RESEARCH ARTICLE

Male accessory gland secretory protein polymorphism in natural populations of *Drosophila nasuta nasuta* and *Drosophila sulfurigaster neonasuta*

K. RAVI RAM[1,2] and S. R. RAMESH[1*]

[1]*Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006, India*
[2]*Present Address: Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853-2703, USA*

Abstract

Male accessory gland secretory protein polymorphism was analysed in natural populations of *Drosophila nasuta nasuta* and *D. sulfurigaster neonasuta* for the first time, using SDS-PAGE to score polymorphism of these proteins in 2788 individuals of *D. n. nasuta* and 2232 individuals of *D. s. neonasuta* from 12 different populations from southern India. A total of 25 and 18 variant protein phenotypes were identified in *D. n. nasuta* and *D. s. neonasuta*, respectively. Protein fractions of group III were more polymorphic than those from groups I and II. The results show that accessory gland secretory proteins show high levels of polymorphism, irrespective of species or habitat. Moreover, we have used the variation in the accessory gland proteins to assess the extent of divergence between the species and to infer their population structure. The study suggests that though both *D. n. nasuta* and *D. s. neonasuta* belong to the same subgroup, they differ in population structure, as far as accessory gland protein polymorphism is concerned.

[Ravi Ram K. and Ramesh S. R. 2007 Male accessory gland secretory protein polymorphism in natural populations of *Drosophila nasuta nasuta* and *Drosophila sulfurigaster neonasuta. J. Genet.* **86**, 217–224]

Introduction

The evolution of internal fertilization has provided ample opportunity for strong selective interactions between males and females, and also among males. In *Drosophila*, there is good evidence that during mating the male transfers a collection of specialized proteins, secreted by male accessory glands along with sperms (Monsma and Wolfner 1988). These accessory gland proteins not only help the sperm to survive in the female genital tract, but also affect the mated female's physiology and behaviour (Ravi Ram and Ramesh 2003). As these proteins are involved in reproduction, they are under strong selection pressure (Chen 1996). Investigations for the past two decades on these proteins have revealed high levels of polymorphism both at the biochemical as well as at the DNA level (Whalen and Wilson 1986; Coulthart and Singh 1988; Aguadé *et al*. 1992; Aguadé 1999; Begun *et al*. 2000; Tsaur *et al*. 2001). However, these investigations have only been carried out in *D. melanogaster* and a few of its most closely related species, namely *D. simulans* and *D. mauritiana*. Because our current population genetic understanding of *Drosophila* is dominated by data from melanogaster subgroup species, it is important to ask whether the patterns of polymorphism and divergence, and the functional biology of reproduction related proteins are similar in other *Drosophila* species too (Wagstaff and Begun 2005). Given the hypothesis that the dynamics of certain male reproduction-related proteins may be driven by male–male and male–female post-copulatory interactions (Eberhard 1996; Rice 1998), it is important to investigate *Drosophila* species having different reproductive biology from *D. melanogaster* and *D. simulans* for further understanding of the evolution of these proteins.

The *nasuta* subgroup of *Drosophila immigrans* group consists of a cluster of closely related, morphologically almost identical species/subspecies and the major reproductive difference with the *D. melanogaster* subgroup is that the members of the *nasuta* subgroup exhibit wide variation in the degree of reproductive isolation between them (Wilson *et al*. 1969; Nirmala and Krishnamurthy 1974). Further,

*For correspondence. E-mail: srramesh2000@yahoo.com.

Keywords. reproduction; seminal proteins; natural variation; *nasuta* subgroup; *D. immigrans* group; accessory gland proteins; protein variability.

nasuta subgroup members differ not only by having almost double the accessory gland protein quantities compared to *D. melanogaster* (Ravi Ram and Ramesh 2002), but also have simple SDS-PAGE patterns consisting of 4–8 major fractions (Ravi Ram and Ramesh 2001), as compared to 40 fractions resolved on 1-dimensional SDS-PAGE in *D. melanogaster* (Stumm-Zollinger and Chen 1985). The fractions in the *nasuta* subgroup can be categorized into three groups, I (60–100 kD), II (40–60 kD) and III (< 40 kD) (Shivanna and Ramesh 1995; Ravi Ram and Ramesh 1999, 2001). However, nothing is known about the extent of variability of these proteins in the natural populations of *nasuta* subgroup members. Two species of *D. nasuta* subgroup — *D. nasuta nasuta* and *D. sulfurigaster neonasuta* — are widely distributed in India (Gai and Krishnamurthy 1983; Hegde *et al*. 1998). In the present study, we have made an attempt to investigate the extent of accessory gland secretory protein polymorphism in natural populations of these two *nasuta* subgroup species.

