

# Racial divergence in the foreleg bristles of four members of the *nasuta*–*albomicans* complex of *Drosophila*

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*Drosophila nasuta nasuta* and *D. n. albomicans* are a pair of sibling allopatric chromosomal races of *nasuta* subgroup of *Drosophila*, and have resulted in the evolution of two new karyotypic strains called Cytorace 1 and Cytorace 2. These two races are considered to be the members of the newly evolved *nasuta*–*albomicans* complex of *Drosophila*, which are allo-sympatric populations useful for understanding the early events of riation under laboratory conditions. These newly developed Cytoraces have shown appreciable divergence in foreleg bristle number, displaying increased foreleg bristle number with reduced foreleg length, in comparison to *D. n. nasuta* and *D. n. albomicans*, which suggests a negative correlation between foreleg bristle number and foreleg length. This racial divergence could be due to the transgressive segregation of genes responsible for foreleg bristle number during the evolution of these Cytoraces.

DURING last two decades, the *nasuta* subgroup of the *immigrans* species group of *Drosophila* has attracted the attention of taxonomists, cytogeneticists, biochemists, molecular biologists and evolutionary biologists. This subgroup of *Drosophila* has certain evolutionary peculiarities, which include little morphological differentiation among species despite their distribution over an enormous territory, and the ability of species to intercross in the laboratory, often producing fertile offspring and substantial chromosomal evolution in the hybrids<sup>1</sup>. These features make this subgroup a potent system to study the genetics of early stages of speciation in *Drosophila*. *D. nasuta nasuta* ( $2n=8$ ) and *D. n. albomicans* ( $2n=6$ ) are a pair of sibling allopatric chromosomal races of the *nasuta* subgroup of *Drosophila*. The cytological distinctness of these two races has been extensively studied<sup>2–7</sup>. Interracial hybridization between *D. n. nasuta* and *D. n. albomicans* followed by the maintenance of hybrid populations for over 20 generations, has resulted in the emergence of two new karyotypic strains called Cytorace 1 and Cytorace 2 (ref. 6). Cytorace 1 is the product of interracial hybridization between the males of *D. n. nasuta* and females of *D. n. albomicans*. It has  $2n=7$  in males ( $2^n 2^a$

$Y^n 3^n X3^a 4^n 4^n$ ) and  $2n=6$  in females ( $2^n 2^a X3^a X3^a 4^n 4^n$ ). Cytorace 2 is the outcome of interracial hybridization between females of *D. n. nasuta* and males of *D. n. albomicans*. Both males and females of Cytorace 2 have  $2n=6$  ( $2^n 2^a X3^a X3^a/Y3^a 4^a 4^a$ )<sup>6</sup> (superscripts  $n$  and  $a$  represent the chromosomes of *D. n. nasuta* and *D. n. albomicans*, respectively). Each of these Cytoraces is the result of hybrid recombination and drift, in turn retaining some specific chromosomes and eliminating certain chromosomes.

These newly created Cytoraces, along with their parental races, constitute a new assemblage of allo-sympatric populations, the *nasuta*–*albomicans* complex of *Drosophila*<sup>8,9</sup>. Earlier studies on cytogenetic differentiation<sup>6,8</sup>, mating preference<sup>10</sup>, a few fitness traits<sup>11</sup>, sternopleural bristle number<sup>12</sup>, body size<sup>13</sup>, body weight<sup>14</sup> and abdominal bristle number<sup>15</sup> have shown significant differences between parental races and Cytoraces. In view of this, we report the early event of racial divergence in foreleg bristle number of four members of the *nasuta*–*albomicans* complex of *Drosophila*.

## Materials and methods

### Experimental populations

The following *Drosophila* stocks were used in the present experiments: (a) *Drosophila nasuta nasuta* (Coorg, India); (b) *Drosophila nasuta albomicans* (Okinawa strain, Texas collection, USA, 3045.11); (c) Cytorace 1 and Cytorace 2 (ref. 6).

We have already reported the karyotypic compositions of these two Cytoraces along with their parents, *D. n. nasuta* and *D. n. albomicans*<sup>6,11</sup>. At the time of the present experiment, Cytorace 1 and Cytorace 2 had passed through 350 generations. In the evolution of each of these Cytoraces, the starting population size was around 10 pairs of flies. In every generation, flies from five replicate cultures were mixed and distributed to five new bottles (~100 flies in each bottle). All the above stocks were cultured in wheat cream agar medium in an uncrowded (~50 flies/bottle) culture condition at  $22 \pm 1^\circ\text{C}$  and relative humidity of 70%.

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### Counting of foreleg bristles

Sixty adult males and females of all four races were etherized and the left foreleg of each of these flies was dissected with a fine needle using physiological saline, under stereomicroscope. These forelegs were mounted on a slide with a drop of DPX, and the various bristle phenotypes of the nine segments in the foreleg were observed, categorized and recorded under a Leica DMRB microscope based on size, shape, location and distribution pattern, as followed by Schubiger<sup>16</sup>.

