

Taxonomic status of *Drosophila pallidosa* and the biological species concept

B. N. Singh

The genus Drosophila is an interesting biological model which shows extensive diversity at the level of species. About 50 years ago, Bock and Wheeler described a new species of Drosophila, D. pallidosa just on the basis of sexual isolation and differences in sex comb teeth number. However, there is similarity in male genitalia and absence of post mating reproductive isolation and it is considered sibling to D. ananassae, which is a domestic and cosmopolitan species. On the other hand, D. pallidosa is endemic to certain islands such as South Pacific Island of Samoa and Fijii where they are sympatric and remain reproductively isolated by sexual isolation.

In these two sibling species, results of various investigations have been reported pertaining to taxonomic status, morphometric traits, metaphase karyotypes, etc. show similarities and differences which is basis of suggestion that D. pallidosa is in statu nascendi. However, its description as a new species fits well with the biological species concept and it provides interesting material for future studies in the area of speciation genetics.

Keywords: Biological species concept, *Drosophila ananassae*, *Drosophila pallidosa*, genetic diversity, speciation genetics.

ALTHOUGH *Drosophila melanogaster* was used for the first time in genetic studies by T. H. Morgan beginning in 1909, several other species were also employed in studies pertaining to genetics, cytogenetics, chromosomal polymorphism, behaviour, ecology, evolution, molecular biology, etc., which includes *Drosophila simulans*, *Drosophila pseudoobscura*, *Drosophila persimilis*, *Drosophila robusta*, *Drosophila willistoni*, *Drosophila subobscura*, *Drosophila ananassae*, *Drosophila nasuta*, *Drosophila bipectinata*, *Drosophila malerkotliana*, *Drosophila funebris*, *Drosophila rubida*, *Drosophila pavani*, Hawaiian species, etc. Initial studies have demonstrated the use of *Drosophila* in research pertaining to evolutionary biology involving reproductive isolating mechanisms and inversion polymorphism¹⁻³. Among these species, *D. ananassae* is genetically unique due to the presence of certain unusual features such as spontaneous male recombination, high mutability, absence of genetic coadaptation, presence of spontaneous genetic mosaic, parthenogenesis, high incidence of translocations and pericentric inversions, etc.⁴⁻⁸. There are extensive studies on inversion polymorphism in the Indian populations of *D. ananassae*, which have demonstrated that the populations are genetically diverged, and three cosmopolitan inversions have been incorporated in the genetic machinery of the species^{3,9-11}.

In 1858, Doleschall¹² described *D. ananassae* as a new species for the first time. This species is domestic and cosmopolitan in distribution. However, it is mainly circumtropical in geographic distribution. The process of speciation in South Pacific populations of *D. ananassae* was studied for the first time by Futch¹³, who reported light (brown-yellow) as well as dark forms (blackish-brown) of the species. Both these forms are morphologically similar, although they differ in pigmentation and not showing post-zygotic reproductive isolation. Both these forms are reproductively isolated units occurring in the same geographical area and thus are sympatric in distribution. Bock and Wheeler¹⁴ separated these two forms into two different species: the dark form is *D. ananassae*, a domestic and cosmopolitan polytypic species, and the light form is *Drosophila pallidosa*, restricted as an endemic species found in South Pacific populations of Samoa and Fiji. Also, their separation into different species is based on behavioural isolation and variation in sex comb teeth number. Behavioural or sexual or ethological isolation between them was studied in detail by Futch¹⁵, who gave the nomenclature of 'sibling species' to them. The common occurrence of sibling species in the genus *Drosophila* was reviewed by Singh¹⁶ with emphasis on their evolutionary significance. This is a unique pair of sibling species having similar male genitalia as well as the lack of post-mating reproductive isolation^{17,18}. A number of studies pertaining to their evolutionary relationship have been conducted in this unique pair of sibling species¹⁷⁻²⁰. Singh and Singh²⁰ compared these two species, showing the similarities and differences between them, which are

B. N. Singh is in the Genetics Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221 005, India.
e-mail: bnsingh@bhu.ac.in