Materials and methods

Collection and categorization

Table 1 includes the list of 12 different localities along with the elevation at which populations of *Drosophila* for the present study were collected. A few scoops of fermented fruit mash were spread in cool, shaded areas and flies were collected after 24 h by sweeping with a fine nylon net. To minimize the differences in collection areas, even in cities like Mysore and Mangalore, the fly populations were collected from forests/fields in the outskirts, well away from densely populated areas. From such collections, *D. n. nasuta* and *D. s. neonasuta* were separated. In order to establish isofemale lines, single gravid females were placed in separate vials containing cream of wheat–agar medium, seeded with yeast. These cultures were maintained at 22 ± 1°C.

Table 1. List of different areas of collection, their altitude, and the number of variant phenotypes observed in *D. n. nasuta* and *D. s. neonasuta.*

Area	Altitude (metres)	No. of variant phenotypes	
		D. n. nasuta	*D. s. neonasuta*
Palani Hills			
(i) S-4	950	1	4
(ii) S-5	1050	1	3
(iii) S-6	1150	1	5
(iv) S-8	1650	1	3
Mysore			
(i) Nazarbad	690	17	No samples
(ii) Bogadi	710	5	No samples
(iii) Chamundi Hills	870	3	No samples
Chilkunda	600	8	No samples
Pattighat	300	8	7
Mangalore			
(i) Konaje	−50	10	No samples
(ii) Dharmasthala	−100	9	No samples
Nagarahole	710	11	16

Samples and electrophoresis

From the progeny of each naturally inseminated female, 8–10 adult males were picked randomly and treated as individual samples since the frequency of remating is known to be quite high in nature, and these samples were analysed for accessory gland secretory protein polymorphism by 13.4% SDS-PAGE (Ravi Ram and Ramesh 1999). A total of 2788 individuals of *D. n. nasuta* and 2232 individuals of *D. s. neonasuta* were analysed. The protein patterns obtained were documented using Gel Doc 1000 (Bio-Rad, Hercules, CA, USA). Marker proteins (PMW-M of Bangalore Genie, India) were used in every electrophoretic run for calculating the molecular weight of polymorphic protein fractions, using the 'Profile Analysis' module of Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA, USA).

Nomenclature of variants

The SDS-PAGE patterns of accessory gland proteins in the laboratory stocks of *D. n. nasuta* (Stock No. 201.001; Coorg, India) and *D. s. neonasuta* (Stock No. 206.001; Mysore, India) present in the *Drosophila* Stock Centre, University of Mysore, were used as standards to recognize the variant phenotypes in the samples prepared from the flies of natural populations. Only those fractions present in all the individuals of the species analysed were considered as monomorphic while the rest were regarded as polymorphic. The variant phenotypes were designated by NA for *D. n. nasuta* and NE for *D. s. neonasuta*, followed by the group to which the polymorphic fraction belonged and a lower case letter to indicate the type within the group. Individuals with a polymorphic fraction in only one of the groups are referred to as single variants. For example, variant phenotype NA3a indicates a *D. n. nasuta* polymorphic fraction belonging to group III (low molecular weight fraction), with the lower case 'a' indicating that it is one of the variant types under group III. Similarly, NA2a&3a indicates a double variant with polymorphic fractions in both groups II and III, whereas NA1a&2a&3a symbolizes a triple variant with polymorphic fractions in all the three groups.

Statistical analysis

A phylogenetic tree was constructed based on the presence/absence of protein fractions in different variant phenotypes among *D. n. nasuta* and *D. s. neonasuta* following neighbour joining method (NJ) using MEGA 2.0 (Kumar *et al*. 2001). Genetic differentiation among populations was evaluated using a hierarchical Bayesian approach developed by Holsinger *et al*. (2002) that does not assume any prior knowledge of the degree of within population inbreeding and is, therefore, not subject to the problems of traditional methods of analysis using dominant markers. We used

the software HICKORY version 0.8 (Holsinger *et al*. 2002) to estimate θ^B, a Bayesian analogue of F_{ST}, across all populations and for each pair-wise combination of populations. The data were run two to three times with the default parameters (burn-in = 50,000, number of samples = 2,50,000, thinning factor = 50) using four models: a full model, a model that assumes no inbreeding within populations (F_{IS} = 0 model), a model that assumes no differentiation among populations (θ^B = 0 model), and a model that does not attempt to estimate F_{IS} (F-free model). Because estimates of F_{IS} derived from dominant marker data may be unreliable (Holsinger and Wallace 2004), we used the F-free analysis as our preferred method to calculate estimates of θ^B. The deviance information criterion (DIC) values for the F_{IS} = 0, θ^B = 0, and full models were used to estimate how well each model fitted the data (a smaller DIC value difference with full model indicates a better fit).

Results

Populations of *D. n. nasuta* occurred in all the localities sampled, with the density being higher at lower altitudes. The distribution of *D. s. neonasuta* was confined to the localities that were at altitudes above 300 m, with the density being greater at higher altitudes. Totally, 25 variant protein phenotypes were identified for *D. n. nasuta*, and all the three groups of fractions were found to be polymorphic, whereas only 18 variant phenotypes were encountered in *D. s. neonasuta*, and only the fractions of groups I and III were found to be polymorphic. In *D. n. nasuta*, the number of variant phenotypes in a given locality varied from a minimum of one (Palani Hills) to a maximum of 17 (Nazarbad, Mysore), whereas in *D. s. neonasuta* it varied from 3 to 16 with the minimum in populations from two locations of Palani Hills, and the maximum in the populations of Nagarahole (table 1).

