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Isozymes and genetic divergence in the *nasuta*–*albomicans* complex of *Drosophila*

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The *nasuta*–*albomicans* complex is a hybrid-zone, artificially created in the environs of the laboratory¹. The members of this complex include *D. nasuta*, *D. albomicans* and a number of Cytoraces. The Cytoraces are the products of hybridization between *D. nasuta* and *D. albomicans* and have been maintained in the laboratory for 350 to 500 generations². The degree of genetic diversity encompassed by the parental races and the four Cytoraces at eleven isozymal loci has revealed the existence of 122 alleles with 52 alleles being common to all the six races, 70 being shared by at least two races and 9 alleles being unique to one of the races. Based on our study, the divergence time between the parental races, *D. nasuta* and *D. albomicans*, is 264,000 years, which is the least divergence time so far postulated between these two chromosomal races from studies with different parameters. On the other hand, the Cytoraces, which are of recent origin, have accumulated extensive genetic variation within a span of 15 years, in comparison with the parental forms. These findings are discussed with reference to the critical role of hybridization in raiation.

INTROGRESSIVE hybridization has been extensively studied and critically reviewed in the plant kingdom^{3–5}. On the other hand, the potential role of hybridization beyond the concept of evolutionary dead end^{6,7} is only now being recognized in animals^{8–10}. It is important to study introgressive hybridization not only for identifying the factors maintaining genetic integrity of the hybridizing form, but

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also for better understanding of the genetic processes involved in speciation. Introgression may lead to increased genetic diversity, transfer of adaptation or even the development of new adaptations¹¹. More recently, the pervasive role of introgressive hybridization has gained support from the re-evaluation of previous hybrid-zone studies as well as new findings^{10,12,13} which emphasize that for the creation of a hybrid-zone, the hybrids need to overcome the genomic incompatibilities and establish themselves as true breeding forms and should be sufficiently reproductively isolated. One such system where karyotypically stabilized introgressants were available, is taken into consideration for the present study.

Drosophila nasuta nasuta ($2n = 8$) and *D. n. albomicans* ($2n = 6$) are allopatric sibling species, belonging to the *nasuta* sub-group of the *Drosophila immigrans* species group¹⁴. These two siblings are morphologically almost identical and are cross-fertile, but they differ with respect to their karyotypic composition. Hence, they are termed as chromosomal races^{15,16}. Interracial hybridization between these two has led to the introgression of their genomes, with parental chromosomes being differentially represented in different hybrids^{2,17-19}. Over the years, varied hybrid lines of *D. n. nasuta* and *D. n. albomicans* with stable karyotypic composition were recognized and subsequently named as Cytoraces. So far, sixteen Cytoraces have been established, which along with their parental races are referred to as *nasuta-albomicans* complex²⁰. The present members of the *nasuta-albomicans* complex provide us a rare opportunity to probe the catalytic impact of hybridization on population differentiation and the emergence of novel genomic set-ups. This complex constitutes an 'allo-sympatric population', which provides a ground for the understanding of racial divergence in an artificial (laboratory-evolved) hybrid-zone¹. Apart from karyotypic analyses^{19,21-24}, a few fitness parameters²⁵, mating preferences^{26,27} and certain morphometric traits²⁸⁻³⁰ have been studied on some of the members of this complex. The present study is a further attempt to determine the extent of genetic divergence of these races at the biochemical level by employing isozymes as markers, in order to get an insight into the impact of introgression in the course of their evolution.