The mean values of foreleg bristle number were subjected to one-way analysis of variance with general linear model. The bristle phenotypes and races were the two factors used for this assessment. Further, Duncan's multiple range test (DMRT) was used for pairwise comparisons. Principal component analysis (PCA) was also applied for mean values of foreleg bristle number to estimate the divergence among these races. Correlation analysis was done between the values of bristle phenotypes and our earlier published data on foreleg length<sup>13</sup>. All analyses were implemented on statistical presentation system software for MS Windows.

### Results

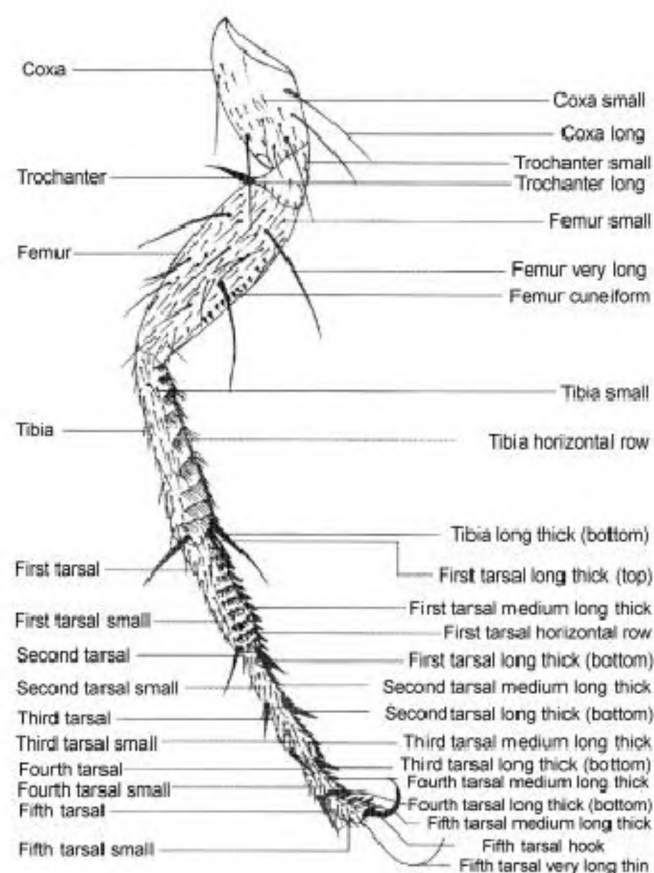
The foreleg of *Drosophila* has nine segments, namely coxa, trochanter, femur, tibia and five tarsal segments, each with distinct type of bristles. The foreleg bristles of *Drosophila melanogaster* are classified into 28 types based on the size, shape and location<sup>16</sup>. In the present study, three of the 28 bristle phenotypes (small bristles of femur, tibia and first tarsal segment) were not considered, since they are numerous and hence not easy to count and record (Figure 1).

Tables 1 and 2 present the mean foreleg bristle number in males and females of all four races of the *nasuta-albomicans* complex of *Drosophila*, respectively. Among the 25 bristle types, four kinds of bristles were similar in number in all four races in males (Table 1). The remaining 21 bristle phenotypes were further categorized into two types based on statistical analysis: bristles with significant differences (BSD) and bristles with insignificant differences (BID). Among the 21 bristle types, 12 had significant differences and were considered BSD, while the remaining nine were BID. The information on pairwise comparisons of foreleg bristles of BSD in the males of four members of the *nasuta-albomicans* complex of *Drosophila* revealed that among the 12 significantly diverged bristles, only three had significant differences between *D. n. nasuta* and Cytorace 1. On the other hand, *D. n. albomicans* and Cytorace 2 had differences in 11 out of 12 types of bristles (11/12). These results indicate that Cytorace 1 is closer in foreleg bristle number to *D. n. nasuta* than *D. n. albomicans*, while Cytorace 2 is more

diverged in bristle number from *D. n. albomicans* than *D. n. nasuta*.

The BSD types in females were different from the BSD types in males. Pairwise comparisons of BSD of foreleg bristles in females of four races of the *nasuta-albomicans* complex of *Drosophila* indicate that among the 12 significantly diverged bristle types in females, *D. n. nasuta* and Cytorace 2 had the greatest divergence, with eleven bristle types (11/12), whereas the difference between Cytorace 1 and Cytorace 2 was the least (5/12), and all other comparisons were in the range of 40 to 70% (Table 2). This indicates that the females of *D. n. nasuta* and Cytorace 2 are more divergent in bristle number than the others.