By comparing the SDS-PAGE patterns in different populations studied, we could recognize two classes of variant phenotypes, namely shared and exclusive. When the same variant phenotype is found in at least two populations, it is regarded as shared and if the variant phenotype is restricted in its occurrence to a specific population, it is regarded as exclusive. In case of *D. n. nasuta*, out of 25 variant phenotypes, 16 variant phenotypes were shared, while the remaining were exclusive (table 2). In *D. s. neonasuta*, out of 18

Table 2. Different variant phenotypes, their frequencies and the polymorphic fractions in *D. n. nasuta.*

Variant phenotype	Protein fractions (kD)			Frequency
	Group I	Group II	Group III	(%)
NA1a&2a[@]	94,92*	45,43,42,41*,40	29*,26	1.20
NA1a&3a	94,92*	45,43,42,40	28*,26	1.43
NA1a&3b[@]	94,92*	45,43,42,40	31*,28*,26	0.14
NA1a&3c	94,92*	45,43,42,40	29,26,24*	0.57
NA1a&3f[@]	94,92*	45,43,42,40	29,28*,26	0.57
NA1a&2a&3b[@]	94,92*	45,43,42,41*,40	31*,28*,26	0.14
NA1a&2a&3c[@]	94,92*	45,43,42,41*,40	29*,26,24*	0.14
NA1a&2a&3c[@]	94,92*	45,43,42,41*,40	31*,28*,26,24*	0.57
NA2a	94	45,43,42,41*,40	29*,26	10.76
NA2b[@]	94	47*,45,43,42,40	29*,26	1.15
NA2a&3a	94	45,43,42,41*,40	28*,26	15.71
NA2a&3b	94	45,43,42,41*,40	31*,28*,26	5.00
NA2a&3c	94	45,43,42,41*,40	29*,26,24*	2.87
NA2a&3d	94	45,43,42,41*,40	28*,26,24*	1.15
NA2a&3f	94	45,43,42,41*,40	29*,28*,26	3.16
NA2a&3g[@]	94	45,43,42,41*,40	29*,28*,26,24*	0.57
NA2a&3h[@]	94	45,43,42,41*,40	31*,29,26,24*	0.14
NA3a	94	45,43,42,40	28*,26	39.02
NA3b	94	45,43,42,40	31*,28*,26	2.44
NA3c	94	45,43,42,40	29*,26,24*	2.15
NA3d	94	45,43,42,40	28*,26,24*	3.30
NA3f	94	45,43,42,40	29*,28*,26	2.22
NA3g	94	45,43,42,40	29*,28*,26,24*	2.15
NA3h	94	45,43,42,40	31*,29,26,24*	1.08
NA3i[#]	94	45,43,42,40	29*,26	2.36

[@] Exclusive variant phenotype; *Polymorphic fraction; [#]Pattern similar to lab stock. No. of localities, 12; No. of individuals analysed, 2788; Altitude, −100 to 1650 m.

Table 3. Different variant phenotypes, their frequencies and the polymorphic fractions in *D. n. nasuta.*

Variant phenotype	Protein fractions (kD)			Frequency
	Group I	Group II	Group III	(%)
NE1a&3f	94,92*	45,43,41,39	25*	0.99
NE1a&3b@	94,92*	45,43,41,39	25*,24[24]	0.99
NE1a&3c@	94,92*	45,43,41,39	30*,26*,24*	1.34
NE1a&3j@	94,92*	45,43,41,39	28,25*,24*	0.36
NE1a&3m@	94,92*	45,43,41,39	28,26*,24*	0.36
NE3a	94	45,43,41,39	30*,28*,24*,22*	28.76
NE3b	94	45,43,41,39	25*,24*	18.46
NE3c	94	45,43,41,39	30*,26*,24*	15.78
NE3d	94	45,43,41,39	30*,28*,26*,24,22*	11.11
NE3e	94	45,43,41,39	30*,28*,26*,25*,24,22*,17*	1.7
NE3f@	94	45,43,41,39	25*	3.94
NE3g#	94	45,43,41,39	28*,24*	6.00
NE3h@	94	45,43,41,39	28*,25*	0.72
NE3i	94	45,43,41,39	28*,25*,24*,22*	2.33
NE3j	94	45,43,41,39	28*,25*,24*	4.4
NE3k@	94	45,43,41,39	28*,25*,22*	0.18
NE31@	94	45,43,41,39	30*, 28*, 26*,24	1.61
NE3m@	94	45,43,41,39	28*,26*,24*	0.99

@ Exclusive variant phenotype; *Polymorphic fraction; # Pattern similar to lab stock. No. of localities, 6; No. of individuals analysed, 2232; Altitude, 300 to 1650 m.

