Drosophila bipectinata species complex

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The Drosophila bipectinata species complex belongs to the ananassae subgroup of the melanogaster species group (Genus Drosophila, Subgenus Sophophora). The members of the complex are: D. bipectinata, D. parabipectinata, D. malerkotliana, and D. pseudoananassae. Of the four species, D. bipectinata is most widely distributed. Females are indistinguishable, but males are distinguishable by their sex-comb teeth number and pattern and by abdominal colouration. Chromosomal inversions have been detected in these species. In natural populations of D. bipectinata the frequency of inversions and the level of inversion heterozygosity were found to be very low but in laboratory stocks inversions persisted for more than 20 generations due to heterotic buffering. On an average 9.3 fixed interspecific inversions separate each species pair. Non-random association between linked inversions indicated epistatic interaction in natural populations of D. bipectinata. Certain spontaneous mutations were detected and mapped for the first time in D. bipectinata. Low frequency of spontaneous male recombination has also been reported in D. bipectinata. Sexual isolation study in the complex indicated strong preference for homogamic mating. The results also indicated incomplete sexual isolation among different members of this complex. The isolation estimate among six different geographic populations of D. bipectinata ranged from 0.54 - 0.92 representing positive assortative mating which is an evidence for incipient sexual isolation. Incipient sexual isolation was also found within D. malerkotliana and D. parabipectinata. Chromosomal, hybridization and allozyme studies revealed close phylogenetic relationship among the four species of the bipectinata complex. Mitochondrial DNA study revealed net nucleotide difference (δ) between these species to be very small (0.0002± 0.0008) reflecting closeness. Evidence for genetic control of sexual activity and existence of sexual selection in D. bipectinata has been shown on the basis of mating propensiy tests carried out on geographic strains, their hybrids and diallel crosses. Significant variation was found among the strains tested with respect to courtship time, duration of copulation and fertility. A positive correlation between duration of copulation and fertility in D. bipectinata was found. Evidence for rare-male mating advantage was also found in D. bipectinata. A positive response to selection for high and low mating activity provided evidence for polygenic control of this phenomenon in D. bipectinata. Bilateral outgrowths on thorax, a unique phenotype, reported for the first time in D. bipectinata has been shown to affect mate recognition ability. Results of the study on pupation site preference (larval behaviour) and oviposition site preference (non-sexual behaviour) have also been included.

Drosophila bipectinata species complex contains 4 species viz. D. bipectinata, D. parabipectinata, D. malerkotliana and D. pseudoananassae. Taxonomically, the bipectinata complex species are members of the subgenus Sophophora of the melanogaster species group and the ananassae species subgroup. The four species are morphologically very similar. Females are indistinguishable and no differences have been found in the genitalia of males or for females. Males are distinguished however by their sex-comb teeth number and pattern and also by abdominal colouration. Males of two species D. malerkotliana and D. parabipectinata have black posterior abdominal tergites. Of the 4 species, the most widespread is D. bipectinata. Its geographic distribution ranges from India, through south-east Asia and New Guinea to Fiji and Samoa in the pacific. The other three species i.e. D. parabipectinata, D. malerkotliana and D. pseudoananassae are less widespread but in parts of south-east Asia all four species are sympatric.

D. bipectinata was first described by Duda from Darjeeling, India. The karyotype of this species comprises of 4 pairs of metacentric chromosomes. The medium sized pair was distinguished as sex-chromosomes. The salivary gland nuclei of this species consist of 6 unequal euchromatic arms radiating from the common chromocentre corresponding to 3 pairs of mitotic chromosomes. The smallest 4th pair of mitotic chromosomes appears to be heterochromatic and remains embedded in chromocentre. Panigrahy also constructed a detailed photomap showing standard banding sequence based on their gross similarity with D. ananassae. The polytene chromosome complement of D. malerkotliana also contains four long autosomal arms.

