

Evolution of a recent neo-Y sex chromosome in a laboratory population of *Drosophila*

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Abstract

In many species of animals, one of the sexes has a chromosome that is structurally and functionally different from its so-called homologue. Conventionally, it is called Y chromosome or W chromosome depending on whether it is present in males or females respectively. The corresponding homologous chromosomes are called X and Z chromosomes. The dimorphic sex chromosomes are believed to have originated from undifferentiated autosomes. In extant species it is difficult to envisage the changes that have occurred in the evolution of dimorphic sex chromosomes. In our laboratory, interracial hybridization between two *Drosophila* chromosomal races has resulted in the evolution of a novel race, which we have called Cytorace 1. Here we record that in the genome of Cytorace 1 one of the autosomes of its parents is inherited in a manner similar to that of a classical Y chromosome. Thus this unique Cytorace 1 has the youngest neo-Y sex chromosome (5000 days old; about 300 generations) and it can serve as a 'window' for following the transition of an autosome to a Y sex chromosome.

[Tanuja M. T., Ramachandra N. B. and Ranganath H. A. 1999 Evolution of a recent neo-Y sex chromosome in a laboratory population of *Drosophila*. *J. Genet.* **78**, 81–85]

Introduction

Drosophila has heteromorphic sex chromosomes (X, Y). Females have two X chromosomes while males have one X and one Y chromosome. Thus the Y chromosome is limited to males only. It is believed that such heteromorphic chromosomes have evolved from a pair of completely homologous autosomes. The evolutionary processes that might have led to dimorphic X and Y chromosomes have been explored in some detail using both experimental and theoretical approaches. The Y chromosome carries fewer active genes than the X chromosome. Such a degenerate Y chromosome could have resulted from accumulation of deleterious mutations, absence of recombination in males, and/or accumulation of transposable elements (Muller 1918; Lucchesi 1978; Charlesworth 1996; Rice 1996a,b; Steinemann and Steinemann 1997). The dimorphism of X and Y chromosomes appears to have evolved slowly over millions of years. Therefore it is almost impossible to retrace the steps by which the Y chromosome evolved from

an ordinary autosome in any one particular lineage. In different species of *Drosophila* evolution of the Y chromosome has occurred independently in different lineages at different points of time. Accordingly, it has been documented that Y-chromosome evolution is at different stages in different extant species of *Drosophila*, which are of different evolutionary ages. By comparing the Y chromosomes of a few extant species of *Drosophila*, it is possible to demonstrate the different stages in the evolution of the Y chromosome—from a primitive stage at which it is almost similar to an autosome, through intermediate stages with various levels of divergence, to the present, almost completely diverged state. The statuses of Y chromosomes of *D. americana americana* (a few hundred thousand years old), *D. miranda* (two million years (Myr) old), *D. pseudoobscura* (13 Myr old) and *D. melanogaster* (60 Myr old) serve as excellent examples to illustrate this point (Bone and Kuroda 1996; Marin *et al.* 1996; Charlesworth *et al.* 1997; Steinemann and Steinemann 1997). In all these cases the origin of the Y chromosome dates back millions of years and the interpretations are made retrospectively. In this communication we report the emergence of a new Y

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Keywords. *Drosophila*; hybridization; *nasuta*–*albomicans* complex; Cytorace 1; neo-Y sex chromosome.

