Genetic Variability Assessed by Competitive Ability and ISSR Markers in the Members of the *Nasuta-albomicans* Complex of *Drosophila*

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Abstract: The *nasuta-albomicans* complex (NAC) of *Drosophila* is an assemblage comprising of two morphologically indistinguishable members of the *nasuta* subgroup of the *immigrans* species group namely, *Drosophila nasuta nasuta*, *Drosophila nasuta albomicans* and 16 cytoraces, which have been evolved through a long range hybridization between *D. n. nasuta* and *D. n. albomicans*. This complex is an artificial hybrid zone of *Drosophila* with "allo-sympatric" populations, which exhibits differences in their cytogenetic differentiation, incipient sexual isolation, body size and fitness. The objectives of our study were to (a) assess 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* (b) examine the DNA polymorphism among these hybrid races and their respective parents based on ISSR markers and (c) bring out the correlation, if any, between the above two. Among the six races, overall competitive ability was higher in *D. n. nasuta*, *D. n. albomicans* and cytorace 1 than other races. Cytorace 1, cytorace 2 and cytorace 4 are with 20-23% of DNA polymorphism while cytorace 3 is with 10.7% of DNA polymorphism with reference to four ISSR profiles. Thus, one can surmise that cytorace 1, cytorace 2 and cytorace 4 with increased genetic variability exhibited better fitness while cytorace 3 with the least DNA polymorphism showed reduced competitive ability. [Nature and Science 2010;8(12):29-42] (ISSN: 1545-0740).

Key words: *Nasuta-albomicans* Complex; Competitive ability, ISSR, Polymorphism

1. Introduction

Drosophila nasuta and *Drosophila albomicans* belongs to the frontal sheen complex of the *nasuta* subgroup of the *immigrans* species group of *Drosophila. D. nasuta* was first described from Seychelles islands (Lamb, 1914) and *D. albomicans* from Paroe, Formosa (Duda, 1923). *D. albomicans* and *D. nasuta* have almost identical morphological description, however they were considered as species in view of the similarities between species of the *nasuta* subgroup, and the remarkable degree of speciation which has taken place in South Asia and the geographical separation ~3000 miles of *D. nasuta*, from the closest known *D. albomicans* (Wilson et al., 1969). *D. albomicans* is reported from islands of Japan and Malaysia (Kitagawa et al., 1982), Thailand (Wilson et al., 1969), Taiwan (Lin et al., 1977) and Shillong, India (Singh, 1977). Karyotype is an important phenotype of a species and an analysis of karyotypic differentiation between species yields a better understanding of evolutionary interrelationship and divergence (Ranganath, 2000). During the evolution of *D. nasuta* from the primitive *Drosophila* karyotype (2n=12), has involved two centric fusions and a pericentric inversion (Ranganath and Hagele, 1981) bringing down the diploid number to 2n=8. It is believed that the karyotype of *D. albomicans* has evolved from that of *D. nasuta* or *nasuta*- like ancestor (Ranganath and Hagele, 1981). During the evolution of karyotype of *D. albomicans*, a centric fusion has occurred between the sex chromosomes and the autosome 3 of *D. nasuta*. Thus in the karyotype evolution of *D. albomicans*, three centric fusions and a pericentric inversion can be seen. *D. nasuta* and *D. albomicans* were treated as two distinct species (Wilson et al., 1969). Preliminary studies of Nirmala and Krishnamurthy (1972) followed by extensive studies of Ranganath and his group (Ranganath, 1975, 1978; Ranganath and Ramachandra, 1987) have shown that these allopatric species are cross-fertile under laboratory conditions. In view of their karyotypic divergence and open

genetic systems, they have been treated as chromosomal races and called *D. nasuta nasuta* and *D. nasuta albomicans* (Nirmala and Krishnamurthy, 1972). *D. n. nasuta* and *D. n. albomicans*, inspite of massive karyotypic divergence are cross-fertile and the hybrid populations of these species can be maintained for any number of generations. Such hybrid populations of *D. n. nasuta* and *D. n. albomicans*, with a stable karyotype are called cytoraces (Ramachandra and Ranganath, 1986, 1990). These cytoraces were generated in three phases. In the first phase, cytorace 1 (males 2n=7, $2^n2^aY^n3^nX3^a4^n4^n$, females 2n=6, $2^n2^aX3^aX3^a4^n4^n$) and cytorace 2 (males 2n=6, $2^n2^aY3^aX3^a4^a4^a$, females 2n=6, $2^n2^aX3^aX3^a4^a4^a$) were generated as a resultant of hybridization between Coorg strain of *D. n. nasuta* and Okinawa strain of *D. n. albomicans* (Ramachandra and Ranganath, 1986). In the second phase, two more karyotypic strains called cytorace 3 (males 2n=8, $2^n2^aX^nY^n3^n3^n4^a4^a$, females 2n=8, $2^n2^aX^nX^n3^n3^n4^a4^a$) and cytorace 4 (males 2n=7, $2^n2^aY3^aX^n3^n4^a4^a$, females 2n=8, $2^n2^aX^nX^n3^n3^n4^a4^a$) were generated through hybridization between Coorg strain of *D. n. nasuta* and Thailand strain of *D. n. albomicans* (Ramachandra and Ranganath, 1990). The third phase generated yet another 12 different karyotypic strains named from cytorace 5 till cytorace 16 (Ramachandra and Ranganath, 1996; Tanuja et al., 2003). *D. n. nasuta* and *D. n. albomicans* and the newly evolved 16 Cytoraces are grouped under a new assemblage called, "*nasuta-albomicans*" complex (NAC) of *Drosophila* (Ramachandra and Ranganath, 1996). This complex is an artificial hybrid zone of *Drosophila* with "allo-sympatric" populations, which exhibits differences in their cytogenetic differentiation, incipient sexual isolation, body size and fitness (Ramachandra and Ranganath, 1996; Tanuja et al., 2001a, 2001b; Harini and Ramachandra, 2003). The complex is an assemblage comprising of two morphologically indistinguishable members of the *nasuta* subgroup of the *immigrans* species group namely, *D. n. nasuta* (males 2n=8: $2^n2^n3^n3^nX^nY^n4^n4^n$, females 2n=8: $2^n2^n3^n3^nX^nX^n4^n4^n$), *D. n. albomicans* (males 2n=6: $2^a2^aX3^aY3^a4^a4^a$, females 2n=6: $2^a2^aX3^aX3^a4^a4^a$) and 16 cytoraces, which have been evolved through long range hybridization between *D. n. nasuta* and *D. n. albomicans*. Interracial hybridization between these two followed by the maintenance of hybrid populations for over 20 generations has resulted in the emergence of two new karyotypic strains called cytoraces (Ramachandra and Ranganath, 1986). Each of these cytoraces is a constituent of recombined genomes of both the parental races, with differential representation of the parental chromosomes but differing in their karyotypic composition. Earlier studies on cytogenetic differentiation (Ramachandra and Ranganath, 1986), mating preference (Tanuja et al., 2001a, 2001b; Ramachandra and Ranganath, 1994), sternopleural bristles number (Harini and Ramachandra, 1999a), body size (Harini and Ramachandra, 1999b), body weight (Harini and Ramachandra, 2000a), abdominal bristle number (Harini and Ramachandra, 2000b), isozymes (Aruna and Ranganath, 2004), glue proteins (Aruna and Ranganath, 2006) and longevity (Ranjini and Ramachandra, 2009) of parental races namely, *D. n. nasuta* and *D. n. albomicans* as well as cytorace 1, cytorace 2, cytorace 3 and cytorace 4 have shown significant differences between parental races and cytoraces.

