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COMPETITIVE ABILITY AMONG THE MEMBERS OF TYPE 2 CYTORACES OF *NASUTA-ALBOMICANS COMPLEX* OF *DROSOPHILA*

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ABSTRACT
The members of *Nasuta-Albomicans Complex* of *Drosophila* are morphologically identical, karyotypically different but cross-fertile and hence are an excellent system to study several dimensions of raciation. Hybridization between *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans,* in the laboratory has given rise to 16 new cytoraces, each having a karyotype that differs from one another and also from those of the parental races. In the present study, we have assessed the competitive ability among the members of type 2 cytoraces along with their parents against a common *D. melanogaster* mutant. Analysis of productivity and population size among them revealed the superiority of the parental races and the members of type 2 cytoraces over the *D. melanogaster* mutant strain at varying time points. Similarly in a comparison between the parental races and type 2 cytoraces, the superiority of cytorace 2 was evident.

KEY WORDS: *Drosophila, Nasuta-Albomicans Complex*, Raciation, Type 2 cytorace, Competitive ability.

INTRODUCTION

The *nasuta* subgroup of *Drosophila* belonging to *immigrans* group consists of an assemblage of morphologically almost identical, closely related species/subspecies that show varying levels of reproductive isolation and includes *D. nasuta nasuta, D. n. albomicans, D. n. kepulauana, D. sulfurigaster sulfurigaster, D. s. bilimbata, D. s. albostrigata, D. s. neonasuta* and others (Wilson *et al*. 1969; Rajasekarasetty *et al*. 1980). This subgroup has been an excellent material for analysis of the patterns and processes of differentiation in closely related species (Ranganath, 1978).

D. n. nasuta (2n = 8) and *D. n. albomicans* (2n = 6) are the cross-fertile chromosomal races that belong to the frontal sheen complex of nasuta subgroup. Cytological distinctness of these two races has been extensively studied (Ranganath and Hagele, 1982; Ramachandra and Ranganath 1986; Ramachandra, 1987; Ranganath, 2002). Hybridization between these two chromosomal races in the laboratory has given rise to new races named cytoraces, which differ in karyotypic composition not only among themselves, but also from those of the parental races (Ramachandra and Ranganath, 1986, 1990, 1996; Tanuja *et al.,* 2001).

The evolution and stabilization of present day cytoraces is a consequence of their successful reproduction for over 650 generations. These cytoraces along with their parents together form the *'Nasuta–Albomicans* complex', which exhibits characters that make them evolutionarily interesting (Ranganath, 2002). As of now, 16 different new karyotypic strains of cytoraces that have been identified and are being maintained in our laboratory. The members of the *Nasuta-Albomicans* complex exhibit distinct characters even though they are genetically closely related and morphologically almost identical.

Differences have been reported in their morphophenotypic trait (Harini and Ramachandra, 2000), fitness parameters (Ramachandra and Ranganath, 1988) and mating preferences (Tanuja *et al*. 2001). Differences between parental races and cytoraces have been documented with respect to various parameters which include isozymes (Aruna and Ranganath, 2004), glue proteins (Aruna and Ranganath, 2006) and longevity (Ranjini and Ramachandra, 2009). Also the competitive ability of four laboratory evolved races - cytorace 1, cytorace 2, cytorace 3, cytorace 4 along with their parents, *D. n .nasuta* and *D. n. albomicans* and the DNA polymorphism among these hybrid races and their respective parents based on ISSR markers were recently studied and have shown significant differences between parental races and the cytoraces (Bijaya and Ramachandra, 2010). Thus, this unique assemblage of closely related races has turned out to be an active entity of evolutionary changes in a laboratory setting.