Among the 25 bristle types, four kinds of bristles were conserved when male and female data were combined (Table 3). In the remaining 21 types of bristles, ten showed significant differences. The pairwise comparisons of these foreleg bristles when male and female data were combined revealed that the difference between *D. n. nasuta* and Cytorace 1 in ten types of BSD was the least (3/10), whereas the differences between *D. n. albomicans* and Cytorace 2 as well as Cytorace 1 and Cytorace 2 were the highest (7/10). This indicates that *D. n. nasuta* and Cytorace 1 are less divergent in bristle number, while *D. n. albomicans* and Cytorace 2 are more divergent.



**Figure 1.** Distribution of foreleg bristle phenotype.

**Table 1.** Mean foreleg bristle number in males of four members of the *nasuta*–*albomicans* complex of *Drosophila* (values are mean  $\pm$  SE of 60 flies) along with statistical analysis

Segment	Bristle phenotype	Race				Analysis of variance		Duncan's multiple range test
		<i>D. n. nasuta</i> (N)	<i>D. n. albomicans</i> (A)	Cytorace 1 (C1)	Cytorace 2 (C2)	<i>F</i> -ratio	<i>P</i> -value	
Coxa	Long	4.71 $\pm$ 0.11	4.53 $\pm$ 0.10	4.53 $\pm$ 0.10	4.75 $\pm$ 0.11	0.8811	$P > 0.4516$	Not significant
	Small	12.31 $\pm$ 0.26	14.15 $\pm$ 0.34	14.01 $\pm$ 0.29	11.98 $\pm$ 0.43	12.1978	$P < 0.0001$	N/A, N/C1, N/C2, C1/C2
Trochanter	Long	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	–	–	Conserved
	Small	9.77 $\pm$ 0.21	9.91 $\pm$ 0.20	9.35 $\pm$ 0.18	9.90 $\pm$ 0.13	2.0752	$P > 0.1042$	Not significant
Femur	Cuneiform	8.40 $\pm$ 0.14	8.50 $\pm$ 0.17	9.17 $\pm$ 0.12	9.37 $\pm$ 0.12	12.0220	$P < 0.0001$	N/C1, N/C2, A/C1, A/C2
	Very long	8.68 $\pm$ 0.20	8.73 $\pm$ 0.16	8.70 $\pm$ 0.22	9.60 $\pm$ 0.21	6.1168	$P < 0.0005$	N/C2, A/C2, C1/C2
Tibia	Horizontal rows	9.10 $\pm$ 0.10	9.17 $\pm$ 0.10	9.10 $\pm$ 0.11	9.21 $\pm$ 0.12	0.2723	$P > 0.8453$	Not significant
	Long thick (bottom)	1.55 $\pm$ 0.06	1.50 $\pm$ 0.06	1.52 $\pm$ 0.06	1.43 $\pm$ 0.06	0.5723	$P > 0.6338$	Not significant
First tarsal	Horizontal rows	10.97 $\pm$ 0.11	10.57 $\pm$ 0.10	10.78 $\pm$ 0.09	10.00 $\pm$ 0.13	14.7437	$P < 0.0001$	N/A, N/C2, A/C2, C1/C2
	Long thick (top)	1.48 $\pm$ 0.06	1.52 $\pm$ 0.06	1.40 $\pm$ 0.06	1.50 $\pm$ 0.06	0.6405	$P > 0.5897$	Not significant
	Long thick (bottom)	1.00 $\pm$ 0.00	1.06 $\pm$ 0.04	1.07 $\pm$ 0.05	1.13 $\pm$ 0.06	0.2425	$P > 0.6371$	Not significant
	Medium long thick	5.03 $\pm$ 0.11	4.45 $\pm$ 0.15	4.82 $\pm$ 0.09	4.78 $\pm$ 0.10	4.5665	$P < 0.0039$	N/A, A/C1, A/C2
Second tarsal	Small	19.20 $\pm$ 0.31	17.12 $\pm$ 0.40	17.42 $\pm$ 0.36	18.47 $\pm$ 0.40	6.7447	$P < 0.0002$	N/A, N/C1, A/C2, C1/C2
	Long thick (bottom)	1.65 $\pm$ 0.06	1.58 $\pm$ 0.06	1.75 $\pm$ 0.06	1.51 $\pm$ 0.06	2.5757	$P < 0.0546$	C1/C2
	Medium long thick	5.03 $\pm$ 0.11	4.45 $\pm$ 0.15	4.82 $\pm$ 0.09	4.78 $\pm$ 0.10	4.5665	$P < 0.0039$	N/A, A/C1, A/C2
Third tarsal	Small	11.95 $\pm$ 0.26	12.51 $\pm$ 0.33	12.21 $\pm$ 0.24	11.62 $\pm$ 0.29	1.5874	$P > 0.1931$	Not significant
	Medium long thick	3.82 $\pm$ 0.11	3.83 $\pm$ 0.11	3.82 $\pm$ 0.12	4.02 $\pm$ 0.10	0.8458	$P > 0.4701$	Not significant
	Long thick (bottom)	1.50 $\pm$ 0.06	1.65 $\pm$ 0.06	1.55 $\pm$ 0.06	1.38 $\pm$ 0.06	3.0173	$P < 0.0306$	A/C2
Fourth tarsal	Small	10.77 $\pm$ 0.23	10.13 $\pm$ 0.18	10.85 $\pm$ 0.21	11.21 $\pm$ 0.18	4.1822	$P < 0.0066$	N/A, A/C1, A/C2
	Medium long thick	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	–	–	Conserved
	Long thick (bottom)	1.37 $\pm$ 0.06	1.43 $\pm$ 0.06	1.52 $\pm$ 0.06	1.53 $\pm$ 0.06	1.4491	$P > 0.2292$	Not significant
Fifth tarsal	Hook	1.71 $\pm$ 0.06	1.65 $\pm$ 0.06	1.83 $\pm$ 0.05	1.97 $\pm$ 0.02	7.5906	$P < 0.0001$	N/C2, A/C1, A/C2
	Small	11.48 $\pm$ 0.28	11.70 $\pm$ 0.28	11.33 $\pm$ 0.33	10.22 $\pm$ 0.18	5.7684	$P < 0.008$	N/C2, A/C2, C1/C2
	Very long thin	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	–	–	Conserved
	Medium long thick	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	–	–	Conserved