Molecular markers like proteins and deoxyribonucleic acids (DNA) are biochemical constituents and macromolecules that play important roles in taxonomy, physiology, embryology, plant breeding, ecology, genetic engineering etc. Among molecular markers, DNA markers are suitable as it is ubiquitous to most of the living organisms and are ideally neutral to the environmental changes (Sharma et al., 2008). Genetic polymorphism is defined as the instantaneous episodes of a trait in the same population with two or more genotypes. Such variations are best identified with DNA sequencing, which in turn is an expensive and painstaking technique (Sharma et al., 2008). In recent years a wide array of PCR based DNA marker techniques has been developed for the detection and exploitation of genetic polymorphism. Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and amplification of tandemly repeated sequences referred to as simple sequence repeats (SSR) are the most reliable and commonly used PCR based techniques for genetic analyses in plants (Williams et al., 1990; Vos et al., 1995; Staub et al., 1996; Gupta and Varshney, 2000). Simple sequence repeats (SSRs) or microsatellites are a class of DNA sequences consisting of simple motifs of 1-6 nucleotides that are tandemly repeated from two to three to a few dozen times at a locus (Vogt, 1990; Gur-Arie et al., 2000). Microsatellites long have been known to be abundantly distributed throughout the genomes of eukaryotes and some prokaryotes. They are highly polymorphic, hypervariable and multi-allelic (Tautz, 1989; Weber, 1990; Goodfellow, 1992; Bell and Ecker, 1994). Evidences on the functional role of microsatellites, affecting gene expression, and that polymorphism of SSR tracts may be important in the evolution of gene regulation has been studied (Rosenberg et al., 1994; Kashi et al., 1997; Moxon and Wills, 1999; Gur-Arie et al., 2000). The major limitations to these techniques are low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers

for microsatellite polymorphism (Wolfe et al., 1998; Nagaraju et al., 2002). Zietkiewics et al., (1994) described inter- simple sequence repeat (ISSR) amplification marker system that overcomes most of these limitations. This technique involves the PCR amplification of regions between adjacent, inversely oriented microsatellites using a single SSR - anchored primers (Awasthi et al., 2004; Verma et al., 2009). ISSR-PCR is simpler to use as prior knowledge of the target genome sequences flanking the repeat regions is not required and is more reliable than RAPD, as the primers used are longer, allowing for more stringent annealing temperatures and provides a higher reproducibility of bands than in RAPD (Hantula et al., 1996; Tsumura et al., 1996; Wolfe et al., 1998; Nagaraju et al., 2002). Although ISSRs have been extensively used by plant biologists for a variety of applications such as cultivar identification and protection of plant variety rights, phylogenetic and diversity analysis, hybrid confirmation, genome mapping and gene tagging for marker assisted selections (Abbot, 2001; Kumar et al., 2001; Pandit et al., 2007), they have been rarely used for animal studies (Reddy et al., 1999; Kostia et al., 2000).

Populations or races that have been recently separated from each other and has not yet attained the status of species are of interests in genetics of speciation as more advanced the stage of speciation of two diverging populations; the more difficult it becomes to delineate the genetic event that has set the process into motion. Thus, it may not be possible to understand the process of speciation by looking at the finished products (Zouros, 1986). Each of the Cytoraces are passing through a phase of racial differentiation in 'genetic isolation' through physical as opposed to behavioral barriers to interbreeding while inhabiting the same area and common set of environmental conditions. In view of this, the following objectives have been taken up in our present study (a) assess 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* (b) examine the DNA polymorphism among these hybrid races and their respective parents based on ISSR markers and (c) bring out the correlation, if any, between the above two.

2. Materials and methods

Drosophila stocks

(i) *D. n. nasuta* (2n=8) Coorg, South India

(ii) *D. n. albomicans* (2n=6) Okinawa, University of Texas collections, 3045.11

(iii) Cytorace 1 (males, 2n=7; $2^n2^aY^n3^nX3^a4^n4^n$, females 2n=6, $2^n2^aX3^aX3^a4^n4^n$) produced by interracial hybridization between males of *D. n. nasuta*, Coorg strain and females of *D. n. albomicans*, Okinawa strain (Ramachandra and Ranganath, 1986).

(iv) Cytorace 2 (males 2n=6, $2^n2^aY3^aX3^a4^a4^a$, females 2n=6, $2^n2^aX3^aX3^a4^a4^a$) produced by interracial hybridization between males of *D. n. albomicans*, Okinawa strain and females of *D. n. nasuta*, Coorg strain (Ramachandra and Ranganath, 1986).

(v) Cytorace 3 (males 2n=8, $2^n2^aX^nY^n3^n3^n4^a4^a$, females 2n=8, $2^n2^aX^nX^n3^n3^n4^a4^a$) produced by interracial hybridization between males of *D. n. nasuta*, Coorg strain and females of *D. n. albomicans*, Thailand strain (Ramachandra and Ranganath, 1990).

(vi) Cytorace 4 (males 2n=7, $2^n2^aY3^aX^n3^n4^a4^a$, females 2n=8, $2^n2^aX^nX^n3^n3^n4^a4^a$) produced by interracial hybridization between males of *D. n. albomicans*, Thailand strain and females of *D. n. nasuta*, Coorg strain (Ramachandra and Ranganath, 1990).

(vii) *D. melanogaster white* eye mutant (Drosophila Stock Centre, University of Mysore, Mysore).

At the time of the present investigation, cytorace 1, cytorace 2, cytorace 3 and cytorace 4 had already crossed 600 generations. All the fly stocks and the experimental cultures were maintained on standard wheat cream agar medium seeded with yeast at 22º ± 1ºC and 70-80% RH.

Inter-genotypic competitive ability assessment -

For the present experiment, synchronized cultures were developed following the modified procedure of Delcour (Ramachandra and Ranganath, 1988). Synchronized eggs of equal numbers (100) were collected and placed in each of the culture bottle and allowed to develop under uniform conditions of temperature, space, amount of food and humidity. Virgin females and unmated males from these cultures were isolated within 4 h of their eclosion and transferred to fresh media vials. Adult flies from these cultures were used for the assessment after aging them for 5-8 days. All the stocks and the experimental cultures were maintained on standard wheat cream agar medium seeded with yeast at 22º ± 1ºC and 70-80% RH.

D. n. nasuta, *D. n. albomicans*, cytorace 1 cytorace 2, cytorace 3 and cytorace 4 were allowed to compete independently against a common white eye mutant strain of *D. melanogaster*. Mixed cultures were established with 24 flies (12 males+12 females) of *D. melanogaster* and 24 flies (12 males+12 females) of one of the four experimental strains. Each set of mixed cultures was maintained in four replicates. The cultures were maintained at 22º ± 1ºC

by adopting the serial transfer technique 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. Every 7 days, they were etherized, counted, and transferred to fresh media bottles. When new flies began to emerge in the bottles where adult flies had deposited eggs, the newly emerged flies were etherized, counted, and added to the bottles with the older flies. This number was taken as the productivity of the race in question. 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. These weekly assessments were expressed in terms of the average of productivity and population size. After 4 weeks each bottle was discarded. The adult ovipositing flies remained 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 eliminates the mutant strain of *D. melanogaster*. Two facets of competitive ability, population size and productivity were calculated.

Statistical analysis- Data obtained from the inter-genotypic competitive ability was individually subjected to one-way Analysis of variance (ANOVA) followed by Duncan's Multiple Range test (DMRT) to analyze the significance of differences.

DNA Extraction

Genomic DNA (gDNA) was extracted from 30 male and female flies separately following the BDGP (Berkeley Drosophila Genome Project) protocol. The quality and quantity of the gDNA were estimated spectrophotometrically at 260/280 nm absorbances as well as by 0.8% agarose gels. DNA was diluted to a uniform concentration of 20ng/µl.