Among these, members of type 2 cytoraces consisting of cytorace 2, cytorace 9, cytorace 11, cytorace 12 and cytorace 13 have been considered for the present study and their karyotypes are listed in Table 1 and diagrammatically represented in figure 1. Among these members, the independence of cytorace 2 from both the parents is supported by mating preference experiments (Tanuja *et al*. 2001b), differences in body weight (Harini and Ramachandra, 2000), copulation duration (Tanuja *et al*. 2001a) and isozyme pattern (Aruna and Ranganath, 2004), thus indicating that cytorace 2 has diverged from both the parents with respect to most of the traits studied so far. In view of this, the intergenotypic competitive ability i.e., the performance between the ebony mutants of *D. melanogaster* and the members of type 2 cytoraces were assessed and evaluated.

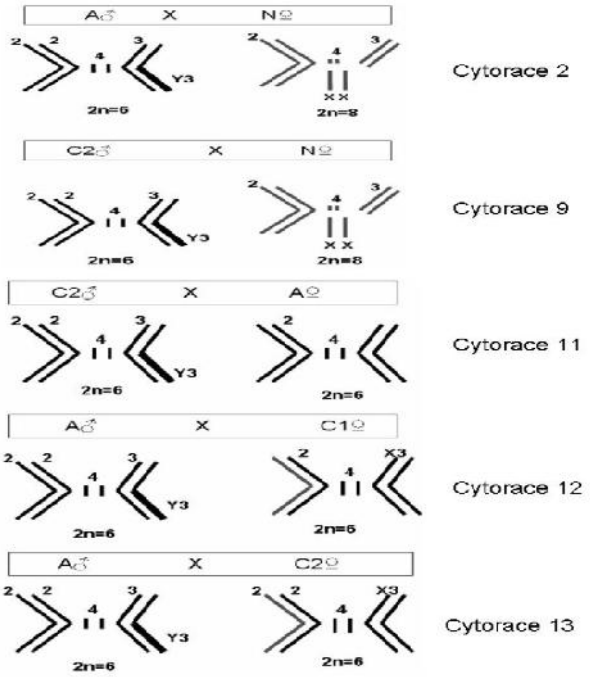

FIGURE 1: The diagramatic representation of the karyotypic composition of the members of type 2 cytoraces – C2, C9, C11, C12, C13.

TABLE 1: Karyotypic composition of the members of type 2 cytoraces along with their parental crosses and the contribution of parental chromosomes in the evolution of the *Nasuta-Albomicans Complex* (NAC) of *Drosophila*. The superscripts 'n' and 'a' indicate the chromosomes derived from '*D. n. nasuta'* and ' *D. n. albomicans'* parents respectively. (A = *D. n. albomicans*; N = *D. n. nasuta*) (Ramachandra and Ranganath 1996).

Parents and the crosses	Races	Karyotypes		Chromosomes of	
				nasuta	*albomicans*
A♂X N♀	Cytorace 2 (C2)	♂-2n = 6 - 2^n 2^a $X3^a$ $Y3^a$ 4^a 4^a	♀-2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^a 4^a	2	10
C2♂ X N♀	Cytorace 9 (C9)	♂-2n = 6 - 2^n 2^a $X3^a$ $Y3^a$ 4^a 4^a	♀-2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^a 4^a	2	10
C2♂ X A♀	Cytorace 11 (C11)	♂-2n = 6 - 2^n 2^a $X3^a$ $Y3^a$ 4^a 4^a	♀-2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^a 4^a	2	10
A♂X C1♀	Cytorace 12 (C12)	♂-2n = 6 - 2^n 2^a $X3^a$ $Y3^a$ 4^a 4^a	♀-2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^a 4^a	2	10
A♂X C2♀	Cytorace 13 (C13)	♂-2n = 6 - 2^n 2^a $X3^a$ $Y3^a$ 4^a 4^a	♀-2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^a 4^a	2	10

MATERIALS AND METHODS

Stocks: The following *Drosophila* stocks obtained from Drosophila Stock Centre, University of Mysore are employed in the present study:

a) *Drosophila nasuta nasuta* (Coorg strain, Stock No.201.009).
b) *Drosophila nasuta albomicans* (Okinawa strain, Stock No.202.001).
c) Members of type 2 cytorace: cytorace 2, cytorace 9, cytorace 11, cytorace 12 and cytorace 13.
d) *ebony* mutant of *D. melanogaster* (Stock No. 3011).

