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Multifaceted approach to evaluate the relationship among closely related forms of *Drosophila*

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Abstract. *Drosophila* is a suitable system to study different facets of population differentiation. *Drosophila sulfurigaster*, *Drosophila bilimbata*, *Drosophila albostrigata*, *Drosophila neonasuta* and *Drosophila pulaua* are morphologically indistinguishable members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila*. They are distributed in different parts of south east Asia. The evolutionary inter-relationship between these closely related forms will be discussed with reference to karyotypes, heterochromatin, satellite DNA, population fitness, ecogenetic divergence and isozyme variations.

Keywords. *Drosophila*; karyotypes; satellite DNA; fitness; resource utilization; isozymes.

1. Introduction

Drosophila is no longer the queen of genetics, as at one time it was. It remains, however, probably the best material for studies on evolutionary and population genetics (Dobzhansky 1970). *Drosophila* has emerged out as a paramount fast breeding diploid bisexual organism for the experimentalist to dissect out and study various facts of evolution. Recently, Templeton (1981) has critically discussed the population genetic mechanisms of speciation. He has cited relevant references to demonstrate that speciation can occur in the absence of, or is uncorrelated in some groups with karyotypic change, DNA sequence divergence, enzyme differentiation, morphological change and shifts in niche or habitat. It has been realized that there is no universal joint pattern relative to speciation. In the words of Dobzhansky (1970) 'the evolution of every phyletic line yields a novelty that never existed before and is unique, unrepeatable and irreversible proceeding'. Therefore one cannot imagine an universal marker to define the process of speciation and it has to be treated as a multidimensional process. In view of this, a multifaceted approach has been made to understand the nature of differentiation among closely related forms of *Drosophila*. The members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila* form the material for the present investigation.

2. Materials and methods

Nasuta subgroup of the *immigrans* species group is an assemblage of morphologically almost identical species and sub-species of *Drosophila*. Taking into cognizance the pioneering work of Wilson *et al* (1969) and their observations, Nirmala and Krishnamurthy (1973) have divided the *nasuta* subgroup into 3 exophenotypic complexes. They are (i) frontal sheen complex which includes the members with silvery sheen over the entire frons; (ii) orbital sheen complex which includes the species with silvery markings confined to the sides of the frontal orbits and (iii) species without such markings.

The present paper deals with the members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila*. The extensive cytological and hybridization investigations of Wilson *et al* (1969), Nirmala and Krishnamurthy (1973) and Ranganath and Krishnamurthy (1976) have resulted in the recognition of the following members of the orbital sheen complex:

(i) *D.s. sulfurigaster* Wilson *et al* 1969 (*D. sulfurigaster* Duda 1923);
(ii) *D.s. bilimbata* Wilson *et al* 1969 (*D. bilimbata* Bezzi 1929);
(iii) *D.s. albostrigata* Wheeler 1969;
(iv) *D.s. neonasuta* Ranganath and Krishnamurthy 1976 (*D. neonasuta* Nirmala and Krishnamurthy 1973);
(v) *D. pulaua* Wheeler 1969;
(vi) *D. nixifrons* Tan, Hsu, Sheng, 1949.

The 4 sub-species of *D. sulfurigaster* are allopatric. *D.s. sulfurigaster* is a dominant member in Papua, New Guinea. *D.s. bilimbata* is widely scattered in many islands of Pacific ocean. *D.s. albostrigata* is a common member in different parts of south east Asia like Ceylon, Thailand, Burma and Philippines. *D.s. neonasuta* is found in some parts of Peninsular India. *D. pulaua* is a common species in Malaysia (Wilson *et al* 1969; Nirmala and Krishnamurthy 1973; Ranganath and Krishnamurthy 1976; Siddaveere Gowda *et al* 1977; Kitagawa *et al* 1982). *D. nixifrons* was reported from China and it was not available for detailed investigations.

The flies of 4 sub-species of *D. sulfurigaster* and *D. pulaua* are morphologically indistinguishable. In this communication an attempt has been made to report and discuss the extent of similarities and differences among these siblings of the orbital sheen complex of *Drosophila* involving different facets of their organization.

