Evolution of a new chromosomal lineage in a laboratory population of *Drosophila* through centric fission

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Structural rearrangements of chromosomes have played a decisive role in the karyotypic evolution of species. It is also known that inversions, translocations, fusions, fissions, heterochromatin variations and other chromosomal changes occur as transient events in natural populations. Herein we report the occurrence of a rare event of centric fission of a metacentric chromosome in a laboratory population of *Drosophila*, called Cytorace 1. This centric fission has been fixed in a sub-population of Cytorace 1, resulting in a new chromosomal lineage called Fissioncytorace-1.

1. Introduction

Research on hybrid zones is a relatively recent development in evolutionary biology. Hybrid zones occur in nature when genetically distinct groups of individuals meet and mate, resulting in at least some offspring of mixed ancestry (Harrison 1990; Barton and Hewitt 1989). The evolutionary significance of hybrid zones, particularly in animals, has been studied in different groups of organisms such as grasshoppers (Hewitt 1993), butterflies (Mallet 1993), crickets (Rand and Harrison 1989), toads (Szymura 1993), birds (Moore 1977) and mammals (Searle 1988, 1991; Patton 1993). This has lead to the realization of its importance which is reflected in treating hybrid zones as "windows on the evolutionary process" (Harrison 1990) and as "natural laboratories" (Hewitt 1988; Barton and Hewitt 1989).

Drosophila nasuta nasuta with 2n = 8 (figure 1a, b) has a pair of metacentrics, representing chromosome 2, a pair of acrocentric chromosomes 3, an acrocentric X, a submetacentric Y and a pair of dot chromosomes. *D. n. albomicans* with 2n = 6 (figure 1c, d) has two pairs of metacentrics and a pair of long dot chromosomes. Of the two pairs of metacentrics, the smaller one represents chromosome 2. The larger metacentric, believed to be a product of centric fusion between autosome 3 and the sex chromosomes of a *nasuta*-like parent, is referred to as X3 and Y3 chromosomes (Ranganath and Hagele 1981). *D. n. nasuta* and *D. n. albomicans* are morphologically indistinguishable members of the *nasuta* subgroup of the *immigrans* species group of *Drosophila*. They are allopatric in distribution (Ranganath and Ramachandra 1987). These karyotypically different sibling forms can be crossed in the laboratory and hybrid progeny produced that can be maintained for many generations. The F_1's of the cross between *D. n. nasuta* and *D. n. albomicans* have 2n = 7, while the F_2's and subsequent hybrid individuals show various karyotypic combinations, referred to as 'karyotypic mosaicism' (Rajasekarasetty *et al* 1979; Ramachandra and Ranganath 1986). In many of the crosses, the karyotypic mosaicism disappears due to fixation of karyotypes, the composition of which differs between crosses. Such recombinant populations, each with a new and a different karyotypic combination are called Cytoraces. Sixteen such Cytoraces have evolved between 1985–1996, differing by the contribution of *D. n. nasuta* and of *D. n. albomicans* chromosomes (Ramachandra and Ranganath 1996).

The assemblage containing *D. n. nasuta, D. n. albomicans* and the 16 Cytoraces is recognized as a new evolving cluster and referred to as the '*nasuta–albomicans* complex' (Ramachandra and Ranganath 1996). Tanuja *et al* (1998) treated this complex as an artificial hybrid zone of *Drosophila* with allo-sympatric populations.

Keywords. *Drosophila*; chromosomes; karyotypes; fission; evolution

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We herein report the emergence of a new karyotypic lineage within this lineage through a very rare event of centric fission.

2. Materials and methods

2.1 *Drosophila stocks*

(i) *D. n. nasuta* (2n = 8) (Coorg, south India), (ii) *D. n. albomicans* (2n = 6) (Okinawa, University of Texas collections, 3045.11) and (iii) Cytorace 1 (males, 2n = 7; females, 2n = 6) (Ramachandra and Ranganath 1986), produced by interracial hybridization between the two races. The metaphase chromosomes from neural ganglion were prepared following the method of Ramachandra and Ranganath (1986). The C-banding technique of Sumner (1972) and the Q-banding procedure adopted by Ranganath *et al* (1982) were followed.