D. n. nasuta, D. n. albomicans and the members of type 2 were allowed to compete independently against a common *ebony* mutant strain of *D. melanogaster*. Mixed cultures were set up with 25 flies consisting of 12 females and 13 males of *D. melanogaster* and 25 flies (12 females and 13 males) of one of the experimental strain (member of type 2 cytorace). These were maintained at constant temperature (22 ± 1ºC), humidity (70 - 80%) and resource by following the serial transfer method of Ayala (1965). The adult flies were introduced into ¼ pint (125 ml) milk bottles containing equal amount of cream of wheat agar medium seeded with yeast. Once in every 7 days they were etherized, counted and transferred to fresh media bottles. When the new flies started emerging from these serially transferred culture bottles, they were etherized, counted and were put back in to the bottle with the other adult flies. These cultures were discarded after four weeks. Four replicates were maintained for every experiment. Two related parameters of adaptedness namely productivity and population size have been considered for studying the population dynamics of the members of type 2 cytoraces. The numbers of newly emerged flies were taken as the productivity of the race under study and the population size of a particular race for that particular week was defined by the total number of the newly emerged flies along with the survivors from the previous week. The adult ovipositing flies were thus always in a single bottle, while other bottles contained flies at different preadult stages. Each experiment was conducted till one of the

competing races completely eliminated the other. The average of population size and productivity was calculated and expressed in terms of Mean ± SE.

Statistical analysis

The results obtained from the assessments of the intergenotypic competitive ability experiments were individually subjected to one-way Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to analyze the significance of differences in the parental races and the members of type 2 cytoraces.

RESULTS

Perusal of table 2 that embodies the mean values of the two components of competitive ability viz., productivity and population size reveals that the parental races namely *D. n. nasuta* and *D. n albomicans* eliminated the mutant strain of *D. melanogaster* in 47 and 45 weeks respectively. Further, among members of type 2 cytoraces, cytorace 2 and cytorace 9 eliminated *ebony* in 49 weeks, much earlier compared to cytorace 11, cytorace 12 and cytorace 13 which was achieved in 57, 61 and 54 weeks respectively. The ebony mutant of *D. melanogater* survived for a long time in the mixed cultures with the competing members of type 2 cytoraces than with *D. n. nasuta* and *D. n. albomicans.* The test for analysis of variance (ANOVA) showed significantly higher values for only the parental races for productivity whereas for the population size it was significant for both parental races and cytorace 2.

TABLE 2: Competitive ability among the members of type 2 cytoraces and their parents of the *Nasuta Albomicans Complex of Drosophila* with *ebony* mutant of *D. melanogaster.* [Values are mean ± SE of the four replicates]

Strains	Parameters	
	Productivity (Mean ± SE)	Population size (Mean ± SE)
D. n. nasuta	163.79 ± 10.25^{a}	249.90 ± 11.89^{a}
D. n. albomicans	184.94 ± 9.36^{b}	217.03 ± 12.08^{b}
Cytorace 2	157.23 ± 7.82^{c}	239.47 ± 11.25^{c}
Cytorace 9	152.62 ± 7.85^{d}	215.18 ± 9.68^{d}
Cytorace 11	158.28 ± 6.11^{e}	204.44 ± 7.79^{e}
Cytorace 12	160.47 ± 6.82^{f}	220.36 ± 7.89^{f}
Cytorace 13	152.10 ± 6.18^{g}	210.22 ± 7.99^{g}
ANOVA	F = 1.898; df = 6, 359 P< 0.02	F = 2.733; df = 6, P< 0.02
DMRT	The difference between a/b is significant at 5% level	The difference between a/b, b/c are significant at 5% level

Of the five cytoraces and two parental races studied in this experiment, the parents showed higher values in terms of productivity compared to type 2 cytoraces employed in the present study, whereas for the population size two cytoraces viz., cytorace 2 and cytorace 12 showed higher values compared to one of its parents, however it was the highest in the parental race, *D. n. nasuta.* Based on productivity and population size, these races can be ranked as follows:

According to the values obtained for the parameters assessed for competitive ability, *D. n. albomicans* > *D. n. nasuta* > C12 > C11 > C2 > C9 > C13 for productivity and *D. n. nasuta* > C2 > C12 > *D. n. albomicans* > C9 > C13 > C11 for population size. The population dynamics of the strains studied has been graphically represented in Fig. 2 to Fig. 8.

