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Linkage disequilibrium in laboratory populations of *Drosophila nasuta*

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Summary. Natural populations of *Drosophila nasuta* are polymorphic for many gene arrangements. Two non-overlapping inversions of the third chromosome, III-2 and III-35, are most common and display extreme linkage disequilibrium. Six randomly mating laboratory stocks, each founded by one gravid female heterozygous in coupling for both III-2 and III-35, were observed after 32 generations. Significant linkage disequilibrium was observed in all stocks. Recombinants were found in only two stocks. The absence of effective recombination in some stocks and its presence in others might be due to different genotypic backgrounds. We suggest that natural selection, influencing recombination rates in several ways, and intrachromosomal epistasis between the two inversions were the main factors for the maintenance of linkage disequilibrium in *D. nasuta*.

Key words: Linkage disequilibrium – Inversions – *Drosophila nasuta*

Introduction

Drosophila nasuta, a member of the immigrans species group, was originally described by Lamb (1914) from the Seychelles Islands in the Indian Ocean. The species is widespread and has been reported from several countries of Southeast Asia and Africa: Sri Lanka, Mauritius Islands, Madagascar, Reunion Islands, India and Kenya (Kitagawa et al. 1982). Cytological studies in this species have revealed 85 inversions in natural populations (Kumar and Gupta 1986a). Among these, two independent linked inversions of the third chromosome, III-2 and III-35, are most common and show extreme linkage disequilibrium (Kumar and Gupta 1986b).

Here, we report the results of an experiment designed to: (a) study whether the linkage disequilibrium between the above inversions persisted in laboratory stocks maintained for 32 generations, and (b) search for possible cause(s) for the maintenance of disequilibrium in *D. nasuta*.

Materials and methods

The stocks used in the present study were founded by a single naturally impregnated female collected from each of six populations, namely, Sarnath, Bhanaur, Jaunpur, Ramnagar, Botanical and Ayurvedic Gardens on the Banaras Hindu University Campus. The analysis of F_1 larvae of each stock revealed that the larvae were double heterozygotes for III-2 and III-35 inversions, i.e., F_1 larvae of all six stocks had only two chromosome types, St St and III-2 III-35. This was confirmed by crossing the F_1 individuals with laboratory individuals homozygous for both inversions and subsequently analysing the third instar larvae. This indicates that the fathers in all six stocks were homozygous for both standard gene arrangements. The stocks were maintained in the laboratory for 32 generations at 25° ± 1 °C by transferring about 50 flies to fresh culture bottles in each generation. Unlike other species of *Drosophila*, multiple matings are not known to occur in this species.

Polytene chromosome preparations of about 100 larvae from each stock were made with the usual lacto-aceto-orcein technique (Strickberger 1962). Hardy-Weinberg equilibrium of each inversion and independence of the two segments were tested by χ^2.

Linkage disequilibrium is defined by $D = f_{11}p - q$ (Hill 1974), where f_{11} is the frequency of double inversion homozygotes and p and q are the frequencies of inversions III-2 and III-35, respectively. The deviation from $D = 0$ measures the disequilibrium existing in a population. To account for the varying magnitudes of p and q, D is standardized to D' by dividing D by $p(1-q)$ or by $(1-p)q$, whichever is smaller (Lewontin 1964).

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Table 1. Observed numbers of combinations of III-2 (=A) and III-35 (=B) inversions in laboratory stocks of *D. nasuta*

Karyotype	Source of stock					
	Sarnath	Bhanaur	Jaunpur	Ramnagar	Botanical Garden	Ayurvedic Garden
St St/St St	20	18	26	22	21	22
/St B	–	–	–	2	10	–
/B B	–	–	–	–	–	–
St A/St St	–	–	–	4	15	–
/St B[a]	62	73	58	65	71	69
/B B	–	–	–	–	–	–
A A/St St	–	–	–	–	1	–
/St B	–	–	–	–	–	–
/B B	16	9	8	7	2	11
Total larvae	98	100	92	100	120	102
Chi-square[b]	196.0	200.0	184.0	170.1	170.3	204.0

[a] Exclusively coupling heterozygotes

[b] All tests have 4 *df*; χ^2 [4, 0.001] = 22.5

Table 2. Observed (D) and standardized (D′) linkage disequilibrium between III-2 and III-35 inversions in laboratory populations of *D. nasuta*

Locality	$\hat{p}^a \pm$SE (%)	$\hat{q}^a \pm$SE (%)	D±SE	D′
Sarnath	48.0±3.6	47.9±3.6	0.2496±0.0015	1.00
Bhanaur	45.5±3.5	45.5±3.5	0.2480±0.0032	1.00
Jaunpur	40.2±3.6	40.2±3.8	0.2404±0.0071	1.00
Ramnagar	41.5±3.5	40.5±3.5	0.2269±0.0085	0.96
Botanical Garden	38.3±3.1	35.4±3.1	0.1767±0.0121	0.81
Ayurvedic Garden	44.6±3.5	44.6±3.4	0.2472±0.0038	1.00

[a] $\hat{p}$, $\hat{q}$ = estimated frequencies of the III-2 and III-35 inversions, respectively

Results

Table 1 shows the observed numbers of combinations of the III-2 and III-35 inversions in the six laboratory stocks. Evidently four stocks (Sarnath, Ayurvedic Garden, Bhanaur and Jaunpur) maintained the initial combinations (St St, III-2 III-35), while the other two (Ramnagar and Botanical Garden) included recombinants as well.