Table 4. Frequency of individuals polymorphic for different groups in *D. n. nasuta* and *D. s. neonasuta.*

Group(s)	Frequency (%)	
	D. n. nasuta	*D. s. neonasuta*
Group I	0.0	0.0
Group II	11.9	0.0
Group III	52.4	90.0
Groups I & II	1.15	0.0
Groups I & III	2.72	4.00
Groups II & III	28.6	0.00
Groups I & II & III	0.86	0.00
Individuals possessing lab stock pattern	2.36	6.00

variant phenotypes, only 9 were confined to specific populations (table 3). The highest frequency variants in *D. n. nasuta* and *D. s. neonasuta* were NA3a and NE3a, respectively. NA1a&3b, NA1a&2a&3a, NA1a&2a&3c and NA2a&3h in *D. n. nasuta*, and NE3k in *D. s. neonasuta* were found at the lowest frequency. Polymorphic variants involving group I alone were absent in both species. In addition, variant phenotypes for group II were absent in *D. s. neonasuta*. The frequencies of polymorphism with respect to different groups are listed in table 4. In *D. n. nasuta*, 52.4% individuals showed polymorphism for group III fractions alone, while only 0.86% individuals were found to be polymorphic for all three groups. In *D. s. neonasuta*, 90% of the individuals when analysed showed polymorphism for group III fractions alone, while only 4% were found to be polymorphic for both groups I and III. Single variants were fewer in *D. n. nasuta* compared to *D. s. neonasuta*. In *D. n. nasuta*, six out of 13 fractions were found to be monomorphic, while the remaining seven were polymorphic (one in group I, two in group II, and four in group III), and gave rise to 25 variant phenotypes in various combinations. Although the fractions that belong to group III were found to be highly variable, the 26-kD fraction of group III, also present in the laboratory stock of *D. n. nasuta*, is seen in all the populations. In case of *D. s. neonasuta*, only five out of 13 fractions were monomorphic, while the remaining eight were polymorphic fractions with one in group I and seven in group III. These fractions occured in different combinations to give rise to 18 variant phenotypes. The polymorphic fractions and their frequencies are listed in table 5.

The tree based on the NJ algorithm formed two clusters, establishing a clear demarcation between variant phenotypes of *D. n. nasuta* and *D. s. neonasuta* (figure 1). The analysis of genetic differentiation among populations through HICKORY 0.8 provided evidence for genetic differences among populations (based on the large difference between full model and $\theta^B = 0$ model, but there is no evidence that the frequencies within populations depart from Hardy–Weinberg expectations as the difference between full model

Table 5. Polymorphic fractions and their frequency in *D. n. nasuta* and *D. s. neonasuta.*

D. n. nasuta		*D. s. neonasuta*	
Polymorphic fraction	Frequency (%)	Polymorphic fraction	Frequency (%)
92 kD	4.73	92 kD	4.03
41 kD	36.4	30 kD	58.3
47 kD	1.15	26 kD	20.4
31 kD	1.79	25 kD	32.4
29 kD	31.1	28 kD	20.6
28 kD	69.6	24 kD	41.9
24 kD	13.3	22 kD	42.4
		17 kD	1.70