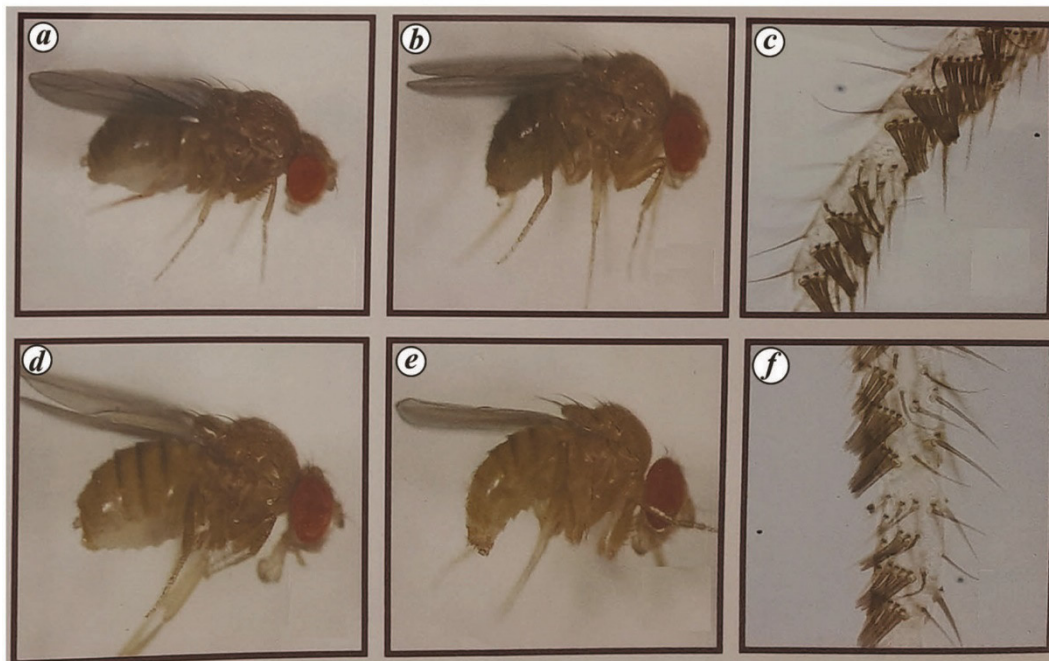


Figure 1. Two sibling species: *Drosophila ananassae* and *Drosophila pallidosa*. (a–c) *D. ananassae*: a, female; b, male and c, sex comb. (d–f) *D. pallidosa*: d, Female; e, Male; f, Sex comb (after Singh¹⁸).

summarized here while adding some new findings related to their phylogenetic relationships. A suggestion is also made that the description of *D. pallidosa* as a new species is valid according to the biological species concept.

Taxonomic status

Futch¹³ reported two forms of *D. ananassae*: light and dark from South Pacific populations. He demonstrated that these forms are cytologically and behaviourally different, although morphologically similar. Bock and Wheeler¹⁴ separated these two forms as separate species: *D. ananassae* (dark form) and *D. pallidosa* (light form). The separation is based on two characteristics: ethological isolation between them and the difference in the number of sex comb teeth. Both species have similar male genitalia, an important taxonomic character for identifying species in *Drosophila*. There is another pair of sibling species, *D. aldrichi* and *D. wheeleri*, belonging to the *mulleri* subgroup. This is also an interesting pair of sibling species with similar male genitalia. Hence, the question was raised about their status as independent species. However, later, based on hybrid sterility and molecular data, their independent status of separate species was substantiated^{21,22}.

However, the case of *D. ananassae* and *D. pallidosa* is different from the others. Singh¹⁸ considers them to be a unique pair of sibling species. In both species, male genitalia are similar, which produce normal and fertile hybrids, indicating the absence of postzygotic reproductive isolation. Further, no molecular data are available to separate them