The six stocks had similar frequencies of inversions III-2 and III-35 (both $\chi^2_5 < 9.2$, $P > 0.1$). Further, compared with Hardy-Weinberg and linkage equilibrium expectations, all stocks had too many heterokaryotypes for the two inversions jointly (all $\chi^2_4 > 170.0$, $P < 0.001$; Table 1) and separately (all $\chi^2_1 > 7.0$, $P < 0.01$), suggesting overdominance. Estimates of D and D′ as well indicate substantial linkage disequilibrium in all stocks between the III-2 and III-35 gene arrangements (Table 2).

Discussion

Our results demonstrate that disequilibrium between the linked III-2 and III-35 inversions persisted in the laboratory stocks even after 32 generations of random mating. However, there is strain variation. Four stocks (Sarnath, Bhanaur, Jaunpur and Ayurvedic Garden) maintained the initial chromosome types, whereas Ramnagar and Botanical Garden stocks included some recombinants, i.e., effective recombination between the two inversions does occur. This variation between stocks suggests that effective recombination may be contingent on background genotype.

The phenomenon of linkage disequilibrium has been reported in certain species of *Drosophila* viz., *D. pavani*, *D. rubida*, *D. paramelanica*, *D. nigromaculata*, *D. euronotus*, *D. robusta*, *D. guaramunu*, *D. nasuta* and *D. subobscura* (for references see Kumar and Gupta 1986b). Among these species, however, only the last four display extreme disequilibrium between linked gene arrangements.

Sperlich and Feuerbach-Mravlag (1974) have reported two cases of linkage disequilibrium in *D. subobscura*, one between two autosomal inversions O_7 and O_{3+4}, and another between two independent inversions A_1 and A_2 of the X chromosome. Their detailed analysis revealed, in the former case, a complete lack of crossing over between the two inversions and in the latter case, intrachromosomal epistasis. In *D. nasuta*, the two inversions III-2 and III-35 comprise respectively, about 7% and 14% of the third chromosome and the distance between them represents about 25% of the total chromosome length. Thus, crossovers should occur frequently and a state of linkage equilibrium should be established in all populations, provided that selection does not favour certain combinations. However, a separate study revealed that the rate of crossing over between the two inversions is surprisingly low at about 3.5% (Kumar and Gupta, unpublished). This is supported by the low percentage of recombinants observed in both natural and laboratory populations of this species (Kumar and Gupta 1986 b). These observations suggest that crossover products may be lethal, or at least sublethal, because of intrachromosomal epistasis regarding fitness as reported by Sperlich and Feuerbach-Mravlag (1974) for A_1 and A_2 inversions of *D. subobscura.* Moreover, Dobzhansky and Spassky (1960) reported that synthetic lethality as a result of gene interaction is not infrequent in *D. pseudoobscura.* Fisher (1930) suggested that if recombination frequency is genetically determined and if recombination products are less fit, selection would tend to reduce recombination between interacting genes. Our observations and those of Sperlich and Feuerbach-Mravlag (1974) on *D. subobscura* suggest the same situation.

It is proposed that such a non-random association between linked inversions is not due to chance. There must be some selection pressure that favours association and suppresses recombination between the two inversions. Thus, the reduced tendency of the two chromosome III inversions to recombine is the product of natural selection acting to enhance the ability of the species to maintain linkage disequilibrium between these two gene arrangements in *D. nasuta.* However, as far as heterotic properties of individual inversions are concerned, Bodmer and Parsons (1962) have argued that these associations are second-order interactions, the primary interactions being those that occur between contents of a single inversion. Inversions contain multiple interacting allelic blocks that are maintained by suppression of crossing over (Lerner 1954; Wallace 1968; Dobzhansky 1970). Levitan (1958) has shown that selection may occur for linked interacting loci that are not part of allelic blocks. Similarly, Sperlich and Feuerbach-Mravlag (1974) have suggested that selection may favour linkage between interacting gene arrangements and the whole chromosome may be considered a functional and selective unit. Our results on *D. nasuta* reflect this situation and are probably the first case of an extreme association of linked inversions in laboratory populations.

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