3. Results

Cytorace 1 is the product of hybridization between the males of *D. n. nasuta* and the females of *D. n. albomicans* (Ramachandra and Ranganath 1986). The females of Cytorace 1 have 2n = 6 (2^n 2^a, $X3^a$ $X3^a$, 4^n 4^n) while the males have 2n = 7 (2^n 2^a, Y^n $X3^a$ 3^n, 4^n 4^n) (the superscripts represent the parent from which the chromosome has been inherited: n = *nasuta*; a = *albomicans*, figure 2).

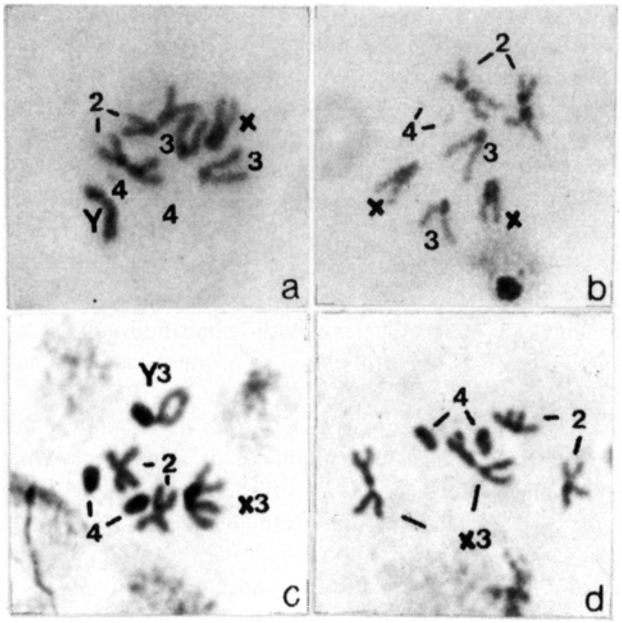


Figure 1. Metaphase chromosomes of *D. n. nasuta* 2n = 8 male (**a**) and female (**b**) and of *D. n. albomicans* 2n = 6 male (**c**) and female (**d**).

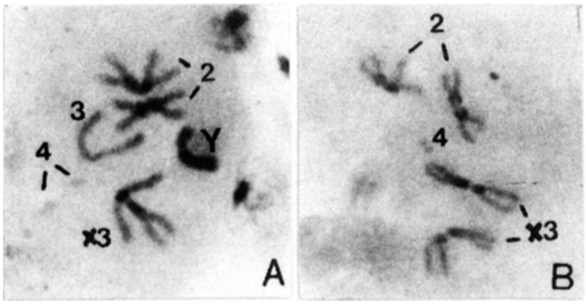


Figure 2. Metaphase chromosomes of Cytorace 1 – males 2n = 7 (**A**) and females 2n = 6 (**B**).

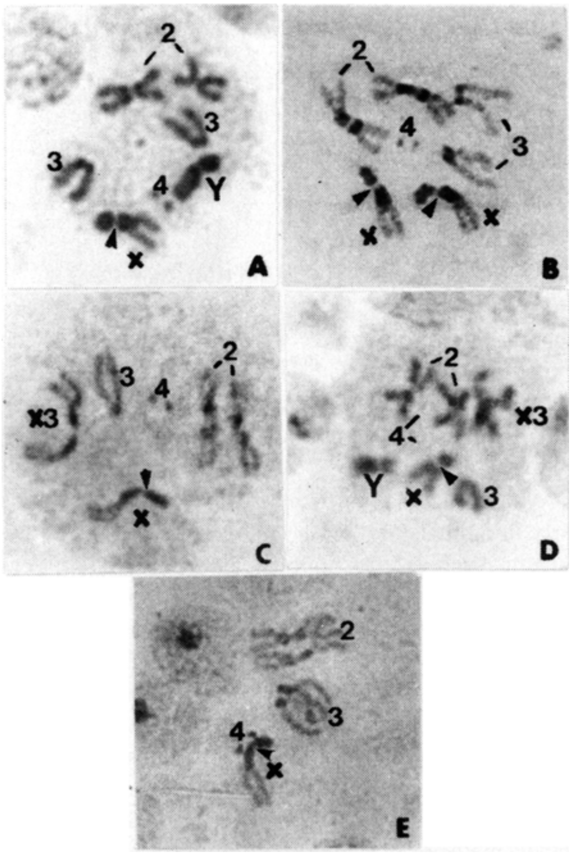
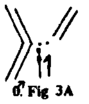