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and two short X-chromosome arms. Male genital structures of these species were described by Gupta. Studies related to taxonomy, morphometric traits, chromosomal polymorphism, hybridization population and behaviour genetics etc. have been carried out by different workers in this complex. A close phylogenetic relationship between D. bipectinata and other members of the bipectinata complex was established based on the results of chromosome analysis, hybridization studies and isozyme analysis. Genetic heterogeneity with respect to certain behavioural traits has also been observed in the members of this complex.

Certain members of the bipectinata species complex are of common occurrence in Indian subcontinent and extensive work on certain aspects in this complex has been done by Indian workers. In view of this, the main objective of the present review is to summarise the work carried out by various workers related to taxonomy, morphometrics, chromosomal polymorphism, allozyme polymorphism, mt. DNA restriction profile, hybridization mutations, non-sexual and sexual behaviour in the bipectinata species complex.

**Taxonomy**

Studies by Kaneshiro and Wheeler and by Bock indicated that the ananassae subgroup consists of two distinct species complexes, the bipectinata complex (in males of which the aedeagus is bifid and bare) and the ananassae complex (in males of which the aedeagus is fused and strongly hirsute).

The bipectinata complex contains D. bipectinata, D. malerkotliana, D. parabipectinata and D. pseudoananassae.

**Drosophila bipectinata**

Distribution — D. bipectinata was first described by Duda from Darjeeling, India. D. bipectinata is most widely distributed in the Oriental Region and known from Reunion Island. D. bipectinata occurs in India, Australia, Indonesia, Malaysia, Cambodia, Celebes, Sri Lanka, Taiwan, Nepal, Pakistan, Japan, Philippines, Ryukyu Islands, Singapore, Fiji, Micronesia, Samoa, New Guinea and Bhutan (New record).

General — Small flies. General body colouration yellow, each abdominal tergite with a dull brown, narrow posterior band.

**Drosophila malerkotliana**

Distribution — D. malerkotliana was first described by Parshad and Pai from Malerkotla, Chandigarh and Pinjore, India. This species is found in India, Indonesia, Malaysia, Celebes, Ivory coast, Thailand, Philippines, Singapore, Brazil, Myanmar and Bhutan.

This species has been introduced in Africa where it is now widespread.

There is evidence of a continuing spread of D. malerkotliana towards North America and to the West Indies. Thus, D. malerkotliana appears to be expanding its ranges.

General — Small flies. Halteres yellow. Abdominal tergites black except first, second and third where only black stripe is present on the posterior margin. Sternite pale and squarish.

**Drosophila parabipectinata**

Distribution — It was first described by Bock from Borneo. D. parabipectinata occurs in the Philippines, Borneo, Christmas Island (Indian Ocean), Celebes, Indo-China and India, with the eastern distribution similar to that of D. malerkotliana.

General — Small flies. Distal portion of male abdomen darkens with age to shiny black. Closely resembles D. bipectinata.

**Drosophila pseudoananassae**

Distribution — It was first described by Bock from Australia. D. pseudoananassae occurs in both Oriental and Australian biogeographic zones from Malaya, Borneo and the Philippines east to New Guinea, northern Australian and the British Solomon Islands.

General — Small, pale brown flies. Distal portion of male abdomen black in specimens from Borneo and Malaya, permitting subspecific classification.

**Morphometrics**

Males of D. bipectinata have two obliquely placed sex-combs on metatarsal segment, upper comb with 5-8 teeth, lower one with 6-9 teeth and 1-2 teeth on distal part of second tarsal segment of the first leg. In D. malerkotliana sex-comb is present in two sets, proximal metatarsal comb of 2 transverse rows of 1 and 3-4 tough bristles and lower tarsal comb with similar rows of 1 and 3 tough bristles. Sex-comb of male of D. parabipectinata consists of two longitudinal rows on distal portion of metatarsal consisting of approximately 5 teeth (proximal row), 7 teeth (distal row), 7 teeth (proximal row), and 7 teeth (distal row).
teeth (distal row) and 1-2 teeth on distal border of second tarsal segment. In *D. pseudoananassae* sex-comb of male is present in 3 transverse rows, two rows on distal portion of metatarsus consisting of approximately 2 teeth (proximal row), 5 teeth (distal row) and one row on distal margin of second tarsal segment of approximately 3 teeth. Hybrid males were found to possess sex-combs intermediate in structure between those of the parental species.

