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LIST OF PUBLICATIONS
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1. 2005 M. C. Shilpa and Ranganath, H. A.
   Evolution of new races by interracial hybridizations of different strains
   of D. n. nasuta (Kenya and Seychelles) and D. n. albomicans (Japan
   and Thailand).

2. 2005 M. C. Shilpa and Ranganath, H. A.
   Mating propensity: an indicator of interracial divergence in the
   nasuta-albomicans complex of Drosophila.
   Dros. Inf. Serv. 88: 89-92.

3. 2006 M. C. Shilpa and Ranganath, H. A.
   Dissection of courtship behaviour elements in a few members of the
   nasuta- albomicans complex of Drosophila.
   Proceedings of the National Academy of Sciences (in press).

4. M. C. Shilpa and Ranganath, H. A.
   Racial divergence in female productivity in a few members of the
   nasuta-albomicans complex of Drosophila.
   (in preparation).
EVOLUTION OF NEW KARYOTYPIC RACES BY INTERRACIAL HYBRIDIZATION OF DIFFERENT STRAINS OF D. N. NASUTA (KENYA AND SEYCHELLES) AND D. N. ALBOMICANS (JAPAN AND THAILAND)

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SUMMARY

Interracial hybridization between different geographic strains of D. n. nasuta (Kenya and Seychelles) and D. n. albomicans (Japan and Thailand) lead to eight hybrid lines. Out of eight hybrid lines, in five hybrid lineages karyotypic mosaicism was lost and such lines with stable karyotypes were termed as Cytoraces. In continuation of earlier hybridization experiments, these new lines were named as Cytorace 26 to Cytorace 30. In the newly evolved Cytoraces, not only the dot chromosomes but even the sex chromosomes of D. n. albomicans have selective advantage to those of D. n. nasuta. The strategies involved in the evolution of new karyotypes have been discussed.

Key Words: Drosophila hybridization, introgression cytorace

INTRODUCTION

Karyotype is a valuable data concerning the origin and nature of the chromosomal differences that in a taxonomic group. It has proved extremely useful in the study of closely related species groups, species complexes and polymorphic species. The karyotype is a basic feature of a species, even with techniques like electrophoresis, blots and chromosomal painting, as Stebbins (1950) states “The because they are bearers of hereditary factors, should be considered as some what more than other structures on which relationship is based”. Over 90 percent of all speciating events accompanied by karyotypic changes and in a majority of these cases, structural chromosomal rearrangement have played primary role in initiating divergence (White, 1978). However, Chang and Carson (1985) are view that in most cases the fixation of particular karyotype is likely to be merely an incidental accom of small population effects and forced selection for reorganization as the species is formed.

Chromosome number is usually considered to be the most important cytological character of interest and evolutionary biologists. A host of investigators have attempted to assess the role played rearrangements in speciation (White, 1973; Futuyama and Mayer, 1980; Bush et al., 1977; 1985; Ramachandra and Ranganath, 1996; King, 1993; Searle, 1998 and Rieseberg, 2001). Many studies on the metaphase chromosome differentiation have been made for the members of the Drosophila in general (Patterson et al., 1942; Patterson and Stone 1952; Matther, 1962; Clayton, 1968; 1975a, b; Lemeunier et al., 1978; Lakhotia and Mishra, 1980; Lakovaara and Saura, 1982; 1982; Wakahama et al., 1983; Chang and Carson, 1985; Mahan and Beck, 1986; Rao and 1991; Shyamala and Ranganath, 1994 and Suma, 1997) and in particular D. nasuta subgroup 1969; Nirmala and Krishnamurthy, 1971, 1972 and 1973; Ranganath and Hagele, 1981; Hagele
and Ranganath 1982; Ranganath and Hägele, 1982; Kitagawa et al., 1982; Ranganath and Ramachandra, 1994; Ramachandra and Ranganath, 1996; Ling and Wang, 1997; Yu et al., 1997; Tanuja et al., 1999 a, b and Tanuja et al., 2003).

*D. n. nasuta* and *D. n. albomicans* based on their open genetic systems and cross fertility have been treated as chromosomal races. They belong to the *nasuta* subgroup of the *immigrans* species groups of *Drosophila* (Wilson et al., 1969; Nirmala and Krishnamurthy, 1973). Interracial hybridization between these two races followed by maintenance of hybrid populations for over 20 to 300 generations in the laboratory has resulted in the evolution of new karyotypic races called Cytoraces. These Cytoraces (Cytorace 1 to 16) along with parental races constitute a new complex, and is called *nasuta-albomicans* complex. The *nasuta-albomicans* complex includes the parental genomes namely *D. n. nasuta* and *D. n. albomicans* as well as the newly evolved hybrid genomes, called Cytoraces (Cytorace 1 to 16) (Ramachandra and Ranganath, 1985; Ramachandra and Ranganath, 1986; Ramachandra and Ranganath, 1990; Ramachandra and Ranganath, 1996; Ranganath, 2002; Tanuja et al., 2003 and Ranganath and Aruna 2003).

In an extension of these studies hybridization experiments were initiated with geographically distantly placed strains of *D. n. nasuta* (Kenya and Seychelles) and of *D. n. albomicans* (Japan and Thailand) and hybrid populations were maintained for many generations. The present paper reports the pattern of karyotypic divergence in the introgressed genomes of *D. n. nasuta* and *D. n. albomicans*.

**MATERIALS AND METHODS**

The geographic strains employed in the present investigation are *D. n. nasuta* from Kenya (0252.21) and Seychelles (0252.22), and *D. n. albomicans* from Thailand (0161.8) and Japan (0231.3).

**Hybridization:**

Five days old males and virgin females of the four strains namely Kenya and Seychelles of *D. n. nasuta* and Japan and Thailand of *D. n. albomicans* were used for reciprocal hybridization experiments. These have yielded the hybrid lines (Table 1). The experimental protocol is depicted in Fig. 1. Ten males and ten females of two different strains were the founders of each hybrid lineage. The culture of the hybrid populations is being maintained by serial transfer technique. The karyotypes of the parents and of *F*<sub>1</sub>, *F*<sub>2</sub>, *F*<sub>4</sub>, *F*<sub>2w</sub>, *F*<sub>3w</sub>, *F*<sub>4w</sub>, *F*<sub>5w</sub>, *F*<sub>6w</sub> and *F*<sub>7w</sub> hybrid generations were screened.

**TABLE 1:** Eight hybrid lines resulted due to interracial hybridization between two geographic strains of the *nasuta* (Kenya and Seychelles) and two strains of *albomicans* (Japan and Thailand) of *Drosophila*. (K=Kenya; S=Seychelles; J=Japan; T=Thailand). In three hybrid populations, karyotypic polymorphism exists (P) while in five lineages a stabilized karyotype is seen (S) and labeled as new Cytoraces (C26 to C30).

<table>
<thead>
<tr>
<th>↓</th>
<th>↑</th>
<th>Kenya ♀</th>
<th>Seychelles ♀</th>
<th>Japan ♀</th>
<th>Thailand ♀</th>
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<td>-</td>
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<td>K♂ X T♀ (P)</td>
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<tr>
<td><strong>Seychelles ♂</strong></td>
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<td>S♂ X J♀ (S)=C27</td>
<td>S♂ X T♀ (P)</td>
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</tr>
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<td><strong>Japan ♂</strong></td>
<td>J♂ X K♀ (S)=C29</td>
<td>J♂ X S♀ (P)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Thailand ♂</strong></td>
<td>T♂ X K♀ (S)=C28</td>
<td>T♂ X S♀ (S)=C26</td>
<td>-</td>
<td>-</td>
<td></td>
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</table>
EXPERIMENTAL PROTOCOL

THE EVOLUTION OF NEW RACES

PARENTAL RACES

\[ D. N. \text{NASUTA} \times D. N. \text{ALBOMICANS} \]

(2n=8) (2n=6)

\[ F_1 \]

(2n=7)

KARYOTYPIC MOSAICISM

\[ F_n \]

GENERATIONS

- IN SOME HYBRID POPULATIONS THE KARYOTYPIC VARIABILITY DISAPPEARS.
- STABLE KARYOTYPE WITH NEW CHROMOSOMAL COMBINATIONS APPEARS.
- REORGANIZATION OF THE KARYOTYPE.
- SELECTIVE ELIMINATION AND/OR SELECTION OF PARTICULAR PARENTAL CHROMOSOMES
- A HYBRID POPULATION WITH A STABILIZED KARYOTYPE IS CALLED A "CYTORACE".

Analysis of Karyotypes:

Hundred larvae were chosen at random from each line for karyotypic analysis and the frequencies of each one of the parental chromosomes in different hybrid generations were recorded. For chromosome preparation, air-dry preparations of the larval brain were made following the technique of Ramachandra and Ranganath (1986). Well-fed third instar larvae maintained at 22°C were dissected out in the physiological saline (0.7% Sodium Chloride). Hypotonic treatment (1% Sodium Citrate solution) was given for 30 min. After the hypotonic treatment, the ganglia were fixed in 3:1 Methanol: Acetic acid fixative for 2 hours. During fixation, 2 or 3 changes with fresh fixative were given. Clean, dry and grease free slides were warmed on slide warmer. A drop of 60% warm Acetic acid was taken on the slide, into which one fixed ganglion was placed. The cells of the neural ganglion were allowed to disperse in Acetic acid. 1-2 drops of fresh fixative were dropped splash on the slide and slides were air-dried. For conventional staining, the air-dried slides were stained with 10% Giemsa in Phosphate buffer of pH 7 for 30 minutes (Hägele, 1977). These slides were washed in distilled water and air-dried.
RESULTS AND DISCUSSION

The metaphase karyotypes of two geographic strains of *D. n. nasuta* and of *D. n. albomicans* of the *nasuta* subgroup of the *immigrans* species group of *Drosophila* were studied.

**Karyotypic description of *D. n. nasuta* strains (Kenya and Seychelles):** In both the strains, the chromosomal complement was $2n=8$, with a pair of metacentric chromosome 2, a pair of acrocentric chromosome 3, a pair of basic dot chromosome 4 and a pair of acrocentric X-chromosome in females while males have one acrocentric X-chromosome and one sub metacentric Y-chromosome (Fig-2a).