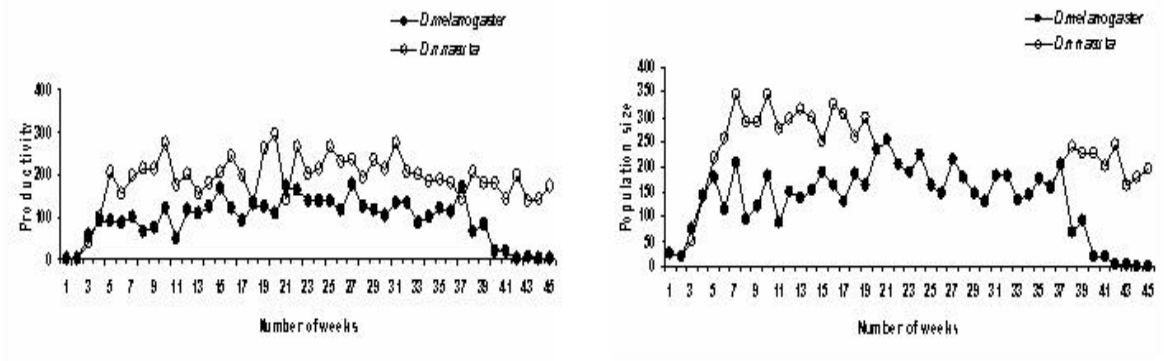

FIGURE 3: Population dynamics of interspecific competition (mean of four replicates) between *D. n. nasuta* and *ebony* mutant of *D. melanogaster.*

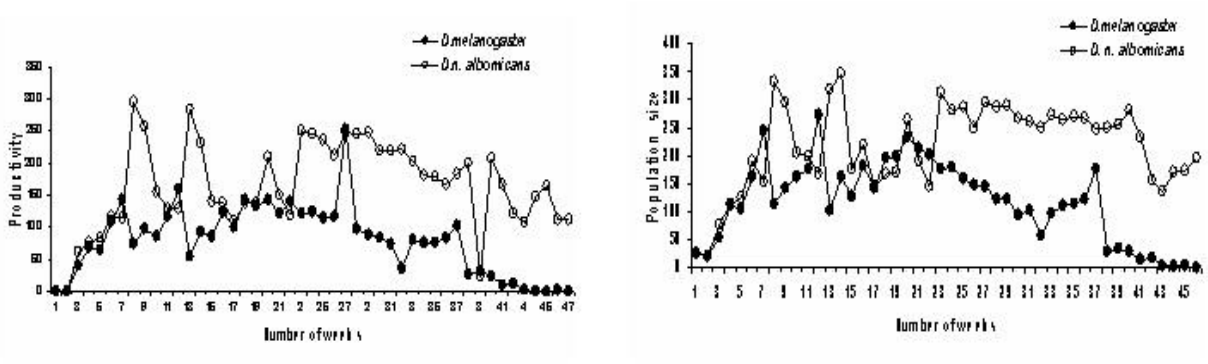

FIGURE 4: Population dynamics of interspecific competition (mean of four replicates) between *D. n. albomicans and ebony* mutant of *D. melanogaster.*