The structure of sex-comb in the males of all the four species of *D. bipectinata* complex was studied by Chatterjee and Singh. They noted variation in the number of sex-comb teeth in the four species of the *bipectinata* complex. Considerable intraspecific variation and also individual variation in the number of teeth within strains of *D. bipectinata* were found suggesting polygenic mode of inheritance. Crossely and Taylor found interspecific variation in the number of sex-comb teeth in males of four species of the *bipectinata* complex and their F1 hybrids suggesting polygenic mode of inheritance on the basis of lack of difference in the mean number of teeth between interspecific hybrids and mid-parent value. A study by Banerjee and Singh demonstrated genetic heterogeneity with respect to sex-comb teeth number in several strains of *D. bipectinata* of different geographic origin.

Evidence for interspecific variation in the number of sternopleural bristles was found by Singh and Lata. It was suggested that sternopleural chaeta number in *Drosophila* is an adaptive trait and the species which are genetically more variable and ecologically more versatile show higher sternopleural chaeta number. Recently, variability in morphological traits in *Drosophila bipectinata* complex was studied by Hegde et al. They found large amount of variation for morphometric characters such as lengths of femur and tibia, wing length, wing width, number of sternopleural bristles and bristles on epandrium among different populations of *D. bipectinata* species group. From these studies, it was inferred that the morphological variations are due to the interplay of both genetic and environmental factors. Also, sexual dimorphism for the same metric traits was analysed in the *bipectinata* complex. The differences between the sexes were found to be significantly variable for lengths of femur and tibia, wing length, wing width and number of sternopleural bristles and it was found that females had significantly larger values for the above mentioned morphological traits.

**Chromosomal polymorphism**

Chromosomal polymorphism in four species of the *bipectinata* complex was studied by Bock. Each species was found to be polymorphic for four to six intraspecific inversions. However, none of the inversions was shared between any two species but have accumulated an average of 9.3 fixed interspecific inversions. Gupta and Panigraphy found nine autosomal paracentric inversions distributed on the left and right arms of chromosomes II and III. In the first interspecific cross between *D. bipectinata* and *D. malerkotliana*, made by Narda, two inversion complexes were found in the salivary chromosomes of F1 larval hybrids, one in II L and other in III R. Jha and Rahman studied the role of chromosomal inversions in the evolution of the *bipectinata* species complex. They found that *D. malerkotliana* and *D. bipectinata* differed in presence of 7 paracentric inversions, 3 on the second chromosome and 4 on third chromosome. The F1 hybrids were sterile when intercrossed. They also reported that hybrid females look like the female parent and hybrid males appear to be intermediate in nature. Inversion polymorphism studied by Singh and Das in laboratory stocks of *D. bipectinata* revealed two linked inversions of the second chromosome (In D in 2L and In C in 2R). These inversions were associated non-randomly providing evidence for linkage disequilibrium between inversions maintained by epistatic gene interaction. Das and Singh analysed the laboratory stocks of *D. bipectinata* for chromosome inversions. Four stocks showed the presence of autosomal paracentric inversions (one in 2L, one in 2R and one in 3L). The inversions, In D 2L and In C 2R were more common and were maintained at considerable frequency. The inversions persisted for more than 20 generations in these stocks which suggested that heterotic buffering is associated with these inversions. The results of interracial hybridization experiments involving chromosomally polymorphic strains derived from different geographical localities indicated decline in the frequency of heterozygous inversions, involving second chromosome inversions, which was due to breakdown of polygene complexes by recombination. However, there was no decline in the frequency of heterozygous inversions in interstrain crosses involving third chromosome inversions. Three inversions were detected in 7 natural
populations of *D. bipectinata* sampled by Banerjee and Singh. In C 2R was most frequent. A low level of inversion heterozygosity and inversion frequency was found in these (*D. bipectinata*) populations. Further, no evidence was found for genetic differentiation in natural populations at the level of inversion polymorphism. Significant non-random associations between inversions indicated epistatic interaction in these natural populations. The above findings provided evidence for rigid chromosomal polymorphism in *D. bipectinata*. When natural population of *D. bipectinata* were transferred to laboratory conditions, an increase in the degree of inversion polymorphism was found by Singh and Banerjee. It was suggested that inversion heterozygotes have selective advantage under stringent competition in laboratory conditions.