For the present study, six chromosomal races of the *nasuta-albomicans* complex, namely the parental races: *Drosophila nasuta nasuta* ($2n = 8$; Coorg strain, India); *Drosophila nasuta albomicans* ($2n = 6$; Okinawa strain, Univ. of Texas collections, 3045.11); and the introgressed systems: Cytorace 1 ($2n = 7$ in males; $2n = 6$ in females)²¹; Cytorace 2 ($2n = 6$)²¹; Cytorace 3 ($2n = 8$)²²; Cytorace 4 ($2n = 7$ in males and $2n = 8$ in females)²² were employed. The age of these Cytoraces is around 350–500 generations. Synchronized cultures were developed following the modified procedure of Delcour²⁶. These cultures were maintained under uniform conditions of relative humidity and $22 \pm 1^\circ\text{C}$ temperature, in wheat cream agar medium seeded

with yeast. Unmated males and virgin females were isolated from the above cultures within 3 h of their eclosion from the pupal case and aged for five days in separate vials (8×2.5 cm). These flies formed the sample. Each fly was homogenized in 30 μl of 40% sucrose solution at 4°C , centrifuged at 4000 rpm for 10 min (Labnet) and the supernatant was loaded onto the gel. These samples were analysed by way of native PAGE (T = 8%; C = 3.5%) following the procedure of Ramesh and Kalisch³¹. Electrophoresis was carried out at 70 V until the tracking dye reached the other end of the gel at 4°C using (i) 0.3 M sodium hydroxide boric acid buffer (pH 8.65) as electrode buffer for non-specific isozymes (1-esterase (1-Est), 2-esterase (2-Est), alkaline phosphatase and acid phosphatase) and (ii) Tris EDTA β -NAD boric acid buffer (pH 8.5) for specific isozymes (xanthine dehydrogenase, 1-glycerophosphate dehydrogenase, superoxide dismutase, octanol dehydrogenase, malate dehydrogenase, alcohol dehydrogenase and glucose-6-phosphate dehydrogenase). After electrophoresis, the gels were stained following the procedure of Ayala *et al.*³² and Ramesh and Rajasekara-setty³³. About 100 individuals from each race were scored for each isozyme. The isozyme patterns were documented using Gel Doc 1000 (Bio-Rad, USA), different alleles was scored and frequencies were determined. These alleles were named following the nomenclature of Ayala *et al.*³². The allelic frequencies were subjected to Z-test, to determine the level of significance. Using allelic frequencies at various isozyme loci, genetic identity [I_{xy}] and genetic distance [D_{xy}] were calculated following Nei's formulae^{34,35}. Based on the genetic distance, the dendrogram was constructed following neighbour joining method (NJ) of MEGA 2.0³⁶.

Isozyme analyses in four Cytoraces (Cyt-1 to Cyt-4) along with the parental races from *nasuta-albomicans* complex revealed the presence of 122 alleles for the 11 isozymes. Among these, nine were unique alleles and 113 were shared alleles. Among the shared alleles, 52 were common to all the six races analysed. Overall, *D. n. nasuta* has 96 alleles, *D. n. albomicans* has 93, and Cyt-1 and Cyt-2 have 92 alleles, while Cyt-3 and Cyt-4 have 83 and 88 alleles respectively, with the alleles being represented at different frequencies in each of the races analysed.

Esterase analysis in six races revealed the presence of nine alleles of which only 1-Est^{1.0(d)} and 1-Est^{1.25} were shared by all. 1-Est⁰ was unique to Cyt 4. The frequency of 1-Est alleles ranged from 0.7 (1-Est⁰) to 60% (1-Est^{1.0(d)}). Genetic distance was highest between Cyt-1 and Cyt-3 (0.78), while the least genetic distance of 0.03 was noticed between Cyt-4 and Cyt-3.

In the case of 2-Est, 14 alleles with frequencies ranging from 0.4 (2-Est^{0.96}) to 52% (2-Est^{1.0}) were recorded. Three alleles, namely 2-Est^{1.3}, 2-Est^{1.54} and 2-Est^{1.61} were found to be unique to *D. n. albomicans*, Cyt-2 and *D. n. nasuta* respectively. 2-Est^{0.84}, 2-Est^{1.0(d)} and 2-Est^{1.25} were

shared among all the six races. When the allelic frequencies were analysed Cyt-1 and Cyt-3 showed 0.75 distance, while the distance was least between Cyt-3 and Cyt-4, with a value of 0.02.