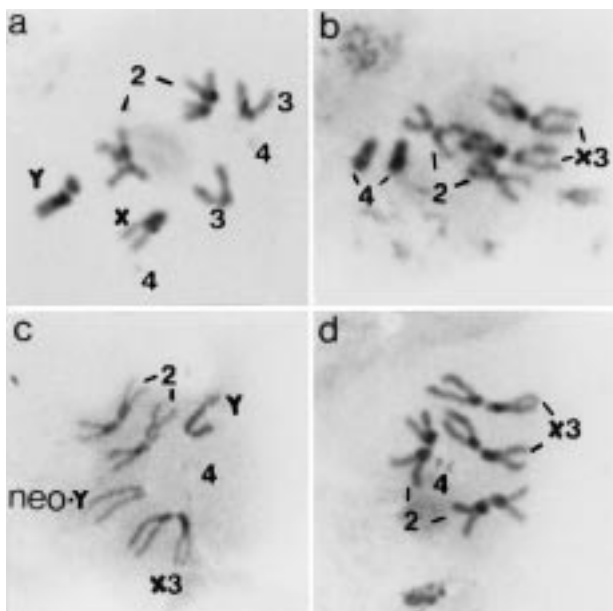


Figure 1. Karyotypes in the *D. nasuta* group. **a**, *D. n. nasuta*, male, $2n = 8$, with a pair of metacentrics (chromosome 2), a pair of acrocentrics (chromosome 3), an acrocentric X chromosome, a submetacentric Y chromosome, and a pair of dots (chromosome 4). **b**, *D. n. albomicans*, female, $2n = 6$, with a pair of small metacentrics (chromosome 2), large metacentrics (chromosome X3), and a pair of long dots (chromosome 4). Hybridization between males of *D. n. nasuta* and females of *D. n. albomicans* has resulted in the evolution of the karyotype of Cytorace 1, which has differential representation of the chromosome of its parents. **c**, Cytorace 1, male, $2n = 7$, a pair of metacentric chromosome 2, a submetacentric Y chromosome, an acrocentric chromosome 3, and a pair of small dot chromosomes (chromosome 4) of *nasuta* parent along with one X3 metacentric chromosome of *albomicans* parent; since the acrocentric chromosome 3 is always restricted to the male genome, it has been called neo-Y chromosome. **d**, Cytorace 1, female, $2n = 6$, a pair of metacentric chromosome 2, and a pair of small dot chromosomes (chromosome 4) of *nasuta* parent along with a pair of metacentric X3 chromosome of *albomicans* parent.

chromosome (neo-Y) that has evolved in a laboratory population of a synthetic *Drosophila* race in a time span of ~ 5000 days (about 300 generations).

Recent neo-Y of Cytorace-1

D. nasuta nasuta and *D. nasuta albomicans* are sibling races of the *nasuta* subgroup of the *immigrans* species group of *Drosophila* (Nirmala and Krishnamurthy 1971; Hagele and Ranganath 1982a; Ranganath and Hagele 1982; Ranganath et al. 1982). The chromosomes of *D. n. nasuta* ($2n = 8$) are shown in figure 1a. Karyotypic evolution in the genus *Drosophila* is assumed to have started from an ancestral karyotype with $2n = 12$. This includes five pairs of acrocentric chromosomes and a pair of dot chromosomes (Patterson and Stone 1952). From such an ancestral karyotype, Ranganath and Hagele (1981) derived the karyotypes of *D. n. nasuta* and *D. n. albomicans*. It is argued that

a centric fusion between two primitive elements followed by a pericentric inversion resulted in the formation of the present-day chromosome 3 of *D. n. nasuta* (see figure 3) (Ranganath and Hagele 1981). Hence the acrocentric chromosome 3 of *D. n. nasuta*, which has two of the six ancestral chromosomes in it, is referred to as double-length rod. The chromosomes of *D. n. albomicans* ($2n = 6$) are seen in figure 1b. On the basis of homology, it is argued that centric fusion between the sex chromosomes and the chromosome 3 of *D. n. nasuta* (*nasuta*-like) ancestor resulted in the evolution of metacentric X3 and Y3 chromosomes (see figure 3), of *D. n. albomicans* (Ranganath and Hagele 1981).

Allopatric *D. n. nasuta* and *D. n. albomicans* are cross-fertile. Therefore they are treated as chromosomal races (Nirmala and Krishnamurthy 1971; Ranganath and Hagele 1982). The hybrid progeny between *D. n. nasuta* and *D. n. albomicans* can be maintained in the laboratory; and in our laboratory they have already passed through 300 generations. The F_1 has $2n = 7$ while the F_2 and the subsequent hybrid generations have karyotypically different types of individuals. In these hybrids the chromosomes of the parents, namely *D. n. nasuta* and *D. n. albomicans*, can be distinguished easily on the basis of heterochromatin blocks in mitotic chromosomes. In some of these crosses, karyotypic variability disappears between 20 and 200 generations with the establishment of a stable karyotype. During this process of the evolution of a stable karyotype, some of the parental chromosomes are eliminated while others are retained (Ramachandra and Ranganath 1986, 1990, 1996).