Inversion polymorphism has also been studied in *D. malerkotliana*. A new paracentric inversion was reported by Naseerulla and Hedge in *D. malerkotliana* population from Varanasi, U.P. The new inversion was named II L C. Earlier, Bock reported two inversions on chromosome II L, one median in position and another in the region between 26 and 46. Jha and Rahman detected two inversions, II L A and II L B. II L A is subterminal in position extending from region 22 to 29 and II L B extending from region 39 to 46. The new inversion II L C is smaller one compared to the above mentioned ones and it lies between the regions 38 and 43.

Allozyme polymorphism

In four species of the *D. bipectinata* complex, electrophoretic analysis was carried out by Yang et al. They studied 21 enzymes controlled by 23 loci. Coefficients of genetic similarity based on frequencies at all loci showed distinctiveness of *pseudoananassae* and the similarity between *D. bipectinata* and *D. malerkotliana*. However, the study revealed that species *D. bipectinata* and *D. parabipectinata* are more similar to each other than either is to *D. malerkotliana* or *D. pseudoananassae*. It was suggested that *D. bipectinata* and *D. parabipectinata* were able to maintain their identities in sympatric populations because of differential niche utilization.

Analysis by polyacrylamide gel electrophoresis of alkaline phosphatase (APH), acid phosphatase (ACPPh) and esterase (EST) activities in the parental species as well as in their hybrids by Hegde and Krishnamurthy revealed certain interesting information. The zymograms of the hybrids of *D. bipectinata* and *D. parabipectinata* exhibited intermediary banding pattern while the hybrids between *D. malerkotliana* and *D. bipectinata* showed peculiar banding pattern resembling one of the parents. On the other hand, the hybrids of *malerkotliana* × *pseudoananassae*, *bipectinata* × *pseudoananassae*, and *parabipectinata* × *pseudoananassae* showed disrupted banding pattern. This study also demonstrated a closer affinity between *D. bipectinata* *D. parabipectinata* and *D. malerkotliana* than with *D. pseudoananassae*. Electrophoretic analysis of alcohol dehydrogenase isozymes by Jha et al. showed co-dominant inheritance of Adh alleles in F1 hybrids and influence of the X-chromosome on the expression of the ADH isozymes in *D. bipectinata* and *D. malerkotliana*.

Substrate specificities for the bands of ADH isozymes were observed in *D. bipectinata* and *D. malerkotliana* by Jha et al. The different ADH isozymes exhibited different substrate affinities and this difference in the sensitivity of ADH to the substrates suggested their possible distinct physiological function. Also, age dependent changes in the ADH activity was noticed in larva, pupa and young adults of *D. bipectinata* and *D. malerkotliana*. Sharma et al. found significant clinal variation in allelic frequency at the Adh locus, which was correlated with increase in latitude. Jha et al. also reported spontaneous occurrence of silent alleles (1-5) for the expression of ADH isozymes in laboratory stocks of *D. malerkotliana* by electrophoresis.

Lattitudinal variation in ethanol tolerance in *D. bipectinata* was observed in different geographical populations by Sharma et al. The northern and southern populations revealed divergence in patterns of resource utilization. They mentioned that ethanol tolerance seems to be maintained by natural selection. Parkash et al. studied patterns of starvation and desiccation tolerance in *D. bipectinata* and *D. malerkotliana*. Sharma et al. found that ethanol utilization indices and the ethanol tolerance threshold values in larval and adult individuals vary latitudinally in different Indian populations of *D. malerkotliana*.