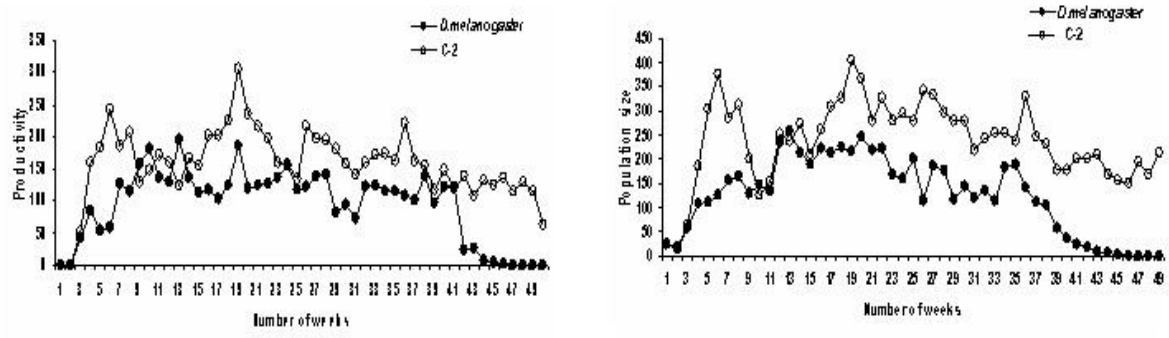

FIGURE 5: Population dynamics of interspecific competition (mean of four replicates) between members of type 2 Cytorace: Cytorace 2 and *ebony* mutant of *D. melanogaster.*

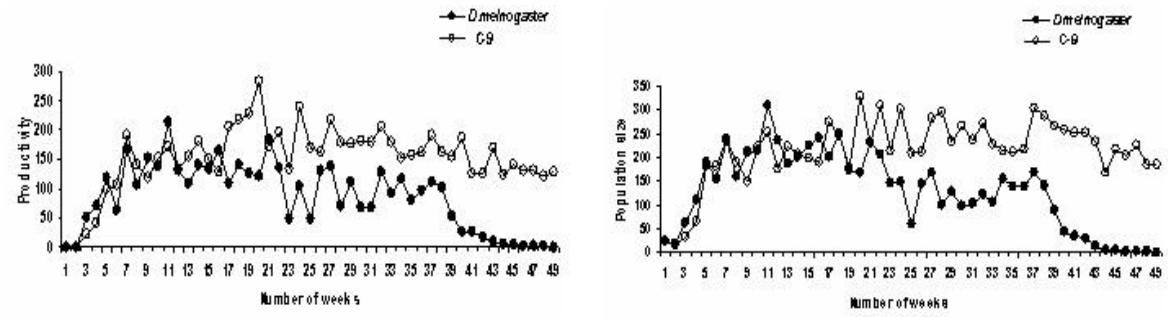

Figure 6: Population dynamics of interspecific competition (mean of four replicates) between members of type 2 Cytorace: Cytorace 9 and *ebony* mutant of *D. melanogaster.*

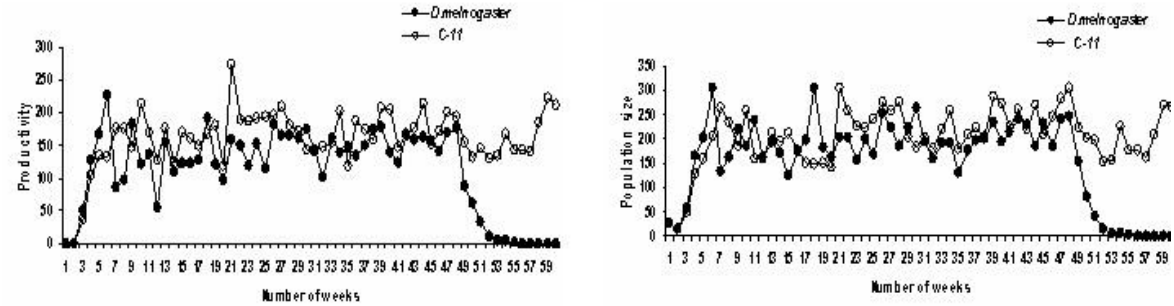

Figure 7: Population dynamics of interspecific competition (mean of four replicates) between members of type 2 Cytorace: Cytorace 11 and *ebony* mutant of *D. melanogaster.*