Cytorace 1 is one such product of hybridization between males of *D. n. nasuta* and females of *D. n. albomicans* (Ramachandra and Ranganath 1986). The males and females of Cytorace 1 have $2n = 7$ and $2n = 6$ respectively (figure 1, c & d). Since 1985, only these karyotypes have been seen in Cytorace 1, with very rare appearance of sterile aneuploids at 1 to 2%. The most crucial event in the transmission of chromosomes from one generation to the next in Cytorace 1 is that of the acrocentric chromosome 3 of the male. As mentioned above, Cytorace 1 has inherited this chromosome 3 from *D. n. nasuta*. This chromosome is exclusively found in the male genome of Cytorace 1 (figure 2). It is inherited from father to son, exactly in the same fashion as a regular Y chromosome although it is not physically linked to it. In other words, this male-trapped chromosome 3 is behaving like yet another Y chromosome (neo-Y) in Cytorace 1. Thus, in the karyotype of Cytorace 1, there are two chromosomes that are restricted to males only. One of them is the original submetacentric Y and the other is the neo-Y chromosome (chromosome 3). Similarly the males of *D. a. americana* have a regular Y chromosome and another male-restricted neo-Y chromosome (Charlesworth et al. 1997). As mentioned earlier, two of the six ancestral chromosomes are represented in chromosome 3 and one of the other ancestral elements is seen in the Y chromosome. Thus

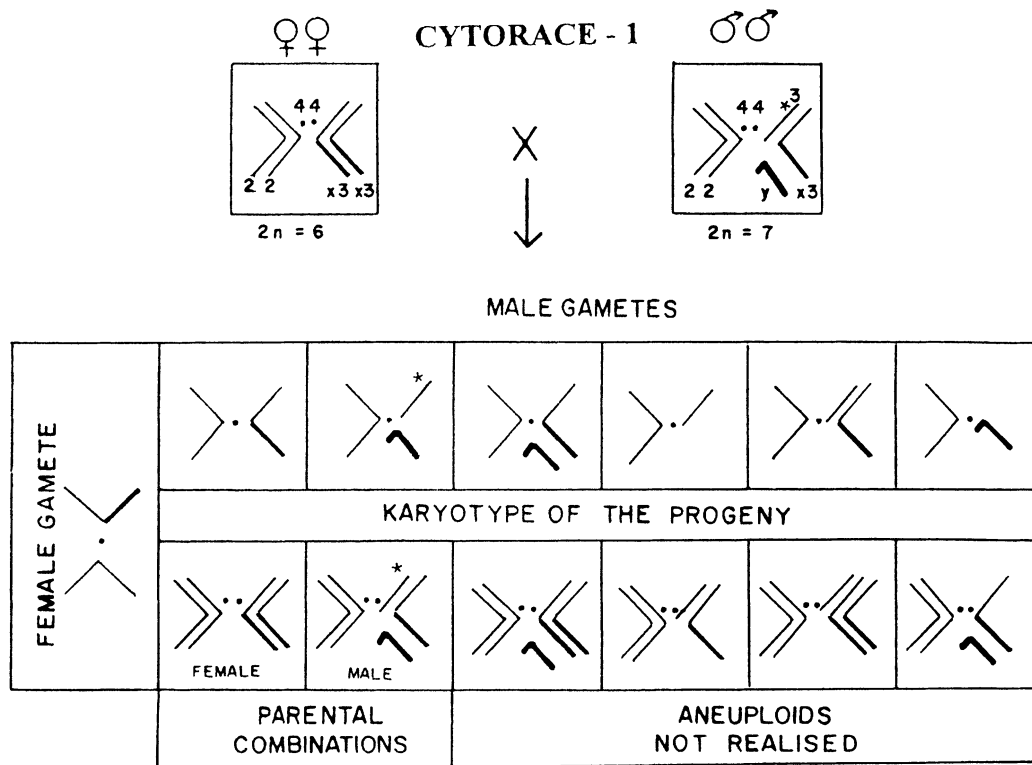


Figure 2. Cytorace 1 is unique, because, in spite of showing a difference between males ($2n = 7$) and females ($2n = 6$) in chromosome number, it breeds true. The mechanism underlying this is given here with diagrammatic representation of chromosomal constitutions of gametes of Cytorace 1 and of progeny. Males with $2n = 7$ can produce six types of gametes while females with $2n = 6$ yield only one type of gamete, to result in six types of individuals. But in reality, only two types of individuals occur in the progeny, wherein all males have $2n = 7$ and all females have $2n = 6$. The other four varieties are extremely rare and are aneuploids. The pattern of inheritance of acrocentric chromosome 3, inherited from *nasuta* parent, being present only in the male genome of Cytorace 1, is interesting (shown with asterisk). This autosome of the *nasuta* parent in Cytorace 1 never gets into the female genome. It is cosegregating with the regular Y chromosome and hence it is named neo-Y chromosome.

three of the six primitive chromosomes (50% of the ancestral genome) of the genus *Drosophila* are restricted to the male genome of Cytorace 1 (figure 3). As this stabilized karyotype of Cytorace 1 evolved in our laboratory during 1985 (Ramachandra and Ranganath 1986), the age of this race and hence of the trapped neo-Y chromosome is about 5000 days (300 generations).