Localization of alkaline phosphatase (Aph) in six races revealed 17 alleles, whose frequencies ranged from 0.3 (Aph^{1.21}) to 43.8% (Aph^{1.0}). Eight alleles, namely Aph^{1.0}, Aph^{0.71}, Aph^{0.76}, Aph^{1.01}, Aph^{1.16}, Aph^{1.02}, Aph^{0.96} and Aph^{1.06} were shared by all. None of the races under study possess unique alleles for Aph loci. The genetic distance based on Aph allelic frequencies indicated a maximum distance of 0.43 between Cyt-1 and Cyt-4, while the least distance was between Cyt-1 and Cyt-2 (0.09).

Localization of acid phosphatase (Acph) resulted in the identification of 21 alleles with a frequency of 0.1 (Acph^{1.05}) to 63% (Acph^{1.0}). Acph^{0.85}, Acph^{0.9}, Acph^{0.95}, Acph^{1.0}, Acph^{1.10}, Acph^{1.17}, Acph^{1.2}, Acph^{1.25}, Acph^{1.3} and Acph^{1.35} were shared among all, while Acph^{1.05} was found to be unique to *D. n. albomicans*. The maximum genetic distance was noticed between Cyt-1 and *D. n. nasuta* (0.34), while it was the least between Cyt-3 and *D. n. albomicans* (0.045).

For xanthine dehydrogenase (XDH) nine alleles were localized, the frequencies of which ranged from 9 (XDH^{1.02}) to 97.5% (XDH^{1.0}). Two alleles, XDH^{0.98} and XDH^{1.06}, were unique to Cyt-1 and *D. n. albomicans* respectively. The allele XDH^{1.02} was noticed only among the Cytoraces. Nei's genetic distance based on allelic frequency showed Cyt-1 and Cyt-2 to be at maximum distance of 0.074, while Cyt-1 and Cyt-3 gave a least genetic distance of 0.007.

For α -glycerophosphate dehydrogenase (α -GPD), nine alleles with a frequency range of 0.6 (α -GPD^{0.91}) to 89.7% (α -GPD^{1.0}) were localized. One allele, α -GPD^{0.91}, was unique to *D. n. albomicans*. The genetic distance value was highest between *D. n. nasuta* and Cyt-2 (0.21), while it was least between Cyt-3 and Cyt-2 (0.018).

In the case of superoxide dismutase (SOD) with a frequency range of 15 (SOD^{0.92}) to 92% (SOD^{1.0}), ten alleles could be localized for SOD. Six alleles were common to all the races, while four were found to be shared at least between two races. Genetic distance was highest between Cyt-3 and Cyt-4 (0.218) and least between Cyt-2 and *D. n. albomicans* (0.0159).

Eight alleles were localized for octanol dehydrogenase (ODH) and all of them were shared, with four alleles being common to all the six races analysed. The frequencies of these alleles ranged from 2 (ODH^{0.93}) to 100% (ODH^{1.0}). It is the only isozyme where a monomorphic allele was noticed, namely ODH^{1.0}. Further, two alleles were found to be shared only among Cytoraces, namely ODH^{0.93} and ODH^{1.05}. Maximum genetic distance was noticed between *D. n. nasuta* and Cyt-1 (0.094) and the least between Cyt-2 and Cyt-3 (0.009).

For malate dehydrogenase (MDH) eight alleles with a frequency range of 0.8 (MDH^{1.10}) to 99.7% (MDH^{1.0})

were noticed. Five alleles were common to all six races, while three were shared at least between two races. A maximum genetic distance of 0.19 was noticed between Cyt-3 and Cyt-4, while the least value of 0.008 was seen between *D. n. nasuta* and Cyt-4.

For alcohol dehydrogenase (ADH) with a frequency range of 0.3 (ADH^{0.96}) to 99.8% (ADH^{1.0}), six alleles were noticed for the ADH loci. There are no unique alleles, with ADH^{0.93} and ADH^{1.0} being common to all six races; the remaining were shared at least between two races. The least genetic distance of 0.014 was noticed between Cyt-4 and *D. n. albomicans*, while a maximum of 0.165 was noticed between Cyt-4 and Cyt-1.