**Karyotypic description of *D. n. albomicans* strains (Japan and Thailand):** In both the strains the

![Fig 2a: Karyotypes of *D. n. nasuta* (Kenya and Seychelles) male and female (2n=8)](image)

![Fig 2b: Karyotype of *D. n. albomicans* (Japan) male and female (2n=6)](image)

![Fig 2c: Karyotype of *D. n. albomicans* (Thailand) male and female (2n=6)](image)
The chromosomal complement was 2n=6, with two pairs of metacentric chromosomes and a pair of dot chromosome. Of the two, the smaller metacentric is chromosome 2, while larger pair of metacentric chromosome is the dimorphic chromosome between sexes. It represents X3, Y3 in males while females have X3, X3 chromosomes. The Japan strain of *D. n. albomicans* has a pair of long dot chromosomes (Fig-2b) while the Thailand strain has a pair of short dot chromosomes (Fig-2c).

The chromosome complements of F₁ hybrids of the eight crosses between *D. n. nasuta* strains and *D. n. albomicans* strains were recorded. The F₁ hybrid males and females had 2n=7. The analysis of the karyotypes of the F₁ and of the succeeding hybrid generations revealed karyotypic polymorphism with altogether new types of chromosome combinations other than those of the parents and the F₁ hybrids.

Out of eight, in five hybrid lineages karyotypic polymorphism was replaced by a stable karyotype. These hybrid populations with stabilized karyotypes will be treated as new Cytoraces and named as Cytorace 26 to Cytorace 30. In the other three hybrid lineages, only the dot chromosomes were stabilized, where as other chromosomes (Sex chromosomes and Chromosome 3) retained the karyotypic polymorphism even after F₉ generations.

A detailed analysis of the incidence of the *D. n. nasuta* and *D. n. albomicans* chromosomes (parental) at F₁, F₂, F₃, F₄, F₅, F₆, F₇, and F₈ generations of these new Cytoraces (Cytorace 26 to Cytorace 30) with stabilized karyotypes have been made. Tables-2a to 2e contain information for chromosomes X* X*, Y* Y*, Y* Y* of *D. n. nasuta* and X3* and Y3* of *D. n. albomicans* while tables-3a to 3e provide information for the dot chromosomes. Summary of the karyotypic composition of the Cytoraces 26 to 30 is given in table-4. The detailed description of the karyotypes of the Cytoraces 26 to 30 is as follows:

**Cytorace 26: (♂ & ♀ 2n=6)**

Cytorace 26 is the product of the hybridization between the females of Seychelles strain of *D. n. nasuta* (2n=8) and the males of Thailand strain of *D. n. albomicans* (2n=6). The karyotypic variability present in the hybrid population has gradually disappeared and by F₈, a stable hybrid population has evolved. The females and males of this karyotypically stable hybrid population had the following features (Fig-3a):

Females: (2n=6) A pair of metacentric representing chromosome 2 (2", 2" or 2", 2", or 2", 2"), another pair of metacentric representing chromosome X3 (X3', X3") and a pair of short dots, representing chromosome 4 (4", 4").

Males: (2n=6) A pair of metacentric representing chromosome 2 (2", 2" or 2", 2", or 2", 2"), another pair of metacentric representing chromosome X3 (X3"), and Y3 (Y3") and a pair of short dots, representing chromosome 4 (4", 4").

**Cytorace 27: ♂ (2n=7) & ♀ (2n=6)**

Cytorace 27 is the product of the hybridization between the males of Seychelles strain of *D. n. nasuta* (2n=8) and the females of Japan strain of *D. n. albomicans* (2n=6). The karyotypic variability present in the hybrid population has gradually disappeared and by F₈, a stable hybrid population has evolved. The karyotypic composition of this race is as follows (Fig-3b):

Females: (2n=6) A pair of metacentric representing chromosome 2 (2", 2" or 2", 2", or 2", 2"), another pair of metacentric representing chromosome X3 (X3", X3") and a pair of short dots, representing chromosome 4 (4", 4").
Males: (2n=7) A pair of metacentric representing chromosome 2 (2*, 2° or 2\(^{\prime}\), one metacentric representing chromosome X3 (X3\(^{*}\)), and one submetacentric Y-chromosome (Y*) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).

Cytorace 28: (♂ & ♀ 2n=6)

Cytorace 28 is the product of the hybridization between the females of Kenya strain of *D. n. nasuta* (2n=8) and the males of Thailand strain of *D. n. albomicans* (2n=6). By F\(_{1}\), karyotypically a stable hybrid population has evolved. The chromosomal configuration of this stable hybrid population is (Fig-3c):

Females: (2n=6) A pair of metacentric representing chromosome 2 (2*, 2° or 2\(^{\prime}\), 2\(^{\prime}\) or 2*, 2\(^{\prime}\)), another pair of metacentric representing chromosome X3 (X3\(^{*}\), X3\(^{\prime}\)) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).

Males: (2n=6) A pair of metacentric representing chromosome 2, another pair of metacentric representing chromosome X3 (X3\(^{*}\)), and Y3 (Y3\(^{\prime}\)) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).

Cytorace 29: (♂ & ♀ 2n=6)

Cytorace 29 is the product of the hybridization between the females of Kenya strain of *D. n. nasuta* (2n=8) and the males of Japan strain of *D. n. albomicans* (2n=6). By F\(_{1}\), karyotypically a stable hybrid population has evolved. The karyotypic configuration of this stable hybrid population had the following features (Fig-3d):

Females: (2n=6) A pair of metacentric representing chromosome 2 (2*, 2° or 2\(^{\prime}\), 2\(^{\prime}\) or 2*, 2\(^{\prime}\)), another pair of metacentric representing chromosome X3 (X3\(^{*}\), X3\(^{\prime}\)) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).

Males: (2n=6) A pair of metacentric representing chromosome 2, another pair of metacentric representing chromosome X3 (X3\(^{*}\)), and Y3 (Y3\(^{\prime}\)) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).

Cytorace 30: (♂ 2n=7 & ♀ 2n=6)

Cytorace 30 is the product of the hybridization between the males of Kenya strain of *D. n. nasuta* (2n=8) and the females of Japan strain of *D. n. albomicans* (2n=6). The karyotypic variability present in the hybrid population has gradually disappeared and by F\(_{10}\) karyotypically a stable hybrid population has evolved. The cytotypes of females and males of this race consists of following elements (Fig-3e):

Females: (2n=6) A pair of metacentric representing chromosome 2 (2*, 2° or 2\(^{\prime}\), 2\(^{\prime}\) or 2*, 2\(^{\prime}\)), another pair of metacentric representing chromosome X3 (X3\(^{*}\), X3\(^{\prime}\)) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).

Males: (2n=7) A pair of metacentric representing chromosome 2 (2*, 2° or 2\(^{\prime}\), 2\(^{\prime}\) or 2*, 2\(^{\prime}\)), one acrocentric chromosome 3 (3\(^{\prime}\)), one metacentric chromosome X3 (X3\(^{\prime}\)), and one submetacentric Y-chromosome (Y*) and a pair of short dots, representing chromosome 4 (4*, 4\(^{\prime}\)).
Figs 3 a-c: Diagrammatic representation of chromosomal complements of the parental races, as well as of the newly evolved Cytoraces of different hybridization experiments. 3A: Chromosomes of *D. nasuta* female (Seychelles), *D. albomicans* male (Thailand) and of the males and females of Cytorace 26. 3B: Chromosomes of *D. nasuta* male (Seychelles), *D. albomicans* female (Japan) and of the males and females of Cytorace 27. 3C: Chromosomes of *D. nasuta* female (Kenya), *D. albomicans* male (Thailand) and of the males and females of Cytorace 28.
**Fig-3**

- **D. nasuta ♀ (Kenya)**
  - Female 2n = 8
  - Male 2n = 6

- **D. albomicans ♂ (Japan)**
  - Female 2n = 8
  - Male 2n = 6

**Figures d-e:**
- **3D:** Chromosomes of *D. nasuta* female (Kenya), *D. albomicans* male (Japan) and of the males and females of Cytarace 29.
- **3l:** Chromosomes of *D. nasuta* male (Kenya), *D. albomicans* female (Japan) and of the males and females of Cytarace 30.
TABLE 2 (A-E): The number of parental and hybrid combinations for the sex chromosome and chromosome 3 in different hybrid generations of the five Cytoraces (C26-C30)

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(TABLE 2a)

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(TABLE 2b)

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(TABLE 2c)
**Sex chromosome and chromosome 3 Cytorace: 29 Parents: D. n. n (K) ♀ X D. n. a (J) ♂**

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<td>F25</td>
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(TABLE 2d)

**Sex chromosome and chromosome 3 Cytorace: 30 Parents: D. n. n (K) ♂ X D. n. a (J) ♀**

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<td>F30</td>
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(TABLE 2e)

**Note:**  
D. n. n = D. n. nasuta; D. n. a = D. n. albomicans; K=Kenya; S=Seychelles; J=Japan; T=Thailand
TABLE 3 (a-c): The number of parental and hybrid combinations for the chromosome 4 in different hybrid generations of the five Cytoraces (C26-C30).