as independent species. It has also been suggested that the rate of evolutionary divergence between this pair is slow in comparison to other species pairs or even races of certain species²³. Schug *et al.*²⁴ studied mate discrimination patterns and DNA polymorphism in different geographic populations of *D. ananassae* and *D. pallidosa*. Based on genetic and behavioural studies, they have raised doubts about the independent status of *D. pallidosa* and suggested that it is not a good species. McEvey and Schiffer²⁵ have also raised similar doubts on the full status of *D. pallidosa* as an independent species based on the similarities in male genitalia between these two *Drosophila* species. Dobzhansky and Spassky²⁶ coined the term ‘*statu nascendi*’ for the cluster of species of *D. paulistorum*, in which different sympatric geographic populations cannot interbreed and exchange genes. Similarly, Singh and Singh²⁰, and Singh^{17,18} suggested that *D. pallidosa* is a species in *statu nascendi*, considered an incipient species and still in the process of speciation. Sex comb tooth number has been studied in the two species and their hybrids. The hybrids have intermediate numbers, but the two species show variation. *D. ananassae* has a significantly higher number of sex comb teeth when compared with *D. pallidosa*²⁷. Figure 1 depicts males, females and sex combs of *D. ananassae* and *D. pallidosa*.

Morphometric traits

Two studies have been published so far in which different morphometric traits were compared between the two *Drosophila* species^{28,29}. There was a significant difference

between them in both sexes with respect to wing length, thorax length, wing-to-thorax ratio, sternopleural bristle number, sex comb tooth number and ovariole number. There was also a significant difference between the hybrids and parental species²⁸. Further, values of all the morphological traits were higher in females than males in both species. When different strains were used, there was intraspecific variation in these morphological traits²⁹. Although sibling species have been defined as morphologically similar, differences in certain morphological traits have been found between them, which act as discriminant markers.

Metaphase karyotypes

Metaphase karyotypes of both the species were identical: 6A+XX female and 6A+XY male. In both species, there were autosomes and sex chromosomes: a pair of medium-size V-shaped metacentric X-chromosomes in females and a J-shaped Y-chromosome in place of X-chromosome in males, two pairs of large V-shaped metacentric autosomes and a pair of small, V-shaped autosomes. This shows the close similarity between the two sibling species¹³.

Hybrid fertility

When light and dark forms were tested, fertility was observed in parental as well as back crosses¹³. Thus, both forms hybridize under laboratory conditions and generate fertile offspring, but the degree of fertility varies in different crosses. Futch¹⁵ has also suggested that occasional hybridization must have occurred in the natural populations of these two species, which are sympatric as well, but such hybridization must have been very low, and the hybrids generated should have been observed in the parental populations. The degree of crossability and productivity by making reciprocal crosses between these two species were studied by Vishalakshi and Singh²⁸, who have shown variation in both parameters. Singh and Singh²³ carried out experiments to score the fertility of male hybrids, morphology of testes and protein content of testes in both the species as well as their hybrids. Their results showed a reduction in the fertility of hybrid males, although statistically insignificant. However, there was no difference in the morphology of testes between the parental species and their hybrids. The protein content was lower in hybrid males than in males of parental species. These findings suggest that both species have not diverged much, and the rate of speciation remains slow. However, these species diverged 1.68 MYA. If this time is compared with other species pairs, evolutionary divergence is more than that of *D. ananassae* and *D. pallidosa*³⁰. Similar testis morphology in both the species and their hybrids, as well as the low level of sterility in hybrid males, support the conclusion that the rate of cladogenesis is slow. They are still in the process of speciation and thus may be designated as incipient species. The

results of Singh and Singh³¹ also extend the evidence for variation in productivity and mate recognition in hybrids and parental species. Further, mate discrimination was found stronger in the ancestral species *D. ananassae*.

