was used to establish these 3 hybrid strains. The female-biased sex ratio may have nothing to do with the Y-like chromosome but is likely caused by the meiotic driver. The Y-like chromosomes extracted from old hybrid strains caused less distortion than those from young hybrid strains probably because suppression had improved. The sex ratio of offspring produced by a fertile F_1 male reverted to normal (Table 2) because the meiotic driver located on the *D. albomicans* neo-X chromosome was not transmitted to the next generation in our crossing scheme. The disappearance of a female-biased sex ratio in F_2 was expected.

Table 1 reveals a significant reduction in the number of F_1 offspring, and this was more serious in the young strains than in the old one. As previously reported (Chang and Ayala 1989), the meiotic driver in the *D. albomicans* strain does not reduce the number of progeny, whereas numbers of F_1 progeny produced by hybrid-strain males crossed with *D. nasuta* females (Table 1) were significantly reduced. This reduction may indicate incompatibility between hybrid-strain males and *D. nasuta* females. There are 2 possibilities: both neo-X- and the (Y+Y-like)-carrying sperm have somehow reduced fertilizing capacity with *D. nasuta* eggs; or both male and female zygotes have developmental problems. The incompatibility in female offspring either between the neo-X carrying sperm and *D. nasuta* eggs during fertilization or between the neo-X and the 3rd and X chromosomes of *D. nasuta* during development does not exist if the neo-X chromosome is directly obtained from *D. albomicans*. Some loss of fitness in F_1 may exist, even though it does not meet the extreme criterion of Coyne and Orr (1997) for "postzygotic isolation". No F_1 reduction was detectable between *D. albomicans* and *D. nasuta*, but "hybrid breakdown" was mentioned in a previous paper (Chang and Ayala 1989). The problem appears significant if the neo-X is from these hybrid strains. Hybrid breakdown may be caused by recombination and complex epistatic interactions after hybridization. After the disturbance, the genomic system could be continually improving through selection. After 20 generations of selection, the hybrid system might still be in an unstable situation, and that might explain why the young hybrid strains were worse than the old one (Table 1). The divergence between *D. albomicans* and *D. nasuta* may have occurred too recently to cause significant hybrid inviability or sterility, which can be explained by the Dobzhansky-Muller incompatibility (Turelli et al. 2001). The reduced number of offspring from the cross between a hybrid strain and *D. nasuta* was unexpected. Each species is canalized for normal fertilization and development while containing underlying genetic variations. Hybridization between genetically divergent but still crossable species may mix up many potential DMIs. Selection against DMIs proceeds, and this may explain the better performance of the old hybrid strain compared to young hybrid strains as shown in table 1. In a young hybrid strain, genetic heterogeneity is higher, but most of the combinations are unfit. When crossed to *D. nasuta*, as long as the F_1 male is viable and fertile, the number of offspring reverted to normal (Table 2). Genomic incompatibility was thus selected against and ceased further genetic transmission. The average numbers of female increased from 31 with a young Y-like chromosome to 114 with an old Y-like chromosome, which was close to the average number of 147 in *D. nasuta*. These results indicate that if selection continues long enough, reproduction of the hybrid system may recover. It might take over 0.5 Ma to achieve fixed DMIs for hybrid sterility or inviability.

The male-biased sex ratio was only observed in flies with 1 Y-like chromosome extracted from the young hybrid strains (Table 2). Due to the small sample size, the significance was marginal. However, the F_2 generation was a mixture of heterozygous Y-like and homozygotes without the Y-like chromosome. The ability to detect a sex ratio distortion should be lower. Under such a circumstance, the significance may be meaningful. In addition, the sex ratio of flies derived from this Y-like chromosome significantly differed from that from the 2 young Y-like chromosomes ($\chi^2 = 5.09$, $p = 0.024$). This phenomenon indicates that sexual antagonism was polymorphic in the young hybrid strains. The same chromosome with sexual antagonism showed less female bias than the other 2 young Y-like chromosomes in the F_1 sex ratios (Table 1), which might also be explained by counteracting forces of the meiotic driver and sexual antagonism. None of the old Y-like chromosomes showed a male-biased sex ratio. Sexual antagonism means a character is favored in 1 sex but disfavored in

the other sex; for example, the lek behavior of *D. albomicans* males (Chang and Tai 2007) might be disadvantageous if it appeared in females (i.e., fighting females often lay fewer eggs). Sexually antagonistic alleles are predicted to accumulate on sex chromosomes (Rice 1984), and an artificially constructed *D. melanogaster* model system demonstrated that sexually antagonistic alleles could indeed accumulate at loci that were tightly linked with nascent gender-determining genes on sex chromosomes (Rice 1992). Sexually antagonistic alleles are expected to accumulate on the entire Y-like chromosome because it lacks recombination. If a sexually antagonistic influence on viability exists, the number of females carrying the Y-like chromosome should decrease. Sexual antagonism was detected (i.e., one among 4 extracted Y-like chromosomes) in the H10 strain using the isozyme APH (alkaline phosphatase) as a genetic marker about 10 yr ago while it was in its 70th generation (Cheng 1999). Moreover, 29 generations after the establishment of a giant sex-linked synthetic chromosome, Rice (1992) found a significant sexual antagonistic effect. These observations suggest that a sexually antagonistic effect may be significant at an early stage.

Nevertheless, after 280 generations, the Y-like chromosome might have become dependent on the neo-X chromosome. Coevolution may occur between Y-linked genes and those located on the X chromosome (Rice and Chippindale 2002). The Y chromosome may evolve strong regulation of its own genome in males and affect the expression of hundreds of X-linked and autosomal genes (Lemos et al. 2008).

Incompatibility between the Y-like chromosome and the 3rd chromosome of *D. nasuta* may affect the viability of heterozygous F_2 males. In the *albomicans-nasuta* hybrid system, a *nasuta* 3rd chromosome (Y-like chromosome) is a partner of the Y chromosome, coevolves with the neo-X chromosome, and becomes neo-X dependent. In our previous study (Lin et al. 2008), we demonstrated the dependence of the neo-Y on neo-X chromosomes of *D. albomicans* by reciprocal crosses. Therefore, when a Y-like chromosome encounters a homologous chromosome from another population, reduced viability may be due to genetic incompatibility. This could explain the fewer heterozygous males with no. 4 and 11 Y-like chromosomes from H10 in F_2 (Table 3). Furthermore, this may be 1 reason explaining why males, the heterogametic sex, might be more vulnerable than females as Haldane's rule suggests (Haldane 1922). In *D. albomicans*, the dependence seems to be fixed, but it remains polymorphic in the H10 hybrid strain.

Our investigation was aimed at detecting gross evolutionary changes over the entire Y-like chromosome rather than on specific genes. The genetic compositions of both the old and young hybrid strains remained polymorphic. Sexual antagonism on the Y-like chromosome favoring males but disadvantageous to females was only weakly supported by the male-biased sex ratio in F_2 from a young hybrid strain, but was undetectable among Y-like chromosomes from the old hybrid strain. On the contrary, that males possessing lower heterozygosity of the 2 old Y-like chromosomes was probably due to the incompatibility of the *D. nasuta* 3rd chromosome and Y-like chromosome. Furthermore, recessive deleterious alleles gradually accumulated on a non-recombining Y-like chromosome during the past 280 generations. Since it is impossible to perform similar experiments on the neo-Y chromosome of *D. albomicans*, molecular approaches are required to uncover the evolution of its neo-Y chromosome.

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