**Table 3a**

<table>
<thead>
<tr>
<th>Chromosome 4</th>
<th>Cytorace: 26</th>
<th>Parents: D. n. n (S) ? X D. n. a (T)♂</th>
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**Table 3b**

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<thead>
<tr>
<th>Chromosome 4</th>
<th>Cytorace: 27</th>
<th>Parents: D. n. n (S)♂ X D. n. a (J)♀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generations</td>
<td>Frequency</td>
</tr>
<tr>
<td>F₁</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>F₂</td>
<td>29</td>
<td>36</td>
</tr>
<tr>
<td>F₃</td>
<td>8</td>
<td>36</td>
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<tr>
<td>F₁₀</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>F₁₅</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F₂₀</td>
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</tr>
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</table>

**Table 3c**

<table>
<thead>
<tr>
<th>Chromosome 4</th>
<th>Cytorace: 28</th>
<th>Parents: D. n. n (K)♀ X D. n. a (T)♂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generations</td>
<td>Frequency</td>
</tr>
<tr>
<td>F₁</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>F₂</td>
<td>11</td>
<td>52</td>
</tr>
<tr>
<td>F₃</td>
<td>28</td>
<td>42</td>
</tr>
<tr>
<td>F₁₀</td>
<td>58</td>
<td>32</td>
</tr>
<tr>
<td>F₁₅</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F₂₀</td>
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<td>0</td>
</tr>
</tbody>
</table>
(TABLE 3d)

<table>
<thead>
<tr>
<th>Chromosome 4</th>
<th>Cytorace: 29</th>
<th>Parents: D. n. n (K) ♀ X D. n. a (J) ♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Generations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₁</td>
<td>F₂</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>20</td>
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<td>100</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Note: D. n. n = D. n. nasuta; D. n. a = D. n. albomicans; K = Kenya; S = Seychelles; J = Japan; T = Thailand; •• = D. n. nasuta dot chromosomes; | = D. n. albomicans (Japan) dot chromosomes; || = D. n. albomicans (Thailand) dot chromosomes)

(TABLE 3e)

<table>
<thead>
<tr>
<th>Chromosome 4</th>
<th>Cytorace: 30</th>
<th>Parents: D. n. n (K) ♂ X D. n. a (J) ♀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Generations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₁</td>
<td>F₂</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>34</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: D. n. n = D. n. nasuta; D. n. a = D. n. albomicans; K = Kenya; S = Seychelles; J = Japan; T = Thailand; •• = D. n. nasuta dot chromosomes; | = D. n. albomicans (Japan) dot chromosomes; || = D. n. albomicans (Thailand) dot chromosomes
EVOIUTION OF NEW KARYOTYPIC RACES

<table>
<thead>
<tr>
<th>Parents</th>
<th>Cytoraces</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D. albomicans (T) ♀ X D. nasuta (S) ♂</strong> (2n=6)</td>
<td>Cytorace 26</td>
</tr>
<tr>
<td></td>
<td>σ=(2n=6) 2a 2b, X3-Y3: 4a-4g</td>
</tr>
<tr>
<td></td>
<td>9=(2n=6) 2a 2b, X3-X3: 4a-4g</td>
</tr>
<tr>
<td><strong>D. nasuta (S) ♀ X D. albomicans (J) ♂</strong> (2n=8)</td>
<td>Cytorace 27</td>
</tr>
<tr>
<td></td>
<td>σ=(2n=7) 2a 2b, X3-Y3: 3a, 4a-4g</td>
</tr>
<tr>
<td></td>
<td>9=(2n=6) 2a 2b, X3-X3: 4a-4g</td>
</tr>
<tr>
<td><strong>D. albomicans (T) ♀ X D. nasuta (K) ♂</strong> (2n=6)</td>
<td>Cytorace 28</td>
</tr>
<tr>
<td></td>
<td>σ=(2n=6) 2a 2b, X3-Y3: 4a-4g</td>
</tr>
<tr>
<td></td>
<td>9=(2n=6) 2a 2b, X3-X3: 4a-4g</td>
</tr>
<tr>
<td><strong>D. albomicans (I) ♀ X D. nasuta (K) ♂</strong> (2n=6)</td>
<td>Cytorace 29</td>
</tr>
<tr>
<td></td>
<td>σ=(2n=6) 2a 2b, X3-Y3: 4a-4g</td>
</tr>
<tr>
<td></td>
<td>9=(2n=6) 2a 2b, X3-X3: 4a-4g</td>
</tr>
<tr>
<td><strong>D. nasuta (K) ♀ X D. albomicans (J) ♂</strong> (2n=6)</td>
<td>Cytorace 30</td>
</tr>
<tr>
<td></td>
<td>σ=(2n=7) 2a 2b, X3-Y3: 3a, 4a-4g</td>
</tr>
<tr>
<td></td>
<td>9=(2n=6) 2a 2b, X3-X3: 4a-4g</td>
</tr>
</tbody>
</table>

Note: Superscripts on each chromosome indicates the parent from which it has been inherited; α D. n. nasuta; α D. n. albomicans (T: Thailand; K: Kenya; J: Japan; and S: Seychelles)

The *nasuta* subgroup of the *immigrans* species group of *Drosophila* is an assemblage of morphologically almost similar forms but with different levels of karyotypic divergence. *D. n. nasuta* and *D. n. albomicans* of this assemblage are a pair of allopatric chromosomal races with 2n=8 and 2n=6 respectively. Ranganath and his coworkers have convincingly documented that the karyotype of *D. n. albomicans* is derived from *D. n. nasuta* by way of centric fusion between sex chromosomes and autosomes 3 (Ranganath and Hägele, 1981). Therefore, in *D. n. nasuta*, the sex chromosomes XX / XY and the autosome 3 exist as independent entities while in *D. n. albomicans*, these elements are held together in metacentric chromosomes called X3 and Y3 chromosomes. In spite of the karyotypic difference, *D. n. nasuta* and *D. n. albomicans* are cross fertile and hybrid progeny can be maintained for many generations. In the hybrids, the parental chromosomes, namely of *nasuta* and *albomicans* are distinguishable. Hence, one can use this model to explore the fate of parental chromosomes in the hybrid generations.

By using two geographic strains of *D. n. nasuta* and *D. n. albomicans* eight hybrid lines were initiated. Of these, in five hybrid lines, karyotypic stability was noticed and these karyotypically stabilized hybrid populations have been called Cytoraces (Cytorace 26 to 30). By taking cognizance of the chromosomes of these five new Cytoraces and of the parents of each of these Cytoraces the following discussion is presented:

J. CYTOL. GENET. VOL. 6 (NS), NO. 2, 31 DECEMBER 2005
Chromosome 2:

A pair of metacentric chromosomes 2 is seen in all the five stabilized Cytoraces (Cytorace 26 to 30). The F₁ males and females of reciprocal crosses had the chromosome 2 in hybrid combinations, that is one of D. n. nasuta and other was from D. n. albomicans parent (2ⁿ and 2'). In each of the Cytorace, three types of individuals are recorded: (1) homozygous for nasuta chromosomes 2 (2ⁿ 2ⁿ); (2) homozygous for albomicans chromosomes 2 (2ⁿ 2ⁿ) and (3) heterozygous for chromosomes 2 (2ⁿ 2ⁿ), suggesting the occurrence of balanced polymorphism and hence balancing selection on this component of the karyotype. Therefore it is inferred that there is no preference for any one type of combination. Such a co-association of chromosomes coming from different parents suggests lack of significant genetic differences between them.

Dot Chromosomes (Chromosome 4):

A pair of dot chromosomes is seen in the karyotypes of all the five Cytoraces. The long dot (Japan) and short dot (Thailand) chromosomes of D. n. albomicans and basic dot of D. n. nasuta (Kenya and Seychelles) are easily distinguished in the hybrid karyotypes. In the present study, all the F₁ hybrids of the crosses between D. n. nasuta and D. n. albomicans strains had one basic dot chromosome of D. n. nasuta (4ⁿ) and one long dot chromosome of D. n. albomicans (4') or small dot D. n. albomicans (4”). In the succeeding generations, for these dot chromosomes, the frequency of individuals with hybrid combination tend to decrease (4ⁿ 4ⁿ) while the incidence of one of the parental (4ⁿ 4ⁿ or 4ⁿ 4ⁿ) combinations increase.

In the present study, in none of the stabilized karyotypes of five Cytoraces (Cytorace 26 to 30), heterozygous condition for the dot chromosomes was recorded. Thus, this part of the karyotype is subjected to another type of selection namely directional selection. In all the lines only the D. n. albomicans dots have been retained. Therefore, the dot chromosomes of D. n. albomicans have selective advantage over the D. n. nasuta dot chromosomes. In view of this, the selection has favored the D. n. albomicans parental combination, over D. n. nasuta and hybrid association.

Sex chromosomes (X and Y), Chromosome 3 of D. n. nasuta and X3 and Y3 chromosomes of D. n. albomicans:

In D. n. nasuta, the sex chromosomes and the chromosome 3 exist as independent entities. In D. n. albomicans, these chromosomes are seen in metacentric X3 and Y3 chromosomes. These chromosomes are differentially represented in the karyotypes of the newly evolved five Cytoraces. One can compare the chromosomal complements of a Cytorace with its male and female parents particularly with reference to X, X or Y, Y and 3, 3 and X3, X3 or X3, Y3 chromosomes. A careful examination of these karyotypes of five Cytoraces reveals two types of trends. In Cytorace 27 and Cytorace 30 the karyotypes of females of these Cytoraces is same as that of their respective female parent (X3* X3*). The males of these Cytoraces have X3*, X*, Y* chromosomes while their respective male parent had X*, Y*, 3*, 3*. The second trend is seen in the evolution of the karyotype Cytorace 26, Cytorace 28 and Cytorace 29. The karyotypes of males of these Cytoraces are similar to their respective male parent (X3* Y3*). The females of these Cytoraces have X3* X3* chromosomes while their respective female parent had X*, X*, 3*, 3*. Therefore during course of evolution of
these five Cytoraces, the chromosomes, namely X, Y, and chromosome 3 of D. n. nasuta, as well as the X3 and Y3 chromosomes of D. n. albomicans have been subjected to two different patterns of segregation, namely (1) retention of the male parent karyotype with a change in the composition of the female karyotype, and (2) retention of the female parent karyotype with a change in the composition of male karyotype. Further it is possible to have three types of hybrid associations for these chromosomes, namely X3*Y"3* (male), Y3*X3* (male) and X3*X3* (Female). It was interesting to see that none of the stabilized hybrid lines have X3*X3* chromosome combination in females. Males have either F1 type (X3*, Y"3*) or D. n. albomicans (X3* Y3*) types. Females of all these five Cytoraces (Cytorace 26 to 30) feature D. n. albomicans pattern sex chromosome X3* X3*, while the males of Cytorace 26, Cytorace 28 and Cytorace 29 have albomicans type (X3* Y3*) and Cytorace 27 and Cytorace 30 were of hybrid type (X3* Y"3*). Therefore none of the new races have the D. n. nasuta pattern. Hence, we feel that these chromosomes are subjected different patterns of sex specific selection in different races.