3. Results and discussion

3.1 *Karyotypic organization*

Karyotype is a useful marker to evaluate the evolutionary relationships between species or groups of species. Variations in metaphase chromosomes are often striking in the genus *Drosophila*. A number of studies have demonstrated that this endophenotype is more variable than other morphological characters in *Drosophila* (Pimpinelli *et al* 1976; Gatti *et al* 1976; Lemeunier *et al* 1978; Baimai 1980; Baimai and Chumchong 1980; Lakhotia and Mishra 1980; Ranganath and Hagele 1982; Singh and Gupta 1982; Baimai *et al* 1983; Gupta and Kumar 1986; Mahan and Beck 1986). Recently, Ushakumari and Ranganath (1986a) have reported the karyotypes of the 5 members of the orbital sheen complex of *Drosophila*, viz *D.s. sulfurigaster*, *D.s. bilimbata*, *D.s. albostrigata*, *D.s. neonasuta* and *D. pulaua*. The diploid number in these forms is $2n = 8$. In general, the metaphase chromosome complement of a female consists of one pair of metacentrics (chromosome 2), two pairs of acrocentrics (chromosomes X and 3) and one pair of dots (chromosome 4). In males, one of the acrocentric X chromosome is replaced by a submetacentric chromosome Y.

Chromosome banding is a useful technique to localize heterochromatin and to study the evolutionary changes, if any, in the architecture of the chromosomes. In the genus *Drosophila*, it is stated that heterochromatin is more divergent than external

morphological characteristics (Holmquist 1975). In the recent years, C-banding has been frequently used as an approach to study variations in amount and distribution of heterochromatin in the karyotypes of related species and has yielded information useful in resolving karyotypic differences (Kaul *et al* 1978). Diagrammatic summary of metaphase chromosomes of the members of the orbital sheen complex of *Drosophila* after C-banding is shown in figure 1. Table 1 provides the data on the quantum of C-band regions present in different chromosomes and in different strains under study. Chromosomewise comparison between these *Drosophila* forms show significant differences. The acrocentric X chromosome of all these forms carry a block of heterochromatin at the centric region, but the quantum of it ranges from about 48%