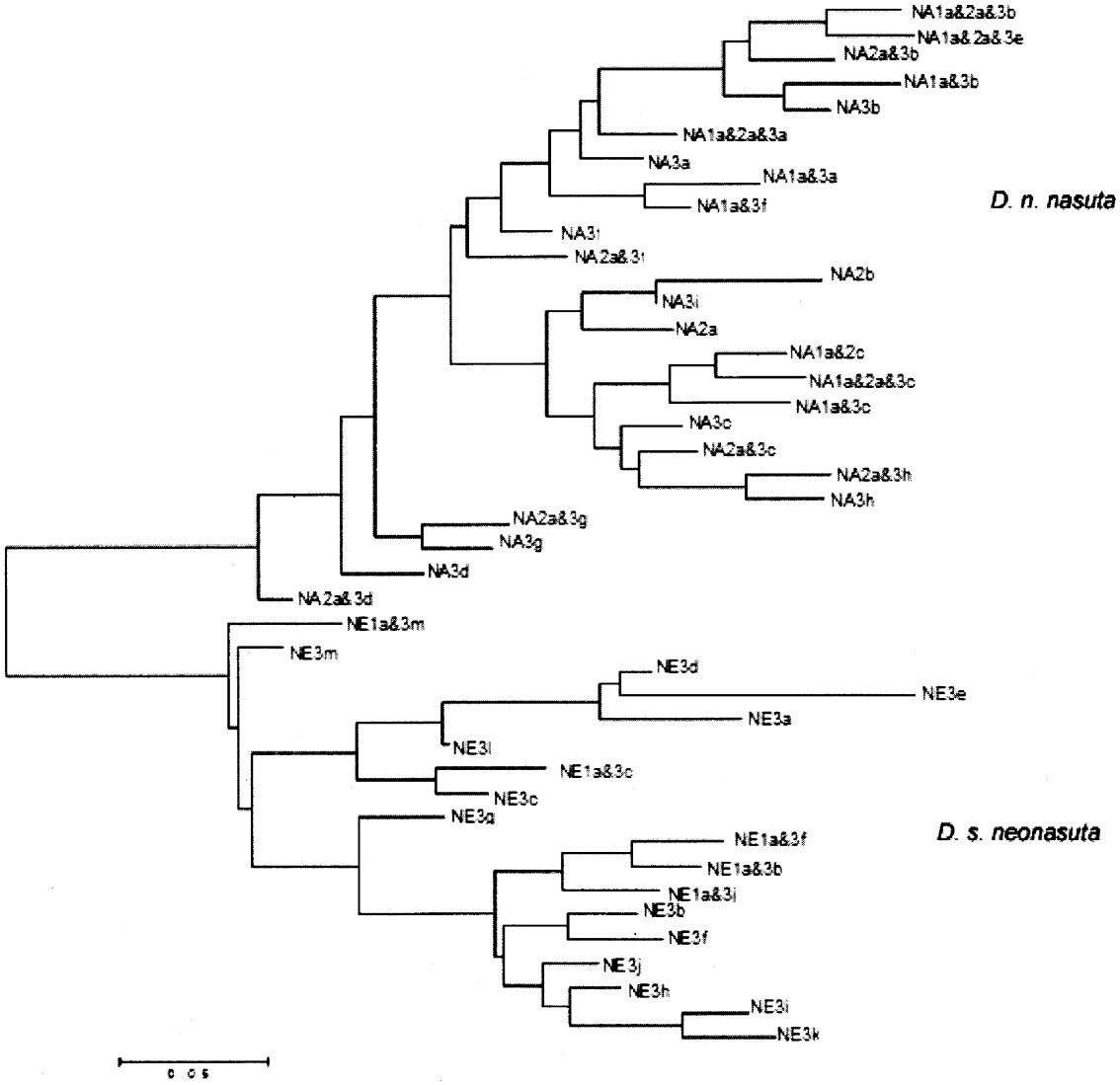

Figure 1. Tree showing the clustering of different variant male accessory gland secretory protein phenotypes in *D. n. nasuta* and *D. s. neonasuta* based on neighbour joining method.

and $F_{IS} = 0$ model is very small. The results suggest that *D. n. nasuta* and *D. s. neonasuta* differ from each other with respect to the extent of diversity and genetic differentiation among their populations (table 6).

Table 6. Results of DIC statistics

	DIC	
Model	*D. n. nasuta*	*D. s. neonasuta*
Full model	305.0927	250.2589
$F_{IS} = 0$	308.6368	252.0624
$\theta^B = 0$	2758.824	1403.4698
GST-B	0.27	0.18

DIC, deviance information criterion; θ^B, estimate of F_{ST}; GST-B, posterior mean of θ^B.

Discussion

In the present study, we have used SDS-PAGE to analyse the extent of accessory gland protein polymorphism in two related species of *D. nasuta* subgroup and such a study has not only provided an insight into the accessory gland protein polymorphism in species other than *D. melanogaster* subgroup but also helped us to understand the extent of biochemical differentiation between *D. n. nasuta* and *D. s. neonasuta* with respect to an adult specific secretory reproductive tissue. Present study revealed the existence of wide spread polymorphism in both the species, while monomorphism was rare (observed only in *D. n. nasuta* populations of Palani Hills), with the shared variant phenotypes occurring in higher frequencies and the exclusive ones being less frequent. We believe that the variant phenotypes in low frequency may be newly arisen. Alternatively, they may also represent the phenotypes that are dwindling due to selective disadvantage. However, at present we do not know whether the shared and exclusive phenotypes are interchangeable and if their preponderance is seasonal. The high levels of polymorphism in Mysore populations as compared to Palani Hills population may be attributed to seasonal variation, as Mysore populations were analysed throughout the year.

In *Drosophila*, da Cunha *et al*. (1950) have shown that the extent of inversion polymorphism is greater in widespread and more abundant forms than in less abundant, closely related forms. *D. n. nasuta* is widespread and occurs abundantly in southern India, and also exhibits extensive chromosomal polymorphism. On the other hand *D. s. neonasuta*, although morphologically very similar, shows much less chromosomal polymorphism, and is sparsely distributed and occurs in smaller numbers (Gai and Krishnamurthy 1983; Hegde *et al*. 1998; Shyamala and Ranganath 1988; Shyamala *et al*. 1989). In the present study, *D. s. neonasuta* could be collected only from six out of 12 locations. Further, wherever *D. s. neonasuta* occurs, it is sympatric with *D. n. nasuta*. Higher level of polymorphism for larval salivary gland proteins has been found in *D. n. nasuta*, as compared to *D. s. neonasuta* (Ramesh and Shivanna 1999) and the present results show the same pattern to be true for accessory gland secretory proteins as well, with the more abundant and widespread species, *D. n. nasuta*, being more polymorphic.