ISSR-PCR

Out of seven ISSR primers screened, 3 di-nucleotide primers namely, UBC-810:5'-GAGAGAGAGAGAGAGAT-3'; UBC-811: 5'-GAGAGAGAGAGAGAGAC-3'; UBC-842: 5'-GAGAGAGAGAGAGAGACG-3' and 1 tetra-nucleotide $(ACTG)_4$: 5'-ACTGACTGACTGACTG-3', primer amplified the DNA of the four races in the present study. Each reaction mixture of 25 µl contained 1 µl of genomic DNA (20 ng), 2 µl of primer (10 pM/ µl), 2.5 µl of 10x buffer (100mM Tris at pH 9.0, 500mM KCl and 1% Triton X-100), 1.5 µl of 1.5 mM $MgCl_2$, 2 µl of 200 µM dNTPs and 1.0 unit of *Taq* polymerase. PCR amplification were performed in a Corbett Research Palm Cycler (Australia) with an initial denaturation of 94ºC for 5 min, followed by 40 cycles of denaturing at 94ºC for 1 min, annealing for 1 min at 55 ºC, extension at 72 ºC for 2 min, and final extension at 72 ºC for 15 min. PCR amplified products were electrophoresed on 1.5% agarose gel containing ethidium bromide (1µg/ml) in 1X TBE buffer at a constant voltage of 60 V. Molecular weight markers, 100 bp and 500 bp (Bangalore Genei, India) were used for band sizing. Gel images were recorded and the band sizes were quantified by Mega bioprint 1000 system (Vilber Lourmat, France). All chemicals used in the experiment were of molecular biology grade and were procured from Bangalore Genei, India.

ISSR data analysis- PCR amplification of each of the ISSR was replicated and only clear and reproducible bands were considered. Amplified ISSR fragments were scored for the presence or absence of bands (1 = present, 0 = absence). Each of the ISSR fragment was considered as a single unique locus. If a relevant band was present in one or more, but not all, the races of the *nasuta-albomicans* complex of *Drosophila*, then it was considered as a polymorphic locus. A two-dimensional matrix was generated for the ISSR marker system. Percentage of polymorphic bands was calculated from the matrix by the numbers of polymorphic bands/total number of bands amplified x 100%. Similarity matrix and distance matrix was computed based on Jaccard's coefficient. To understand the genetic relationships among the six races under study, a dendrogram was constructed using unweighted pair-group method (UPGMA online free version) and the Tree was viewed by TREEVIEW (online free version).

3. Results

The dynamics of interspecific competition (mean population size of four replicates) between the six experimental races are given in Figure 1(a-f). In each of the mixed cultures, the *white* eye mutant strain of *D. melanogaster* was eliminated. The six members of the *nasuta-albomicans* complex of *Drosophila* exhibited competitive superiority over *D. melanogaster* strain, but the time taken to achieve this was strikingly different. *D. n. nasuta* and *D. n. albomicans* competitively eliminated *D. melanogaster* following 40 and 44 weeks respectively. On the other hand, cytorace 1 achieved the same result in 52 weeks, cytorace 2 at 60 weeks, cytorace 3 at 69 weeks and cytorace 4 at 75 weeks. The mutant strain of *D. melanogaster* survived for a longer period in mixed culture with the four cytoraces than in the mixed culture with *D. n. nasuta* or *D. n. albomicans*. The mean values of the two components of competitive ability i.e., productivity and population size, are given in Table 1. ANOVA revealed that the six races exhibited statistically significant differences. The gene

pool that maintains a larger population size may be said to be performing better than the one having a smaller population size. Of the six races under study, the parental races had significantly higher values for the two parameters of competitive ability than the newly evolved cytoraces. Among the cytoraces, cytorace 1 showed a significantly higher productivity and population size while cytorace 3 showed the lowest. Cytorace 1 was equally productive as its parent, *D. n. nasuta* and the rest of the cytoraces equals with their *D. n. albomicans* parent.

Four primers of ISSR generated 63 differently sized fragments ranging from 0.25 to 1.65 kb in total in the four races of the NAC of *Drosophila*. Out of these, 55 were polymorphic (87%). The number of bands varied from 9 to 20 with an average of 15 bands per primer. Out of the 3-dinucleotide primers, UBC-810 and UBC-811 generated 17 fragments and UBC-842 generated 20 fragments, whereas the tetra-nucleotide primer could amplify only 9 fragments. The highest number of polymorphic fragments was 19, generated with UBC-842 and the minimum number was 6, generated with $(ACTG)_4$. UBC-810 and UBC-811 generated 14 and 16 polymorphic fragments respectively.

With the UBC-810 ISSR primer, 8 differently sized fragments (Figure 2a) were generated in cytorace 1 and cytorace 2. Out of these 8 fragments, 4 were shared with both the parents. Besides these, one 0.68 kb fragment unique to *D. n. nasuta* was inherited. Three fragments of the size, 1.2 kb, 0.88 kb and 0.8 kb generated in cytorace 1 and cytorace 2 were found to be absent in both the parents suggesting that these fragments are novel fragments. Two fragments (0.95 kb and 0.82 kb) of *D. n. nasuta* and 5 fragments (1kb, 0.9 kb, 0.85 kb, 0.7 kb and 0.25 kb) of *D. n. albomicans* were not inherited in cytorace 1 and cytorace 2. In cytorace 3, the same primer generated 7 fragments, out of which 6 fragments were shared with both the parents. A 0.9 kb fragment was inherited from the *D .n. nasuta* parent. Two fragments (1 kb and 0.6 kb) of *D. n. albomicans* were not inherited. In cytorace 4, 8 fragments were generated, out of these, fragments 6 were shared with both the parents. Of the remaining, a 0.9 kb fragment was inherited from the *D. n. nasuta* parent; however two fragments (1 kb and 0.6 kb) of *D. n. albomicans* were not in cytorace 4. A 1.2 kb fragment was found to be novel in cytorace 4.

The UBC-811 ISSR primer generated 8 different fragments in cytorace 1 (Figure 2b). Out of these, 5 were shared with both the parents. Two fragments (1.48 kb and 1.38 kb) of *D. n. albomicans* were inherited. One fragment (0.4 kb) was found to be a novel fragment not found in either of the parents. A 1.3 kb fragment of *D. n. nasuta* and 0.65 kb fragment of *D. n. albomicans* were not inherited in cytorace 1. In cytorace 2, out of the 8 fragments generated, 5 were shared with both the parents. A 1.48 kb fragment was inherited from *D. n. albomicans* parent and a 1.3 kb fragment was inherited from *D. n. nasuta* parent. As in cytorace 1, a 0.4 kb fragment was new in cytorace 2. Two fragments (1.38 kb and 0.65 kb) of *D. n. albomicans* and one fragment (0.37 kb) of *D. n. nasuta* were not inherited. The same primer amplified 6 fragments in cytorace 3, out of which only one (1.2 kb) fragment was shared with both the parents. Besides this, three fragments (1.25 kb, 0.95 kb and 0.85 kb) of *D. n. nasuta* and two fragments (1.5 kb and 1.3 kb) of *D. n. albomicans* were inherited in cytorace 4. However, a 0.55 kb fragment unique to *D. n. nasuta* and three fragments (1.4 kb, 0.98 kb and 0.8 kb) unique to *D. n. albomicans* were not inherited.