Catalytic functional differences with respect to specific activities, pH dependent activity profile, Vmax values, Km values (Michaelis constant) and KI (Inhibition constant) were observed by Parkash among three common allozymes of ACPH in *D. malerkotliana*. 
Naseerulla and Hegde\textsuperscript{41} compared populations from microclimatic regions of each of \textit{D. malerkotliana} and \textit{D. bipectinata} with regard to electrophoretic variation in two enzymes, alkaline phosphatase (APH) and glucose 6-phosphate dehydrogenase (G6-PD). In \textit{D. malerkotliana} the allelic frequencies and heterozygosity per individual for Aph locus showed significant variation, while in \textit{D. bipectinata} only one allele (Aph 0.95) showed significant interpopulation variation. For G6-PD, differences in allelic frequencies and heterozygosity were insignificant in both \textit{D. malerkotliana} and \textit{D. bipectinata}. Da Lage et al.\textsuperscript{42} located multiple amylase genes in \textit{D. ananassae} subgroup by molecular methods. In \textit{D. bipectinata} they showed the location of amylase genes on chromosomes 2L and 3L.

\textbf{Mitochondrial DNA (mt-DNA) study}

Analysis of mt-DNA restriction profile in the two species of \textit{D. bipectinata} species complex—\textit{D. bipectinata} and \textit{D. malerkotliana} was carried out by Gupta \textit{et al.}\textsuperscript{43}. The study revealed altogether 8 mt-DNA haplotypes with respect to presence or absence of restriction site in mt-DNA in the sample of 18 geographic strains. Only one haplotype (IB) was shared by the two species. The net nucleotide substitution per site calculated between these species was 0.0002. This value appeared to be relatively much lower than expected at the interspecific level, even lower than that reported between two subspecies of \textit{Drosophila}, despite being good species. Thus, from mt-DNA study it appears that the two species have not diverged at the level of mt-DNA, though they have diverged to a greater extent at the level of morphology, chromosomes, isozymes and reproductive isolation after descending from common ancestor population. According to Gupta \textit{et al.}\textsuperscript{43} the most plausible explanation for the closeness of mt-DNA between \textit{D. bipectinata} and \textit{D. malerkotliana} seems to be that these species exhibit poly- or paraphyletic relationship in mt-DNA sequence.

\textbf{Hybridization study}

Bock\textsuperscript{49} studied interspecific hybridization among four species of the \textit{bipectinata} complex and found a high degree of crossability in one direction with production of large number of sterile males and fertile females in a 1:1 ratio (\textit{bipectinata} × \textit{parabipectinata}) to almost complete isolation in both directions. A close phylogenetic relationship among \textit{D. bipectinata}, \textit{D. parabipectinata} and \textit{D. malerkotliana} was suggested based on the results of this study. A considerable degree of divergence between \textit{D. pseudoananassae} and the three species was found which suggested \textit{D. pseudoananassae} to be distantly related. Natural hybridization has been reported between \textit{D. bipectinata} and \textit{D. malerkotliana} but it is rare\textsuperscript{44}.

Sexual isolation among three species of the \textit{D. bipectinata} species complex (\textit{D. bipectinata}, \textit{D. malerkotliana} and \textit{D. parabipectinata}) was studied by Singh \textit{et al.}\textsuperscript{45}. The three species were found to be incompletely isolated. However, the degree of isolation varied in different crosses. The deviation from random mating was highly significant in 5 of the 6 crosses examined, which indicated a strong preference for homogamic matings. Singh and Chatterjee\textsuperscript{46} found evidence for incipient sexual isolation within \textit{D. bipectinata}. Intraspecific sexual isolation among wild strains of \textit{D. malerkotliana}, \textit{D. parabipectinata} and \textit{D. pseudoananassae} was studied by Singh and Chatterjee\textsuperscript{47}. In this study, evidence for incipient sexual isolation was found only within \textit{D. malerkotliana} and \textit{D. parabipectinata}. In \textit{D. pseudoananassae}, only two strains were tested, which showed random mating and isolation estimate was close to one indicating no sexual isolation. No evidence for the existence of character displacement (average isolation index between sympatric and allopatric populations) for sexual isolation between \textit{D. bipectinata} and \textit{D. malerkotliana} was found by Singh and Chatterjee\textsuperscript{48}. Singh \textit{et al.}\textsuperscript{49} found that the females of \textit{D. bipectinata} and \textit{D. malerkotliana} were able to discriminate between their own and alien males in absence of antennae which suggested that mate recognition in these species seems to depend on contact chemoreceptors.