Table 4 provides the total mean of all the foreleg segment bristles in four members of the *nasuta*–*albomicans* complex of *Drosophila*. Except the males of Cytorace 1, the males of the other three races had shown decreased number of foreleg bristles compared to their females. Males of Cytorace 1 were found to have a greater number of foreleg bristles than their females, but the difference between them was insignificant. In males, females, and in both males and females together, *D. n. nasuta* had the lowest number of foreleg bristles, while Cytorace 2 had the highest number of foreleg bristles. The DMRT for foreleg bristles is significant between the males of *D. n. nasuta* with Cytorace 1 and Cytorace 2. In females, Cytorace 2 had differences with *D. n. nasuta*, *D. n. albomicans*, and Cytorace 1. The difference was also significant between females of *D. n. nasuta* with *D. n. albomicans* as well as Cytorace 1. In both males and females together, Cytorace 2 had significant differences with *D. n. nasuta*, Cytorace 1 and *D. n. albomicans*, and also differences were significant between *D. n. nasuta* and Cytorace 1. Thus, the order of ranking is as follows:

Males: Cytorace 2  $\geq$  Cytorace 1  $\geq D. n. albomicans \geq D. n. nasuta$ ; Females: Cytorace 2  $> D. n. albomicans \geq$  Cyto-

race 1  $> D. n. nasuta$ ; both males and females together: Cytorace 2  $>$  Cytorace 1  $\geq D. n. albomicans > D. n. nasuta$ .

PCA is a method that allows one to summarize the information of many correlated measures extracted through linear combinations. The results of PCA for the 21 significant foreleg bristle phenotypes in males and females (Table 5) revealed that (i) *D. n. nasuta* differs from the other three races with less per cent variation in males as well as in females and, (ii) Cytorace 2 showed less variation with *D. n. albomicans* and Cytorace 1, but the per cent variance is maximum with *D. n. nasuta*.

Our earlier studies<sup>13</sup> showed that *D. n. albomicans* and Cytorace 1 had the highest and the lowest mean values for foreleg length, respectively in males, females and in both males and females together. Thus, the order of ranking is as follows: males as well as both males and females together – *D. n. albomicans*  $> D. n. nasuta >$  Cytorace 2  $>$  Cytorace 1; females – *D. n. albomicans*  $\geq D. n. nasuta >$  Cytorace 2  $>$  Cytorace 1. The present analysis of correlation between the foreleg bristle number and foreleg length of *D. n. nasuta* (males,  $-0.756$ ; females,  $-0.784$ ), *D. n. albomicans* (males,  $-0.836$ ; females,  $-0.862$ ), Cytorace 1 (males,  $-0.732$ ; females,  $-0.997$ ) and Cytorace 2

**Table 2.** Mean foreleg bristle number in females of four members of the *nasuta*–*albomicans* complex of *Drosophila* (values are mean  $\pm$  SE of 60 flies) along with statistical analysis