The *D. n. nasuta* populations analysed in the present study includes individuals collected from different localities whose altitudes range from −100 m (below sea level) to +1650 m, whereas the *D. s. neonasuta* populations came from localities with altitudes ranging from +300 to +1650 m. The localities from where these collections were made differ not only in the altitude but also in climatic conditions: cool and arid (Palani Hills with evergreen forest), semi-arid (Chamundi Hills, Mysore), or humid (localities in and around Mangalore). These areas from where collections were made are separated by at least 100 km. From the present study, it is evident that in spite of the altitudinal, geographic and climatic differences, some variant phenotypes namely, NA3a of *D. n. nasuta* and NE3a of *D. s. neonasuta*, occur in the entire range of their distribution. In *D. n. nasuta*, the individuals with variant phenotypes NA3a and NA2a&3a were always found to occur together, except in the populations of Palani Hills and Chamundi Hills; while such instances of coexistence of variant phenotypes is absent in case of *D. s. neonasuta*. Further, the genetic differentiation detected among populations, as well as between the two species, by the Bayesian approach in the present study might reflect the extent of genetic variability at the loci that are involved in accessory gland protein synthesis, which might be a consequence of species-specific genetic responses to variations in the microclimatic conditions prevailing in the localities from where collections have been made.

Molecular studies in different animal taxa suggest that genes involved in reproduction evolve at an accelerated rate relative to other genes (reviewed in Swanson and Vacquier 2002), and positive selection has been inferred for proteins coded by some such genes (Swanson and Vacquier 1995; Metz and Palumbi 1996; Sutton and Wilkinson 1997; Wyckoff *et al*. 2000; Torgerson *et al*. 2002). Even within *D. nasuta* subgroup, Ramesh and Shivanna (1999) found only eight variant phenotypes for glue proteins that are not involved in any reproductive function, whereas we have recorded about 25 variant phenotypes for proteins involved in reproduction in the present study. In any case, rapid phenotypic/molecular evolution of reproductive characters/genes is consistent with the notion that male–male and male–female interactions may contribute to the rapid divergence between populations and the evolution of reproductive isolation (Eberhard 1996; Rice 1998). In plants, reproductive traits like floral anatomy were given greater credibility in grouping plants, as compared to other traits like stem anatomy based on the adaptive hypothesis for taxonomic relationships (Niklas 1997). Since *D. nasuta* subgroup members show different levels of reproduc-

tive isolation, we used the accessory gland protein polymorphism data to analyse the genetic differentiation between natural populations of these species and found that though these two species are taxonomically related, there is species-wise clustering of accessory gland protein variant phenotypes (figure 1), reflecting their difference at the biochemical level.

Rapid evolution of proteins may occur due to (i) lack of strong functional or structural constraints (Kimura 1968; Kimura and Ohta 1974), (ii) positive selection for sequence divergence, e.g. in proteins involved in reproduction (Swanson and Vacquier 2002), or (iii) combination of the first two: neutral evolution of some residues and positive selection of others (Schmid *et al*. 1999). A bimodal distribution of polymorphism i.e., either loci are highly polymorphic or weakly polymorphic, is strongly suggestive that different forces are in action on different loci (Buchanan and Johnson 1983). In the present study, the ultimate distribution of polymorphism seems to be bimodal with groups I and II being less polymorphic, and high degree of polymorphism in group III, a feature that is consistent with the notion that different selective forces are probably acting on these different loci. Further, the low molecular weight fractions being highly polymorphic in both the species, mediate much of the variability in the natural populations.

Civetta and Singh (1998) have shown that, at least in *D. melanogaster, D. simulans* and *D. pseudoobscura*, the evolution of sex-related genes is associated with the operation of directional selection. Previous studies on accessory gland proteins in *D. melanogaster* and related species at the protein as well as at DNA level have shown that a significant proportion of these proteins are rapidly evolving and are under positive selection (Cirera and Aguadé 1997; Coulthart and Singh 1988; Tsaur *et al*. 1998; Aguadé 1999; Begun *et al*. 2000; Swanson *et al*. 2001). Even in the present study, we have observed high level of polymorphism but at present we do not know the significance of these high levels of accessory gland protein variability in the natural populations of *D. n. nasuta* and *D. s. neonasuta*, or the nature of selective forces that maintain them. However, it seems clear that accessory gland proteins are highly polymorphic, irrespective of *Drosophila* species group or their habitat.

Our earlier studies on the male accessory secretory proteins have shown that some protein fractions are synthesized by autosomal and some by X-linked genes, and that these secretions are glycosylated (Ravi Ram and Ramesh 2001). The nature of SDS-PAGE dictates that the variation seen in proteins is solely due to differences in their mobility in the gel. These differences could be due to substitutions, deletions or insertions in the structural genes that code for these proteins, or due to variation in sequence resulting in posttranslational modifications such as glycosylation (Dunbar 1987). In addition, certain types of single-site mutations involving noncharged amino acid substitutions can alter the mobility of proteins on SDS-PAGE (Seeburg *et al*. 1984). Further biochemical and genetic analyses of accessory gland proteins in *D. nasuta* subgroup species would help us to understand the basis for the difference in mobility and also to unravel the biological significance of high levels of polymorphism among these closely related species.