By considering all the chromosomes, further analysis can be made as to the number of D. n. nasuta and D. n. albomicans chromosomes retained in the karyotypes of five Cytoraces. A total 62 chromosomes are seen in these newly evolved five Cytoraces. Of these, only 14 chromosomes are of D. n. nasuta while 48 chromosomes are of D. n. albomicans. This clearly indicates that the chromosomes of D. n. albomicans were more preferred than those of D. n. nasuta, particularly with reference to dot and sex chromosomes.

Karyotypic divergence due to differences in the diploid number and composition of the karyotype is a major distinct feature of these Cytoraces. Different strategies of selection namely, balancing selection, directional selection and sex specific selection have been documented. Similar strategies of selection and pattern of karyotypic evolution have been reported by earlier workers (Ramachandra and Ranganath, 1996 and Tanuja et al., 2003) during the evolution of other Cytoraces (Cytorace 1 to Cytorace 16). Thus, the karyotypic evolution among the members of the nasuta-albomicans complex has illustrated differential fate of parental chromosomes in the introgressed genomes as well as the interplay of different strategies of selection on the components of the evolving karyotype.

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REFERENCES


Mating propensity: an indicator of interracial divergence in the nasuta-albomicans complex of Drosophila.

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1Corresponding author (e-mail: drosrang@sancharnet.in).

Introduction

Interracial hybridization between Drosophila nasuta and Drosophila albomicans belonging to nasuta sub group of Drosophila immigrans species group has led to introgression of their genomes, with parental chromosomes being differentially represented in different hybrids (Ranganath, 1978; Ranganath, 2002; Tanuja et al., 2003). Over years, varied hybrid lines of D. n. nasuta and D. n. albomicans with stable karyotypic composition were recognized and subsequently named as Cytoraces. Cytoraces along with parental races are referred to as nasuta-albomicans complex (Ramachandra and Ranganath, 1996). This hybridogenetic complex constitutes allo-sympatric populations, which provides a ground for understanding of racial divergence in an artificial hybrid zone. Apart from karyotypic divergence (Tanuja et al., 1999a, b, 2003), differences have been reported for different parameters like morphophenotypic traits (Harini and Ramachandra, 1999a, b, 2000), fitness parameters (Ramachandra and Ranganath, 1988), differential mating preferences (Tanuja et al., 2001), and analyses of isozyme (Aruna and Ranganath, 2004) and have shed light on the genetic differences among the members of the nasuta-albomicans complex.

Mating propensity is a complex trait based on interaction of both sexes. The mating propensity is defined as the proportion of flies mated during an observation period (Koepfer, 1987). Studies on mating propensity in various species of Drosophila are well documented. It is widely recognized that deviation from random mating can be caused by two different biological factors, namely discrimination and mating propensity (Ringo, 1986). Differentiating between these two factors is of paramount importance for the evolutionary implication of these tests, since differences in mating propensity between mutants, strains, and wild populations occur very often (Spiess, 1970). Thus mating propensity was taken to measure sexual receptivity of females and mating ability in males among six races of the nasuta-albomicans complex.
Material and Methods

During the course of the present study, we tested the mating propensity in six races of the *nasuta-albomicans* complex of *Drosophila*, namely *D. n. nasuta*, *D. n. albomicans*, Cytorace 1, Cytorace 2, Cytorace 3, and Cytorace 4. All these flies were maintained at 22 ± 1°C under uniform conditions. For experimental purposes, virgin females and males were collected from synchronized cultures and aged for seven days. By using these flies, experiments were carried out by direct observation in an empty vial plugged with cotton, between 7-11 A.M. For each race (intra-racial crosses) five replicates were set up. In each replicate fifteen males and fifteen females were placed, and the number of matings was recorded for 60 min. When a pair commenced mating, it was aspirated out. [Intra crosses involve 6 crosses; these 6 crosses were grouped into homo-parental (2 sets of crosses) and homo-cytoraces (4 sets of crosses)]. Mating propensity was also recorded in mixed cultures (inter-racial crosses) of six races; this involves 30 crosses. For each cross, five replicates, each with fifteen males and fifteen females, were used. These 30 crosses are grouped into hetero-parental (2 sets of crosses), hetero-cytoraces (12 sets of crosses) and hetero-mixed (16 sets of crosses) (Tanuja et al., 2001). The mean values were subjected to one-way ANOVA and also diallel analysis were done by following procedure of Singh (1999), to measure sexual receptivity of females and male mating ability.

Results

During the course of the present study, we tested the mating propensity in six races of *nasuta-albomicans* complex. Of the six races, *D. albomicans* and Cytorace 2 have the highest (13.0 ± 0.7) and the lowest (8.0 ± 1.0) mean number of matings, respectively (Table 1). For testing variation in mean number of matings in different races, analysis of variance was performed, which indicates significant variations among six races (F = 5, 4.53, P = 0.005).

The results are subjected to another type of analysis. Based on mating type, the 36 crosses were grouped in to five groups (Tanuja et al., 2001). For testing variation in mean number of matings in five groups, analysis of variance was performed, which indicates, significant variations among five groups (F = 4, 7.75, P = 0.000) (Table 2). Further, among five groups, pair-wise comparisons showed hetero-cytoraces have the least mating propensity (8.6 ± 0.3), while homo-parentals have the highest mating propensity (11.9 ± 0.5).

Table 1. Mean numbers of matings along with standard error in 36 crosses of the *nasuta-albomicans* complex. Note: (N-D. *nasuta*, A- *D. albomicans*; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3; C4-Cytorace 4)

<table>
<thead>
<tr>
<th>Races of female parent</th>
<th>Races of male parent</th>
<th>NN</th>
<th>AA</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>10.8 ± 0.5</td>
<td>9.6 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>11.2 ± 0.4</td>
<td>10.6 ± 0.6</td>
<td>8.4 ± 0.4</td>
<td>57.4</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>13.8 ± 0.7</td>
<td>13.0 ± 0.7</td>
<td>12.6 ± 0.5</td>
<td>9.2 ± 0.3</td>
<td>12.4 ± 0.6</td>
<td>12.0 ± 0.6</td>
<td>73.0</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>10.0 ± 0.4</td>
<td>9.4 ± 0.5</td>
<td>11.6 ± 0.8</td>
<td>10.2 ± 0.2</td>
<td>5.6 ± 0.5</td>
<td>7.8 ± 0.4</td>
<td>54.6</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>10.6 ± 0.8</td>
<td>7.6 ± 0.6</td>
<td>8.8 ± 0.3</td>
<td>8.0 ± 1.0</td>
<td>3.0 ± 0.3</td>
<td>4.6 ± 0.4</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>9.6 ± 0.6</td>
<td>9.2 ± 0.5</td>
<td>11.2 ± 0.5</td>
<td>11.6 ± 0.2</td>
<td>10.0 ± 0.3</td>
<td>8.2 ± 0.3</td>
<td>59.8</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>8.0 ± 0.4</td>
<td>9.4 ± 0.6</td>
<td>11.8 ± 0.8</td>
<td>11.0 ± 0.5</td>
<td>9.6 ± 0.4</td>
<td>11.8 ± 1.1</td>
<td>61.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52.8</td>
<td>58.2</td>
<td>62.8</td>
<td>61.2</td>
<td>51.2</td>
<td>62.8</td>
<td>61.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 presents the results of diallel crosses, which were carried out to assess the relative sexual activity of males and females. Analysis of variance was carried out to measure the degree of variation in male mating ability and female receptivity in different races. ANOVA shows highly
Table 2. Mean values along with standard of mating propensity for the pooled data of 36 crosses based on different types of homo-and heterogamic matings among six races of the *nasuta-albomicans* complex, along with the summary of tukey’s test.

<table>
<thead>
<tr>
<th>Categories</th>
<th>Total pairs</th>
<th>Mating propensity (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogamic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homo-parental</td>
<td>150</td>
<td>11.9 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homo-Cytorace</td>
<td>300</td>
<td>10.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heterogamic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hetero-parental</td>
<td>150</td>
<td>11.7 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hetero-Cytorace</td>
<td>900</td>
<td>8.6 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hetero-mixed</td>
<td>1200</td>
<td>9.8 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-value, df</td>
<td>7.138*, 4</td>
<td></td>
</tr>
</tbody>
</table>

Following pair-wise comparisons showed significant different at 5% level
Mating propensity: d/c, d/a

Discussion

During the course of the present investigation, 6 races of *nasuta-albomicans* complex were tested for mating propensity. There is significant variation in mean number of matings among the races tested, which is attributable to genetic heterogeneity among the races resulting from hybridization and genetic drift during laboratory rearing. To assess the relative sexual activity of the two sexes, among six races, diallel crosses were also made. Based on the analysis of data of diallel crosses by ANOVA and pair-wise comparisons, it was demonstrated that the contribution of females is greater to the observed variation.

It is known that male activity and female receptivity are the main factors responsible for successful mating in *Drosophila*. Intra-specific variation in mating activity has been reported in *D. pseudoobscura*, *D. ananassae*, and *D. montana*. The dependence of successful mating on a particular sex varies between species and within species between genotypes, such that males may often be more important if mating is so rapid, while if mating is slow, females play a progressively more important role. In *D. pseudoobscura*, mating is so rapid that variation in female receptivity may be relatively unimportant (Parsons, 1973). Kesseler (1968) has shown that the contribution of females to the total variance of mating propensity was greater than males in *D. pseudoobscura*; Singh (1999) has shown greater variation in female receptivity than males in few strains of *D. ananassae*; and Suvanto et al. (2000) have shown that mating propensity was influenced more by the females than males in a few strains of *D. montana*. In *D. persimilis* it has been found that females are critical over a one hour period, because of an interaction between copulation and avoidance tendencies. Thus, the results concerning the contribution of a particular sex to variation in sexual activity as well as dependence of mating success on a particular sex may vary within a species depending upon the genetic constitution. In different species it has been demonstrated that sexual activity of males and female receptivity have genetic basis.