For glucose-6-phosphate dehydrogenase (G6PD) with a frequency range of 0.006 (G6PD^{1.20}) to 56% (G6PD^{1.0}), 11 alleles could be localized for G6PD. Six alleles were common to all the races and four were found to be shared at least between two races, while one allele, G6PD^{1.20}, was found to be unique to *D. n. nasuta*. Genetic distance was highest between Cyt-2 and *D. n. nasuta* (0.398) and least between Cyt-1 and *D. n. albomicans* (0.0559).

For these alleles of 11 isozymes, inter-parental, parent vs Cytorace and inter-Cytorace comparisons were made to unravel the pattern of introgression of alleles.

Let us consider inter-parental comparison. *D. n. nasuta* harbours 96 alleles and *D. n. albomicans* has 93 alleles. Sixteen alleles of *D. n. nasuta* were not recorded in *D. n. albomicans*, while 13 alleles of *D. n. albomicans* were not represented in *D. n. nasuta*.

Further, among the 16 alleles, 2-Est^{1.61} and G6PD^{1.20} were unique to *D. n. nasuta*, while among the 13 alleles found in *D. n. albomicans*, four were unique to *D. n. albomicans*, namely 2-Est^{1.3}, Acph^{1.05}, XDH^{1.06} and α -GPD^{0.91}. Among the 80 alleles that were found to be shared by both the parents, 40 gave significant values in pair-wise comparison at 5% level of significance [Z-test].

Now consider parent vs Cytorace comparison. Cyt-1 and Cyt-2 both harbour 92 alleles each, of which Cyt-1 shares 79 alleles with *D. n. nasuta* and 77 alleles with *D. n. albomicans*, while Cyt-2 shares 76 alleles with *D. n. nasuta* and 74 alleles with *D. n. albomicans*. Cyt-3 and Cyt-4 harbour 83 and 88 alleles respectively, among which Cyt-3 shares 73 alleles and Cyt-4 shares 75 alleles with *D. n. nasuta*, while Cyt-3 and Cyt-4 share 70 and 73 alleles with *D. n. albomicans* respectively.

Pair-wise comparison of the level of significance for frequency distribution at 5% level revealed that among the 79 and 77 alleles that Cyt-1 shares with each of its parent, 39 and 37 alleles were significant. Among the 76 alleles that Cyt-2 shares with *D. n. nasuta*, 44 were found to be significant and among the 74 alleles it shares with *D. n. albomicans*, 36 showed significance for frequency distribution. Forty-two of the 73 alleles that Cyt-3 shares with *D. n. nasuta* gave significant value for the frequency distribution, while 38 of 70 alleles which it shares with *D. n. albomicans* were significant. Similarly, 38 of the 75

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alleles that Cyt-4 shares with *D. n. nasuta* gave significant value, while 33 of the 73 alleles shared with *D. n. albomicans* were significant at 5% level of significance [Z-test].

Among the 16 alleles noticed in *D. n. nasuta*, 14 were found to have introgressed into the genomes of Cytoraces with different frequencies. Not all 14 were common to all Cytoraces; Cyt-1 shared 8 of the 14 alleles, while Cyt-2, Cyt-3 and Cyt-4 shared 10, 8 and 9 alleles respectively. And of the 13 alleles of *D. n. albomicans*, four were unique and of the remaining nine alleles, six were also represented in Cyt-1 and 8, 5 and 6 alleles were found to have introgressed into the genomes of Cyt-2, Cyt-3 and Cyt-4 respectively.

A similar attempt to assess alleles common to both parents and the pattern of their introgression in Cytoraces revealed 35 alleles to be common to both *D. n. nasuta* and *D. n. albomicans*. Among these, two are exclusive to parental races, namely 2-Est^{0.87} and 2-Est^{0.96}, while among the 33 alleles Cyt-1 shares 19 alleles, Cyt-2 shares 13, Cyt-3 shares 13 and Cyt-4 shares 16 alleles.