Krishna and Hegde\textsuperscript{50} made an attempt to study the effect of bottlenecks on sexual isolation between control and bottleneck lines of \textit{D. malerkotliana}. They also compared the effect of bottleneck on chromosomally monomorphic and polymorphic populations of \textit{malerkotliana}. They found that after 10 generations of laboratory breeding, control and bottleneck lines of chromosomally monomorphic and polymorphic populations showed incipient sexual isolation. The polymorphic populations being more versatile were able to adapt and evolve more quickly even after a demographic decline. Hegde and Krishna\textsuperscript{51} also studied effect of bottlenecks on incipient sexual isolation, mating activity and fertility in \textit{D. malerkotliana}. After 10 generations of
laboratory breeding flies showed incipient sexual isolation between control line and bottleneck lines and also between bottleneck lines. The courtship latency and mating speed increased with increasing size of bottlenecks whereas copulation duration and fertility decreased with increasing size of bottlenecks. In another study by Hegde and Krishna 52, effect of bottleneck on intra – and interspecific competition in *D. malerkotliana* and *D. nasuta nasuta* was studied. In this study intra – and interspecific competition experiments involving control and bottleneck lines of monomorphic and polymorphic populations of *D. malerkotliana* with *D. nasuta nasuta* were carried out. Productivity and population size were evaluated in these lines. The results showed that control line had higher relative fitness and adaptedness (productivity and population size) under both intra- and interspecific competition than their respective bottleneck lines and *D. nasuta nasuta*. These studies thus suggest the effect of bottlenecks on competition in *D. malerkotliana*.

**Mutations**

In laboratory stocks of *D. bipectinata* a few spontaneous mutations have been detected. Hegde and Krishna 53 detected *brown* eye colour mutation which is an autosomal recessive mutation. Singh *et al.* 54 reported two spontaneous mutations—*sepia* eye colour and *cut* wing in laboratory stocks raised from females collected from different localities in India. Banerjee and Singh 55 detected *black* body colour mutation in *F*₁ progeny of a cross involving Trivendrum and Kottayam stocks. The *sepia* eye and *black* body colour were found to be autosomal recessive mutations whereas *cut* wing was found to be a sex-linked recessive mutation. Singh *et al.* 56 reported bilateral outgrowths on thorax of *sepia* mutants for the first time which also appeared to be a case of autosomal recessive mutation. The *sepia* eye and *black* body loci are located on chromosome 2 and are 33.75 map units apart. Bilateral outgrowth mutation and *sepia* eye are 35.71 map units apart on the same chromosome 2 57.

Spontaneous recombination in males of *D. bipectinata* using the markers *sepia* eye and *black* body 58 and *sepia* eye and thoracic outgrowth57 were reported for the first time. The results indicated spontaneous but low male recombination rate in *D. bipectinata*. Also, interstrain variation in male crossing over rate was found which was assumed to be due to genetic heterogeneity among the strains tested. Crosses between wild type and *cut* wing provided evidence for the interaction of selection and genetic drift in the laboratory populations of *D. bipectinata* 59. Quick elimination of *cut* gene in all the populations was assumed due to selection and the fluctuations in the *cut* gene frequency in different generations and in different populations were due to genetic drift.

**Behaviour genetics**

*Drosophila* is a very suitable material for the study of different aspects of behaviour. In different *Drosophila* species, different aspects of behaviour have been extensively studied. Certain aspects of behaviour which have been studied in *D. bipectinata* complex by different workers are oviposition site preference, larval pupation behaviour and sexual activity.

