Incipient sexual isolation in the *nasuta-albomicans* complex of *Drosophila*: No-choice experiments

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*Drosophila nasuta nasuta* and *Drosophila nasuta albomicans* are cross-fertile races of *Drosophila*. Hybridization between these races in the laboratory has given rise to new races (Cytoraces), among which karyotypic composition differs from one another and also from those of the parental races. In this study, we search for the evidence of incipient reproductive isolation among the parental races and four Cytoraces by assessing the fraction of no-matings, mating latency and copulation duration in all possible types of homo- and heterogamic crosses (*N*= 4184). In no-choice conditions, the latency time (time to initiation of copulation) is lower in homogamic crosses than in heterogamic crosses for both parental races and Cytoraces. Latency time and copulation duration are negatively correlated, whereas fraction of no matings is positively correlated with latency time. Thus these six closely related races of the *nasuta-albomicans* complex show the initiation of the earliest stages of pre-zygotic isolation, manifested as a tendency for matings to be initiated earlier and more often, and for a longer duration, among homogamic rather than heterogamic individuals.

1. Introduction

*Drosophila nasuta nasuta* (*2n*= 8) and *Drosophila nasuta albomicans* (*2n*= 6) are a pair of allopatric sibling chromosomal races of the *nasuta* subgroup of *Drosophila* (Nirmala and Krishnamurthy 1972; Ranganath and Hägele 1981). Interracial hybridization of these races followed by the maintenance of hybrids for many generations has resulted in the evolution of populations whose karyotypic composition is different from those of parents and these hybrid populations have been termed Cytoraces (Ramachandra and Ranganath 1986, 1990). The assemblage consisting of the parental races, namely *D. n. nasuta* and *D. n. albomicans* and the newly evolved Cytoraces are treated as ‘*nasuta-albomicans* complex’ of *Drosophila* (Ramachandra and Ranganath 1996). Cytogenetic and morpho-phenotypic divergence has been recorded among a few members of this new complex (Tanuja et al 1998, 1999a, b). Now we report the divergence for three important components of prezygotic reproductive isolation, namely the incidence of no-mating, mating latency and duration of copulation among six members of the *nasuta-albomicans* complex of *Drosophila*.

2. Materials and methods

2.1 Fly stocks

The following six chromosomal races of the *nasuta-albomicans* complex have been used: (i) *D. n. nasuta* (*2n*= 8; Coorg strain, India); (ii) *D. n. albomicans* (*2n*= 6; Okinawa strain, University of Texas collections, 3045·11); (iii) Cytorace 1 (*2n*= 7 in males; 2*n*= 6 in females; Ramachandra and Ranganath 1986); (iv) Cytorace 2 (*2n*= 6; Ramachandra and Ranganath 1986); (v) Cytorace 3 (*2n*= 8; Ramachandra and Ranganath 1990); (vi) Cytorace 4 (*2n*= 7 in males and 2*n*= 8 in females; Ramachandra and Ranganath 1990).

2.2 Mating experiments

Fifty eggs were collected in fresh half-pint-milk bottles with wheat cream agar medium to avoid larval competition during the development, and incubated at 22°C. Once the flies started emerging from these bottles, virgin...
females and males from each of these bottles were isolated and transferred to fresh media vials within 6 h of their eclosion and maintained separately at 22°C. After aging these virgin flies for 5 days, they were used for no-choice mating experiments in which one male was allowed to mate with a female of either its own race (homogamic) or of a different race (heterogamic).

The matings were carried out in the empty vials plugged with cotton. Flies were aspirated into the vials, to avoid etherization before the experiment. The mating activities of these flies were recorded for about 5 h starting at 07:00 h. Mating latency was measured as time (in minutes) taken from the introduction of a male and a female together into an empty vial until the initiation of copulation. Copulation duration was measured as time (in minutes) taken from the initiation to the termination of copulation. For each of the cross, the fraction of vials in which no matings occurred during the 5 h of observation was also recorded.

In the present study, with six different races, 36 crosses were made. The total number of matings attempted was 4184. The following statistical comparisons were made for the three traits studied: (i) Among homogamic matings of six races. (ii) Among males of six races, with trait values averaged over all matings with females of other races. (iii) Among females of six races, with trait values averaged over all matings with males of other races. (iv) Among homo-parental (homogamic matings of parental races); homo-cytorace (homogamic matings for each of the four Cytoraces); hetero-parental (cross between two parental races); hetero-cytorace (cross among any two Cytoraces); hetero-mixed (heterogamic matings between a parental race and a Cytorace) types of matings. (v) Correlation coefficients between three measures of reproductive isolation namely mating latency, copulation duration and the fraction of no-matings have been computed.

Statistical analyses for (i) through (iv) above was by means of analysis of variance (ANOVA) followed by Tukey’s HSD test for unequal sizes (Spjotvoll-Stoline test) for multiple comparisons. All statistical analysis were done on Statistica for Windows release 5-0B.

3. Results

In the present study with six races of *nasuta-albomicans* complex, a total of 4184 crosses were attempted. Of this, 2715 crosses resulted in matings. Table 1 provides the mean values of mating latency, copulation duration and per cent of no-matings in each of the 36 crosses of the present experiment. Of the six races, Cytorace 2 and *D. n. albomicans* have the highest (113.72 min) and the lowest (68.06 min) mean mating latency respectively. Similarly, the mean maximum duration of copulation was in *D. n. albomicans* (20.4 min) while the lowest was in Cytorace 2 (15 min). The differences among six races for mating latency (*F* = 4.85; *P* = 0.00024) and for copulation duration (*F* = 24.01; *P* = 0.0001) are found to be significant. The table 2 has the means of the three parameters for a male of a particular race across the females of six races as well as of female of a race across the males of the other races. These means would help us to compare the performance of males and females of six races under study on the parameters analysed. The two-way ANOVA computed to these means shows the significant difference among males and among females for the parameters mating latency and copulation duration (table 2). Further, pair-wise comparisons were made with the help of Tukey’s HSD test and based on the level of differences, the six races under study can be organized in a hierarchical pattern, wherein the races within a cluster do not show significant differences. The hierarchy of the males of six races for mating latency could be represented as

\[ N \ C4 \ C3 \ C1 \ A \ C2 \ . \]

Similarly the males of six races copulate for different duration and it is hierarchically represented as

\[ C2 \ A \ C1 \ C3 \ C4 \ N \ . \]

The sequence of females for mating latency is

\[ A \ C4 \ C3 \ N \ C1 \ C2 \ . \]

while the relative position of females of six races for duration of copulation is

\[ C2 \ C1 \ N \ C3 \ C4 \ A \ . \]

The results are subjected to another type of analysis. Based on the mating type, the 36 crosses can be grouped into homogamic and heterogamic categories. Similarly, based on the identity of the races involved in each cross, the 36 crosses can be organized into five groups, such as homo-parental, homo-cytorace, hetero-parental, hetero-cytorace and hetero-mixed. The mean values of the three parameters for each of these groups are presented in table 3. The overall mating latency of homogamic matings is significantly less than that of heterogamic matings (*F* = 16.48; *P* = 0.00005) while insignificant difference is seen for the duration of copulation (*F* = 0.52; *P* = 0.4730). Similarly, the two-way ANOVA has shown significant impact of race identity (parental vs Cytoraces vs mixed of both homogamic and heterogamic matings) on mating latency (*F* = 15.14; *P* = 0.0000003) and copulation duration (*F* = 23.75; *P* = 0.0000003). Further, among five groups, pair-wise comparisons

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