Among Cytoraces, 13 alleles are novel to the introgressed systems. Three alleles, namely 1-Est⁰, 2-Est^{1.54} and XDH^{0.98} are unique to Cyt-4 (frequency, 4.8%), Cyt-2 (3.7%) and Cyt-1 (12.7%) respectively. Ten alleles are shared among Cytoraces. The allele Aph^{0.97} was noticed in all four Cytoraces analysed (is common to Cytoraces), with an overall frequency of 26.6% for Cytoraces.

Next, let us consider inter Cytorace comparison. Cyt-1 shares 79, 71 and 74 alleles with Cyt-2, Cyt-3 and Cyt-4 respectively, among which 39, 39 and 35 alleles gave significant values for the frequency distribution at 5% level of significance [Z-test]. Similarly, Cyt-2 shares 75 alleles with Cyt-3 and 74 alleles with Cyt-4, among which 35 and 44 alleles gave significant values for the frequency distribution in pair-wise comparison respectively. Cyt-3 and Cyt-4 share 70 alleles, among which 31 gave significant values. Out of the ten alleles that are shared among Cytoraces, Aph^{0.97} is common to all the Cytoraces and the remaining nine alleles showed differential representation in different Cytoraces. Among these,

Cyt-1 has eight alleles and Cyt-2, Cyt-3 and Cyt-4 have 9, 5 and 8 alleles respectively.

Genetic distance calculated based on the frequency of 122 alleles among six races ranged from 0.091 to 0.219, with the maximum distance between Cyt-1 and Cyt-3 and the least between Cyt-1 and *D. n. albomicans* (Table 1). The dendrogram based on genetic distance obtained by combined results of eleven isozymes using the NJ tree (Figure 1) gave two clusters. In one clade, Cyt-1 and *D. n. albomicans* cluster with *D. n. nasuta*, while in the other clade Cyt-2 and Cyt-3 cluster with Cyt-4.

In the present analysis based on distribution, the alleles are classified as common, shared and unique. The basis for their classification is as follows: the alleles which are distributed in all the six races analysed are termed as common, those which are found in at least two races are termed as shared and those that are specific to any one of the races are unique. In the present investigation, overall 122 alleles could be identified, among which 52 were common, 9 were unique, whereas the remaining 70 alleles were shared.

The unique alleles and those which are shared between only two races occur in lower frequencies; so also the null alleles. The unique alleles are a reflection of divergence among the races. And the differential representation of allelic frequencies between *D. n. nasuta* and *D. n. albomicans* is a measure of extent of polymorphism in intra-race comparison and a measure of divergence in the inter-race comparison. The differential allelic frequencies among the introgressed hybrids of *D. n. nasuta* and *D. n. albomicans* (the Cytoraces) are a measure of genetic variability, incorporated due to hybridization and introgression. As reported by Riesberger and Wendel³⁷, the introgressed population is found to exhibit alleles of both parents as well as new single and multi-locus genotypes. Those alleles which are unique to Cytoraces, also called as hybridzymes³⁸, may be recombined products of either the genes inherited from both the parents or the products of genes obtained through recombination or the reflection of gene expression in a co-adapted hybrid genetic background.

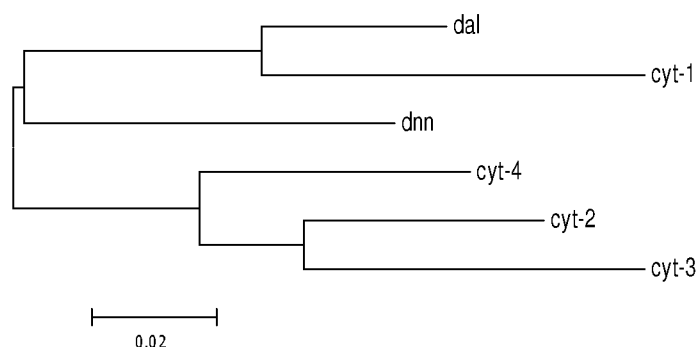


Figure 1. Dendrogram based on 11 isozymal genetic distance data (NJ tree from MEGA 2.0 software).