Table 2. Mean values along with standard of mating propensity for the pooled data of 36 crosses based on different types of homo-and heterogamic matings among six races of the *nasuta-albomicans* complex, along with the summary of tukey’s test.

significant differences in sexual activity of both sexes. However, variation is greater for females (F = 5, 17.95, P = 0.000) than in males (F = 5, 3.528, P = 0.005). Thus, females of newly evolved races of *nasuta-albomicans* complex contribute more variation in sexual receptivity than males.

By using the data [marginal total: A method generally used to estimate mating propensity (Anderson and Ehrman, (1969)] of diallel analysis, 15 pair-wise comparisons between races have been made to test the differences between male mating ability and female receptivity in different races. These pair-wise comparisons are presented in Table 3, which shows the range of variations and differences for male sexual activity and female receptivity. Out of 15 comparisons, 10 showed greater variation in female receptivity than in male sexual activity. Only in 4 comparisons was there greater variation in male mating ability than in female receptivity. However, differences in male mating activity and female receptivity are nearly identical only in one comparison.

Discussion

During the course of the present investigation, 6 races of *nasuta-albomicans* complex were tested for mating propensity. There is significant variation in mean number of matings among the races tested, which is attributable to genetic heterogeneity among the races resulting from hybridization and genetic drift during laboratory rearing. To assess the relative sexual activity of the two sexes, among six races, diallel crosses were also made. Based on the analysis of data of diallel crosses by ANOVA and pair-wise comparisons, it was demonstrated that the contribution of females is greater to the observed variation.

It is known that male activity and female receptivity are the main factors responsible for successful mating in *Drosophila*. Intra-specific variation in mating activity has been reported in *D. pseudoobscura*, *D. ananassae*, and *D. montana*. The dependence of successful mating on a particular sex varies between species and within species between genotypes, such that males may often be more important if mating is so rapid, while if mating is slow, females play a progressively more important role. In *D. pseudoobscura*, mating is so rapid that variation in female receptivity may be relatively unimportant (Parsons, 1973). Kesseler (1968) has shown that the contribution of females to the total variance of mating propensity was greater than males in *D. pseudoobscura*; Singh (1999) has shown greater variation in female receptivity than males in few strains of *D. ananassae*; and Suvanto et al. (2000) have shown that mating propensity was influenced more by the females than males in a few strains of *D. montana*. In *D. persimilis* it has been found that females are critical over a one hour period, because of an interaction between copulation and avoidance tendencies. Thus, the results concerning the contribution of a particular sex to variation in sexual activity as well as dependence of mating success on a particular sex may vary within a species depending upon the genetic constitution. In different species it has been demonstrated that sexual activity of males and female receptivity have genetic basis.
Table 3. Pairwise comparisons to test the differences between male activity and female receptivity based on the marginal total of mean number of matings in diallel crosses.

<table>
<thead>
<tr>
<th>Pairs of races</th>
<th>Range of variation</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>N V/s A</td>
<td>Male activity</td>
<td>62.8-58.2</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>57.4-73</td>
</tr>
<tr>
<td>N V/s C1</td>
<td>Male activity</td>
<td>62.8-62.8</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>57.4-54.6</td>
</tr>
<tr>
<td>N V/s C2</td>
<td>Male activity</td>
<td>62.8-61.2</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>57.4-42.6</td>
</tr>
<tr>
<td>N V/s C3</td>
<td>Male activity</td>
<td>62.8-51.2</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>57.4-59.8</td>
</tr>
<tr>
<td>N V/s C4</td>
<td>Male activity</td>
<td>62.8-52.6</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>57.4-61.4</td>
</tr>
<tr>
<td>A V/s C1</td>
<td>Male activity</td>
<td>58.2-62.8</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>73-54.6</td>
</tr>
<tr>
<td>A V/s C2</td>
<td>Male activity</td>
<td>58.2-61.2</td>
</tr>
<tr>
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<tr>
<td>A V/s C3</td>
<td>Male activity</td>
<td>58.2-51.2</td>
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<tr>
<td></td>
<td>Female receptivity</td>
<td>73-59.8</td>
</tr>
<tr>
<td>A V/s C4</td>
<td>Male activity</td>
<td>58.2-52.6</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>73-61.4</td>
</tr>
<tr>
<td>C1 V/s C2</td>
<td>Male activity</td>
<td>62.8-61.2</td>
</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>54.6-42.6</td>
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<td>C1 V/s C3</td>
<td>Male activity</td>
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</tr>
<tr>
<td></td>
<td>Female receptivity</td>
<td>59.8-61.4</td>
</tr>
</tbody>
</table>

Note: (N-D. nasuta; A-D. albomicans; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3; C4-Cytorace 4)

The ‘Sexual selection’ model predicts that the females could be selective about their mates (Anderson, 1994; Bateman, 1948), since females have a high proportion of their total reproductive effort at stake and so should avoid unfit matings with conspecifics and heterospecifics alike. Thus, one would predict that female would show a stronger conspecific mating preference than males. Clearly the prediction of greater female choosiness under sexual selection model supports our data, where female receptivity had played the main role than male mating ability in mating propensity analysis.

The present study has revealed the existence of genetic variability for the trait mating propensity among six races and greater divergence among females receptivity, which plays a predominant role during mating. Thus, the analysis of mating propensity has shown yet another facet of inter-racial divergence among the closely related members of the nasuta-albomicans complex of Drosophila.

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To Prof. H.A. Ranganath  
Vice Chancellor (Bangalore University)  
Department of Studies in Zoology  
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Dear Prof. Ranganath,

Your paper entitled “Dissection of courtship... Drosophila” Index No. B/2 5.2.2006 has been accepted for Vol. 76 part IV, 2006 of the Proceedings of the National Academy of Sciences of India.

With regards

Yours sincerely

(Niraj Kumar)
Dissection of courtship elements in a few members of the *nasuta-albomicans* complex of *Drosophila*

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**Short running title:** Courtship behaviour elements in *Drosophila*.

**Keywords:** *D. n. nasuta*, *D. n. albomicans*, Hybridization, Cytoraces,  

*nasuta-albomicans* complex, Courtship behaviour.
Dissection of courtship behaviour elements in a few members of the
nasuta-albomicans complex of Drosophila

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Abstract:

The nasuta-albomicans complex is an assemblage of allo-sympatric races consisting of \textit{D. n. nasuta}, \textit{D. n. albomicans} and Cytoraces. Cytoraces are the products of interracial hybridization between \textit{D. n. nasuta} and \textit{D. n. albomicans} in the environs of laboratory. In each of these Cytoraces parental genomes are differentially represented. In the present study by taking six races of the nasuta-albomicans complex, courtship behaviour studies have been made. The detailed qualitative and quantitative analysis of courtship behaviour elements has given us insights into the symptoms of behavioural divergence marking the very early stages of raciation.

Keywords: \textit{D. n. nasuta}, \textit{D. n. albomicans}, Hybridization, Cytoraces, nasuta-albomicans complex, Courtship behaviour.

Introduction:

The nasuta subgroup of the immigrans species group of \textit{Drosophila} with members distributed in different parts of south east Asia, is an excellent model for evolutionary analysis\textsuperscript{1,5}. Females of different members are almost identical, while males exhibit differences as to the pollinosity in the head region\textsuperscript{6}. \textit{D. n. nasuta}, \textit{D. n. albomicans} \textit{D. n. kepulauana}, \textit{D. kohkoa} and \textit{D. nevifrons} forms the frontal sheen complex; \textit{D. s. sulfurigaster}, \textit{D. s. bilimbata}, \textit{D. s. albostrigata}, \textit{D. s. neonasuta} and \textit{D.
pulaua constitute orbital sheen complex; while, *D. pallidifrons*, taxon *F, J, and I* with no pollinosity is the other complex\(^7\)-\(^8\). During the last four decades, the evolutionary biology of this assemblage has been analyzed by many investigators. Of these members, *D. n. nasuta* and *D. n. albomicans* of the frontal sheen complex are allopatric, cross fertile races with \(2n=8\) and \(2n=6\) respectively\(^9\)-\(^10\). The hybrid progeny of *D. n. nasuta* and *D. n. albomicans* maintained under laboratory conditions resulted in the evolution of karyotypically stable hybrid lineages in which parental chromosomes were differentially represented in different hybrid lineages. Such hybrid lineages have been called Cytoraces\(^11\)-\(^13\). Over the years, 16 Cytoraces were established by Ranganath and his coworkers. *D. n. nasuta*, *D. n. albomicans* and Cytoraces were organized in to a new assemblage called *nasuta-albomicans* complex\(^8\), \(^11\), \(^12\), \(^13\), \(^14\). Of these, *D. n. nasuta* and *D. n. albomicans* are the products of evolution in nature while Cytoraces are the products of hybridization in laboratory. These cytogenetically closely related members of the *nasuta-albomicans* complex maintained in different cages under normal laboratory environment are referred to as allo-sympatric races\(^14\). These races have been studied for different parameters such as karyotype\(^11\)-\(^13\), fitness\(^15\), mating preference\(^16\), \(^17\), morphometric traits\(^18\)-\(^20\) and isozyme variations\(^21\) and interracial divergence has been documented. Thus, *nasuta-albomicans* complex has proved to be a potential system to analyze the process and pattern of racial divergence under controlled conditions. In continuation of this, the present report targets to measure another important facet of divergence related to the courtship behaviour among six members of the *nasuta-albomicans* complex of *Drosophila*.
**Materials and Methods:**

(a) Stocks:

In the present study, courtship behaviour elements was observed in *D. n. nasuta* (Coorg), *D. n. albomicans* (Okinawa) and Cytorace 1, Cytorace 2, Cytorace 3 and Cytorace 4\[^{11,12}\]. The karyotypic composition of these members is diagrammatically represented in fig-1.