Segment	Bristle phenotype	Race				Analysis of variance		Duncan's multiple range test
		<i>D. n. nasuta</i> (N)	<i>D. n. albomicans</i> (A)	Cytorace 1 (C1)	Cytorace 2 (C2)	F-ratio	P-value	
Coxa	Long	4.63 $\pm$ 0.11	4.45 $\pm$ 0.08	4.53 $\pm$ 0.09	4.60 $\pm$ 0.09	0.6716	$P > 0.5702$	Not significant
	Small	13.76 $\pm$ 0.19	16.10 $\pm$ 0.32	15.73 $\pm$ 0.33	14.66 $\pm$ 0.26	13.6902	$P < 0.0001$	N/A, N/CI, N/C2, A/C2, C1/C2
Trochanter	Long	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	–	–	Conserved
	Small	8.80 $\pm$ 0.19	10.28 $\pm$ 0.28	9.58 $\pm$ 0.17	10.78 $\pm$ 0.22	14.8676	$P < 0.0001$	N/A, N/CI, A/C1, A/C2, C1/C2
Femur	Cunieform	8.15 $\pm$ 0.12	9.38 $\pm$ 0.13	10.33 $\pm$ 0.14	9.28 $\pm$ 0.13	42.0020	$P < 0.0001$	N/CI, N/C2, A/C1, A/C2
	Very long	8.75 $\pm$ 0.18	9.05 $\pm$ 0.19	9.76 $\pm$ 0.21	9.78 $\pm$ 0.23	6.3885	$P < 0.0004$	N/C2, A/C1, A/C2
Tibia	Horizontal rows	9.53 $\pm$ 0.07	9.56 $\pm$ 0.10	9.36 $\pm$ 0.08	9.91 $\pm$ 0.12	5.1728	$P < 0.0018$	N/C2, A/C2, C1/C2
	Long thick (bottom)	1.30 $\pm$ 0.05	1.35 $\pm$ 0.06	1.46 $\pm$ 0.06	1.43 $\pm$ 0.06	1.4713	$P > 0.2230$	Not significant
First tarsal	Horizontal rows	11.03 $\pm$ 0.09	10.95 $\pm$ 0.09	10.76 $\pm$ 0.12	10.53 $\pm$ 0.13	3.8995	$P < 0.0096$	N/C2, A/C2
	Long thick (top)	1.48 $\pm$ 0.06	1.45 $\pm$ 0.06	1.50 $\pm$ 0.06	1.50 $\pm$ 0.06	0.1315	$P > 0.9413$	Not significant
	Long thick (bottom)	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.20 $\pm$ 0.07	1.00 $\pm$ 0.00	0.2115	$P > 0.7121$	Not significant
	Medium long thick	5.81 $\pm$ 0.11	5.66 $\pm$ 0.10	5.71 $\pm$ 0.12	5.70 $\pm$ 0.12	0.2993	$P > 0.8259$	Not significant
Second tarsal	Small	14.26 $\pm$ 0.32	16.10 $\pm$ 0.40	15.98 $\pm$ 0.40	15.48 $\pm$ 0.49	4.1165	$P < 0.0072$	N/A, N/CI, N/C2
	Long thick (bottom)	1.81 $\pm$ 0.05	1.88 $\pm$ 0.04	1.56 $\pm$ 0.06	1.46 $\pm$ 0.06	12.4580	$P < 0.0001$	N/CI, N/C2, A/C1, C1/C2
	Medium long thick	5.06 $\pm$ 0.11	5.16 $\pm$ 0.10	5.10 $\pm$ 0.08	5.13 $\pm$ 0.09	0.1868	$P > 0.9053$	Not significant
Third tarsal	Small	10.33 $\pm$ 0.22	10.53 $\pm$ 0.20	10.05 $\pm$ 0.22	10.43 $\pm$ 0.22	0.8961	$P < 0.4439$	Not significant
	Medium long thick	3.51 $\pm$ 0.06	3.71 $\pm$ 0.08	3.30 $\pm$ 0.05	3.15 $\pm$ 0.04	14.1910	$P < 0.0001$	N/A, N/CI, N/C2, A/C1, A/C2
	Long thick (bottom)	1.51 $\pm$ 0.06	1.40 $\pm$ 0.06	1.30 $\pm$ 0.05	1.46 $\pm$ 0.06	2.1826	$P < 0.0908$	N/CI
Fourth tarsal	Small	9.58 $\pm$ 0.16	10.01 $\pm$ 0.10	9.65 $\pm$ 0.11	9.71 $\pm$ 0.20	1.6014	$P > 0.1898$	Not significant
	Medium long thick	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	–	–	Conserved
	Long thick (bottom)	1.60 $\pm$ 0.06	1.21 $\pm$ 0.05	1.45 $\pm$ 0.06	1.38 $\pm$ 0.06	6.6697	$P < 0.0002$	N/A, A/C1, N/C2
Fifth tarsal	Hook	1.60 $\pm$ 0.06	1.70 $\pm$ 0.05	1.63 $\pm$ 0.06	1.38 $\pm$ 0.06	4.8230	$P < 0.0028$	N/A, N/C2, A/C1, A/C2, C1/C2
	Small	10.08 $\pm$ 0.25	10.55 $\pm$ 0.25	10.45 $\pm$ 0.27	10.38 $\pm$ 0.22	0.1631	$P > 0.3471$	Not significant
	Very long thin	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	–	–	Conserved
	Medium long thick	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	–	–	Conserved

(males,  $-0.956$ ; females,  $-0.815$ ) is negative, suggesting that these two traits behave antagonistically.