Out of 7 fragments generated in cytorace 1 with the UBC-842 primer, 6 were shared with both the parents (Figure 2c). One fragment (0.68 kb) was inherited from the *D. n. albomicans* parent. One fragment (1.28 kb) of *D. n. nasuta* and two fragments (1.4 kb and 1.3 kb) of *D. n. albomicans* were not inherited in cytorace 1. In cytorace 2, 8 fragments were amplified, out of these, 4 were shared with both the parents. Two fragments (0.75 kb and 0.68 kb) were inherited from *D. n. albomicans* parent. Two fragments (1.2 kb and 1.1 kb) were new to cytorace 2 not seen in either of the parents. Two fragments (1.4 kb and 1.3 kb) of *D. n. albomicans* and two fragments (1.28 kb and 0.78 kb) of *D. n. nasuta* were not inherited in cytorace 2. In cytorace 3, the same primer generated 8 fragments, out of which, 4 were shared with the parents. Two fragments (1.25 kb and 0.55 kb) of *D. n. albomicans* and one fragment (1.6 kb) of *D. n. nasuta* were inherited in cytorace 3. A 0.8 kb fragment was a novel fragment found only in cytorace 3 and not generated in either of the parents. Four fragments (1.45 kb, 1.4 kb, 1.3 kb and 0.85 kb) of *D. n. nasuta* were not inherited. In cytorace 4, 8 fragments were amplified, out of which two fragments (1.5 kb and 0.7 kb) were shared with both the parents. Two fragments (1.6 kb and 1.45 kb) were inherited from *D. n. nasuta*. Two fragments of *D. n. albomicans* and three fragments (1.4 kb, 1.3 kb and 0.85 kb) of *D. n. nasuta* were not inherited. Four of the fragments (1.65 kb, 0.9 kb, 0.8 kb and 0.6 kb) were unique to cytorace 4 not amplified in either of the parents.

The primer $(ACTG)_4$ generated 6 fragments (Figure 2d) each in cytorace 1 and cytorace 2. Out of these fragments, three fragments were shared with the parents. Three fragments (0.68 kb, 0.58 kb and 0.4 kb) were inherited from *D. n. nasuta* parent. Two fragments (0.6 kb and 0.35 kb) of *D. n. albomicans* were not inherited in both the cytoraces. In cytorace 3 and cytorace 4, the same primer generated 7 fragments; six fragments were shared with both the

parents. A 0.78kb fragment unique to cytorace 3 and cytorace 4 was not amplified in either of the parents. One fragment (0.35 kb) of *D. n. albomicans* was not inherited in either of the two cytoraces.

The similarity matrix based on Jaccard's coefficient is shown in Table 2. The highest value of 0.788 was between cytorace 1 and cytorace 2, and the lowest value was 0.261, between females of *D. n. albomicans* (Okinawa) and females of *D. n. albomicans* (Thailand). The distance matrix based on Jaccard's coefficient is shown in Table 3. The highest value of 0.755 was between *D. n. albomicans* (Okinawa) and cytorace 4, and the lowest value was 0.212, between cytorace 1 and cytorace 2. A dendrogram was constructed based on online free version UPGMA (Figure 3). Cytorace 1 and cytorace 2 clustered together with their parents, *D. n. nasuta* (Coorg), *D. n. albomicans* (Okinawa). On the other hand, cytorace 3 and cytorace 4 clustered together with their *D. n. albomicans* (Thailand) parent.

4. Discussions

Hybrid zones in any natural populations contain diverse genotypes that are resultant of several generations of recombination (Harrison, 1990). The potentiality of a hybrid zone lies in its ability to provide insights into the mechanisms of speciation revealing the genetic differences that have accumulated during the early steps of speciation. Studies on hybrid zone can yield information about the possible state and degree of divergence between populations that may be 'on the way' to differentiating into races/species (Hewitt, 1988). Hybridization plays important creative roles in evolution of both plants and animals; however limited work has been done in animals (Rieseberg et al., 1999; Ramachandra and Ranganath, 1986, 1990, 1996; Harini and Ramachandra, 2003). Because of open genetic system of *D. n. nasuta* and *D. n. albomicans* brought about by force sympatry followed by interracial hybridization has resulted in the formation of new hybrid lineages which are unique but represent a differential composition of the parental chromosomes with an admixture of the parental genomes.

Interspecific competitive fitness is an important attribute in any population which will determine its success in a sympatric association of different species. Population fitness can be assessed by evaluating the inter-genotypic competitive ability of particular strain either with strains of a different species or with strains of the same species (Ayala, 1965; Futuyama, 1970; Goodman, 1979; Ramachandra and Ranganath, 1994). Zimmering (1948) has demonstrated that a 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. In the present study, *D. n. nasuta* had the maximum productivity and population size than *D. n. albomicans*. Among cytoraces, cytorace 1 had the highest productivity and population size which almost equals to its *D. n. nasuta* parent whereas cytorace 3 had the lowest productivity and population size which is also almost similar to its *D. n. albomicans* parent. Cytorace 2 and cytorace 4 also have similar competitive ability like *D. n. albomicans*. These different degrees of competitive ability suggest divergence between the cytoraces and its parents and among the cytoraces themselves also.

Available genetic mapping tools have made the complete exploitation of the genotypic diversity in hybrid zones and the utility of hybrid zones for analyzing the genetic architecture have been reported (Rieseberg et al., 1999). In the present study, to understand the level of introgression, four ISSR markers were employed which generated 63 discrete fragments out of which 87% were found to be polymorphic. The fragments amplified from all the four ISSR primers in males of *D. n. nasuta*, females of *D. n. albomicans*, and the four cytoraces suggest the following:

1) The UBC-842 amplifies more polymorphic fragments, followed by UBC-811 and UBC-810 in all the six members of the *nasuta-albomicans* complex of *Drosophila*. This result could be suggestive of the high variability of di-nucleotide repeats in the genome of *Drosophila.*

2) $(ACTG)_4$ generated the least number of polymorphic fragments which could be suggestive of the low variability of the tetra- nucleotide repeats in the *Drosophila* genome.

3) Cytorace 1 and cytorace 2 forms a cluster with their parents *D. n. nasuta* (Coorg) and *D. n. albomicans* (Okinawa). If we consider the number of parental chromosomes inherited in the cytoraces, *D. n. nasuta* (n) chromosomes are more in cytorace 1 (n=8, a=5), while *D. n. albomicans* (a) chromosomes are more in cytorace 2 (n=2, a=10) (Ramachandra and Ranganath, 1986, 1990). Cytorace 1 with more number of *nasuta* chromosomes is closely related with *D. n. nasuta*; however cytorace 2 with more number of *albomicans* chromosomes is closely related to *D. n. nasuta* than *D. n. albomicans* indicating that in these races, the parental chromosomes and the genetic content are not the same.

4) Cytorace 3 and cytorace 4 clusters together with *D. n. albomicans* (Thailand). In both cytorace 3 (n=10, a=6) and cytorace 4 (n=8, a=7), despite of more number of *D. n. nasuta* chromosomes, they are closely related with *D. n. albomicans* (Thailand). This suggests that even though they carry more of *D. n. nasuta* chromosomes, the genome in these chromosomes is more of *D. n. albomicans* type than

D. n. nasuta which highlight the role of recombination leading to differential introgression of the parental genomes in the cytoraces.

If we consider the number of parental chromosomes inherited in cytoraces *D. n. nasuta* (n) chromosomes are more in cytorace 1 (n=8, a=5) (Ramachandra and Ranganath, 1986, 1990) , cytorace 3 (n=10, a=6) and cytorace 4 (n=8, a=7) (Ramachandra and Ranganath, 1990) while *D. n. albomicans* (a) chromosomes were more in cytorace 2 (n=2, a=10) (Ramachandra and Ranganath, 1986, 1990). Although cytorace 1 harbors more of *D. n. nasuta* chromosomes and exhibit almost similar competitive ability like *D. n. nasuta*, while other two cytoraces, namely cytorace 3 and cytorace 4 exhibit *D. n. albomicans* type of competitive ability. This suggests that, one can make a positive correlation with the number of parental chromosomes in cytoraces and their competitive ability in these races.

