RESEARCH NOTE

Chromosomal localization of autosomal mutations in *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans*

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Introduction

We studied two eye colour and two wing mutants (sepia, brown, crossveinless and Curly) in *Drosophila nasuta nasuta* and four eye colour mutants (brown, carmine, purple and bright eyes) in *Drosophila nasuta albomicans*. Most mutations were isolated from natural populations whereas two (crossveinless and Curly in *D. n. nasuta*) were induced in laboratory populations. From the pattern of inheritance in the F_1 and backcross generations, all the mutant genes analysed were seen to be recessive, except Curly in *D. n. nasuta*, which was dominant. Further, based on the inheritance pattern in the F_1 and backcross progeny of interspecific crosses, we could show that all the mutant genes are located on chromosome 2, except the gene for purple in case of *D. n. albomicans*, which is located on chromosome 3.

The *nasuta* subgroup of *Drosophila immigrans* group includes an assemblage of morphologically almost identical, closely related species (Wilson *et al*. 1969). *D. n. nasuta* was first described by Lamb (1914) from the Seychelles, while *D. n. albomicans* was first described by Duda (1923) from Paroe, Taiwan. These two subspecies are widely distributed in the Southeast Asian region, enjoy a special status among other members of the subgroup because of their open genetic system and differing karyotypic composition and have been used as parents in the evolution of novel cytoraces in the laboratory (Mather and Pope 1972; Nirmala and Krishnamurthy 1972; Ranganath and Krishnamurthy 1972; Wakahama and Kitagawa 1972; Clyde 1977; Gai and Krishnamurty 1983; Shyamala *et al*. 1987; Ranganath and Ramachandra 1994; Tanuja *et al*. 1998). In earlier studies, X-linked mutations in these two subspecies could easily be identified based on the typical criss-cross pattern of inheritance of the phenotype in question. However, autosomal mutations could not be localised due to absence of genetic markers. In the present study, we have exploited the differences in karyotypic composition between these two subspecies, and their cross-fertility, in order to localize some autosomal genes.

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Materials and methods

Two eye colour (sepia, *se*, and brown, *bw*) and two wing (Curly, *Cy*, and crossveinless, *cv*) mutants of *D. n. nasuta* and four eye colour (brown, *bw*, carmine, cm, bright eyes, *be* and purple, *pr*) mutants of *D. n. albomicans* were chosen for the present study. All the mutants were spontaneous, except crossveinless and Curly which were induced mutations. The wild stocks of *D. n. nasuta* (Coorg, South India) and *D. n. albomicans* (Okinawa) were obtained from *Drosophila* Stock Centre, Department of Studies in Zoology, University of Mysore, Mysore.

Unmated males and virgin females of both mutants as well as wild types were isolated every 4 h after eclosion and were aged for 5 days before using them for crosses. To determine the dominant or recessive nature of mutations, crosses were conducted between mutant strains and wild type individuals of the same species. However, for chromosomal localization of mutations in *D. n. nasuta*, crosses were conducted between the females of *D. n. nasuta* mutants and the wild type males of *D. n. albomicans*. Similarly, for chromosomal localization of *D. n. albomicans* mutations, *D. n. albomicans* mutant females were crossed to

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the wild type males of *D. n. nasuta*. In all the crosses, the F_1 progeny were scored for the phenotypes. Further, the F_1 males were backcrossed to the females of parental mutant stocks, the progeny of which were sexed and their phenotypes recorded. All crosses were conducted in 8 × 2.5 cm vials containing standard wheat cream agar medium seeded with yeast. All the parental and experimental cultures were maintained at 22 ± 1°C.