## Discussion

Evolutionary biology is currently the scene of debate around the tempo and mode of morphological evolution. However, the mechanisms underlying the evolution of morphology are poorly understood<sup>17,18</sup>. Morphology is a structural and functional consequence at all levels, from cells to organisms, of activities of the genetic material. The degree of genetic similarity and differences between diverging populations can be assessed in different ways<sup>10,11,19–26</sup>, and constitutes an important tool in studying the genetics of speciation. Many investigators have studied various quantitative morphological characters of *Drosophila* to elucidate genetic variability and differentiation in natural populations<sup>27–31</sup>. However, in *Drosophila*, studies on foreleg bristles are limited. Schubiger<sup>16</sup> has studied in detail the various kinds of leg bristles of *D. melanogaster* present on different leg segments, namely coxa, trochanter, femur, tibia and the tarsals.

In the present study, 21 of the 28 bristles types of the foreleg have shown variation in the mean foreleg bristle number. The Cytoraces have more foreleg bristles than the parental races. The pairwise comparison of foreleg bristles of BSD demonstrates that the males of *D. n. albomicans* and Cytorace 2 are more divergent in bristle number, whereas the males of Cytorace 1 and *D. n. nasuta* are more similar. Similarly, the females of *D. n. nasuta* and Cytorace 2 are more diverged than the others. The application of PCA method in the present study also revealed the greater differences between the males and females of *D. n. nasuta* than males and females of the other three races. Taking together the overall analysis on foreleg bristles of these four races, one can point out that (i) Cytoraces have more bristles than their parents, and (ii) Cytorace 2 is diverging/evolving faster than Cytorace 1 with regard to foreleg bristles.

Carson and Teramoto<sup>32</sup> have reported that the bristles on the tibia of male foreleg in *D. silvestris* are used as a brush to stimulate the females during courtship. There are also observations of more homogamic matings of Cytorace 2, in the mating choice experiments with the paren-

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**Table 3.** Mean foreleg bristle number in both males and females of four members of the *nasuta*–*albomicans* complex of *Drosophila* (values are mean  $\pm$  SE of 60 flies) along with statistical analysis