If we compare, body size of the cytoraces and the competitive ability wherein all these four cytoraces are smaller in size than the parental races (Harini and Ramachandra, 1999, 2003) indicating that although cytorace 1 is smaller like the other three cytoraces, exhibit better competitive ability than the other cytoraces. This indicates that body size and competitive ability cannot be correlated in this situation. However, in the life history traits, lifetime fecundity and lifetime fertility, all the four cytoraces had better fecundity and fertility than the parental races indicating that the smaller the better (Harini and Ramachandra, 2003). In the present analysis, competitive ability of these races is inversely proportional to their life history traits.

Isozyme and genetic divergence study in these four races and the parents revealed that, cytorace 1, cytorace 2 and cytorace 3 are closer to *D. n. albomicans* than *D. n. nasuta* (Aruna and Ranganath, 2004). This suggests that genetic distance using isozyme markers is not correlating with the competitive ability. The studies on glue proteins in these cytoraces revealed that *D. n. nasuta* is closer to cytorace 3 and cytorace 4 than *D. n. albomicans* (Aruna and Ranganath, 2006). Our earlier studies on the chromosomes (Ramachandra and Ranganath, 1986), body size and fitness (Harini and Ramachandra, 1999b, 2003), and the longevity (Ranjini and Ramachandra, 2009) revealed the extent of divergence among the cytoraces.

Cytorace 1, cytorace 2 and cytorace 4 are with 20-23% of DNA polymorphism while cytorace 3 is with 10.7% of DNA polymorphism with reference to four ISSR profiles. Thus, one can surmise that cytorace 1, cytorace 2 and cytorace 4 with increased genetic variability exhibited better fitness while cytorace 3 with the least DNA polymorphism showed reduced competitive ability. Taking into consideration of all the studies done so far on cytoraces one can suggests that all these traits are controlled by many genes. These are located on different places in different chromosomes of the parents. Recombination followed by hybridization brought about the reshuffling of the genomic units and exhibit polymorphism in their genomes. Accordingly, differential response and ability were exhibited by these cytoraces. Therefore, this study suggests that these cytoraces are evolving through recombinational speciation which is yet another evidence.

Table 1: Competitive ability of six members of the *nasuta-albomicans* complex of *Drosophila* during interspecific competition with *white* eye mutant strain of *D. melanogaster* [Values are mean ± SE of four replicates].

Strains	Parameters	
	Productivity	Population size
D. n. nasuta	235.39 ± 16.62^{a}	331.61 ± 22.14^{a}
D. n. albomicans	187.81 ± 13.07^{b}	266.03 ± 19.43^{b}
Cytorace 1	234.17 ± 14.86^{c}	332.50 ± 18.71^{c}
Cytorace 2	181.42 ± 7.94^{d}	273.46 ± 11.66^{d}
Cytorace 3	179.08 ± 6.94^{e}	255.24 ± 9.42^{e}
Cytorace 4	188.29 ± 6.85^{f}	276.76 ± 9.41^{f}
ANOVA	$F = 5.759$; df = 5, 334; $P < 0.05$	$F = 5.172$; df = 5, 334; $P < 0.05$
DMRT	The difference between a/b, a/d, a/e, b/c, c/d, c/e and c/f are significant at 5% level	The difference between a/b, a/d, a/e, a/f, b/c, c/d, c/e and c/f are significant at 5% level

Table 2: Similarity matrix computed with Jaccard's coefficient in six members of the *nasuta-albomicans* complex of *Drosophila* with four ISSR primers [N = *D. n. nasuta*, A = *D. n. albomicans* (Okinawa), A (T) = *D. n. albomicans* (Thailand), C1 = Cytorace 1, C2 = Cytorace 2, C3 = Cytorace 3 and C4 = Cytorace 4].

Races	**N ♂**	**N ♀**	**A ♂**	**A ♀**	**A(T) ♂**	**A(T) ♀**	**C1 ♂**	**C1 ♀**	**C2 ♂**	**C2 ♀**	**C3 ♂**	**C3 ♀**	**C4 ♂**	**C4 ♀**
N ♂	1	0.625	0.436	0.436	0.378	0.333	0.588	0.588	0.571	0.571	0.395	0.395	0.310	0.310
N ♀		1	0.450	0.450	0.472	0.421	0.514	0.514	0.500	0.500	0.571	0.571	0.425	0.425
A ♂			1	1.000	0.295	0.261	0.538	0.538	0.452	0.452	0.311	0.311	0.245	0.245
A ♀				1	0.295	0.261	0.538	0.538	0.452	0.452	0.311	0.311	0.245	0.245
A(T) ♂					1	0.893	0.341	0.341	0.400	0.400	0.543	0.543	0.474	0.474
A(T) ♀						1	0.302	0.302	0.357	0.357	0.571	0.571	0.462	0.462
C1 ♂							1	1.000	0.788	0.788	0.390	0.390	0.341	0.341
C1 ♀								1	0.788	0.788	0.390	0.390	0.341	0.341
C2 ♂									1	1.000	0.381	0.381	0.304	0.304
C2 ♀										1	0.381	0.381	0.304	0.304
C3 ♂											1	1.000	0.611	0.611
C3 ♀												1	0.611	0.611
C4 ♂													1	1.000
C4 ♀														1

Table 3: Distance matrix based on Jaccard's coefficient in six members of the *nasuta-albomicans* complex of *Drosophila* with four ISSR primers [N = *D. n. nasuta*, A = *D. n. albomicans* (Okinawa), A (T) = *D. n. albomicans* (Thailand), C1 = Cytorace 1, C2 = Cytorace 2, C3 = Cytorace 3 and C4 = Cytorace 4].

Races	**N ♂**	**N ♀**	**A ♂**	**A ♀**	**A(T) ♂**	**A(T) ♀**	**C1 ♂**	**C1 ♀**	**C2 ♂**	**C2 ♀**	**C3 ♂**	**C3 ♀**	**C4 ♂**	**C4 ♀**
N ♂	0	0.375	0.564	0.564	0.622	0.667	0.412	0.412	0.429	0.429	0.605	0.605	0.690	0.690
N ♀		0	0.550	0.550	0.528	0.579	0.486	0.486	0.500	0.500	0.429	0.429	0.575	0.575
A ♂			0	0.000	0.705	0.739	0.462	0.462	0.548	0.548	0.689	0.689	0.755	0.755
A ♀				0	0.705	0.739	0.462	0.462	0.548	0.548	0.689	0.689	0.755	0.755
A(T) ♂					0	0.107	0.659	0.659	0.600	0.600	0.457	0.457	0.526	0.526
A(T) ♀						0	0.698	0.698	0.643	0.643	0.429	0.429	0.538	0.538
C1 ♂							0	0.000	0.212	0.212	0.610	0.610	0.659	0.659
C1 ♀								0	0.212	0.212	0.610	0.610	0.659	0.659
C2 ♂									0	0.000	0.619	0.619	0.696	0.696
C2 ♀										0	0.619	0.619	0.696	0.696
C3 ♂											0	0.000	0.389	0.389
C3 ♀												0	0.389	0.389
C4 ♂													0	0.000
C4 ♀														0