I. Non-sexual behaviour

A) Oviposition site preference—Oviposition site preference was studied in four species of *Drosophila* by Srivastava and Singh 60, which included *D. bipectinata* and *D. malerkotliana* of the *bipectinata* complex. It was found that females preferred peripheral area to the central area of culture medium for oviposition and females of all the species inserted the eggs into the surface of the medium. Interspecific variation with respect to oviposition site preference was also found. Srivastava and Singh 61 also found significant variation with respect to total fecundity in different species. Srivastava and Singh 62 also studied the effect of illumination conditions on oviposition pattern and found that egg laying was more under light condition by the females of all the four species studied as compared to dark condition. Experiments were also conducted to test the effect of different chemicals (ethanol, ethyl acetate and lactic acid) on oviposition in four Indian species of *Drosophila*, which included *D. bipectinata* and *D. malerkotliana*. From the results, it became evident that there was variation in oviposition pattern for high and low concentrations of different chemicals 63. Srivastava and Singh 64 also studied the effect of temperature on oviposition in four species of the *melanogaster* species group of *Drosophila*, which again included *D. bipectinata* and *D. malerkotliana*. Of the three different temperatures (19°, 24° and 30° C) at which eggs laid by females were counted for 4 days at 24hr interval, it was found that females laid low number of eggs at low temperature (19° C). Thus, oviposition in
these species was significantly reduced at low temperature. Females of *D. bipectinata* and *D. malerkotliana* show preference for oviposition on surface of medium when given choice between surface of medium and paper positioned vertically in the medium.

**B) Larval pupation behaviour**—Pupation site preference is an important aspect of *Drosophila* larval behaviour. Pupation height was scored in different strains of *D. bipectinata* and *D. malerkotliana* by Singh and Pandey. The mean pupation height of different strains ranged from 0.41-0.75 mm in *D. bipectinata* and from 1.1-1.3 mm in *D. malerkotliana*. Significant variation both at the intra- and interspecific level was shown by t-test and analysis of variance. Pupation site preference study in F1 hybrids obtained by reciprocal hybridization between *D. bipectinata* and *D. malerkotliana* showed no difference in pupation height.

Effects of different biotic and abiotic factors such as sex, density, larval development time, temperature and light on pupation height were tested in *D. bipectinata* and *D. malerkotliana* by Pandey and Singh. The results indicated that there was no effect of sex difference in pupation height, however, moisture content of the food medium and density of larvae strongly influenced pupation height. Larval development time, light and temperature were found to have effect on pupation height. Also, there were intra- and interspecific variations in response to these factors for pupation site preference. Thus, different biotic and abiotic factors influence pupation height in these species.

II Sexual behaviour

Courtship and mating behaviour were studied by Hegde and Krishnamurthy in six species and subspecies of the *bipectinata* complex. It was found that courtship behaviour did not vary significantly between different geographical populations within a species, except for the circling behaviour of males and extruding by females in *D. malerkotliana*. On the other hand, significant differences were found between species and subspecies. Similarly, the duration of copulation did not vary significantly between populations within a species, though there were significant differences between species and subspecies. From this study, it was inferred that *D. bipectinata* and *D. parabipectinata* are possibly the most similar of the four species and *D. pseudoananassae* the most distant. The results of this study corroborate the findings of cytological, isozyme and hybridization studies.