Acknowledgements

Department of Science and Technology, Government of India, funded this work. KRR is grateful to Council for Scientific and Industrial Research, Government of India, for the award of senior research fellowship. We thank the Chairman of our department for the facilities, and Prof. H. A. Ranganath for his valuable suggestions. We are also thankful to Drs M. S. Krishna, V. Shakunthala and J. S. Ashadevi of the Drosophila Stock Center, University of Mysore, for their help during collection tours, and to Profs A. G. Clark and M. F. Wolfner of Cornell University, USA, for discussions as well as suggestions during manuscript preparation.

References

Aguadé M. 1999 Positive selection drives the evolution of the *Acp29AB* accessory gland protein in *Drosophila. Genetics* **152**, 543–551.

Aguadé M., Miyashita N. and Langley C. H. 1992 Polymorphism and divergence in the *Mst26A* male accessory gland gene region in *Drosophila. Genetics* **132**, 755–770.

Begun D. J., Whitley P., Todd B. L., Waldrip-Dail H. M. and Clark A. G. 2000 Molecular population genetics of male accessory gland proteins in *Drosophila. Genetics* **56**, 1879–1888.

Buchanan B. A. and Johnson D. L. E. 1983 Hidden electrophoretic variation at the xanthine dehydrogenase locus in a natural population of *Drosophila melanogaster. Genetics* **104**, 301–315.

Chen P. S. 1996 The accessory gland proteins in male *Drosophila*: structural, reproductive, and evolutionary aspects. *Experientia* **52**, 503–510.

Cirera S. and Aguadé M. 1997 Evolutionary history of the sex-peptide (*Acp70A*) gene region in *Drosophila melanogaster. Genetics* **147**, 189–197.

Civetta A. and Singh R. S. 1998 Sex related genes, directional sexual selection and speciation. *Mol. Biol. Evol*. **15**, 901–909.

Coulthart M. B. and Singh R. S. 1988 Differing amounts of genetic polymorphism in testes and male accessory glands of *Drosophila melanogaster* and *Drosophila simulans. Biochem. Genet*. **26**, 153–164.

da Cunha A. B., Burla H. and Dobzhansky T. 1950 Adaptive chromosomal polymorphism in *Drosophila willistoni. Evolution* **4**, 212–235.

Dunbar B. S. 1987 Basic principles of post-translational modification of proteins and their analysis using high resolution two dimensional polyacrylamide gel electrophoresis. In *Two dimensional electrophoresis and immunological techniques*, pp 77–101. Plenum Press, New York.

Eberhard W. G. 1996 Female control: *sexual selection by cryptic female choice*. Princeton University Press, Princeton, NJ.

Gai P. G. and Krishnamurthy N. B. 1983 Studies on the *Drosophila* fauna from Sampaje and Shiradi ghats, Karnataka, India. *Dros. Inf. Serv*. **59**, 36–37.

Hegde S. N., Vasudev V., Shakunthala V. and Krishna M. S. 1998 *Drosophila* fauna of Palani hills, Tamil Nadu, India. *Dros. Inf. Serv*. **81**, 138–139.

Holsinger and Wallace 2004. Bayesian approaches for the analysis of population structure: an example from *Platanthera leucophaca* (orchidaceae). *Molecular Ecology* **13**, 887–894.

Holsinger K. E., Lewis P. O. and Dey D. K. 2002 A Bayesian approach to inferring population structure from dominant markers. *Mol. Ecol*. **11**, 1157–1164.

Kimura M. 1968 Genetic variability maintained in a finite population due to mutational production of neutral and nearly neutral isoalleles. *Genet. Res*. **11**, 247–269.

Kimura M. and Ohta T. 1974 On some principles governing molecular evolution. *Proc. Natl. Acad. Sci. USA*. **71**, 2848–2852.

Kumar S., Tamura K., Jakobsen I. B. and Nei M. 2001 MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

Metz E. C. and Palumbi S. R. 1996 Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. *Mol. Biol. Evol*. **13**, 397–406.

Monsma S. A. and Wolfner M. F. 1988 Structure and expression of a *Drosophila* male accessory gland gene whose product resembles a peptide pheromone precursor. *Genes Dev*. **2**, 1063–1073.

Niklas K. J. 1997 *The evolutionary biology of plants*. University of Chicago Press, Chicago.

Nirmala S. S. and Krishnamurthy N. B. 1974 Cytogenetic studies on *Drosophila neonasuta* - a member of *nasuta* subgroup. *J. Mysore Univ*. **26**, 162–167.

Ramesh S. R. and Shivanna N. 1999 SDS-PAGE pattern polymorphism of X-chromosomal glue proteins in natural populations of two *Drosophila nasuta* subgroup species. *Biochem. Genet*. **37**, 1–21.