Segment	Bristle phenotype	Race				Analysis of variance		Duncan's multiple range test
		<i>D. n. nasuta</i> (N)	<i>D. n. albomicans</i> (A)	Cytorace 1 (C1)	Cytorace 2 (C2)	F-ratio	P-value	
Coxa	Long	4.68 $\pm$ 0.11	4.49 $\pm$ 0.09	4.58 $\pm$ 0.09	4.68 $\pm$ 0.10	1.513	$P > 0.210$	Not significant
	Small	12.95 $\pm$ 0.22	15.13 $\pm$ 0.33	14.88 $\pm$ 0.31	13.33 $\pm$ 0.34	24.48	$P < 0.01$	N/A, N/C1, A/C2, C1/C2
Trochanter	Long	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	–	–	Conserved
	Small	9.28 $\pm$ 0.20	10.10 $\pm$ 0.24	9.47 $\pm$ 0.17	10.34 $\pm$ 0.17	12.133	$P < 0.001$	N/A, N/C2, A/C1, C1/C2
Femur	Cunieform	8.28 $\pm$ 0.13	8.94 $\pm$ 0.15	9.75 $\pm$ 0.13	9.32 $\pm$ 0.12	41.035	$P < 0.01$	N/A, N/C1, N/C2, A/C1, A/C2, C1/C2
	Very long	8.72 $\pm$ 0.19	8.89 $\pm$ 0.17	9.23 $\pm$ 0.21	9.73 $\pm$ 0.22	9.848	$P < 0.001$	N/C1, N/C2, A/C2, C1/C2
Tibia	Horizontal rows	9.32 $\pm$ 0.08	9.37 $\pm$ 0.10	9.23 $\pm$ 0.09	9.57 $\pm$ 0.12	3.626	$P < 0.013$	N/C2, C1/C2
	Long thick (bottom)	1.43 $\pm$ 0.05	1.43 $\pm$ 0.06	1.49 $\pm$ 0.06	1.43 $\pm$ 0.06	0.508	$P > 0.677$	Not significant
First tarsal	Horizontal rows	11.00 $\pm$ 0.10	10.76 $\pm$ 0.09	10.78 $\pm$ 0.12	10.27 $\pm$ 0.12	15.591	$P < 0.001$	A/C2, C1/C2
	Long thick (top)	1.48 $\pm$ 0.06	1.48 $\pm$ 0.06	1.45 $\pm$ 0.06	1.50 $\pm$ 0.06	0.386	$P > 0.760$	Not significant
	Long thick (bottom)	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.13 $\pm$ 0.06	1.06 $\pm$ 0.03	0.227	$P > 0.67$	Not significant
	Medium long thick	5.52 $\pm$ 0.11	5.53 $\pm$ 0.10	5.47 $\pm$ 0.10	5.37 $\pm$ 0.10	0.888	$P > 0.447$	Not significant
Second tarsal	Small	16.73 $\pm$ 0.31	16.61 $\pm$ 0.40	16.70 $\pm$ 0.38	16.98 $\pm$ 0.44	0.318	$P > 0.812$	Not significant
	Long thick (bottom)	1.73 $\pm$ 0.05	1.73 $\pm$ 0.05	1.66 $\pm$ 0.06	1.49 $\pm$ 0.06	7.407	$P < 0.001$	N/C2, A/C2, C1/C2
	Medium long thick	5.05 $\pm$ 0.11	4.81 $\pm$ 0.12	4.96 $\pm$ 0.08	4.96 $\pm$ 0.09	1.771	$P > 0.152$	Not significant
Third tarsal	Small	11.14 $\pm$ 0.24	11.53 $\pm$ 0.26	11.13 $\pm$ 0.23	11.06 $\pm$ 0.25	1.373	$P > 0.250$	Not significant
	Medium long thick	3.67 $\pm$ 0.08	3.78 $\pm$ 0.09	3.56 $\pm$ 0.08	3.58 $\pm$ 0.07	2.454	$P < 0.063$	A/C1
	Long thick (bottom)	1.50 $\pm$ 0.06	1.52 $\pm$ 0.06	1.42 $\pm$ 0.06	1.42 $\pm$ 0.06	2.599	$P > 0.660$	Not significant
Fourth tarsal	Small	10.18 $\pm$ 0.19	10.07 $\pm$ 0.14	10.25 $\pm$ 0.16	10.42 $\pm$ 0.19	1.291	$P > 0.277$	Not significant
	Medium long thick	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	3.00 $\pm$ 0.00	–	–	Conserved
	Long thick (bottom)	1.48 $\pm$ 0.06	1.33 $\pm$ 0.05	1.48 $\pm$ 0.06	1.46 $\pm$ 0.06	2.910	$P < 0.034$	N/A, A/C1, A/C2
Fifth tarsal	Hook	1.66 $\pm$ 0.06	1.68 $\pm$ 0.05	1.73 $\pm$ 0.06	1.68 $\pm$ 0.06	0.672	$P > 0.570$	Not significant
	Small	10.78 $\pm$ 0.26	11.13 $\pm$ 0.26	10.89 $\pm$ 0.30	10.30 $\pm$ 0.20	9.078	$P < 0.003$	N/A, A/C1, A/C2
	Very long thin	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	–	–	Conserved
	Medium long thick	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00	–	–	Conserved

**Table 4.** Mean of foreleg bristles (of all nine segments) in four members of the *nasuta*–*albomicans* complex of *Drosophila* (values are mean  $\pm$  SE of 60 flies) along with statistical analysis

Races	Mean foreleg bristles in		
	Male	Female	Both male and female
<i>D. n. nasuta</i> (N)	109.36 $\pm$ 7.06	112.22 $\pm$ 9.78	110.79 $\pm$ 8.42
<i>D. n. albomicans</i> (A)	112.27 $\pm$ 9.21	115.67 $\pm$ 11.25	113.47 $\pm$ 10.23
Cytorace 1 (C1)	114.27 $\pm$ 8.43	113.24 $\pm$ 8.94	113.76 $\pm$ 8.68
Cytorace 2 (C2)	114.50 $\pm$ 9.92	118.51 $\pm$ 9.63	116.50 $\pm$ 9.77
Analysis of variance	$F = 37.086$	$F = 35.911$	$F = 43.586$
	$d.f. = 3,236$	$d.f. = 3,236$	$d.f. = 7,472$
	$P < 0.06$	$P < 0.001$	$P < 0.001$
Duncan's multiple range test	N/C1, N/C2	N/A, N/C1, N/C2, A/C2, C1/C2	N/C1, N/C2, A/C2, C1/C2

tal races<sup>10</sup> (unpublished data). In the present study, Cytorace 2 has shown maximum differences from the parental races in the foreleg bristle phenotypes. Therefore, one can speculate that the foreleg bristles might play an important role during the sexual selection and isolation in the *nasuta* subgenus also.