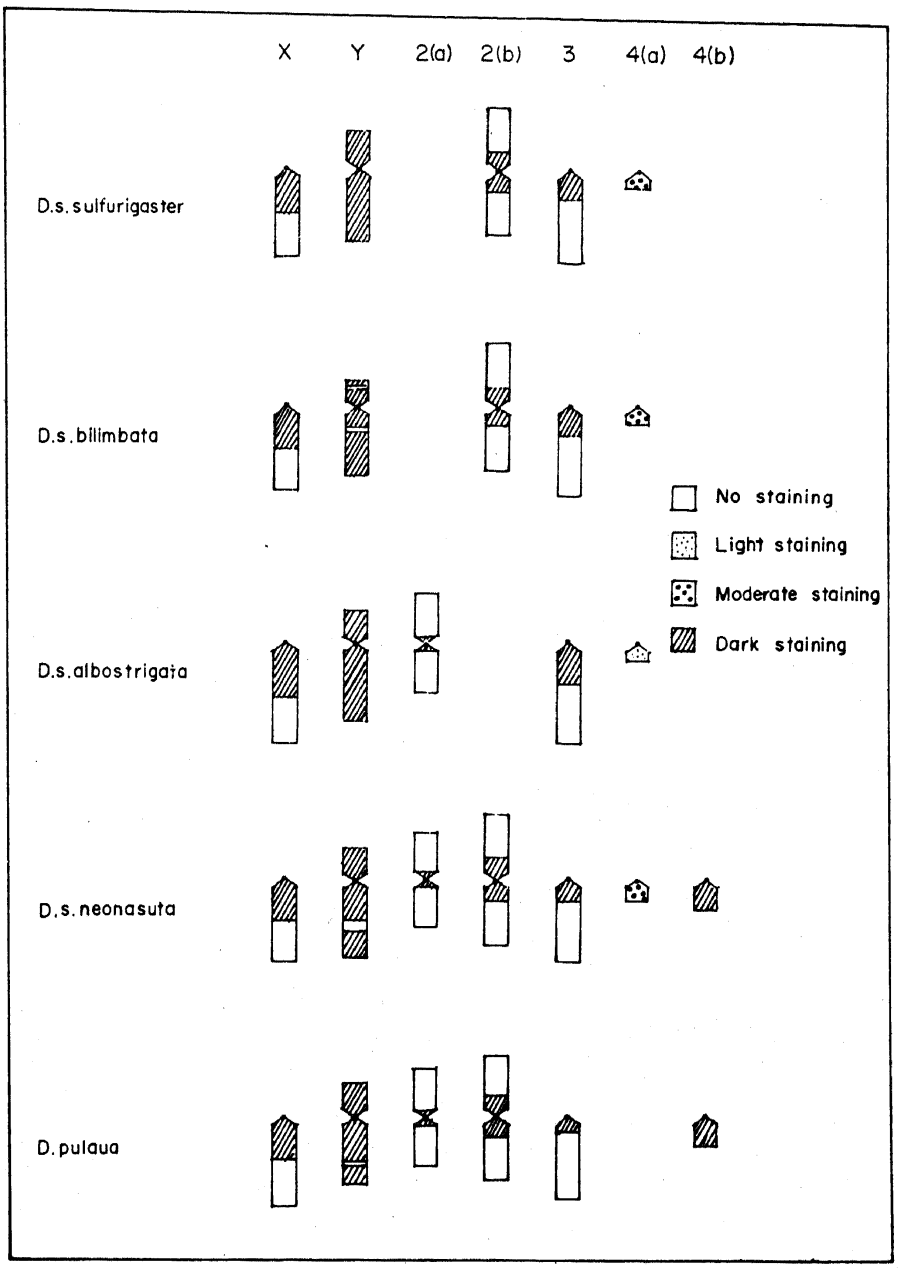

Figure 1. Metaphase chromosomes of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* as seen after C-banding.

heterochromatin regions recognized by C-banding. These C-band regions fluoresce uniformly in all chromosomes with the exception of chromosome Y. The differentiation of the heterochromatin of the Y chromosome is interesting. The Y chromosome of *D.s. sulfurigaster* shows 3 Q-banded regions, of which one is lighter than others; while in *D.s. bilimbata* two brightly fluorescing segments are seen. In *D.s. albostrigata* the short arm of the Y chromosome is brighter than the long arm. The Y chromosome of *D.s. neonasuta* has revealed a clear linear differentiation into 4 fluorescing regions separated by non-fluorescing ones. Differential fluorescing regions are also seen in the Y chromosome of *D. pulaua*. At the outset it can be seen that no two forms of the orbital sheen complex of *Drosophila* have Y chromosomes which have a similar response to A-T specific dye, namely Quinacrine. The differences reported for the chromosome 2 and the dot chromosomes, after C-banding, among the members under study, are very much distinct after Q-banding.

Thus, the karyotypic differentiation within the orbital sheen complex of *Drosophila* has occurred and it appears to be associated with changes in the heterochromatin of its chromosomes.

3.2 *Satellite DNA*

A molecular dimension of heterochromatin was provided when *in situ* hybridizations showed that highly repeated DNA sequences were predominantly located in this form of chromatin (Pardue and Gall 1970; Jones and Robertson 1970). In the light of notable differences in the amount and distribution of heterochromatin in metaphase chromosomes of the members of the orbital sheen complex, the satellite DNAs of these forms were analysed. The DNA extracted from the larval neural ganglia of these members were analysed by CsCl density gradient centrifugation and the buoyant density profiles are shown in figure 3. Varying quantities of A-T rich satellite DNA is seen in different members under study. No two profiles resemble one another. Four distinct A-T rich satellite fractions are seen in *D.s. sulfurigaster* (1·651, 1·661, 1·665 and 1·675 g/cm^3). In *D.s. bilimbata*, even though the quantum of satellite DNA is more than that in *D.s. sulfurigaster*, the number of clearly separable satellite fractions were only two. On the other hand, in *D.s. albostrigata*, *D.s. neonasuta* and *D. pulaua*, the amount of satellite DNA is less and also separation is not as clear as it is in other forms. These preliminary studies on satellite DNA do demonstrate the differences among these closely related forms of *Drosophila*.

Perusal of literature reveals that there exist conflicting reports with regard to the role of heterochromatin and satellite DNA in evolution (Miklos *et al* 1980; Miklos and Gill 1981). In view of these, it is premature to say anything about the impact of differences in heterochromatin and satellite DNA on the evolutionary biology of the orbital sheen complex of *Drosophila*.