Ethological isolation

Dobzhansky³² coined the term 'isolating mechanisms' for the first time. This is an important aspect of speciation in particular and evolution in general. It has been considered that without isolation, evolution is not possible. It has also been emphasized that reproductive isolating mechanisms are a pre-requisite for speciation³³. Isolating mechanisms are defined as factors that prevent gene flow between different Mendelian populations by preventing interbreeding between them. Different types of isolating mechanisms in animals have been described with suitable examples^{1,2,32-34}. In reproductive isolating mechanisms, two types have been extensively studied in the genus *Drosophila*: sexual, behavioural or ethological isolation and hybrid sterility. There are numerous examples in *Drosophila*^{1,2}. Sexual isolation is premating or prezygotic isolation, while hybrid sterility is post-mating or post-zygotic isolation. Interestingly, the separation of light and dark forms of *D. ananassae* was done on the basis of ethological or sexual isolation into different species, viz. *D. ananassae* and *D. pallidosa*. Futch^{13,15} observed sexual isolation between light and dark forms as well as between *D. ananassae* and *D. pallidosa*. He also referred to them as sibling species. Vishalakshi and Singh³⁵ reported sexual isolation between these two *Drosophila* species using different experimental techniques. They observed strong sexual isolation between them and that different experimental techniques did not affect the pattern and degree of sexual isolation. Singh and Singh³⁶ also studied sexual isolation between these two species by employing different strains of both species. These authors presented evidence for both intra- and interspecific ethological isolation between them. Although *D. pallidosa* is an endemic species, it showed a higher degree of intraspecific sexual isolation compared to its sibling *D. ananassae*. With the help of molecular techniques, it has been shown that certain loci are involved in mate discrimination and willingness of females to mate, which are present on 2L, XL and 3R (refs 37, 38). It shows that ethological isolation between these two species has a genetic basis. It has also been suggested by Singh^{2,39} that these two species have an ancestral and derived relationship: *D. pallidosa* is derived from *D. ananassae* (ancestral). Thus, the pattern and degree of ethological isolation, which is premating isolation, have been used to elucidate the phylogenetic relationship and direction of evolution between these two species². *D. ananassae* and *D. pallidosa*, which are sympatric and sibling species, maintain their independent status through behavioural isolation, which is the main basis of their separation as independent species¹⁴.

Fluctuating asymmetry

Vishalakshi and Singh⁴⁰ studied the fluctuating asymmetry (FA) in this pair of sibling species and their hybrids. FA is a measure of developmental stability which refers to the ability of organisms to buffer their developmental processes against environmental and genetic perturbations⁴¹. Different morphological traits were used to study FA in these two species and their hybrids. It was found that FA in hybrids was trait- and sex-specific. Further, a comparison among parental species and hybrids showed that the level of FA was not higher in hybrids compared to parental species, suggesting that hybridization does not cause a reduction in the level of developmental stability of the hybrids⁴⁰. This is evidence that these two species have not diverged much genetically.

Parthenogenesis

Futch¹³ reported parthenogenesis in light and dark forms of *D. ananassae* for the first time. He also established an entirely parthenogenetic line by selection. Automictic type of parthenogenesis was reported by Carson *et al.*⁴² in *D. mercatorum*. Parthenogenesis found in *D. ananassae* was similar to that reported in *D. mercatorum*. Few flies of both forms were found to carry the *Parth* gene, causing parthenogenesis. The occurrence of *Parth* gene in both the forms shows that this gene is derived from their ancestral species and has been conserved in both forms. It also shows the substantial gene flow between them. Interestingly, the hybrid females between the two species also generate impaternal progeny efficiently, similar to parthenogenetic strain of both parental species. The *Parth* gene of both sibling species showed homology, and there was no incompatibility between them, which demonstrates similarity between *D. ananassae* and *D. pallidosa*.

Allozyme and chromosomal polymorphism

Chromosomal and allozyme polymorphisms have been studied in both light and dark forms of *D. ananassae*^{13,43}. Johnson *et al.*⁴³ studied allozyme polymorphism in light and dark forms of *D. ananassae* from American and Western Samoa. No hybrid bands were found for Est C and APH, and there was a difference in allelic frequencies between the two forms of *D. ananassae*. Chromosomal polymorphism in light and dark forms of *D. ananassae* was studied by Futch¹³, employing different stocks derived from different localities, and making crosses between them. A large number of pair matings involving dark flies from Tutuila, American Samoa, showed the presence of standard gene arrangement of *D. ananassae*. One pair mating produced larvae heterozygous of basal inversion in 3R. Another pair showed one larva with an asymmetric pericentric inversion in the third chromosome. A large number of pair