(b) Behavioural procedures:

For courtship behavioural observations, 7-day-old virgin females and unmated males were used. These experiments were performed at 22 ± 1°C, and for each race observations were made from fifty replicates (300 crosses).

The behaviour of each pair was observed in Perspex experimental chamber. The courtship behaviour elements were recorded from the moment of introduction of male and female into the chamber till the initiation of copulation. The mating activities of these flies were recorded between 7 to 11 AM. The experimental procedure of Tanuja et al.,\[^{16,17}\] and the terminology of courtship behaviour elements of Spieth\[^{22}\], Cobb\[^{23}\] and Hoikkala\[^{24}\] were adopted. The description of each courtship element is as follows:

**MALE COURTSHIP ELEMENTS:**

**Approach:**

1. **POSTERIOR APPROACH:**
   - The male stands behind the female, with his head under wing.

2. **TRANSVERSE APPROACH:**
   - The male stands diagonally to the female.

3. **ANTERIOR APPROACH:**
   - The male stands in front just opposite to the female head.
4. **Tapping:**

The male initiate courtship with foreleg motion. The male partially extends one foreleg and then strikes downwards, thus bringing the ventral surfaces of the tarsus in contact with the female.

**Circling:**

4. **ANTERIOR CIRCLING:**

The male move in front just opposite to the female head i.e., in semicircle arc (180°)

5. **POSTERIOR CIRCLING:**

The male move behind the female with head behind the wing in semicircle arc (180°)

6. **LEFT CIRCLING:**

The male move in a semicircle fashion on the left side of the courting female

7. **RIGHT CIRCLING:**

The male moves in a semicircle fashion on right side of the courting female.

8. **FULL CIRCLING:**

The male moves completely around courting female in an arc of 360°.

**Wing actions:**

9. **WING EXTENSION:**

The courting male engages in special wing posturing movements as he circles. Wing is extended extensively in front of the female.

10. **WING FLUTTERING:**

The wings are slightly elevated, separated from contact with each other and then slightly moved laterally and vibrated rapidly.

11. **WING FLICKING:**

In flicking, the wing vane is perpendicular to the substratum with the posterior edge of the wing directed downwards.

12. **WING SCISSORING:**

Courting male some times open and close both the wings in a scissor like movements.

13. **WING WAVING:**

Courting male extends both the wing laterally in 90°.
15. ATTEMPT TO COPULATE: The male forcibly lifts the female wing and curls his abdomen and then tries to mount the female.

FEMALE COURTSHIP ELEMENTS:

1. DECAMPING: Non receptive female often attempts to escape the male overtures by running, jumping, flying or walking away from the immediate vicinity of the male.

2. KICKING: Non-receptive female often attempts to escape the male overtures by kicking the courting male with her leg.

3. IGNORE: Non-receptive female some times when courted, simply keeps on whatever activity she has been engaged in and apparently ignoring male actions.

4. WING FLUTTERING: The wings are slightly elevated separated from the contact with each and then slightly moved laterally and vibrated rapidly.

5. FEMALE WING FLICKING: In flicking, the wing vane is perpendicular to the substratum with the posterior edge of the wing directed downwards.

6. WING SPREADING: Female spreads wing outward and upward and holds them extended until male mounts.

COURTSHIP ELEMENTS DUE TO BOTH SEXES:

1. COURTSHIP LATENCY: The time elapsing from introduction of male and female in to mating chamber till male approaches the female.

2. COURTSHIP DURATION: The time elapsed from approach till initiation of copulation (Irrespective of break in courtship).
3. COPULATION DURATION: The time from the initiation to the termination of copulation.

The courtship acts include a number of postures as listed above. When a male encounters the female, he approaches her from side (transverse approach), front (anterior approach) and back (posterior approach) and enter the phase of courtship rituals. Often at the start of the courtship, the male taps the female with his front legs. While approaching the female, the male may make wing waving, wing flicking, wing fluttering and wing scissoring or with out doing these wing actions it directly circles with the extension of the wings (wing postures extensively in front of the female) and attempts to mount. The sequence may break down at any stage. The female can influence the behaviour of the courting male by her responses. Decamp, ignore, kicking, wing fluttering and wing flicking shows her disinterest towards courting male while when she is receptive and ready to mate she spreads her wings as a sign of acceptance. Among the courting pairs, if the courtship breaks, the male may immediately start the courtship rituals or it takes time to initiate the courtship again. During this time, when it is away from the female, some time wing fluttering and wing flicking were noticed. And even in female, female wing fluttering and female wing flicking were also seen.

(d) Statistical analysis:

The data is analyzed by one-way ANOVA followed by Tukey’s test. The frequency of each courtship element was determined and was subjected to Z-test, to determine the level of significance. Based on frequency distribution of each courtship element and frequency of each courtship act, genetic Identity [Ixy] and Distance [Dxy] was
determined following Nei’s formula. Dendrograms were constructed using UPGMA, by means SPSS 10.0 software.

**Results:**

Based on the pattern of occurrence (presence or absence), the 24 behavioural elements recorded in six races under study, can be categorized as “common elements” for those, which have been present in all races and “shared elements” for those which were detected in at least two races (Table-1). Of the six races, Cytorace 1 and Cytorace 4 are the unique races with all the 24 elements while *D. n. nasuta*, Cytorace 2, and Cytorace 3 had 23 elements. On the other hand, *D. n. albomicans* revealed 21 elements. Of the 24, 20 elements come under the “common category” while only 4 elements namely, anterior approach, wing waving, female wing fluttering, and female wing flicking belongs to the “shared category”. The female wing fluttering, female wing flicking and male wing waving were absent in *D. n. albomicans*, while in *D. n. nasuta* male wing waving was not recorded. The male anterior approach was not seen in Cytorace 2 and Cytorace 3. The element namely wing waving, which was not expressed in parental races namely *D. n. nasuta* and *D. n. albomicans* but was seen in all the four Cytoraces. This novel courtship element ‘wing waving’ is unique to Cytoraces (Table-1).

The frequencies in percent for each of the elements are compiled in table-1, which indicate differential occurrence of the same elements in different races under investigation. For instance ninety four percent of individuals of Cytorace 1 showed the feature posterior approach of males while in other races such as *D. n. albomicans* and Cytorace 3, it was recorded in eighty and eighty four percent respectively. Similarly, for the character wing scissoring, the range was 78% (Cytorace 1) to 26% (*D. n. albomicans*)
and for the feature female wing flicking it was from 34% (Cytorace 1) to 8% (*D. n. nasuta*). Based on these values by applying Nei’s formula, genetic identity and genetic distance were calculated for these six races. The maximum distance of 0.0290 was recorded between *D. n. albomicans* and Cytorace 1, while the least distance of 0.0045 was noticed between *D. n. nasuta* and Cytorace 4 (Table-2). And the same was used to construct the dendrogram (Fig-2). Two clusters are recognized of which Cytorace 1 form an independent cluster, while other cluster *D. n. nasuta*, Cytorace 4, Cytorace 2 and Cytorace 3 constitute one clade, while *D. n. albomicans* forms an independent clade.

In the next step of analysis, for quantitative comparisons, the mean value for each of the twenty four elements for all six races were calculated and same is presented in tables-3a to 3d along with the summary of ANOVA. Of the 24 elements, only 15 elements showed significant differences between races. A wide range of variability was noticed for some of the elements. For instance, the males of Cytorace 1 had a mean of 147.6±27.5 while those of *D. n. albomicans* had 50.3 ± 7.1 for the character ‘wing extension’. Similarly, the females of Cytorace 1 have the maximum mean of 4.9±0.9 while those of *D. n. nasuta* had the least mean of 1.4 ± 0.4 for the element kicking. The maximum courtship duration of 53.4 ± 6.6 min was seen in Cytorace 4 while the least of 25.1 ± 4.6 min was recorded for *D. n. albomicans*.

To understand the pattern of interracial divergence for each behaviour parameter among the races under study, the mean values were subjected to Tukey’s test, which gave five patterns of groupings. The races within a cluster or within overlapping segment of two or more clusters have insignificant differences, while races placed in two distinct clusters have significant differences. Within a cluster, the names of the races are arranged
from left to right in an ascending order of the values of these elements. ('A' represents
*D. n. albomicans*; N = *D. n. nasuta*; C1 = Cytorace 1; C2 = Cytorace 2; C3 = Cytorace 3;
& C4 = Cytorace 4)

Pattern 1: For nine elements namely, posterior approach, anterior approach, posterior
circle, left circle, full circle, wing fluttering, wing flicking, decamp, and courtship latency
six races form a single cluster.

<table>
<thead>
<tr>
<th>Pattern 1</th>
<th>Pattern 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A C3 C2 C4 C1 N</td>
<td>A C1 C2 C4 C3 N</td>
</tr>
<tr>
<td>Posterior approach</td>
<td>Anterior approach</td>
</tr>
<tr>
<td>CC2 A C1 C4 C3 N</td>
<td>N C4 A C4 C3 C1</td>
</tr>
<tr>
<td>Left circle</td>
<td>Full circle</td>
</tr>
<tr>
<td>A C3 C1 C2 N C4</td>
<td>C1 C3 C2 A N C4</td>
</tr>
<tr>
<td>Wing flicking</td>
<td>Decamp</td>
</tr>
<tr>
<td>A C1 C2 C4 C3 N</td>
<td>C1 C4 C3 C2 N A</td>
</tr>
<tr>
<td>Posterior circle</td>
<td>Courtship latency</td>
</tr>
</tbody>
</table>

Pattern 2: Two overlapping clusters were organized for the seven elements namely,
transverse approach, tapping, right circle, attempt, ignore, wing spread and courtship
duration.
Courtship duration

Pattern 3: Three overlapping clusters were organized for the element, female wing-flicking.

Female wing flicking

Pattern 4: For the six behaviour elements namely, anterior circle, wing extension, wing scissor, wing waving, female wing fluttering and kicking gave two independent clusters. Out of six elements for 5, the Cytorace 1 had significantly more value than the rest of the races.
Pattern 5: Three clusters were formed, for the copulation duration element, of which, two clusters overlap with each other, while other form an independent cluster. The race belonging to independent cluster has significant differences with the races of two overlapping clusters.