3.3 *Population fitness*

Differential reproduction is the essence of Neo Darwinian natural selection. The biological success of a species population can be measured in terms of its reproductive success in relation to other populations. It is a cumulative assessment of fecundity, rate of development, viability and adaptedness. Each one of these com

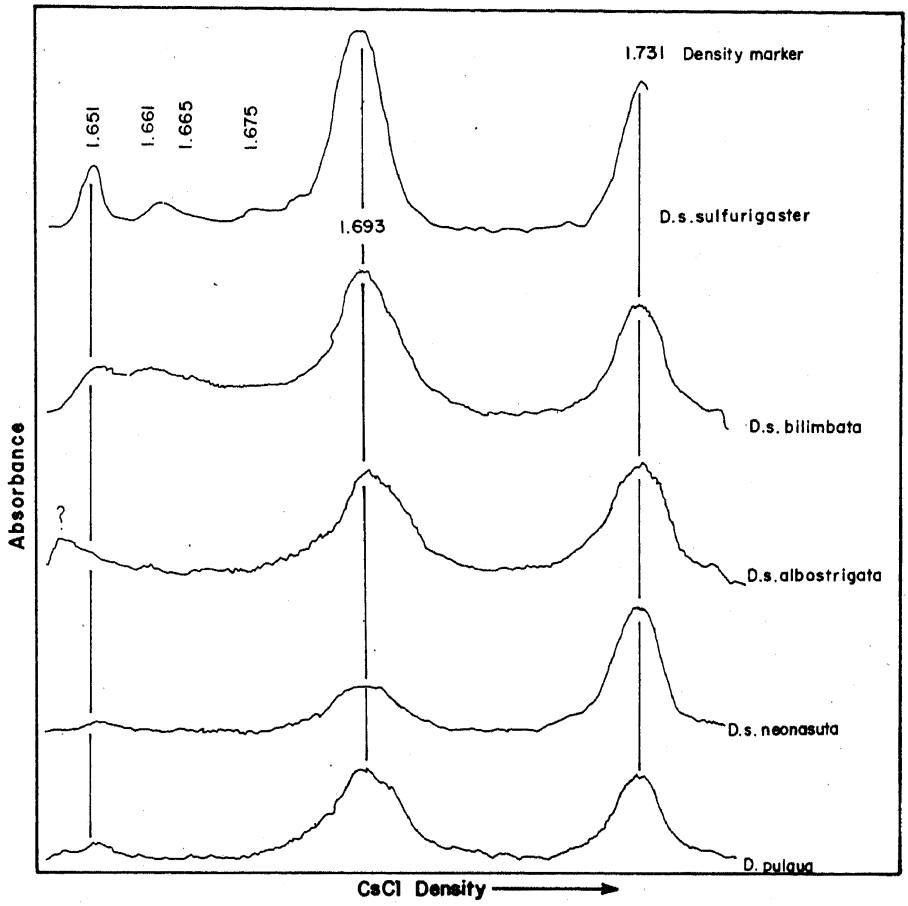

Figure 3. Density profile of DNA (from larval brains) of 5 strains of the orbital sheen complex of *nasuta* subgroup, after analytical CsCl equilibrium/density gradient centrifugation. The densities of the various DNA fractions were calculated relatively to *Micrococcus lysodeictius* according to the method of Szybalski (1968).

ponents contribute to the overall reproductive performance of a population. The differences in the reproductive abilities of different populations reflect the extent of fitness divergence and hence the underlying genetic differences between the concerned populations.

The fitness phenotypes of the members of the orbital sheen complex of *Drosophila* were compared by means of assessing certain parameters of fitness (tables 2 and 3). Of the 5 members under study, *D.s. neonasuta* was more fecund and also its eggs were more viable than others. The eggs of *D.s. albostrigata* had the fastest rate of egg to adult rate of development while it had the least degree of fecundity. *D.s. sulfurigaster* had the slowest rate of development while the eggs of *D.s. bilimbata* had the least degree of viability.

Populations of organisms must live and reproduce in order to be designated 'adapted'. Adaptedness refers to the ability of the carriers of a genotype or a group of genotypes to survive and reproduce in a given environment (Dobzhansky 1968). Four components of adaptedness namely population size, productivity, mortality and flies per bottle were estimated and the mean values of these are set forth in table 3. For all the components of adaptedness, *D.s. neonasuta* tops the list with

Table 2. Three pre-adult parameters of fitness, namely fecundity, egg to adult rate of development and egg to adult viability of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila*.