matings of light flies from Tutuila produced larvae heterozygous for overlapping inversion in 2R, larvae heterozygous for overlapping inversion in 2L and larvae for a small, single inversion in 2L (2LB). These inversions were found frequently in heterozygous conditions in hybrid larvae obtained by crossing dark and light flies. Hybrid larvae were always heterozygous for inversion in 3R (3RB) and XL (XLA). Both these inversions were found to be homozygous in the light forms. The same basic gene arrangement was found in the dark flies when the larvae were produced by crossing two forms from Tutuila and flies from other stocks of *D. ananassae*, except those from Papua New Guinea. Two stocks from Papua were found to be homozygous for XLA, and one of these two was homozygous for 3RB. The chromosome II inversions, viz. 2RA, 2LB and 2LC, found in Tutuila light form, were also present in these flies. There is an evolutionary relationship between light Tutuila flies and the Papuan flies. Futch¹⁵ studied ethological isolation between *D. ananassae* and *D. pallidosa* and chromosomal variability. All the stocks of *D. pallidosa* were found to be homozygous for the XLA arrangement. The polymorphic nature of chromosome II was found to be a general phenomenon in the Samoan *D. pallidosa* populations. Interestingly, the small median inversion 2LB in the left arm of *D. pallidosa* chromosome II was detected in *D. ananassae* collected from Micronesia and Formosa, as well as in the sample from Papua New Guinea¹³. In the collections of Micronesia and New Guinea, two pairs of overlapping inversions 2LC and 2RA have also been found. The presence of overlapping inversions shows evolutionary significance, and it has been used to determine the structure of the second chromosome intermediate between these unique *D. pallidosa* sequences and the standard *D. ananassae* sequence¹⁵. Inversion 3RB has been commonly found in all the Samoan populations of *D. pallidosa*. However, the standard *D. ananassae* third chromosome arrangement was also detected in the stocks from the two Samoan western islands. This was taken as the basis for interbreeding caused by introducing the standard arrangement of *D. ananassae* into the populations of *D. pallidosa*¹⁵. The sharing of inversions was also noted by Matsuda *et al.*¹⁹ among *D. ananassae*, *D. pallidosa* and Papua New Guinea endemics resulting from introgression of the chromosomes. When these two species were crossed, Singh *et al.*⁴⁴ found heterozygous loops in certain regions of the chromosomes, indicating the difference in gene arrangements between the two species. However, there was a complete synapsis of homologous chromosomes in the F1 hybrid larvae, demonstrating homology between chromosomes of these two species. Singh and Singh²⁰ suggested that these studies on inversion polymorphism showing differences as well as sharing of inversions reveal that they are of recent evolutionary divergence and are characterized by a certain degree of gene flow, although they are reproductively isolated in sympatric situations.

Interpulse interval and courtship song

Bennet-Clark and Ewing⁴⁵ have demonstrated that the interpulse interval (IPI), which is a parameter of courtship song in *Drosophila*, plays a crucial role in female preference among closely related species. This parameter has a genetic basis⁴⁶. It has been found that these two sibling species of *Drosophila* have identical IPI and intrapulse frequency⁴⁷. It is unique that both species have identical IPI values. This finding clearly shows that *D. ananassae* and *D. pallidosa* have not diverged genetically with respect to IPI. Males vibrate their wings prior to copulation, and this occurs in a species-specific manner. Spieth⁴⁸ found a striking difference between *D. ananassae* and *D. pallidosa* males with regard to wing display. The pattern of variation in wing vibrations showed minimal differences, clearly indicating that their separation is of recent origin. It has been demonstrated that the acoustic signals produced by male wing vibrations are important in gaining ethological isolation between them. Females of both species discriminate the courting males primarily on the basis of acoustic cues^{37,47}. This proves that the divergence of mating signals and the mate recognition system have played a crucial role in separating these two sibling species²⁰.

Nucleolus organizing region variations

In *D. melanogaster*, the nucleolus organizing region (NOR) is located on the X- and Y-chromosomes. However, *D. ananassae* has NOR on the Y- and fourth chromosomes, whereas *D. pallidosa* has NOR on the fourth chromosome only. This is a striking difference between the two sibling species²⁰.

Molecular phylogeny

Only one study has been conducted so far on the molecular phylogeny between this pair of sibling species belonging to the *ananassae* subgroup¹⁹. The study employed mitochondrial COI and Y-chromosome K/2 loci because they do not show recombination, so they are more useful in such studies on phylogeny. The results of the study revealed the presence of common sequences in both species at the COI locus, and no evidence of differences either within the species or between them at the K/2 locus. This proves that the divergence between these two sibling species is of recent origin²⁰.