Interracial hybridization between *D. n. nasuta* and *D. n. albomicans* has given rise to the genome of Cytorace 1. The neo-Y, i.e. chromosome 3, and the Y chromosome of Cytorace 1 are of *D. n. nasuta* parent. The counterparts of these chromosomes are from *D. n. albomicans*, and are present in the metacentric X3 chromosome. The euchromatic neo-Y chromosome of Cytorace 1 has its homologue in one arm of the X3 chromosome while the counterpart of the heterochromatic Y chromosome is seen in the other euchromatic arm of the X3 chromosome. The structural and functional hemizygoty is clearly seen between the Y chromosome and the X arm of the X3 chromosome, while this is yet to be established between the neo-Y and its homologue-3 arm of the X3 metacentric in the males of Cytorace 1.

Hybridization between *D. a. americana* and *D. a. texana* of the *virilis* group of *Drosophila* produced fertile F_1 s

(Throckmorton 1982). A backcross of the F_1 males of this cross with the females of *D. a. americana* results in progeny in which the autosome 4 inherited from *D. texana* grandparent is restricted to the male genome. Therefore this chromosome may be treated as a new neo-Y chromosome. But such a hybrid lineage has limitations, because the chromosomes of the *americana* and *texana* parents will be segregating in these individuals, and due to permutation and combination between these chromosomes the cytogenetic backgrounds of the individuals differ. Therefore it is not a true-breeding lineage. On the other hand, the status of Cytorace 1 is distinctly different from that of the backcross progeny of the F_1 hybrids of *D. a. americana* and *D. a. texana*. Cytorace 1 is isogenized for the parental chromosomes and therefore is a 'true-breeding' hybrid lineage.

Interracial hybridization between *D. n. nasuta* and *D. n. albomicans* followed by the evolution of a hybrid lineage called Cytorace 1 is a sort of evolutionary experimentation and karyotypic race formation under laboratory conditions. During these 300 generations since it evolved, Cytorace 1 has passed through a phase of population differentiation. The extent of difference between Cytorace 1 and its parents is seen in terms of its karyotypic organization (Ramachandra

and Ranganath 1986, 1996; Ranganath and Ramachandra 1987; M. T. Tanuja, N. B. Ramachandra and H. A. Ranganath, unpublished), population fitness (Ramachandra and Ranganath 1988), mating preference (Ramachandra and

Ranganath 1994), and morphophenotypic variations (Harini and Ramachandra 1999). The present report of neo-Y chromosome of Cytorace 1 has added one more novel feature of genetic differentiation.

Implications

The present model, i.e. the Cytorace 1 with the youngest neo-Y chromosome in its genome, provides a unique opportunity to study the process of transition of an autosome to a Y sex chromosome from the day of its commitment to the male genome. It is tempting to consider the following three long-range evolutionary implications of the karyotype of Cytorace 1. (i) Phylogenetically, the karyotype of *D. n. albomicans* is a product of three centric fusions (figure 3) (Ranganath and Hagele 1981). Again, this 'fusion tendency' was noticed in an individual of *D. n. albomicans* during 1981, wherein the fusion had occurred between two dot chromosomes (Hagele and Ranganath 1982b). If the same tendency were to prevail in Cytorace 1, it may promote fusion between the neo-Y (chromosome 3) and the Y chromosome to result in a metacentric chromosome, mimicking the 'Y3' chromosome of *D. n. albomicans*. (ii) The genomes of *D. n. nasuta* and *D. n. albomicans* have more heterochromatin than their hybrid progeny, Cytorace 1. The heterochromatinization of neo-Y, if it happens in due course, can add to the amount of heterochromatin of Cytorace 1. (iii) Rice (Rice 1996a, b, 1998) through his elegant experiments has created a synthetic Y chromosome in *D. melanogaster* by a combination of artificial selection and visible genetic markers. He has recorded the impact of the transmission of chromosomes from mother to daughter as well as from father to son. These experiments have thrown light on the degeneration of the nonrecombining Y sex chromosome. H. A. Ranganath and W. E. Kalisch (unpublished data) have observed that, like in many other species of *Drosophila*, meiotic recombination is absent in the males of *D. n. nasuta*, *D. n. albomicans* and their hybrids. Therefore the male-restricted neo-Y chromosome of Cytorace 1, now deprived of meiotic recombination, is a suitable candidate for further