Figure 3. Karyotypic polymorphism in one of the replicate cultures of Cytorace 1. In addition to normal Cytorace 1 karyotypes, males with 2n = 7 (figure 2A) and females with 2n = 6 (figure 2B), the following 5 new karyotypes were recorded. Arrowhead indicates the new X chromosome. (**A**) Male: 2n = 8 with pairs of chromosomes 2, 3, and 4, a new X chromosome and a Y chromosome. (**B**) Female: 2n = 8 with pairs of chromosomes 2, 3, and 4, and a pair of new X chromosomes. (**C**) Female: 2n = 7 with pairs of chromosomes 2 and 4, one X3 chromosome, one 3 and a new X chromosome. (**D**) 2n = 8 with pairs of chromosomes 2 and 4, a metacentric X3, 3, Y and a new X chromosome (aneuploid). (**E**) 2n = 7 with pairs of chromosomes 2, 3 and 4, a new X chromosome (aneuploid).

The uniqueness of Cytorace 1 is that although different diploid numbers are present in males and females, it breeds true for these karyotypes. Females of Cytorace 1 produce only one type of egg with 2, $X3^{a}$, 4^{n} chromosomes, while the males can produce 6 types of sperm. Of these, only two carry haploid sets of chromosomes, namely 2, $X3^{a}$, 4^{n} and 2, Y^{n}, 3^{n}, 4^{n}, while the others are aneuploid for either chromosome 3 or the sex chromosomes. The eggs with 2, $X3^{a}$, 4^{n} chromosomes, if fertilized by a sperm with the 2, $X3^{a}$, 4^{n} complement, result in a female with $2n = 6$ (2 2, $X3^{a}X3^{a}$, $4^{n}\ 4^{n}$); on the other hand, if this egg is fertilized by a sperm with 2, Y^{n}, 3^{n}, 4^{n}, it results in a male with $2n = 7$ (2 2, $Y^{n}\ X3^{a}\ 3^{n}$, $4^{n}\ 4^{n}$). Since 1985, the karyotype of Cytorace 1 has been regularly screened, and except for the very rare occurrence of one or two aneuploids, all males and females showed the $2n = 7$ and $2n = 6$ karyotypes respectively as described above.

Table 1. Karyotypic polymorphism in one of the replicate cultures of Cytorace 1 during 1996–1998. At each screening, 100 individuals were scored and the frequencies of the 7 different karyotypes are given below.

Karyotypes	1996 I	1996 II	1996 III	1997 IV	1997 V	1998 VI	1998 VII
	Expected karyotypes						
 ♂ Fig 2A	36	44	27	22	32	20	24
 ♀ Fig 2B	21	15	24	03	08	07	04
	New karyotypes						
 ♂ Fig 3A	14	13	26	34	21	34	27
 ♀ Fig 3B	10	08	05	21	26	28	25
 ♀ Fig 3C	13	17	17	20	13	11	20
 ♂ Fig 3D	04	02	01	–	–	–	–
 ♀ Fig 3E	02	01	–	–	–	–	–