Strains	Fecundity (eggs/day/ individual)	Mean Developmental time (days)	Egg to adult viability. (for 500 eggs)
D.s. sulfurigaster	7·23	16·42 ± 0·08	148 (29·60%)
D.s. bilimbata	4·67	13·46 ± 0·14	83 (16·60%)
D.s. albostrigata	4·30	11·79 ± 0·12	185 (37%)
D.s. neonasuta	12·34	13·14 ± 0·09	303 (60·60%)
D. pulaua	7·82	15·17 ± 0·14	271 (54·20%)

Table 3. Mean values of population size, productivity, mortality and flies per bottle—4 components of adaptedness recorded in 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila*.

Strains	Population size	Productivity	Mortality	Flies per bottle
D.s. sulfurigaster	112·95 ± 4·21	93·87 ± 6·06	64·77 ± 6·37	32·86 ± 1·22
D.s. bilimbata	123·43 ± 7·85	82·78 ± 5·56	62·78 ± 3·70	35·91 ± 2·28
D.s. albostrigata	132·68 ± 7·31	109·45 ± 5·33	84·48 ± 8·26	38·60 ± 2·13
D.s. neonasuta	243·29 ± 11·37	146·38 ± 6·12	115·35 ± 5·10	70·78 ± 3·33
D. pulaua	133·89 ± 6·06	109·95 ± 8·82	82·37 ± 7·47	38·95 ± 1·53

significantly more values than those of others. The ranking is as follows: *D.s. neonasuta* > *D. pulaua* > *D.s. albostrigata* > *D.s. bilimbata* > *D.s. sulfurigaster*.

3.4 *Ecogenetic divergence*

Although many authors regard animal speciation as primarily the development of reproductive isolating mechanisms, Parsons (1981) has argued that an ability to use the resources of the environment in such a way as to be protected against niche competitors (Mayr 1977) is an equally, if not more important component. For a coherent evolutionary analysis of species and populations, an integration of genetics at the levels of populations and ecology is needed (Ushakumari and Ranganath 1986b). *Drosophila* has been used as a representative system to understand genetic basis of ecological differentiation (Parsons and Spence 1981; Powell and Andjelkovic 1983; Taylor and Condra 1983; Ramachandra and Ranganath 1986). Parsons and Spence (1981) have shown resource utilization divergence among 6 closely related *Drosophila* species of *melanogaster* subgroup in the utilization of ethanol and acetic acid as energy sources. In the present study, 3 types of sugars, sucrose, glucose and fructose were used as different resources and the response of the 5 members of the orbital sheen complex to these different sugars was evaluated in terms of rate of development, viability and population size. Table 4 gives the mean rate of development in days of the 5 strains in different media. The average rate of development ranges between 13 and 17 days in the media with sucrose, 12–16 days in the media with glucose and 12–13 days in the media with fructose. Thus, there is not much of a difference in the egg to adult rate of development of the 5 strains under study in the

Table 4. Egg to adult rate of development in days (mean ± SE) of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* in media with different sugars.

Strains	Sucrose	Glucose	Fructose
D.s. sulfurigaster	14·49 ± 0·12	15·36 ± 0·14	13·93 ± 0·12
D.s. bilimbata	17·87 ± 0·10	12·16 ± 0·11	13·36 ± 0·07
D.s. albostrigata	13·85 ± 0·11	13·25 ± 0·19	12·04 ± 0·04
D.s. neonasuta	13·92 ± 0·11	12·56 ± 0·12	12·85 ± 0·11
D. pulaua	17·37 ± 0·25	16·66 ± 0·22	13·94 ± 0·20

Table 5. Egg to adult viability (for 500 eggs) of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* in media with different sugars.

Strains	Sucrose	Glucose	Fructose
D.s. sulfurigaster	121 (24·20%)	55 (11%)	116 (23·20%)
D.s. bilimbata	78 (15·60%)	119 (23·80%)	140 (28%)
D.s. albostrigata	106 (21·20%)	32 (6·40%)	25 (5%)
D.s. neonasuta	253 (50·60%)	230 (46%)	238 (47·60%)
D. pulaua	117 (23·40%)	80 (16%)	114 (22·80%)

Table 6. Average population size (mean of 4 replicates) of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* in media with different sugars.