Table 1. Genetic distance (upper diagonal) and genetic identity (lower diagonal) for the combined 11 isozymes

Overall 11 isozymes	<i>D. n.</i>					
	<i>D. n. nasuta</i>	<i>albomicans</i>	Cytorace-1	Cytorace-2	Cytorace-3	Cytorace-4
<i>D. n. nasuta</i>	0	0.132	0.153	0.152	0.173	0.118
<i>D. n. albomicans</i>	0.867	0	0.091	0.128	0.17	0.162
Cytorace-1	0.847	0.909	0	0.163	0.22	0.186
Cytorace-2	0.848	0.872	0.837	0	0.093	0.108
Cytorace-3	0.827	0.830	0.780	0.907	0	0.105
Cytorace-4	0.882	0.838	0.814	0.891	0.894	0

Hewitt⁹ reported that both exogenous (extrinsic) and endogenous (intrinsic) selection operate on the hybrids. If selection against hybrids is strong or if the genes that contribute to reduced hybrid fitness are distributed throughout the genome³⁹, such gene combinations may be rare⁴⁰ or nonexistent for some loci. A favourable combination would initially arise at low frequencies and could be lost⁴¹ or fixed by genetic drift^{11,42}. Further genetic drift or population bottleneck is also found to decrease variability among the introgressants that are reproductively isolated from their parental taxa⁴³. During the course of evolution of these Cytoraces, the founder events following hybrid or recombinational speciation⁴⁴, in terms of differential chromosomal representation of the parental genomes led to the establishment of new gene pools with different genomic compatibility. Thus, the lower number of alleles harboured by Cytoraces in comparison with both the parental races is likely to be a reflection of the genetic drift in action.

The neutral mutations give widespread enzyme polymorphism in *Drosophila*⁴⁵, but the same alleles being found in similar frequencies among different sub-populations of a species indicates operation of balancing selection³⁴. Gellipsei and Kojima⁴⁶ found that the loci encoding enzymes which utilize substrates originating from the external environment are far more polymorphic than loci whose enzymes utilize internal metabolites, suggesting that genetic polymorphism reflects a physiological response to the environmental variation. Since environmental variation in our experimental system is minimized (for the entire experimental set-up is in laboratory environment), the extensive polymorphism we noticed could be mainly due to the neutral mutation and also the overlapping substrate specificity of the isozymes, because of which similar variations escape natural selection. Among Cytoraces, the introgression of parental genomes has not only led to novel chromosomal complement but also to the varied cytoplasmic milieu in which they lie. The interaction of reshuffled parental genome and the cytoplasm could probably be one of the causative factors for the high level of polymorphism observed in these laboratory hybrids.

Genetic distance, when calculated based on allelic frequencies among these races, was least between Cyt-3 and Cyt-4 and highest between Cyt-1 and Cyt-4. Based on the