During 1996–1998, in one of the replicate cultures of Cytorace 1, in addition to the expected $2n = 6$ female and $2n = 7$ male karyotypes, five new karyotypic combinations were recorded (figure 3), the frequency of which is provided in table 1. Among these new karyotypes, two are aneuploids (figure 3), which occurred in very low frequency and only during 1996. The other three types are euploid, more frequent and have been consistently recorded since 1996. Of these, one was a male with $2n = 8$ (figure 3) with a pair of metacentric chromosomes (chromosome 2), a pair of acrocentric chromosomes 3, a submetacentric X and a Y chromosome, along with a pair of dot chromosomes. The other two new karyotypes were of females. One had $2n = 8$ (figure 3) with a pair of metacentric chromosomes 2, a pair of acrocentric chromosomes 3, a pair of submetacentric X chromosomes and a pair of dots. The other female had $2n = 7$ (figure 3), with a pair of chromosomes 2, one chromosome 3, one submetacentric X chromosome, one metacentric X3 chromosome and a pair of dots. The submetacentric nature of the new X chromosome is resolved by looking at its anaphase configuration (figure 6E, F). Thus, this culture of Cytorace 1 is polymorphic with two expected and three new karyotypes. The interesting observation is that the frequency of the expected female karyotype with $2n = 6$, gradually declined to 4% while each of the four other types was represented with a frequency above 20% (table 1).

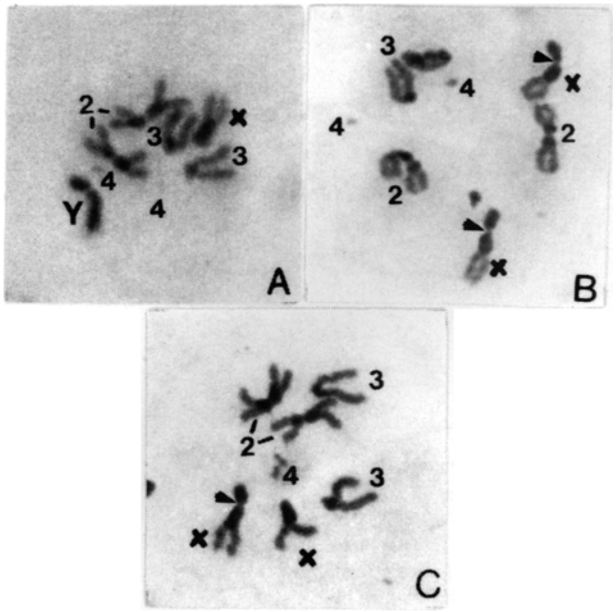


Figure 4. Comparison between the acrocentric X chromosome of *D. n. nasuta* and the new submetacentric X chromosome found in a few individuals of Cytorace 1. A cross between males of *D. n. nasuta* with $2n = 8$ (**A**) and females of Cytorace 1 which have $2n = 8$ with new X chromosomes (**B**) produced a F_1 female with $2n = 8$ (**C**). In **C**, the new X chromosome and the X chromosome of *D. n. nasuta* are compared. Arrowhead indicates the new submetacentric X chromosome.

4. Discussion

The segregation pattern of chromosomes found in Cytorace 1 males with 2n = 7 and females with 2n = 6 cannot produce these new karyotypes, as the latter have a submetacentric X chromosome which is absent in Cytorace 1.

What is the source of this 'submetacentric X' chromosome? One possibility is that this culture was contaminated with individuals of *D. n. nasuta* which have unfused X chromosomes. To resolve this, a cross was made between *D. n. nasuta* males and Cytorace 1 females with 2n = 8, to provide F_1's in which a direct comparison between the X chromosomes could be made (figure 4). A marked difference is seen between these chromosomes, wherein the acrocentric X chromosome of *nasuta* is much shorter with significantly less heterochromatin than that of the "new submetacentric X" chromosome. Therefore, the appearance of the latter in Cytorace 1 is not due to contamination with *D. n. nasuta* flies.