In addition to these, one of the important attempts made in this study is the establishment of key/marker bristles to distinguish these closely-related four races of the *nasuta*–*albomicans* complex of *Drosophila*. The key/marker foreleg bristles identified in males are coxa small and fifth tarsal small bristles; while in females, coxa

**Table 5.** Total variance for principal component of foreleg bristles in males and females of four races of the *nasuta*–*albomicans* complex of *Drosophila*

Trait	Total				Per cent of variance				Cumulative per cent			
	N	A	C1	C2	N	A	C1	C2	N	A	C1	C2
In males												
Coxa long	2.88	3.63	3.23	5.07	13.1	17.4	16.2	17.3	13.1	17.4	16.2	17.3
Coxa small	2.61	2.44	2.54	2.35	11.9	12.8	12.7	11.7	25.0	34.8	32.4	34.6
Trochanter small	2.29	2.15	2.24	1.75	10.4	8.8	11.2	10.3	35.4	47.6	45.1	46.3
Femur cuneiform	2.12	2.08	1.97	1.63	9.6	8.2	9.9	10.0	45.1	56.4	56.3	56.6
Femur long	2.03	1.74	1.53	1.42	9.3	7.1	7.7	8.3	54.3	64.6	66.2	66.6
Tibia horizontal rows	1.48	1.55	1.32	1.24	6.8	6.2	6.6	7.4	61.10	71.7	73.9	74.9
Tibia long thick	1.22	1.22	1.23	1.07	6.6	5.4	6.2	5.8	67.7	77.9	80.5	82.3
First tarsal small	1.05	1.05	1.10	1.03	5.6	5.0	5.5	5.0	73.2	83.3	86.7	88.1
In females												
Coxa long	3.97	3.46	4.73	4.07	12.73	13.60	13.50	15.10	12.73	27.20	13.50	15.10
Coxa small	3.63	3.04	3.98	3.35	11.11	11.20	12.50	13.20	25.46	38.40	27.00	30.20
Trochanter small	2.81	2.45	2.71	2.75	10.13	10.95	11.70	11.90	36.57	49.35	39.50	43.40
Femur cuneiform	2.00	1.89	2.10	1.63	9.00	9.61	9.00	11.20	46.70	58.96	52.20	55.30
Femur long	1.93	1.71	1.92	1.42	8.60	8.14	8.10	10.60	55.70	67.10	61.20	66.50
Tibia horizontal rows	1.68	1.67	1.71	1.29	7.80	6.42	8.00	9.40	64.30	73.52	69.30	76.50
Tibia long thick	1.73	1.20	1.60	1.07	6.10	5.11	6.70	7.80	72.10	78.63	77.30	85.90
First tarsal small	1.06	1.07	1.31	1.01	5.30	4.09	4.80	5.24	78.20	82.70	84.00	93.70

small and cuneiform bristles. By looking into the range of bristle number in at least 30 flies, one can distinguish these four races (data not shown). One can surmise that four types of foreleg bristles significantly diverged in the laboratory created Cytoraces.

In *Drosophila*, size is generally estimated by some linear measurements of size-related traits, such as wing, thorax and leg length. Foreleg tibia length was ascertained to provide a measure of body size, as leg segment is significantly correlated with body mass in males of *D. melanogaster*<sup>33–36</sup>. The foreleg length reported in our earlier studies<sup>13</sup> revealed that *D. n. albomicans* and Cytorace 1 have the highest and the lowest foreleg length, respectively. The order of ranking is: *D. n. albomicans* > *D. n. nasuta* > Cytorace 2 > Cytorace 1, which suggests that the parental races are bigger in size than the Cytoraces. Our earlier studies have also shown that the newly evolved Cytoraces have more sternopleural bristles<sup>12</sup> and greater body weight<sup>14</sup> than *D. n. nasuta*. One of the possibilities of reduction in the body weight of *D. n. nasuta* could be due to the presence of less ovariole number than other races under study. On the other hand, abdominal bristle number<sup>15</sup>, wing length, wing width and foreleg length<sup>13</sup> are greater in the parental races than the newly evolved races. The body size and body weight are two different traits and are negatively correlated in these four members of *nasuta*–*albomicans* complex of *Drosophila*.

Females of all the races in the present study are bigger in size than the males, and consequently have more bristles than the males, which indicates that bristle number is correlated with body size. However, such a correlation

was not found when the parental races were compared with the Cytoraces, whereby the parental races have an increased body size and less bristles compared to the newly evolved Cytoraces. In addition, Cytorace 2 has significantly more bristles than Cytorace 1 and the parental races. Although Cytorace 2 and *D. n. albomicans* have almost the same karyotype, they differ in their quantitative trait expression. This could be due to the hybrid recombination that leads to transgressive segregation of genes as well as the genetic drift that occurred during the evolution of these Cytoraces.

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