Effect of age on mating propensity

Mating propensity was tested in both species employing different geographic strains. There was significant variation in different strains of both species. Further, flies of different age groups (4, 8, 12, 16 and 20 days) were used. *D. ana-*

nassae had a higher mating propensity than *D. pallidosa*. Interestingly, 12-day-old flies had the highest mating propensity in both species. Age-wise variation was more apparent than strain-wise variation in *D. ananassae*, but the findings were just the opposite in the case of *D. pallidosa*⁴⁹.

Cuticular hydrocarbons

The two *Drosophila* species showed variation in cuticular hydrocarbons (CHCs), which play an important role in species isolation^{50,51}. It has been shown that CHCs have an important role in sexual communication as sex attractants and cues for species, gender and individual recognition⁵². Certainly, the obvious differences in CHCs between *D. ananassae* and *D. pallidosa* may be important in the behavioural isolation between them. Further, the differences in CHC level arise at the species level but not at the sex-specific level, which shows that their separation is of recent origin in the process of speciation²⁰.

Conclusion

Futch¹³ conducted an extensive study pertaining to the process of speciation in the South Pacific populations of *D. ananassae* and reported two forms, viz. light and dark. He also found cytogenetic and behavioural differences between the two forms¹³. Subsequently, Bock and Wheeler¹⁴ identified the light form as a new species, which was named *D. pallidosa*. The two species, viz. *D. ananassae* (dark form), cosmopolitan and domestic species and *D. pallidosa* (light form), having restricted distribution in certain South Pacific Islands, occur sympatrically. Interestingly, they are identified as separate species on the basis of ethological isolation and variation in sex comb teeth number. It is important to note that both species have identical male genitalia, which is a taxonomic feature. Further, these species can be crossed in the laboratory, generating fertile hybrids of both sexes. The post-mating reproductive isolation is absent between them. Futch¹⁵ designated them as sibling species. As suggested by Singh¹⁸, they are a unique pair of sibling species. Numerous studies performed on this pair of sibling species discussed here clearly demonstrate differences and similarities between the species. Schug *et al.*²⁴ suggested that *D. pallidosa* is not a good species, and they doubted the independent status of this species. It may be in *statu nascenti*^{17,20}. In conclusion, it is suggested that *D. pallidosa* is still undergoing the process of cladogenesis, and its separation is a recent event. Further, phylogenetically *D. ananassae* and *D. pallidosa* are close to each other, showing ancestral-derived relationships.

Bock and Wheeler¹⁴ described *D. pallidosa* as a new species about 50 years ago, originally named as the light form of *D. ananassae* by Futch¹³. These *Drosophila* species also constitute a unique pair of sibling species¹⁸. Although

D. pallidosa shows similarity in male genitalia with *D. ananassae* and lacks post-zygotic reproductive isolation with its sibling species because it produces fertile hybrids when hybridized in the laboratory, its description as a new species is valid according to the definition based on the biological species concept proposed by Theodosius Dobzhansky and Ernst Mayr in the previous century. In the literature, a number of species concepts have been described, and a species, which is the basic unit of biological classification and taxonomic rank, is defined differently under different species concepts (for references, see Singh⁵³). The biological species concept is the most widely accepted, although it has certain limitations in application⁵⁴. Under the biological species concept, a species is defined as a group of potentially or actually interbreeding natural populations which are reproductively isolated from other such groups⁵⁵. Dobzhansky⁵⁶ defined species as a reproductive community of sexually and cross-fertilizing individuals sharing a common gene pool. Dobzhansky, being a population geneticist, used the term 'gene pool'. Thus, *D. ananassae* and *D. pallidosa* occurring sympatrically in certain American Samoan Islands are separate species and maintained as separate gene pools by sexual isolation, which is an important class of prezygotic reproductive isolation that does not allow gene flow between these two separate species, even though they are geographically sympatric in distribution. It may be noted that there are criticisms about the biological species concept. As suggested by Wang *et al.*⁵⁷, it is time to abandon the biological species concept and accept the genic concept of species. However, Butlin and Stankowski⁵⁸ have argued that there is no difference between the genic and biological species concepts, unless the latter is associated with allopatric accumulation of reproductive isolation and the former is not. Thus, this unique pair of *Drosophila* sibling species provides good material for further studies in speciation genetics.

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