The other possibility involves a centric fission of the metacentric X3 chromosome present in Cytorace 1 resulting in a submetacentric X chromosome and an acrocentric chromosome 3 (figure 5). Centric fission would have been followed by addition of heterochromatin in the form of a short arm to produce a submetacentric X chromosome. The original metacentric 'X3' chromosome was inherited from *D. n. albomicans*. The analysis of the karyotypic phylogeny of the *nasuta* subgroup has shown that the X3 and the Y3 metacentric chromosomes of

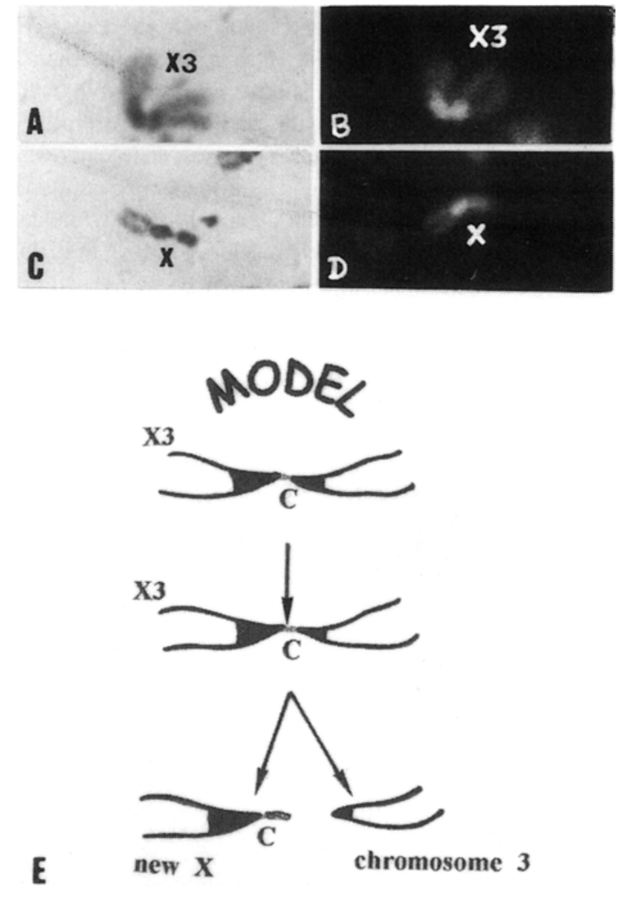


Figure 5. Comparison and derivation of the submetacentric X chromosome of the Fissioncytorace-1 through centric fission of the metacentric X3 chromosome of *D. n. albomicans*. (**A, B**) C- and Q-banded X3 chromosomes. (**C, D**) C- and Q-banded submetacentric X chromosomes. (**E**) Model to explain the origin of the submetacentric X chromosome through a centric fission of the X3 metacentric chromosome. After centric fission, additional heterochromatin is added in the form of a short arm to form the new submetacentric X chromosome. The intensity of the Q-band of this added heterochromatin is lighter than the other segment of heterochromatin.