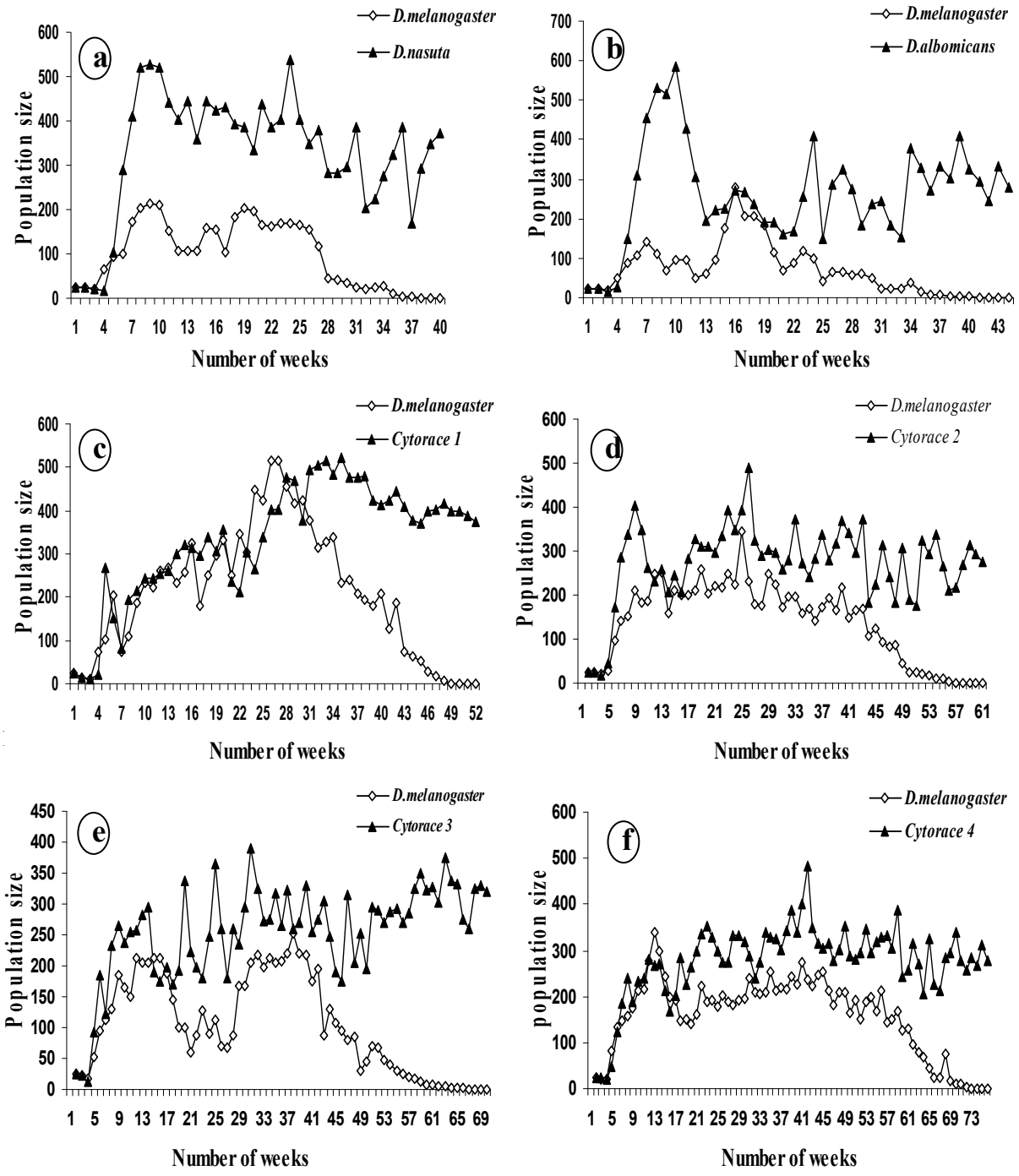

Figure 1: Population dynamics of interspecific competition (mean of four replicates) between (a) *D. n. nasuta* and *white* eye mutant of *D. melanogaster*, (b) *D. n. albomicans* and *white* eye mutant of *D. melanogaster* (c) Cytorace 1 and *white* eye mutant of *D. melanogaster* (d) Cytorace 2 and *white* eye mutant of *D. melanogaster* (e) Cytorace 3 and *white* eye mutant of *D. melanogaster* and (f) Cytorace 4 and *white* eye mutant of *D. melanogaster*.

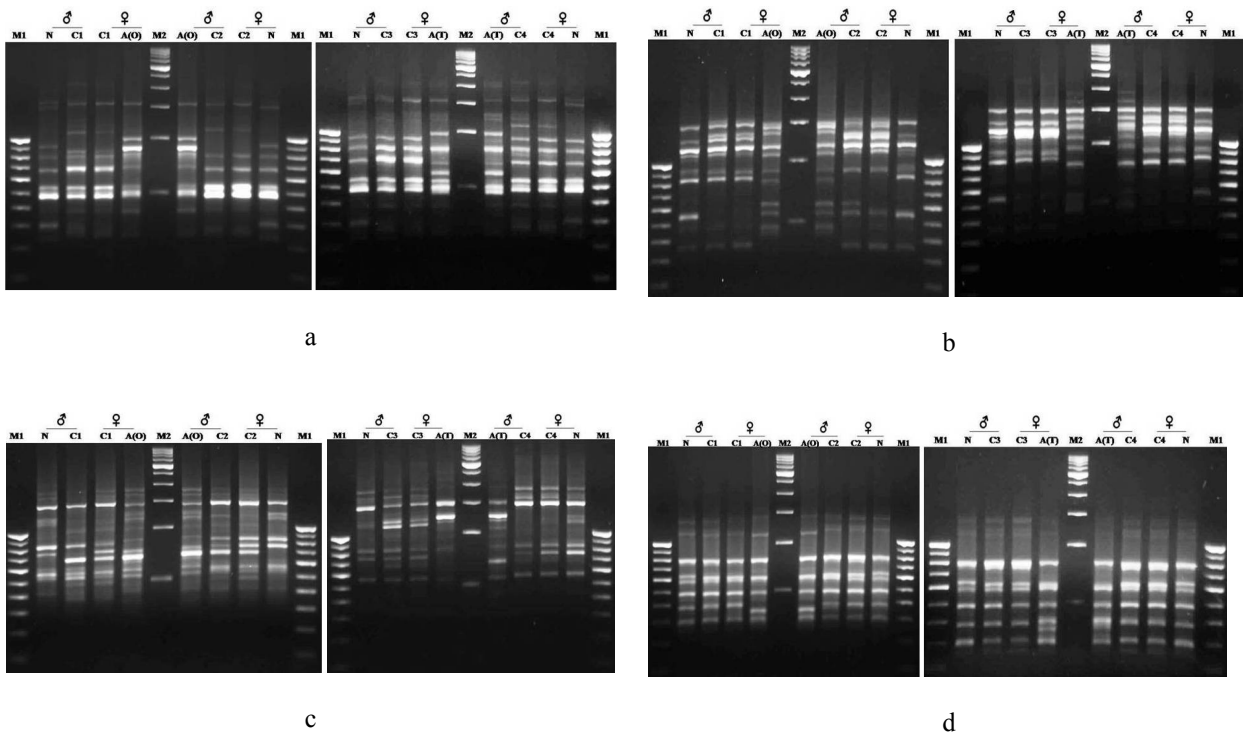

Figure 2: ISSR profile of *D. n. nasuta*, *D. n. albomicans* (Okinawa), *D. n. albomicans* (Thailand), Cytorace 1, Cytorace 2, Cytorace 3 and Cytorace 4 generated with the primers (a) UBC-810 (b) UBC-811 (c) UBC-842 and (d) $(ACTG)_4$ [M1 and M2 are 100 bp and 500 bp DNA markers].