Strains	Sucrose	Glucose	Fructose
D.s. sulfurigaster	93·63 ± 2·33	64·14 ± 4·84	42·77 ± 3·96
D.s. bilimbata	89·22 ± 2·62	56·34 ± 3·02	58·50 ± 3·60
D.s. albostrigata	75·92 ± 5·27	34·97 ± 2·32	57·47 ± 1·34
D.s. neonasuta	188·13 ± 5·81	147·97 ± 6·64	73·82 ± 2·30
D. pulaua	75·98 ± 3·48	31·86 ± 2·51	68·88 ± 2·39

media with fructose, but the differential response of these strains to media either with sucrose or glucose is revealing. Similarly, the response of the eggs of any one strain to different media reveal that the eggs of *D.s. albostrigata* and *D.s. neonasuta* have completed egg to adult development within a span of 12–13 days in 3 types of media, while it was 12–17 days for the eggs of *D.s. bilimbata* and 13–17 days for the eggs of *D. pulaua.*

The findings on egg to adult viability of the 5 strains of *Drosophila* in different media is shown in table 5. In all the 3 types of media, eggs of *D.s. neonasuta* were found to be more viable than the eggs of other strains. The range of variation in the extent of viability in the media with sucrose was 15–50%, in the media with glucose it was 6–46% and in the media with fructose it was 5–47%. Intrastrain comparison shows that except *D.s. bilimbata*, remaining 4 strains had more viability in the media with sucrose than in other media, while the eggs of *D.s. bilimbata* were more viable in the media with fructose and it had the least viability in the media with sucrose.

In addition to these, the overall average population size maintained by these 5 strains in different media has been estimated and the same is given in table 6. *D.s. neonasuta* has emerged as the most successful strain in all the 3 types of media as

indicated by its highest mean values. *D.s. neonasuta* had a mean population size of 188, 147 and 73 in the media with sucrose, glucose and fructose respectively.

These studies with different sugars have exposed differential abilities of these strains to exploit the media with different sugars. This is an indication of inherent divergence of these strains to exploit a resource material present in the ambient system.

3.5 *Isozyme variations*

The electrophoretic revolution of the last two decades has proved that electrophoretic technique is a powerful tool to assess genetic divergence and/or identity between populations/races/species (Throckmorton 1977). Recently, Buth (1984) has critically evaluated the application of electrophoretic data in systematic studies. Ramesh and Rajasekarasetty (1980) have analysed the genetic variability at loci concerned with acid phosphatase, alkaline phosphatase, α-esterase, β-esterase, α-glycerophosphate dehydrogenase and tetrazolium oxidase in these 5 members of the orbital sheen complex of *Drosophila*. The summary of their findings in terms of genetic identity and genetic distance among these members are compiled in table 7. These values suggest the level of genetic differentiation at these loci studied in the *Drosophila* members under study. The maximum genetic distance of 0·705 was noticed between *D.s. neonasuta* and *D. pulaua*, while the least genetic distance of 0·378 was between *D.s. albostrigata* and *D.s. sulfurigaster*.

4. Conclusion

These morphologically similar and taxonomically and phylogenetically parsimonious members of the orbital sheen complex of *Drosophila* have striking differences in their karyotypic organization, satellite DNA, fitness phenotypes, ecogenetic divergence and isozyme variations. Evolutionary divergence is a dynamic process and each one of the above said features represents different facets of differentiation. Further, it is difficult to establish and to universalise the correlations between different phenotypes of evolving populations. This is especially important, when considering the fauna on a world wide scale, after all, evolutionary patterns, rates and pressures should not be expected to produce uniform results at all places, at all times and in all aspects (Wheeler 1981). Therefore, the genetic history and evolutionary fate of each population is unique to itself and it is beyond the range of predictability.

Table 7. Values of genetic identity, Ixy (above diagonal separation) and distance, Dxy (below diagonal separation) estimated among different members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila* (Ramesh and Rajasekarasetty 1980).

DXY \ IXY	*D. pulaua*	*D.s. neonasuta*	*D.s. sulfurigaster*	*D.s. bilimbata*	*D.s. albostrigata*
D. pulaua	—	0·494	0·571	0·639	0·678
D.s. neonasuta	0·705	—	0·549	0·612	0·573
D.s. sulfurigaster	0·561	0·600	—	0·574	0·685
D.s. bilimbata	0·448	0·491	0·555	—	0·680
D.s. albostrigata	0·389	0·557	0·378	0·386	—

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