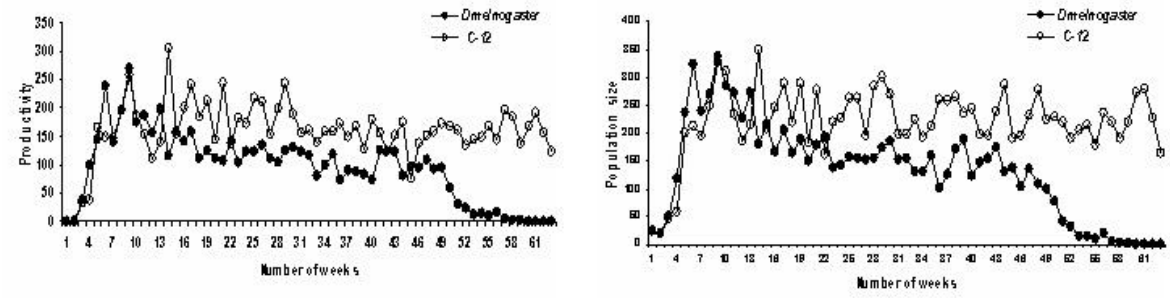

FIGURE 8: Population dynamics of interspecific competition (mean of four replicates) between members of type 2 Cytorace: Cytorace 12 and *ebony* mutant of *D. melanogaster.*

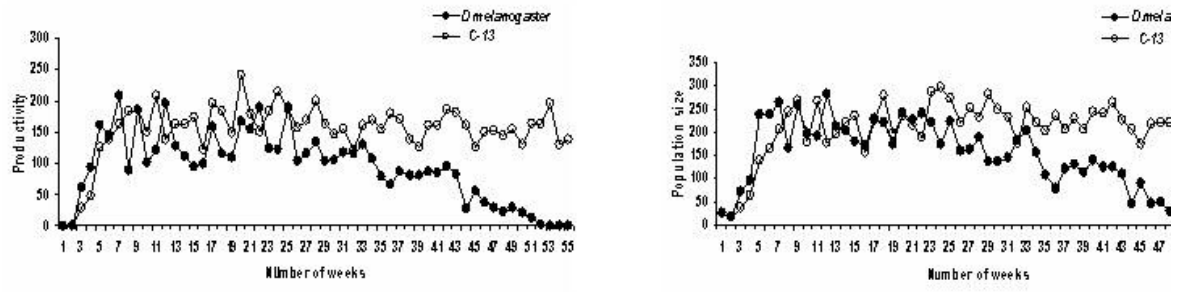

FIGURE 9: Population dynamics of interspecific competition (mean of four replicates) between members of type 2 Cytorace: Cytorace 13 and *ebony* mutant of *D. melanogaster.*

DISCUSSION

Drosophila is one of the most potent eukaryotic model systems to explore many aspects of population and evolutionary genetics (Ranganath, 1978). Species that are closely related phylogenetically and ecologically have frequently been observed to co-exist in the same habitat, apparently exploiting the same resources and there will be competitive interactions between them. The performance in competition may be used as a measure of population fitness of the species concerned and the laboratory populations can be utilized as biological models to study the dynamics and the process of competition (Ranganath *et al*. 1985). The understanding of competitive relationship between closely related species and its appreciation is of considerable evolutionary importance (Ranganath and Krishnamurthy, 1986). Further, the estimation of fitness is considered to be the first step towards understanding the adaptive evolution of a population (Ramachandra, 1987). Interspecific competitive fitness is an important attribute in any population which will determine its success in a sympatric association of two or more species. Population fitness can be assessed by evaluating the inter-genotypic competitive ability of particular strains either with strains of a different species or with strains of the same species and the mutant strain of *Drosophila* can be used as an interspecific competitor to determine the relative fitness of different species or strains of the same species (Zimmering, 1948; Ayala, 1965; Futuyama, 1970; Goodman, 1979, Ramachandra and Ranganath, 1986, Bijaya and Ramachandra, 2010).