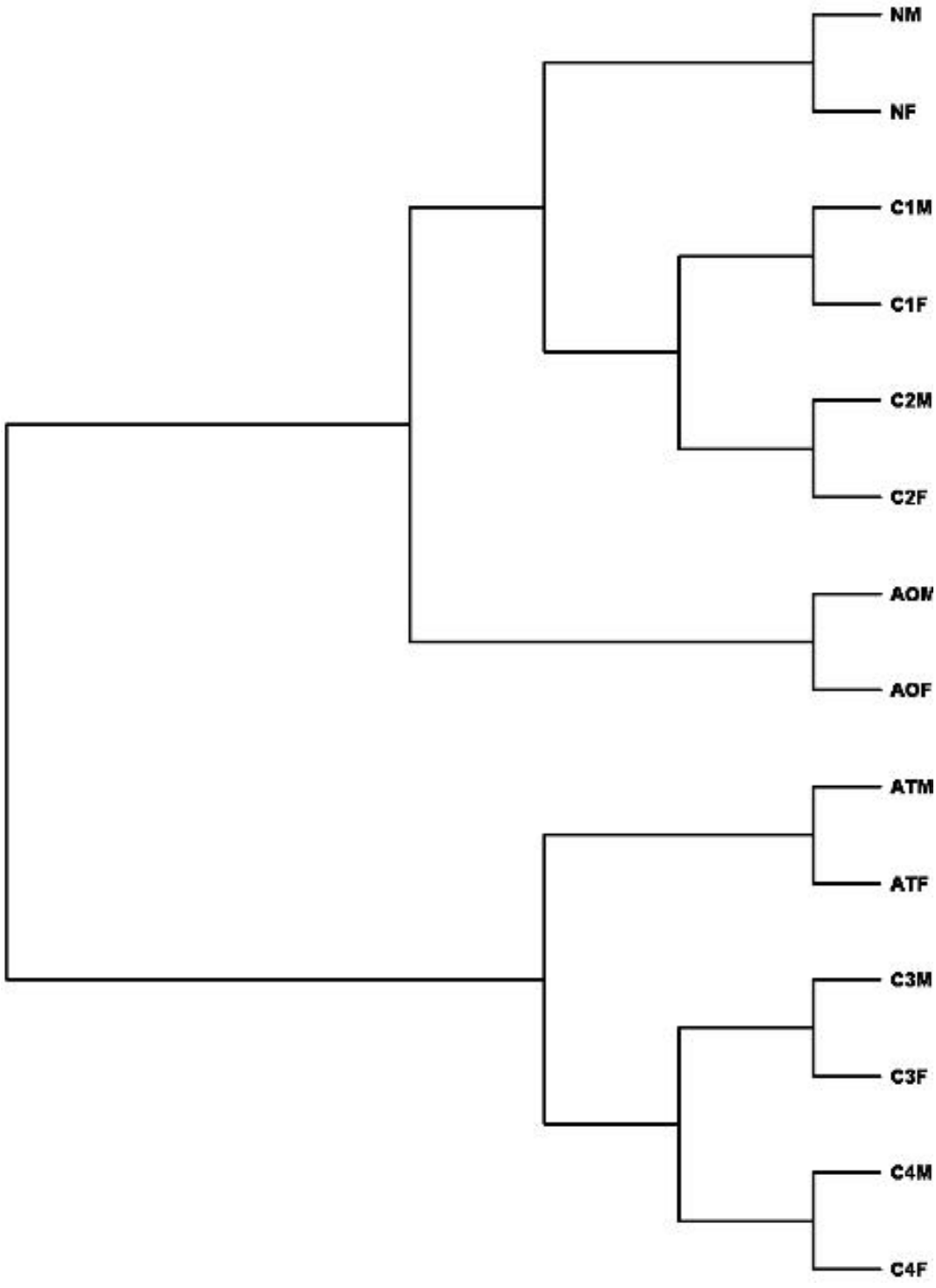

Figure 3: UPGMA based cluster tree of the genotypes of six members of the *nasuta-albomicans* complex of *Drosophila.*

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References

[1] Lamb CG. Diptera: *Heteroneuridae,Ortalidae, Trypetidae, Sepsidae, Micropezidae, Drosophilidae, Geomysidae, Milichiidae of Scyehelles*. Trans. Linn. Soc. London 1914; 16: 307-372.

[2] Duda O. Die orientalischen and australischen *Drosophiliden*- Arten des ungarischen National Musuems Zu Budapest. Ann. Mus. Nat. Hung 1923; 20: 24-59.

[3] Wilson FD, Wheeler MR, Harget M, Kambysellis M. Cytogenetics relations in the *Drosophila nasuta* subgroup of the *immigrans* group of species. Univ. Texas Publ 1969; 6918: 207-254.

[4] Kitagawa O, Wakahama K, Fuyama Y, Shimada Y, Takanashi E, Hatsumi M, Mita Y. Genetic studies of the *Drosophila nasuta* subgroup, with notes on distribution and morphology. Jpn. J. Genet 1982; 57: 113-141.

[5] Lin FJ, Tseng HC, Chiang W. Chromosomal polymorphisms in *Drosophila albomicans*. Dros. Infor. Serv 1977; 52: 153.

[6] Singh BK. Studies on the systematic and Cytogenetics of Indian *Drosophilids*. Ph. D., Thesis, Banaras Hindu University, Varanasi, 1977.

[7] Ranganath HA. Evolutionary biology of *Drosophila nasuta* and *Drosophila albomicans*. Proc. Indian Natn. Sci. Acad. (PINSA) 2000; 68(3): 255-272.

[8] Ranganath HA, Hagele K. Karyotypic orthoselection in *Drosophila*. Naturwisssenschaften., 1981, 68(10): 527-528.

[9] Nirmala SS, Krishnamurthy NB. *Drosophila albomicans* – a race of *Drosophila nasuta*. Dros. Infor. Serv 1972; 49: 60.

[10] Ranganath HA. Genetic integrity, population density and selection studies in a few members of the *immigrans* group of *Drosophila*. Ph.D., Thesis, University of Mysore, Mysore, India, 1975.

[11] Ranganath HA. Population Genetics of *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans* and their hybrids. Science Academy Medals for Young Scientists Lectures. Proc. Ind. Natl. Sci. Acad. (New Delhi) 1978: 124-139.

[12] Ranganath HA, Ramachandra NB. Chromosomal basis of raciation in *Drosophila*: A study with *Drosophila nasuta* and *Drosophila albomicans*. Proc. Ind. Acad. Sci. (Anim. Sci) 1987; 96(5): 451-459.

[13] Ramachandra NB, Ranganath HA. The chromosomes of two races: *Drosophila nasuta nasuta* and *Drosophila nasuta albomicana*: IV. Hybridization karyotype repatterning. Chromosoma 1986; 93(3): 243-248.

[14] Ramachandra NB, Ranganath HA. The chromosomes of two *Drosophila* races: *Drosophila nasuta nasuta* and *Drosophila nasuta albomicana*: V. Introgression and the evolution of new karyotypes. Z. Zool. Syst. Evolut-forch (Germany) 1990; 28(1): 62-68.

[15] Ramachandra NB, Ranganath HA. Evolution of the *nasuta-albomicans* complex of *Drosophila*. Curr. Sci 1996; 71: 515-517.

[16] Tanuja MT, Ramachandra NB, Ranganath HA. Hybridization and introgression of the genomes of *Drosophila nasuta* and *Drosophila albomicans*: Evolution of new karyotypes. Genome 2003; 46(4): 605-611.

[17] Tanuja MT, Ramachandra NB, Ranganath HA. Incipient sexual isolation in the *nasuta-albomicans* complex of *Drosophila*: No-choice experiments. J. Biosci 2001a; 26(1): 71-76.

[18] Tanuja MT, Ramachandra NB, Ranganath HA. Incipient sexual isolation in the *nasuta-albomicans* complex of *Drosophila*: mating preference in male-, female- and multiple-choice mating experiments. J. Biosci 2001b; 26(3): 365-371.

[19] Harini BP, Ramachandra NB. Evolutionary experimentation through hybridization under laboratory condition in *Drosophila*: Evidence for Recombinational Speciation. BMC. Evol. Biol 2003; 3: 1-19.

[20] Ramachandra NB, Ranganath HA. Interspecific competition of the parental races (*D. n. nasuta* and *D. n. albomicana*) and of the newly evolved Cytoraces (I and II). Z. Zool. Syst. and Evolut-forsch (Germany) 1994; 32: 73-78.