genetic distance, when the divergence time between *D. n. nasuta* and *D. n. albomicans* was calculated as postulated by Carson⁴⁷, it comes out to 264,000 yrs, which is in sharp contrast to the 500,000 yrs as given by Chang *et al.*⁴⁸, on the basis of mtDNA analysis. However, when similar calculations were applied to the genetic distance of Cytoraces, the divergence time ranged from 182,000 yrs to 346,000 yrs between different Cytoraces and parents, and about 184,000 to 438,000 yrs among Cytoraces. Gaggiotti and Excoffier⁴⁹ opined that the conventional genetic distances are not proportional to the divergence time. Cariou⁵⁰ argued that large discrepancies exist between different values depending on different assumptions and felt that such inferences of divergence time are highly speculative. However, the allozyme estimates (using Nei values), the lowest values established by Adh DNA sequencing⁵¹ and palaeobiographic arguments⁵² are consistent with each other and thus may correspond to the true divergence times. But the actual time of origin of the Cytoraces under investigation is ≈ 15 yrs. Thus within a span of one and a half decades, a new assemblage of *Drosophila* races has evolved in the environs of the laboratory, which is comparable to other assemblages of the *Drosophila nasuta* subgroup that are the products of evolution in nature. The cause for such a vast divergence within a span of such a short duration could be hybridization and introgression of the parental genomes. As reported by Jiang *et al.*⁵³, genomic interactions do not always favour host chromatin; rather, it appears that favourable hetero-specific interactions may encourage the introgression of certain chromosomal blocks from one taxon to another. However, at this instance the critical role of hybrid founder events during the establishment of new hybrid lineages are not to be underestimated, for they are the major contributors to the new introgressed gene pool.

The catalytic role of introgression following hybridization was put forth by Anderson¹¹, which was time and again strengthened by various investigators⁵⁴⁻⁵⁷. However, Hercus and Hoffman⁷ have felt that these studies are only indirect arguments and there was uncertainty due to lack of direct evidence. However, it was opined that direct evidence could be obtained from experimental evolution, where the impact of hybridization could be directly compared with that of parental species or races. In this

regard the *nasuta*–*albomicans* complex offers an opportunity to understand and unravel the sequence of evolutionary consequences among the introgressed populations over 350–500 generations. Earlier studies among members of these karyotypically differentiated and closely related races have shown detectable divergence at different levels of organization, such as morphometric, karyotypic, parameters of fitness, and for a few components of pre-mating reproductive isolation. The present dendrogram based on genetic distance (Figure 1) of isozymes indicates clustering of Cyt-1 and *D. n. albomicans*, which supports the karyotypic data^{21,22} and studies on morphometric traits, such as abdominal bristle number, sternopleural bristle number, body size, etc.^{28–30}. Analysis of courtship element has revealed that Cyt-1 has diverged from *D. n. albomicans* (M. C. Shilpa and H.A. Ranganath, under preparation). The clustering of Cyt-2 along with Cyt-3 does not support the karyotypic data; however it supports findings on morphometric traits. The clustering of Cyt-4 with Cyt-3 and Cyt-2 could neither be correlated with morphometric traits nor with the mating preference. Even though Cyt-3 and Cyt-4 are found to be closer when these parameters are taken into consideration, Cyt-2 is found to deviate. With respect to mating latency and incidence of lack of mating, Cyt-2 deviates the most from *D. n. albomicans* and when the copulation duration is taken into consideration *D. n. nasuta* deviates the most²⁶ from Cyt-2. This is in accordance with the present findings, wherein Cyt-2 is represented in a different clade, while *D. n. nasuta* and *D. n. albomicans* are represented in another.

Thus the pattern of introgression among these neo-races for different sets of characters gives a deviating picture reflecting lack of correlation for unrelated characters, enshrining population diversification to be a multi-dimensional process with drift, perhaps, playing an important role. Hence, a precise formulation for these events cannot be designed. Finally, the present study also highlights the predominant role of introgressive hybridization in shaping the extent of genetic diversity, interracial differences and population structuring among a few members of the *nasuta*–*albomicans* complex of *Drosophila*.

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On the occurrence of *Chordodes cf. furnessi* (Nematomorpha) from praying mantis in India, and a note on Indian nematomorph species

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Two worms collected from a praying mantis, *Hierodula* sp., in India were investigated using light and scanning electron microscopy for species determination. Three types of cuticular structures (areoles) were distinguishable on the cuticle with characteristic distribution patterns. The observed characteristics of the specimens studied are very close to those of *Chordodes furnessi*, which was reported from Borneo in 1898. Nevertheless, a difference is observed in one type of areole along both sides of the ventral midline. There is no mention of this type of areole in the specimens described from Borneo. We presume this character

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