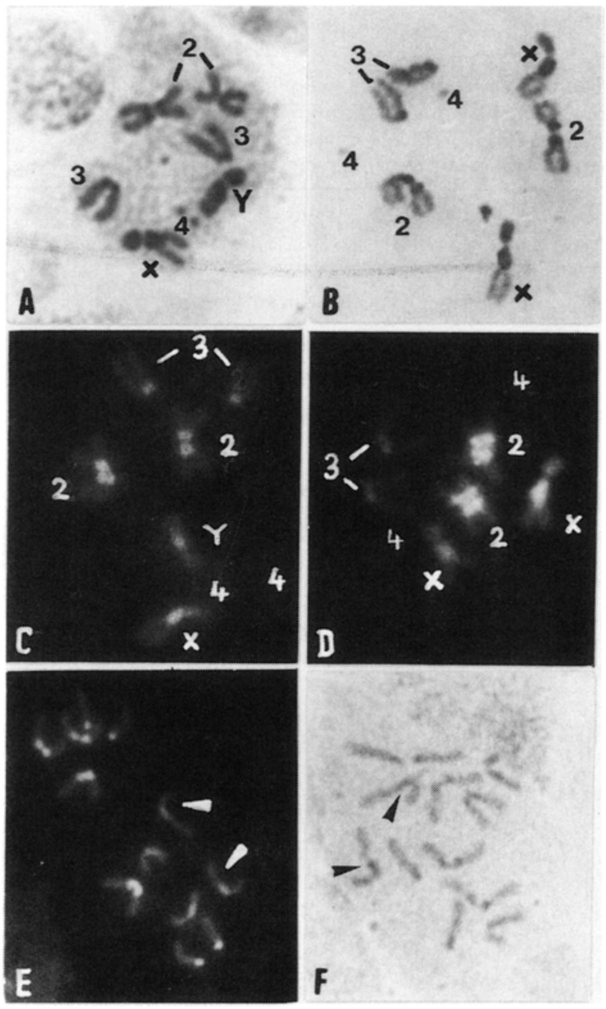


Figure 6. Male (2n = 8) and female (2n = 8) karyotypes of Fissioncytorace-1. (**A, B**) Giemsa stained chromosomes. (**C, D**) Q-banded chromosomes. (**E, F**) Q-banded and Giemsa stained anaphase chromosomes. Arrow heads point to the submetacentric J-shaped X chromosomes.

D. n. albomicans have evolved through centric fusion between the acrocentric chromosomes 3 and the sex chromosomes (X and Y) of a *D. n. nasuta* like karyotype (Ranganath and Hagele 1981). The new X chromosome derived through fission has a nearly 59% content of heterochromatin whereas that of *D. n. nasuta* contains about 47% (figure 4). Since these new karyotypes with submetacentric X chromosomes appeared during the early part of 1996, the fission in one of the X3 metacentric chromosomes of either a male or a female of Cytorace 1 might have occurred during 1995. The products of this fission were retained in the population of Cytorace 1, sometimes at a frequency higher than that of others (table 1).

During 1998, about 200 isofemale lines from this polymorphic culture of Cytorace 1 were established. One of the isofemale lines thus established had males and females with 2n = 8 only (figure 6), indicating that the fixation of the new submetacentric X chromosome derived from the fission of the X3 chromosome had occurred. This new chromosomal strain is named "Fissioncytorace-1" and its karyotypic phylogeny is depicted in figure 7. With the submetacentric X chromosome, the karyotype of Fissioncytorace-1 (2n = 8) differs from that of *D. n. nasuta* (2n = 8) which has acrocentric X chromosomes. However, Lakhotia and Roy (1981) have reported the occurrence of a submetacentric X chromosome in the Varanasi strain of *D. n. nasuta.* But for this rare report, studies made by our group since 1975 as well as those of Professor O Kitagawa's group in Japan (Wakahama and Kitagawa 1980; Wakahama *et al* 1983) and of Wilson *et al* (1969), have shown only acrocentric X chromosomes in *D. n. nasuta.*

The present finding is unique in that a fission not only has occurred but also that the products of this fission have been fixed in a new chromosomal lineage of *Drosophila.* To our knowledge, this is a first report for the genus *Drosophila.* Further, the X3 chromosome of *D. n. albomicans*, an earlier product of centric fusion, and the X chromosomes, the present products of centric fission, are suitable candidates to probe the status of centromeres. Similarly, Imai *et al* (1994) have shown the importance of chromosomal fission during the karyotypic evolution of the *Myrmecia pilosula* species complex of ants. The cytogenetic relationship between Fissioncytorace-1 and its parent namely the Cytorace 1 as well as with its grand-