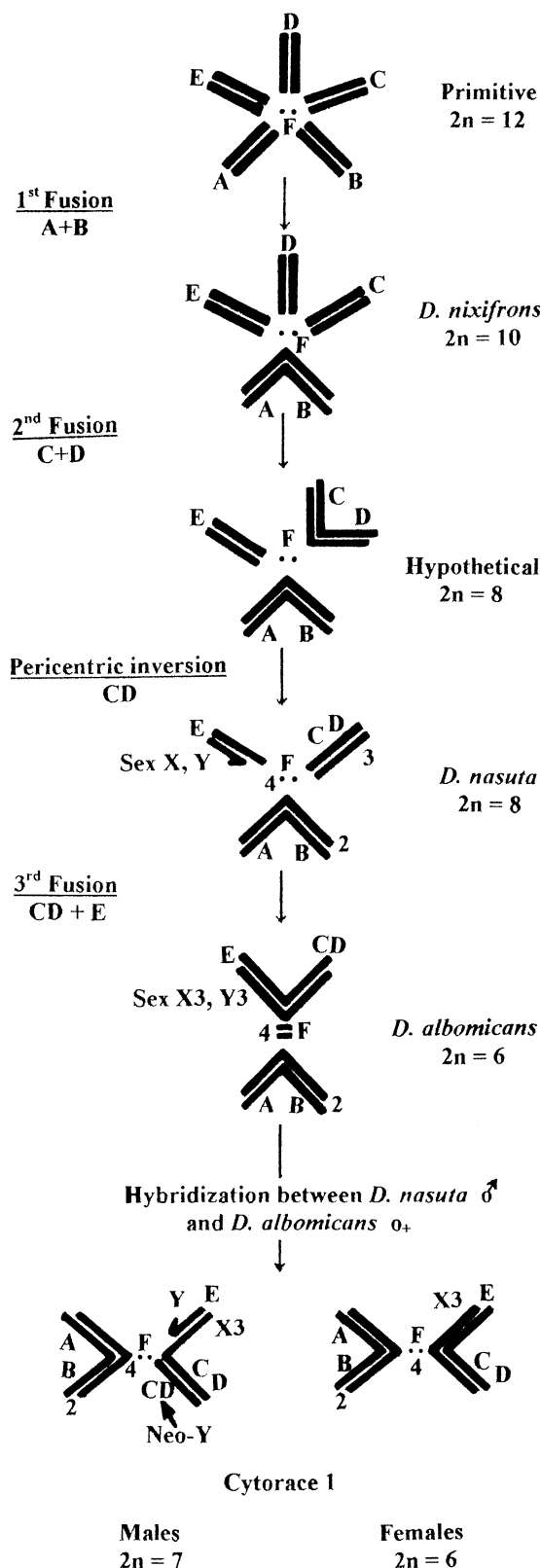


Figure 3. Phylogeny of the neo-Y chromosome of Cytorace 1. The primitive karyotypic constitution of *Drosophila* has $2n = 12$ (A to F). From this primitive setup Ranganath and Hagele (1981) have drawn the successive stages in the karyotypic evolution of the members of the *nasuta* subgroup. In this lineage, the karyotype of *D. nasuta* is a product of two centric fusions and a pericentric inversion. A third centric fusion has resulted in the evolution of the karyotype of *D. albomicans*. Cytorace 1 is a product of hybridization between males of *D. nasuta* and females of *D. albomicans*. The males and females of Cytorace 1 have $2n = 7$ and $2n = 6$ respectively. The neo-Y chromosome of Cytorace 1 (labelled 'CD') is an autosome (chromosome 3) in *D. nasuta*. This 'CD' chromosome phylogenetically is a product of centric fusion between C and D chromosomes of the primitive karyotype, which was followed by a pericentric inversion to result in an acrocentric chromosome, which is referred to as double-length rod.

studies. Experiments are in progress to selectively induce mutations and introduce transposable elements into the neo-Y of Cytorace 1 to simulate the probable changes that the differentiated heteromorphic Y chromosomes might have undergone during their evolution. With this, we hope to hasten the otherwise slow evolutionary events and thus attempt to witness the various transitional stages in the metamorphosis of an autosome into the Y chromosome.

Acknowledgements

Financial assistance received from the Department of Biotechnology and the Department of Science and Technology of the Government of India is gratefully acknowledged.

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Received 11 December 1998; in revised form 30 April 1999