Copulation duration

Quantitatively, to understand the pattern of interracial divergence, overall mean frequency of courtship behaviour acts of each race was taken into consideration. Based on these mean values genetic identity and genetic distance were calculated among these six races by applying Nie’s formula. The maximum distance of 0.0970 was between D. n. albomicans and Cytorace 1, while the least distance of 0.0020 was noticed between Cytorace 2 and Cytorace 3 (Table-4). And the same was used to construct the dendrogram (Fig-3). Two clusters are recognized of which Cytorace 1 form an independent cluster, while in other cluster D. n. nasuta, Cytorace 4, Cytorace 2 and Cytorace 3 form one clade, while D. n. albomicans forms an independent clade.

Discussion:

A allosympatric members of the nasuta-albomicans complex are at different levels of differentiatation and analysis of the anagentic changes in these has illuminated a few novel patterns. In the present analysis based on the pattern of occurrence, the courtship elements are classified as ‘common’ and ‘shared’. In the present investigation 24 elements could be seen, among which 20 were common, and remaining 4 elements were shared. For these 24 elements, inter-parental, parental v/s Cytoraces and inter-
Cytorace comparisons were made to reveal the pattern of divergence among these races under study.

Inter-parental comparisons:

*D. n. nasuta* has 23 elements while *D. n. albomicans* revealed 21 elements. Two elements of the *D. n. nasuta* were not recorded in *D. n. albomicans* while all the 21 elements of *D. n. albomicans* were represented in *D. n. nasuta*. 2 elements namely, female wing flicking and female wing fluttering was unique to *D. n. nasuta*, while none of elements were unique to *D. n. albomicans*. Among the 21 elements that were shared by both parents, only for 3 elements (right circle, wing fluttering, and wing scissoring) significant differences for frequency distribution was noticed (Z-test). In quantitative comparisons only 1 courtship element (right circle) showed significant differences (Tukey’s test).

Parental v/s Cytoraces comparisons:

Cytorace 1 and Cytorace 4 possess all the 24 elements, and share 23 elements with *D. n. nasuta* and 21 elements with *D. n. albomicans*, while Cytorace 2 and Cytorace 3 revealed 23 elements and share 22 elements with *D. n. nasuta*, and 20 elements with *D. n. albomicans*. Further, pair wise comparisons were made for frequency distribution for both qualitative and quantitative values. Such a study revealed that among 23 and 21 elements that Cytorace 1 shares with each of its parents, only 7 elements have shown significant differences. The 7 elements which significantly differed from *D. n. nasuta* are full circle, wing flicking, wing scissoring, attempt, ignore, kicking and female wing flicking, while 7 elements which showed significant differences with *D. n. albomicans* are posterior approach, full circle, wing fluttering, wing flicking, wing scissoring, ignore and kicking. Quantitative comparisons revealed 6 (anterior circle, wing extension, wing
scissoring, kicking, female wing flicking and copulation duration) and 7 (transverse approach, tapping, anterior circle, wing extension, wing scissoring, kicking, and copulation duration) elements to vary significantly with each of its parents. Cytorace 2 shares 22 elements with *D. n. nasuta* and 20 elements with *D. n. albomicans*, of these, 4 (right circle, wing flicking kicking and female wing flicking) and 3 (full circle, wing flicking and wing fluttering) elements showed significant differences for frequency distribution. Quantitatively, none of the 22 elements gave significant differences with *D. n. nasuta*, while only one (wing spread) element gave significant differences with *D. n. albomicans*. 4 (Ignore, kicking, female wing fluttering and female wing flicking) of the 22 elements that Cytorace 3 shares with *D. n. nasuta* showed significantly different values for frequency distribution, while 5 (right circle, wing fluttering, wing flicking, ignore and decamp) of the 20 elements, which it shares with *D. n. albomicans* were significantly different. Quantitatively 2 elements namely, female wing fluttering and female wing flicking varied significantly from those of *D. n. nasuta*, while none of the elements significantly differed from those of *D. n. albomicans*. Similarly, one (wing flicking) of the 23 elements that Cytorace 4 shares with *D. n. nasuta*, while 4 (left circle, right circle, wing fluttering and wing flicking) of the 21 elements shares with *D. n. albomicans* were showed significant values for frequency distribution. In quantitative comparisons none of the 23 elements was significantly different from *D. n. nasuta*, while only 1 element (courtship duration) had significant values with *D. n. albomicans*. The most important unique observation in the occurrence of wing waving only in the hybrid genomes of Cytorace 1 and Cytorace 4, but not in either of the parents namely *D. n. nasuta* and *D. n. albomicans*
Inter-Cytorace comparisons:

Cytorace 1 shares all 24 elements with Cytorace 4, while 23 with Cytorace 3 and Cytorace 2. Of these, 6 (left circle, full circle, wing scissor, ignore, kicking and female wing flicking), 5 (right circle, full circle, wing flicking, wing scissoring and kicking) 6 (full circle, wing scissor, attempt, ignore wing waving and female wing flicking) elements, of Cytorace 1 differed significantly from those of Cytorace 4, Cytorace 3 and Cytorace 2 respectively. While quantitatively 8 (attempt, ignore, anterior circle, wing extension, wing scissoring, wing waving, kicking and copulation duration), 7 (anterior circle wing extension, wing scissoring, wing waving, female wing fluttering, kicking and copulation duration), 6 (anterior circle, wing extension, wing scissoring, wing waving, kicking and copulation duration) elements gave significant values with Cytorace 4, Cytorace 3 and Cytorace 2 respectively. Similarly, Cytorace 2 shares 23 elements with both Cytorace 3 and Cytorace 4, among which 3 elements gave significant values for frequency distribution in pair wise comparisons with both races (Right circle, full circle and female fluttering with Cytorace 3, while left circle, right circle and kicking with Cytorace 4). Quantitative comparison revealed only 1 (female wing fluttering) element with Cytorace 3 and 2 elements (wingspread and copulation duration) with Cytorace 4 showed significantly different values. Cytorace 3 and Cytorace 4 share 23 elements among which 2 (Ignore and female wing fluttering) elements gave significant values for frequency distribution, while 3 elements namely courtship duration, female wing fluttering and female wing flicking showed significantly quantitative differences.

One of the events that is expected to happen during interracial divergence is the gradual acquisition of reproductive isolation by pre or post-zygotic means resulting in the
emergence of biologically valid species between which hybridization is no longer possible. In natural populations it is extremely difficult or almost impossible to trace the events right from the initial stages as well as to follow it through successive stages. In this regard, the *nasuta-albomicans* complex is an excellent laboratory model to undertake such investigations. Tanuja et al.\textsuperscript{16, 17} conducted male choice, female choice, multiple choice and no choice experiments among a few members of the *nasuta-albomicans* complex and reported that even though mating occurs between different races, there was significantly more of homogamic mating than heterogamic matings. In continuation of these, qualitative and quantitative divergence for a few elements of courtship behaviour is recorded in the study which represents facet of pre-zygotic/pre-mating isolation.

In the present dendrograms, based on differences (Fig-2 and 3) of courtship elements (qualitative and quantitative), clustering of three Cytoraces (Cytorace 2, Cytorace 3, Cytorace 4) along with *D. n. nasuta* (Parental race) indicates lack of divergence of these races with *D. n. nasuta* parent. Association between Cytorace 4 and *D. n. nasuta* supports the karyotype data\textsuperscript{11, 12} while clustering of Cytorace 2 with Cytorace 3 does not support the karyotypic data; however it supports the findings on morphometric traits and isozyme analysis \textsuperscript{18-21}. Clustering of Cytorace 3 with Cytorace 4 is found to be closer when the parameters like karyotype, fitness, mating preference and isozyme variations are taken consideration\textsuperscript{11, 12, 18-21}. With respect to mating latency and incidence of lack of matings Cytorace 2 deviates from *D. n. albomicans*, which is in accordance with the present findings where in Cytorace 2 is represented in a different clade while *D. n. albomicans* in another\textsuperscript{16, 17}. In the present study, Cytorace 1 forms an independent unit, which is also
reflected in mating choice experiments\textsuperscript{16, 17} where it had more homogamic matings against other members of the \textit{nasuta-albomicans} complex.

Thus in depth qualitative and quantitative dissection of the components of courtship elements of the parental races (\textit{D. n. nasuta} and \textit{D. n. albomicans}) and of their introgressed products (Cytoraces) has uncovered the very early phases in the development of courtship behavioural isolation among cytogenetically closely related members of the \textit{nasuta-albomicans} complex of \textit{Drosophila}.

**Conclusions:**

The \textit{nasuta-albomicans} complex is an assemblage within the \textit{nasuta} subgroup of the genus \textit{Drosophila}. It consists of \textit{D. n. nasuta}, \textit{D. n. albomicans} and their introgressed hybrid lineages called Cytoraces. The present investigation on the dissection of the behavioural steps of the courtship process has revealed 24 elements. The qualitative and quantitative comparison of these elements among six members of the \textit{nasuta-albomicans} complex has shown detectable level of divergence for different sets of features suggesting the onset of the pre-mating isolation among the closely related races.

**Acknowledgements:**

Authors are grateful to the Department of Science and Technology, New Delhi, for financial assistance.
References:


Legends for Figures:

**Figure 1:** Karyotypic composition of the six races of the *nasuta-albomicans* complex.

**Figure 2:** Dendrogram based on frequency of courtship (Qualitative) elements in six races of the *nasuta-albomicans* complex of *Drosophila*.