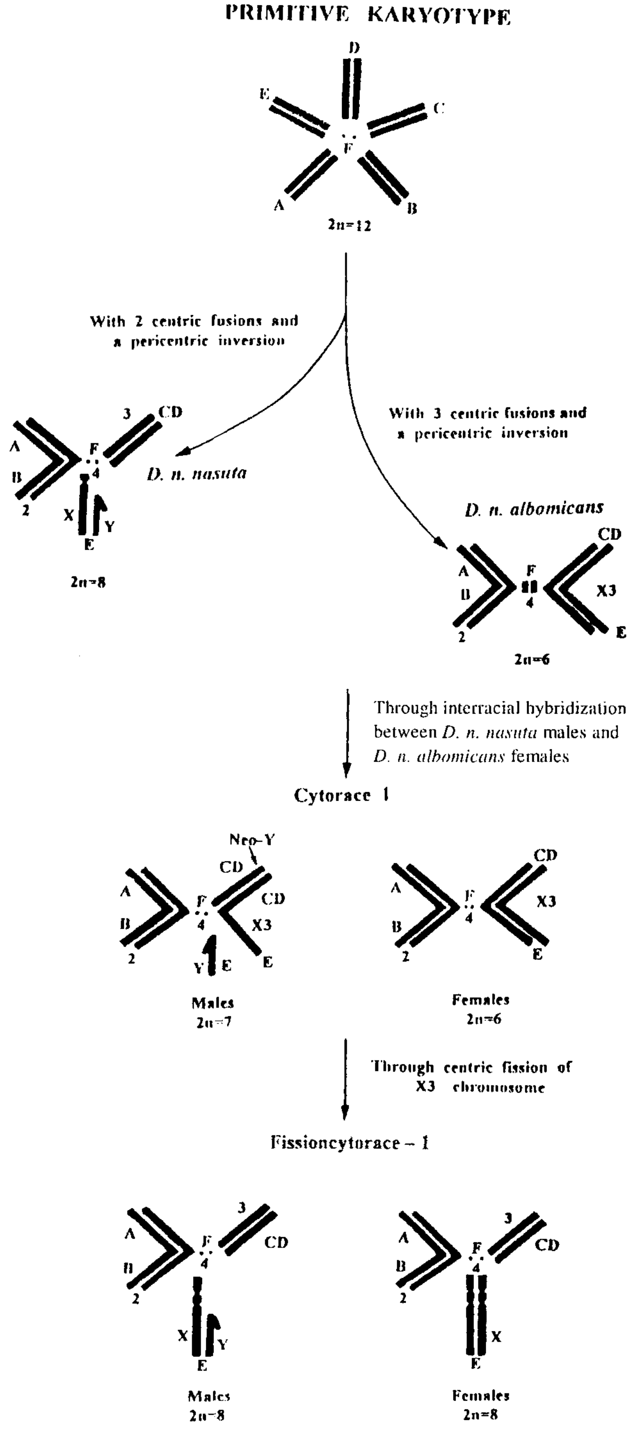


Figure 7. Karyotypic phylogeny of the new race Fissioncytorace-1. The primitive karyotype of the genus *Drosophila* has A to F elements (2n = 12) (Patterson and Stone 1952). The karyotype of *D. n. nasuta* (2n = 8) is derived through 2 centric fusions, one between A and B elements and another between C and D elements. Subsequently, a pericentric inversion in the latter has resulted in the CD acrocentric chromosome. The karyotype of *D. n. albomicans* (2n = 6) is derived through yet another centric fusion between the element E (sex chromosome) and the CD acrocentric to result in a metacentric chromosome X3 or Y3 (Ranganath and Hagele 1981). Interracial hybridization in the laboratory between males of *D. n. nasuta* and females of *D. n. albomicans*, followed by the maintenance of hybrid populations for many generations has lead to the evolution of Cytorace 1 with males 2n = 7 and females 2n = 6 (Ramachandra and Ranganath 1986). Subsequently, in one of the replicate cultures of Cytorace 1, a centric fission in one of the X3 chromosomes has resulted in a new lineage called Fissioncytorace-1 with 2n = 8.

parents namely *D. n. nasuta* and *D. n. albomicans* is being investigated.

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