Ravi Ram K. and Ramesh S. R. 1999 Male accessory gland secretory proteins in *nasuta* subgroup of *Drosophila*: nature and SDS-PAGE patterns. *Ind. J. Exp. Biol*. **37**, 767–773.

Ravi Ram K. and Ramesh S. R. 2001 Male accessory gland secretory proteins in a few members of *Drosophila nasuta* subgroup. *Biochem. Genet*. **39**, 99–115.

Ravi Ram K. and Ramesh S. R. 2002. Male accessory gland secretory proteins in *nasuta* subgroup of *Drosophila*: Synthetic Activity of Acp. *Zoological Science* **19**, 513–518.

Ravi Ram K. and Ramesh S. R. 2003 Male accessory gland proteins in *Drosophila*: a multifaceted field. *Ind. J. Exp. Biol*. **41**, 1372–1383.

Rice W. R. 1998 Intergenomic conflict, interlocus antagonistic coevolution, and the evolution of reproductive isolation. In *Endless forms: species and speciation* (ed. by D. J. Howard and S. H. Berlocher), pp. 261–270. Oxford University Press, New York.

Schmid K. J., Nigro L., Aquadro C. F. and Tautz D. 1999 Large number of replacement polymorphisms in rapidly evolving genes of *Drosophila*: implications for genome-wide surveys of DNA polymorphism. *Genetics* **153**, 1717–1729.

Seeburg P. H., Colby W. W., Capon D. J., Goeddel D. V. and Levinson A. D. 1984 Biological properties of human *C-Ha-ras 1* genes mutated at codon 12. Nature **312**, 71–75.

Shivanna N. and Ramesh S. R. 1995 Quantitative and qualitative analysis of accessory gland secretory proteins in a few species of *Drosophila immigrans* group. *Ind. J. Exp. Biol*. **33**, 668–672.

Shyamala B. V. and Ranganath H. A. 1988 Inversion polymorphism in natural populations of *Drosophila nasuta nasuta*. *Proc. Ind. Acad. Sci*. **97**, 471–477.

Shyamala B. V., Rao P. M. and Ranganath H. A. 1989 Inversion polymorphism and linkage disequilibrium in *Drosophila sulfurigaster neonasuta*. *J. Heredity* **80**, 488–490.

Stumm-Zollinger E. and Chen P. S. 1985 Protein metabolism of *Drosophila melanogaster* male accessory glands. 1. Characterization of secretory proteins. *Insect. Biochem*. **15**, 375–381.

Sutton K. A. and Wilkinson M. F. 1997 Rapid evolution of a homeodomain: evidence for positive selection. *J. Mol. Evol*. **45**, 579–588.

Swanson W. J. and Vacquier V. D. 1995 Extraordinary divergence and positive Darwinian selection in a fusagenic protein coating the acrosomal process of abalonespermatozoa. *Proc. Natl. Acad. Sci. USA* **92**, 4957–4961.

Swanson W. J. and Vacquier V. D. 2002 The rapid evolution of reproductive proteins. *Nature Rev. Genet*. **3**, 137–144.

Swanson W. J., Clark A. G., Waldrip-Dail H. M., Wolfner M. F. and Aquadro C. F. 2001 Evolutionary EST analysis identifies rapidly evolving male reproductive tract proteins in *Drosophila. Proc. Natl. Acad. Sci. USA*. **98**, 7375–7379.

Torgerson D. G., Kulathinal R. J. and Singh R. S. 2002 Mammalian sperm proteins are rapidly evolving: evidence of positive selection in functionally diverse genes. *Mol. Biol. Evol*. **19**, 1973–1980.

Tsaur S. -H., Ting C. -T. and Wu C. -I. 1998 Positive selection driving the evolution of a gene of male reproduction, *Acp26Aa* of *Drosophila. Mol. Biol. Evol*. **15**, 1040–1046.

Tsaur S. -H., Ting C. -T. and Wu C. -I. 2001 Sex in *Drosophila mauritiana*: a very high level of amino acid polymorphism in a male reproductive protein gene, *Acp26Aa. Mol. Biol. Evol*. **18**, 22–26.

Wagstaff B. J., and Begun D. J. 2005 Comparative genomics of accessory gland protein genes in *Drosophila melanogaster* and *D. pseudoobscura. Mol. Biol. Evol*. **22**, 818–832.

Whalen M. and Wilson G. T. 1986 Variation and genomic localization of genes encoding *Drosophila melanogaster* accessory gland proteins separated by SDS-PAGE. *Genetics* **114**, 77–92.

Wilson F. D., Wheeler M. R., Harget M. and Kambysellis M. 1969 Cytogenetic relations in the *Drosophila nasuta* subgroup of the *immigrans* group of species. *Univ. Texas Publ*. **6918**, 207–253.

Wyckoff G. J., Wang W. and Wu C. -I. 2000 Rapid evolution of male reproductive genes in the descent of man. *Nature* **503**, 304–309.

Received 24 November 2006