D. n. nasuta has 2n = 8 with a pair of metacentrics (chromosome 2) two pairs of acroccentrics (chromosomes X and 3) and a pair of dot chromosome (chromosome 4). *D. n. albomicans* has 2n = 6 with a pair of metacentric chromosomes (chromosome 2), a pair of long dot chromosomes (chromosome 4) and another pair of metacentrics representing fused products of chromosome 3 and chromosome X in females and chromosome 3 and chromosome Y in male (Nirmala and Krishnamurthy, 1971; Wakahama and Kitagawa, 1972) (Fig. 9) Under laboratory conditions they are cross-fertile but in natural populations no hybrids are found. They are allopatric in distribution isolated from each other by more than 4,800 Km. Ranganath and Hagele (1981) have demonstrated that the karyotype of *D. n. albomicans* is a recent product of karyotypic orthoselection involving successive centric fusions. The production of cytoraces was carried out in three phases; in the first phase, cytorace 1 and 2 were formed through hybridization of *D. n. nasuta* (Coorg strain) and *D. n. albomicans* (Okinawa strain) (Ramachandra and Ranganath, 1986). The second phase yielded two more hybrids called cytorace 3 with males and females having 2n=8 and cytorace 4 where males have 2n = 7 and females have 2n = 8 that were derived from the hybridization between *D. n. nasuta* (Coorg strain) and *D. n. albomicans* (Thailand strain) (Ramachandra and Ranganath, 1990). The third phase that included inter-racial hybridization among *D. n. nasuta, D. n. albomicans,* cytorace 1, cytorace 2, cytorace 3 and cytorace 4 resulted in 12 new stabilized karyotypic strains viz., cytoraces 5 to 16 (Ramachandra and Ranganath, 1996).

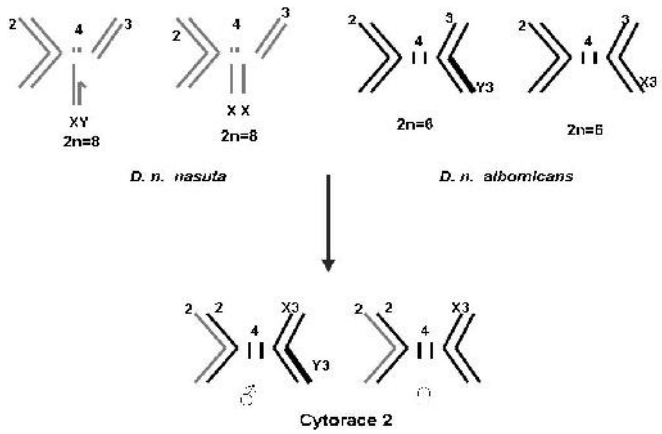

FIGURE 9: The karyotypic composition of cytorace 2 obtained during the first phase of hybridization experiments (From Ramachandra and Ranganath, 1986).