Results and discussion

Unlike the case of *D. melanogaster* in which the availability of large number of genetic markers makes mapping easy, *D. n. nasuta* and *D. n. albomicans* do not offer many markers for convenient mapping. However, since these two subspecies are cross-fertile, we could localise the mutant genes based on the phenotype of backcross progeny. Based on the phenotypes in F_1 progeny of the reciprocal crosses, we could infer that none of the mutations under investigation are X-linked. Only *Cy/Cy* in *D. n. nasuta* was found to be a dominant mutation, since all the individuals of F_1 generation possessed mutant phenotype. The remaining, namely *se/se*, *bw/bw* and *cv/cv* in *D. n. nasuta* as well as *bw/bw*, *pr/pr*, *cm/cm* and *be/be* in *D. n. albomicans* were found to be recessive; as these phenotypes typically appeared in the ratio of 3 wild type: 1 mutant in the F_2 generation. For the autosomal localization of these mutations, we used the differences in karyotypes and the chromosomal associations arising from parental crosses and back cross progeny.

The chromosomal complement of *D. n. nasuta* consists of a pair of metacentrics (chromosome 2), two pairs of acrocentrics (chromosome 3 and X) and a pair of dots (chromosome 4) in females, whereas in males, the Y-chromosome is submetacentric (Ranganath and Ramachandra 1994). The chromosomal complement of *D. n. albomicans* consists of two pairs of metacentrics and a pair of long dots. Here, the chromosome 3 is fused with the sex chromosomes (3X or 3Y), thus forming a pair of metacentrics (Wilson *et al*. 1969; Wakahama *et al*. 1971; Ramachandra and Ranganath 1986; Ranganath 2002). Based on the karyotypic differences in the parents of each cross, we could predict the karyotypic composition of the F_1 and backcross progeny along with their phenotype, when mutant genes are present on the major autosomes, namely chromosomes 2 and 3. The pattern of inheritance expected for a recessive mutation in chromosome 2 *D. n. nasuta* is illustrated in figure 1. Similarly, we could predict the karyotypic composition, possible varieties of gametes, the phenotypes of F_1 and backcross progeny for all other cases.

In crosses involving *se/se*, *bw/bw* & *cv/cv* mutants of *D. n. nasuta* as one of the parents, all the F_1 individuals had wild type phenotype (table 1). Hence these mutant alleles are recessive. Both sexes of wild type as well as mutant phenotypes appeared in backcross progeny in 1 : 1 : 1 : 1 ratio (c^2-test non significant). Hence, it could be inferred that all these three mutant genes are located on chromosome 2 of *D. n. nasuta*. Further, Curly alleles were found to be dominant since all the F_1 individuals of reciprocal crosses involving Curly as one of the parents had mutant phenotype (table 1). The backcross progeny consisted of males and females of both wild type and Curly phenotypes, indicating that Curly mutation in *D. n. nasuta* is dominant and is on chromosome 2.

In crosses involving *bw/bw*, *be/be*, *cm/cm* & *pr/pr* mutant females of *D. n. albomicans* and wild type *D. n. nasuta* males, all the F_1 individuals possessed wild type phenotype (table 1). Hence, these mutations are recessive. Further, both the sexes of wild type and mutant phenotypes appeared in the backcross progeny of crosses involving brown, bright eyes & carmine mutants of *D. n. albomicans* in the ratio of 1 : 1 : 1 : 1 (χ^2-test non significant), indicating that all these three mutant alleles are located on