**Figure 3:** Dendrogram based on frequency of each courtship (Quantitative) act in six races of the *nasuta-albomicans* complex of *Drosophila*. 
I. 2X Y Male 2n=8
II. 2X3 Y3 Male 2n=6
III. 2X Y3 Male 2n=7
IV. 2X3 Y3 Male 2n=6
V. 2X Y Male 2n=8
VI. 2X3 Y3 Male 2n=7

D. n. nasuta
D. n. alblockans
Cytorace 1
Cytorace 2
Cytorace 3
Cytorace 4

Figure 1
Cluster Tree

Figure 2

(Note: NN- D. n. nasuta; AA- D. n. albomicans; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4)
Figure 3

Note: NN- *D. n. nasuta*; AA- *D. n. albomicans*; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4
Table-1: The pattern of occurrence (presence \( '+' \) or absence \( '-' \)) and frequencies of different courtship elements in six races of *nasuta-albomicans* complex of *Drosophila*. Significant differences for pair wise comparisons between races for different components are indicated as follows: ‘\( \dagger \)’: against N; ‘\( \ddagger \)’: against A; ‘\( \star \)’ : against C1; ‘\( \overline{\star} \)’ : against C2; ‘\( \beta \)’: against C3; ‘\( \approx \)’: against C4.

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Note: N: *D. n. nasuta*; A: *D. n. albomicans*; C1: Cytorace-1; C2: Cytorace-2; C3: Cytorace-3 and C4: Cytorace-4
<table>
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<tr>
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<th>C2</th>
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**Table-2:** Genetic Distance (upper diagonal) and Genetic Identity (lower diagonal) for courtship elements (Qualitative data) of six races of the *nasuta-albomicans* complex *Drosophila*.

*(Note: NN- *D. n. nasuta*; AA- *D. n. albomicans*; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4)*
### Table 3a

<table>
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<tr>
<th>Sl No.</th>
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<th>Transverse approach</th>
<th>Anterior approach</th>
<th>Tapping</th>
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<th>Posterior circle</th>
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### Table 3b

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<th>Full circle</th>
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<th>Wing Fluttering</th>
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<th>Wing waving</th>
<th>Wing Scissor</th>
<th>Attempt</th>
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<td>5,294</td>
</tr>
</tbody>
</table>

**Table 3a and 3b:** Mean values with standard error and along with analysis of variance for male courtship acts among six races of the *nasuta-albomicans* complex of *Drosophila.*

**Note:** NN- *D. n. nasuta*; AA- *D. n. albomicans*; CI-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4

* * significance at 5% level
Table-3c: Mean values with standard error and along with analysis of variance for female courtship acts among six races of the *nasuta-albomicans* complex of *Drosophila*.

**Note:** NN- *D. n. nasuta*; AA- *D. n. albomicans*; Cl-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4

<table>
<thead>
<tr>
<th>SI No</th>
<th>Races</th>
<th>Decamp</th>
<th>Ignore</th>
<th>Kick</th>
<th>Female wing fluttering</th>
<th>Female wing flicking</th>
<th>Wing spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NN</td>
<td>3.9±0.5</td>
<td>1.3±0.1</td>
<td>1.4±0.4</td>
<td>0.5±0.2</td>
<td>0.6±0.3</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>2</td>
<td>AA</td>
<td>3.8±0.6</td>
<td>1.6±0.3</td>
<td>1.8±0.3</td>
<td>2.2±0.5</td>
<td>1.5±0.2</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>3</td>
<td>C1</td>
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<td>0.4±0.1</td>
<td>2.3±0.3</td>
<td>0.2±0.1</td>
<td>1.5±0.5</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>4</td>
<td>C2</td>
<td>3.7±0.4</td>
<td>1.1±0.2</td>
<td>0.8±0.3</td>
<td>3.0±0.8</td>
<td>1.5±0.1</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>5</td>
<td>C3</td>
<td>3.5±0.4</td>
<td>0.7±0.1</td>
<td>2.5±0.3</td>
<td>3.0±0.8</td>
<td>1.5±0.1</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>6</td>
<td>C4</td>
<td>4.2±0.6</td>
<td>1.9±0.6</td>
<td>2.3±0.4</td>
<td>0.3±0.1</td>
<td>1.0±0.3</td>
<td>2.3±0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.667</td>
<td>5.294</td>
</tr>
<tr>
<td>2.326*</td>
<td>5.294</td>
</tr>
<tr>
<td>5.298*</td>
<td>5.294</td>
</tr>
<tr>
<td>5.408*</td>
<td>5.294</td>
</tr>
<tr>
<td>4.771*</td>
<td>5.294</td>
</tr>
<tr>
<td>4.207*</td>
<td>5.294</td>
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</tbody>
</table>

Table-3d: Mean values with standard error along with analysis of variance for each courtship act due to both sexes among six races of the *nasuta-albomicans* complex of *Drosophila*.

**Note:** NN- *D. n. nasuta*; AA- *D. n. albomicans*; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4

<table>
<thead>
<tr>
<th>SI No</th>
<th>Races</th>
<th>Courtship latency</th>
<th>Courtship duration</th>
<th>Copulation duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NN</td>
<td>33.8±6.0</td>
<td>42.2±5.9</td>
<td>18.8±0.4</td>
</tr>
<tr>
<td>2</td>
<td>AA</td>
<td>34.0±6.2</td>
<td>25.1±4.6</td>
<td>18.6±0.4</td>
</tr>
<tr>
<td>3</td>
<td>C1</td>
<td>23.6±4.6</td>
<td>40.9±4.8</td>
<td>21.0±0.4</td>
</tr>
<tr>
<td>4</td>
<td>C2</td>
<td>32.9±5.7</td>
<td>35.6±5.7</td>
<td>17.6±0.3</td>
</tr>
<tr>
<td>5</td>
<td>C3</td>
<td>31.5±5.7</td>
<td>30.8±5.3</td>
<td>18.8±0.3</td>
</tr>
<tr>
<td>6</td>
<td>C4</td>
<td>26.7±4.5</td>
<td>53.4±6.6</td>
<td>19.5±0.4</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>F-value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.603</td>
<td>5.294</td>
</tr>
<tr>
<td>3.128*</td>
<td>5.294</td>
</tr>
<tr>
<td>8.680*</td>
<td>5.294</td>
</tr>
</tbody>
</table>

"*" significance at 5% level
Table-3c: Mean values with standard error and along with analysis of variance for female courtship acts among six races of the *nasuta-albomicans* complex of *Drosophila*.

**Note:** NN- *D. n. nasuta*; AA- *D. n. albomicans*; Cl-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4

\*\* significance at 5% level

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Races</th>
<th>Decamp</th>
<th>Ignore</th>
<th>Kick</th>
<th>Female wing fluttering</th>
<th>Female wing flicking</th>
<th>Wing spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NN</td>
<td>3.9±0.5</td>
<td>1.3±0.1</td>
<td>1.4±0.4</td>
<td>0.5±0.2</td>
<td>0.6±0.3</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>2</td>
<td>AA</td>
<td>3.8±0.6</td>
<td>1.6±0.3</td>
<td>1.8±0.3</td>
<td>0</td>
<td>0</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>3</td>
<td>C1</td>
<td>2.9±0.4</td>
<td>0.4±0.1</td>
<td>4.9±0.9</td>
<td>0.9±0.3</td>
<td>2.2±0.5</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>4</td>
<td>C2</td>
<td>3.8±0.4</td>
<td>1.1±0.2</td>
<td>2.1±0.3</td>
<td>0.2±0.1</td>
<td>1.5±0.5</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>5</td>
<td>C3</td>
<td>3.5±0.4</td>
<td>0.7±0.1</td>
<td>2.1±0.3</td>
<td>2.1±0.6</td>
<td>3.0±0.8</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>6</td>
<td>C4</td>
<td>4.2±0.6</td>
<td>1.9±0.6</td>
<td>2.3±0.4</td>
<td>0.3±0.1</td>
<td>1.0±0.3</td>
<td>2.3±0.3</td>
</tr>
</tbody>
</table>

**F-value** | 0.667 | 2.326* | 5.298* | 5.408* | 4.771* | 4.207* |

**df** | 5,294 | 5,294 | 5,294 | 5,294 | 5,294 | 5,294 |

Table-3d: Mean values with standard error along with analysis of variance for each courtship act due to both sexes among six races of the *nasuta-albomicans* complex of *Drosophila*.

**Note:** NN- *D. n. nasuta*; AA- *D. n. albomicans*; Cl-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4

\*\* significance at 5% level

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Races</th>
<th>Courtship latency</th>
<th>Courtship duration</th>
<th>Copulation duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NN</td>
<td>33.8±6.0</td>
<td>42.2±5.9</td>
<td>18.8±0.4</td>
</tr>
<tr>
<td>2</td>
<td>AA</td>
<td>34.0±6.2</td>
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<td>18.6±0.4</td>
</tr>
<tr>
<td>3</td>
<td>C1</td>
<td>23.6±4.6</td>
<td>40.9±4.8</td>
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<td>4</td>
<td>C2</td>
<td>32.9±5.7</td>
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<tr>
<td>5</td>
<td>C3</td>
<td>31.5±5.7</td>
<td>30.8±5.3</td>
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<tr>
<td>6</td>
<td>C4</td>
<td>26.7±4.5</td>
<td>53.4±6.6</td>
<td>19.5±0.4</td>
</tr>
</tbody>
</table>

**F-value** | 0.603 | 3.128* | 8.680* |

**df** | 5,294 | 5,294 | 5,294 |
<table>
<thead>
<tr>
<th></th>
<th>NN</th>
<th>AA</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
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<td>0.0646</td>
<td>0.0148</td>
<td>0.0136</td>
<td>0.0105</td>
</tr>
<tr>
<td>AA</td>
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<tr>
<td>C1</td>
<td>0.9353</td>
<td>0.9029</td>
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<td>0.0286</td>
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</tr>
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<tr>
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<td>0.0222</td>
</tr>
<tr>
<td>C4</td>
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<td>0.9467</td>
<td>0.9825</td>
<td>0.9777</td>
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</tr>
</tbody>
</table>

**Table-4:** Genetic Distance (upper diagonal) and Genetic Identity (lower diagonal) for each courtship act (Quantitative data) of six races of the *nasuta-albomicans* complex of *Drosophila*.

(**Note:** NN- *D. n. nasuta*; AA-*D. n. albomicans*; C1-Cytorace 1; C2-Cytorace 2; C3-Cytorace 3 and C4-Cytorace 4)