[21] Harini BP, Ramachandra NB. Racial divergence in sternopleural bristles among the parental races and the newly evolved Cytorace 1 and Cytorace 2 of the *nasuta-*

albomicans complex of *Drosophila*. Curr. Sci 1999a; 76(7): 1017-1019.

[22] Harini BP, Ramachandra NB. Does evolution reduces the body size? A study in the four members of newly evolved *nasuta-albomicans* complex of *Drosophila*. Genetica 1999b; 105: 1-6.

[23] Harini BP, Ramachandra NB. Racial divergence in body weight: A study in the four members of newly evolved *nasuta-albomicans* complex of *Drosophila*. Curr. Sci 2000a; 78(3): 342-344.

[24] Harini BP, Ramachandra NB. Racial divergence in abdominal bristles among the parental races and the newly evolved Cytoraces of *nasuta-albomicans* complex of *Drosophila*. Indian J. Exp. Biol 2000b; 38(12): 1263-1266.

[25] Aruna S, Ranganath HA. Isozymes and genetic divergence in the *nasuta-albomicans* complex of *Drosophila*. Curr. Sci 2004; 86(7):1017-1023.

[26] Aruna S, Ranganath HA. Introgressive hybridization and evolution of a novel protein phenotype: glue protein profiles in the *nasuta-albomicans* complex of *Drosophila*. J. Genet 2006; 85(1): 25-30.

[27] Ranjini MS, Ramachandra NB. Evolution of short-lived and long-lived races of *Drosophila* in the environs of laboratory. Ind. J. Geron 2009; 23: 381-398.

[28] Sharma A, Namdeo AG, Mahadik KR. Molecular markers: new prospects in plant genome analysis. Phcog. Rev 2008; 2(4): 23-34.

[29] Williams JGK, Kubelik AR, Livak K, Rafalski JA. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990; 18(22): 6531- 6535.

[30] Vos P, Hogers R, Bleeker M, Van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 1995; 23(21): 4407- 4414.

[31] Staub JE, Serquen FC, Gupta M. Genetic markers, map construction, and their application in plant breeding. Hortscience 1996; 31: 729-739.

[32] Gupta PK, Varshney RK. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 2000; 113(3): 163-185.

[33] Vogt P. Potential genetic functions of tandemly repeated DNA sequence blocks in the human genome are based on a highly conserved "chromatin folding code:" Human Genet 1990; 84(4): 301-336.

[34] Gur-Arie R, Cohen CJ, Eitan Y, Shelef L, Hallerman EM, Kashi Y. Simple sequence repeats in *Escherichia coli*: abundance, distribution, composition, and polymorphism. Genome Res 2000; 10: 62-71.

[35] Tautz D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 1989; 17(16): 6463-6471.

[36] Weber JL. Informativeness of human poly $(GT)_n$ polymorphisms. Genomics 1990; 7(4): 524-530.

[37] Goodfellow PN. Variation is now the theme. Nature 1992; 359: 777-778.

[38] Bell CJ, Ecker JR. Assignment of 30 microsatellites loci to the linkage map of Arabidopsis. Genomics 1994; 19(1): 137-144.

[39] Rosenberg SM, Longerich S, Gee P, Harris. Adaptive mutation by deletions in small mononucleotide repeats. Science 1994; 265(5170): 405-407.

[40] Kashi Y, King D, Soller M. Simple sequence repeats as a source of quantitative genetic variation. Trends Genet 1997; 13(2): 74-78.

[41] Moxon ER, Wills. DNA microsatellites: Agents of evolution? Sci. Am 1999; 280: 94-99.

[42] Wolfe AD, Xiang QY, Kephart SR. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable inter-simple sequence repeat (ISSR) bands. Mol. Ecol 1998; 7(9): 1107-1126.

[43] Nagaraju J, Kathirvel M, Kumar RR, Siddiq EA, Hasnain SE. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci. (USA) 2002; 99(9): 5836-5841.

[44] Zietkiewics E, Rafalski A, Labuda D. Genome fingerprint by sequence repeat (SSR) – anchored polymerase chain reaction amplification. Genomics 1994; 20(2): 176-183.

[45] Awasthi AK, Nagaraja GM, Naik GV, Kanginakudru S, Thangavelu K, Nagaraju J. Genetic diversity and relationships in mulberry (genus Morus) as revealed by RAPD and ISSR marker assays. BMC. Gen 2004; 5: 1-9.

[46] Verma S, Rana TS, Ranade SA. Genetic variation and clustering in *Murraya paniculata* complex as revealed by single primer amplification reaction methods. Curr. Sci 2009; 96(9): 1210-1216.
[47] Hantula J, Dusabenygasani M, Hamelin RC. Random amplified microsatellites (RAMS) – a novel method for characterizing genetic variation within fungi. Eur. J. For Path 1996; 26(3): 159-166.
[48] Tsumura Y, Ohba K, Strauss SH. Diversity and inheritance of inter-simple sequence repeat polymorphism in Douglas-fir (*Pseudotsuga menziesii*) and Sugi (*Cryptomeria japonica*). Theor. Appl. Genet 1996; 92(1): 40-45.
[49] Abbot P. Individual and population variation in invertebrates revealed by Inter- simple Sequence Repeats (ISSRs). J. Insc. Sci 2001; 1.8: 1-3.
[50] Kumar LD, Kathirvel M, Rao GV, Nagaraju J. DNA profiling of disputed chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. Foren. Sci. Inter 2001; 116(1): 63-68.
[51] Pandit SS, Mitra S, Giri AP, Pujari KH, Patil BP, Jambhale ND, Gupta VS. Genetic diversity of mango cultivars using inter simple sequence repeat markers. Curr. Sci 2007; 93(8): 1135-1141.
[52] Reddy KD, Nagaraju J, Abraham EG. Genetic characterization of the silkworm *Bombyx mori* by simple sequence repeat (SSR) - anchored PCR. Heredity 1999; 83: 681- 687.
[53] Kostia S, Ruohonen – Lehto M, Vainola R, Varvio SL. Phylogenetic information in inter-SINE and inter- SSR fingerprints of the Artiodactyla and evolution of the BovtA SINE. Heredity 2000; 84(1): 37-45.
[54] Zouros E. A model for the evolution of asymmetrical male hybrid sterility and its implications for speciation. Evolution 1986; 40(6): 1171-1184.
[55] Ramachandra NB, Ranganath HA. Estimation of population fitness of parental races (*Drosophila nasuta nasuta*, *Drosophila nasuta albomicana*) and of the newly evolved Cytoraces (I and II) - the products of parental interracial hybridization. Genome 1988; 30: 58-62.
[56] Ayala FJ. Relative fitness of populations of *Drosophila serrata* and *Drosophila birchii*. Genetics 1965; 51: 527-544.
[57] Harrison RS. Hybrid zones: windows on evolutionary process. Oxford Surv. Evol. Biol 1990; 7: 158-167.
[58] Hewitt GM. Hybrid zones-natural laboratories for evolutionary studies. Trends Ecol. Evol 1988; 3(7): 158-167.
[59] Rieseberg LH, Archer MA, Wayne RK. Transgressive segregation, adaptation and speciation. Heredity 1999; 83: 363-372.
[60] Futuyama DJ. Variation in genetic response to interspecific competition in laboratory populations of *Drosophila*. Am. Nat 1970; 104(937): 239-252.
[61] Goodman D. Competitive hierarchies in laboratory *Drosophila*. Evolution 1979; 33(1): 207-219.
[62] Ramachandra NB, Ranganath HA. Pattern of sexual isolation between parental races (*Drosophila nasuta nasuta* and *D. n. albomicans*) and the newly evolved races (Cytoraces 1 and 2). Indian J. Exp. Biol 1994; 32(2): 98-102.
[63] Zimmering S. Competition between *Drosophila pseudoobscura* and *Drosophila melanogaster* in population cages. Am. Nat 1948; 82: 326-330.

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