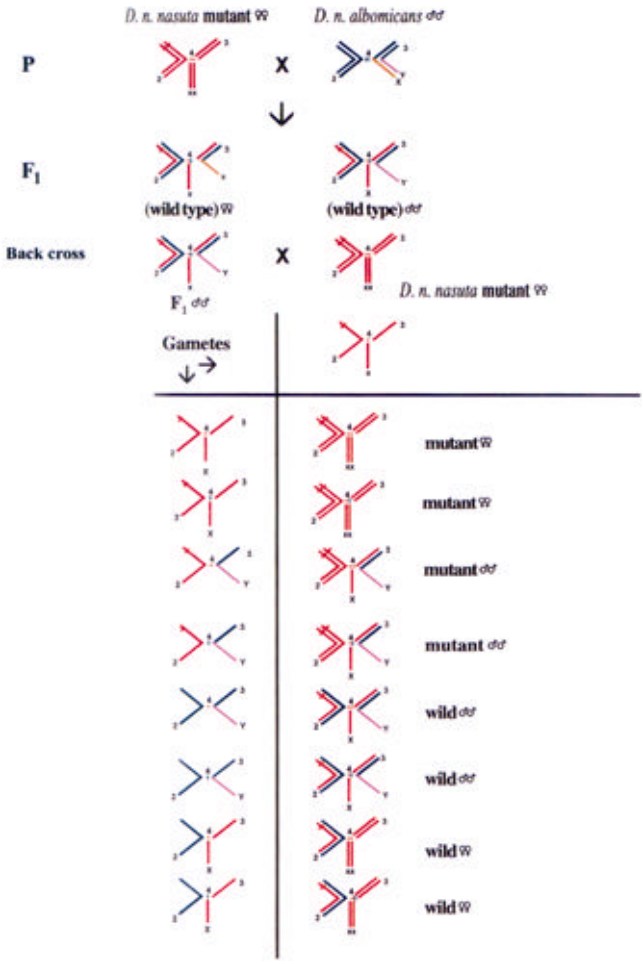

Figure 1. Schematic illustration of the interspecific crosses showing possible karyotypes in F_1 and backcross progeny along with their phenotypes if the mutation is recessive and the gene is located on chromosome 2 in case of *D. n. nasuta*.

Table 1. Results of interspecific crosses and back crosses involving *D. n. nasuta* and *D. n. albomicans* mutant females.

	F_1 Phenotype	Back cross	Total no. of flies analysed	Back cross progeny numbers				c^2
				Mutants		Wild types		
				Male	Female	Male	Female	
D. n. nasuta crosses								
se/se F X *Dna* M	Wild type	F_1 M X *se/se* F	1254	306	324	294	330	0.209
bw/bw F X *Dna* M	Wild type	F_1 M X *bw/bw* F	1152	294	270	306	282	0.216
cv/cv F X *Dna* M	Wild type	F_1 M X *cv/cv* F	1440	366	348	372	354	0.162
Cy/Cy F X *Dna* M	Curly	F_1 M X *Dnn* F	1398	348	336	366	348	0.0935
D. n. albomicans crosses								
bw/bw F X *Dnn* M	Wild type	F_1 M X *bw/bw* F	1752	447	401	475	429	0.394
cm/cm F X *Dnn* M	Wild type	F_1 M X *cm/cm* F	1100	284	276	252	288	0.258
be/be F X *Dnn* M	Wild type	F_1 M X *be/be* F	900	236	224	204	236	0.479
Pr/pr F X *Dnn* M	Wild type	F_1 M X *pr/pr* F	776	–	364	412	–	0.764

F = Female and M = Male.