Tanuja *et al.* (2003) have categorized sixteen cytoraces into six types based on karyotypic homology, namely, **Type 1** (♂:2n = 7 - 2^n 2^a $X3^a$ Y^n 3^n 4^n 4^n ; ♀: 2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^n 4^n), **Type 2** (♂: 2n = 6 - 2^n 2^a $X3^a$ Y^{3a} 4^a 4^a ; ♀: 2n = 6 - 2^n 2^a $X3^a$ $X3^a$ 4^a 4^a) , **Type 3** (♂: 2n = 8 - 2^n 2^a X^n Y^n 3^n 3^n 4^a 4^a; ♀: 2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^a4^a), **Type 4** (♂: 2n = 7 - 2^n 2^a Y^{3a} X^n 3^n 4^a 4^a ; ♀: 2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^a 4^a), **Type 5** (♂:2n = 7 - 2^n 2^a $X3^a$ Y^n $3^n4^a4^a$; ♀: 2n = 6-2^n2^a $X3^a$ $X3^a4^a4^a$) and **Type 6** (♂ : 2n = 7 - 2^n 2^a Y^{3a} X^n 3^n 4^n 4^n ; ♀: 2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n). The cytoraces 9, 11, 12, and 13 had similar type of karyotype as cytorace 2 and hence were grouped under type 2 cytoraces. The competitive fitness i. e., the adaptedness of the members of type 2 cytoraces were achieved at different time points, indicating that the genetic constitution of the competing species under study can also affect the duration of coexistence of the competing species. Accordingly, the results indicated the competitive superiority of the parental races wherein they competitively excluded the *ebony* strain of *D. melanogaster* much earlier to that of the members of type 2 cytoraces, at 45th and 47th week, whereas the same was achieved at 49th, 57th, 61st and 54th weeks for cytorace 2, 9, 11, 12 and 13 respectively. The different degrees of competitive ability suggest the extent of divergence in fitness among the cytoraces and from its parents. However, comparison among the members of type 2 cytoraces indicate that there is no significant divergence, which suggests that these members are much closely related and have further not shown much hybrid recombination from that of the parental races, presumably due to similarity in karyotypic composition. Rieseberg *et al*. (2003) and Llopart *et al*. (2005) are of the opinion that novel genotype of hybrids may confer on them a unique ecological tolerance or preference beyond the range of parental species, as is seen in several plant hybrids.

Adaptedness refers to the ability of the individuals with a genotype or a group of genotypes to survive and reproduce in a given environment. In the present study, the experimental cultures were maintained till the elimination of one of the competing species. The total number of flies emerged was considered as the productivity of the race and the population size of a race for a particular week was defined by the total number of the newborn flies plus the survivors from the previous week. This was further expressed in terms of the average of productivity and population sizes in order understand the population dynamics of the members of type 2 cytoraces.

Hybridization and introgression of genomes can lead to genesis of novel genotypes. Hybridization, an evolutionary catalyst, can lead to increased quantum of genetic variability and thereby could be a source for the origin of new races/species (Harini and Ramachandra, 2003). No race is the best for all the components of fitness and also the fitness hierarchy is reversed when considering different parameters, which could be an evolutionary strategy to generate diversity phenotypes in adaptedness of the species so as to promote coexistence of different species with variable levels of divergence for fitness components (Bijaya and Ramachandra, 2010). Present study has disclosed a narrow range of degree of divergence in competitive ability, implying the absence of greater racial divergence among the members of type 2 cytoraces, which have similiarity at chromosomal level as well. However, cytorace 2 is found to have better competitive ability compared to other members of type 2 cytoraces, suggesting of an early event of recombinational raciation in their evolution under laboratory conditions. While in other cytoraces of type 2, many more generations are probably needed for stabilization of adaptedness. The evolution of cytoraces has occurred due to a series of events of hybrid recombination and interracial hybridization under laboratory environment. These cytoraces are the representatives of novel genetic variations and an admixture of the parental genomes which is a consequence of 'mixing' of parental chromosomes. Some of the cytoraces despite of sharing same chromosome number, do not exhibit similarities in their body size, reproductive fitness and competitive ability. Thus, the rapid divergence recorded in the chromosomes, karyotypes, body size, bristle number, fitness traits, and competitive ability is suggestive of an early event of recombinational raciation in their evolution in the laboratory environment which is a rare observation in animal system illustrating the increase in the tempo of evolution following hybridization (Bijaya and Ramachandra, 2010). Though there is significant variation in competitive ability between parental races and cytorace 1, cytorace 2, cytorace 3, and cytorace 4 that are

chromosomally different, there is no significant variation among the members of type 2 cytoraces. Present study that involved chromosomally similar type 2 cytoraces revealed marginal differentiation in competitive ability parameters (population size and productivity) and such a situation may also exist in other chromosomally similar cytorace types which need to be investigated to visualize which chromosomal combinations might promote divergence or might neutralize divergence in competitive abilities.

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