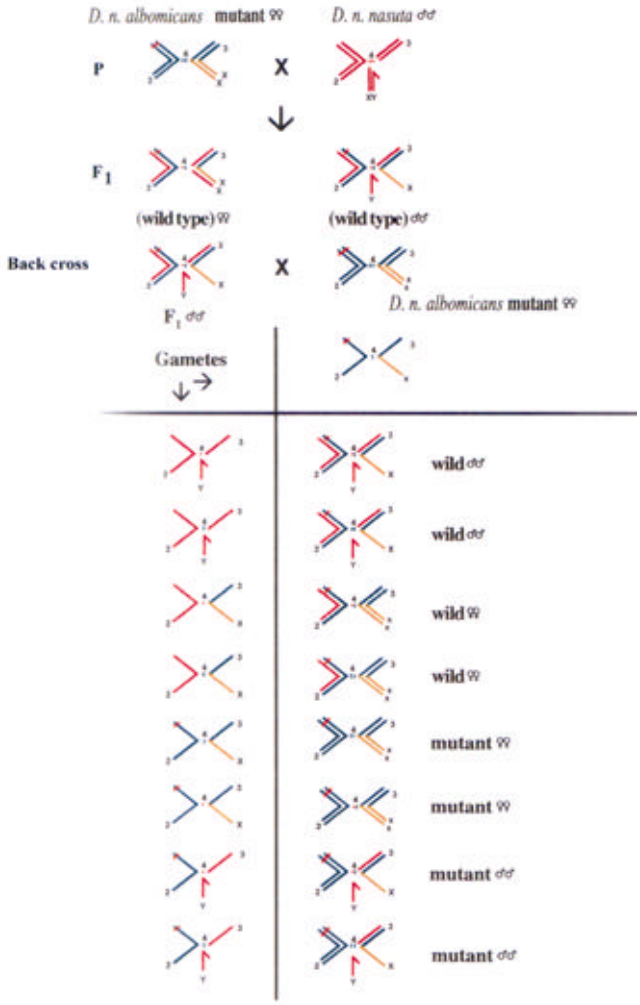

Figure 2. Schematic illustration of the interspecific crosses showing possible karyotypes in F_1 and backcross progeny along with their phenotypes if the mutation is recessive and the gene is located on chromosome 2 in case of *D. n. albomicans*.

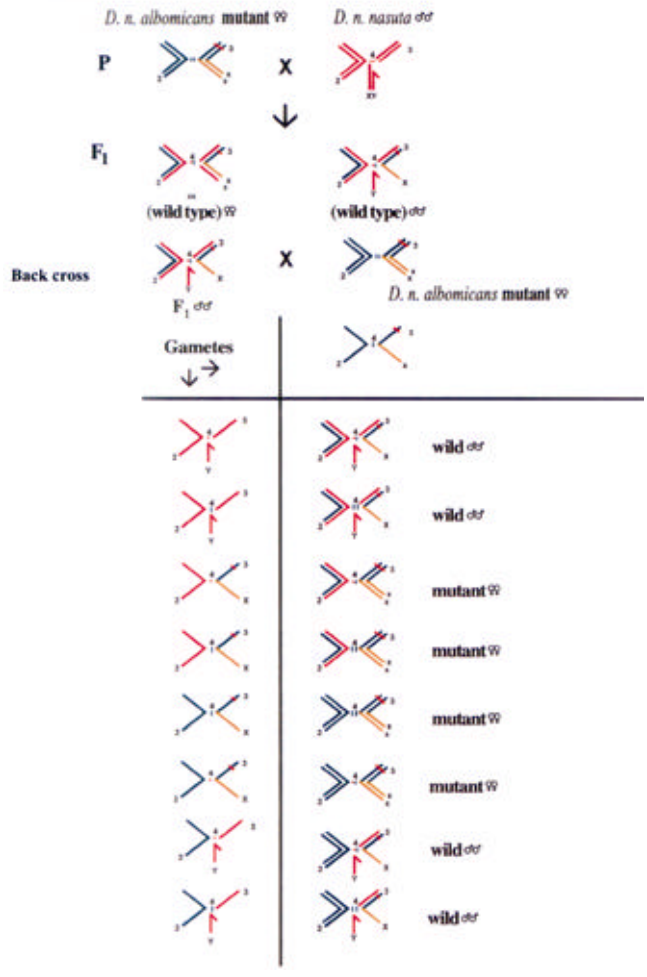

Figure 3. Schematic illustration of the interspecific crosses showing possible karyotypes in F_1 and backcross progeny along with their phenotypes if the mutation is recessive and the gene is located on chromosome 3 in case of *D. n. albomicans*.

chromosome 2 (figure 2). In the crosses involving *pr/pr* mutant of *D. n. albomicans*, only purple females were seen in the backcross progeny and wild type females were never seen. Such an outcome could be expected if the gene for purple mutation in *D. n. albomicans* is recessive and is located on chromosome 3 (figure 3).

In case of *D. melanogaster* the genes carmine and crossveinless are sex-linked, while the genes brown eye, purple eye, bright eyes and Curly wing are on chromosome 2, and sepia eye is on chromosome 3. Further, Curly is dominant and lethal in homozygous condition in *D. melanogaster* (Lindsley and Zimm 1990). All the mutations of *D. n. nasuta* and *D. n. albomicans* analysed in the present study are on chromosome 2, except purple of *D. n. albomicans*, which, is on chromosome 3. It is quite interesting to note that Curly mutation of *D. n. nasuta*, though dominant as in *D. melanogaster*, is not lethal in homozygous state.

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