The courtship sounds and associated behaviours of species in the *D. bipectinata* complex were studied by Crossley. All the four species showed similar but distinct courtship patterns. Males of all species sing two songs, long and short song during early and late in courtship respectively. Due to differences in at least one song parameter, each species was found to have a unique song profile. According to Crossley, sound functions to maintain sexual isolation within this complex and has been described as a circumstantial evidence. Crossley and Bennet-Clark studied the response to simulated courtship songs in *D. parabipectinata*. This study demonstrated that two male songs when played in the naturally occurring sequence in courtship enhanced female receptivity. An attempt was made by Hegde and Naseerulla to correlate mating speed and other metric characters like length of wing, femur, tibia and first tarsus in *D. malerkotliana*. They found that flies mating first had longer wings and forelegs. Also, mating flies had lower coefficient of variability in wing length than non-mating ones. Age seemed to have no detectable effect on the relationship between these metric traits and mating speed. Similarly, Hegde and Krishna showed that long-winged flies courted and mated more successfully than short-winged flies. In *Drosophila* wings are important in courtship because species-specific auditory signals are generated by male’s wing vibrations. In another study by Krishna & Hegde mating success of large and small flies of *D. malerkotliana* and *D. bipectinata* was studied using multiple, male and female-choice methods. From this study it became evident that preferential mating exists for body size in *D. bipectinata* species complex and larger flies are advantageous.

Mating propensity which is an important component of fitness, was tested in various wild strains of *D. bipectinata* by Singh and Sisodia. Significant variation in the frequency of matings among the wild strains was found which was attributed to genetic heterogeneity among the stocks. Males of *D. bipectinata* showed more variation in sexual activity than females and were more subject to intrasexual selection. Sisodia and Singh studied the mating success of interstrain hybrids of *D. bipectinata*. The results indicated that mating propensity is under genetic control and there is sexual selection in *D. bipectinata*. Sisodia and Singh also
found significant variation among the strains of *D. bipectinata* with respect to courtship time, duration of copulation and fertility. Positive correlation between duration of copulation and fertility was also reported.

Singh and Sisodia studied the effect of cut wing mutation on mating propensity. From the investigation it became evident that cut wing mutation has no effect on the sexual activity of females and males of *D. bipectinata*, as wild type and mutant flies were equally successful in mating. Further, no evidence was found for sexual isolation between the mutant flies and wild type flies as the numbers of homogamic and heterogamic matings showed no significant differences and isolation estimate remained close to one in all the crosses. In *D. bipectinata* thoracic outgrowth mutation also does not affect mating propensity although thoracic outgrowths affect the mate recognition system, leading to behavioural reproductive isolation. However, evidence for positive assortative mating was found when mating propensity was tested between wild type flies and flies with bilateral outgrowths on thorax.

Mating success of wild type and cut males at different ratios was studied by Singh and Sisodia and it was found that males which were rare in mating chamber were more successful in mating which provided evidence for rare-male mating advantage in *D. bipectinata*. Also, significant difference in mean mating frequency between fast and slow lines was found by Singh and Sisodia in an artificial selection experiment to test the effect of selection on mating propensity. When the effect of *sepsia* eye colour mutation (autosomal recessive) on mating propensity was tested by Singh and Sisodia, it was found that *sepsia* mutation diminishes the sexual activity of males. However, no effect on receptivity of females was found. Singh and Sisodia also reported that sex-ratio has no effect on mating success of two types of flies, though it has been found to be influenced by choice-situation.

In a study by Naserulla and Hegde, lack of correlation between mating activity and Est-1 polymorphism was found in three natural and laboratory populations of *D. bipectinata* unlike Est-6 which has significance with respect to copulation duration and mating propensity. Trejan and Gill tested the effect of inbreeding on wing morphology in *D. malerkotliana* and found that inbreeding not only induces wing abnormalities but also drastically reduces the fertility in *malerkotliana*.

**Conclusion**

Studies related to taxonomy, population and behaviour genetics and speciation in the *D. bipectinata* complex revealed the existence of many interesting phenomena of evolutionary genetics such as balanced polymorphism, rigid chromosomal polymorphism, linkage disequilibrium, heterosis, genetic coadaptation, epistasis, male crossing-over, incipient sexual isolation, genetic control of mating propensity and sexual selection, genetic drift and natural selection. All the four species: *D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoananassae* show incomplete sexual isolation. Results of studies on chromosomal polymorphism, hybridization, isozyme polymorphism and courtship and mating behaviour revealed that *D. bipectinata* and *D. parabipectinata* are phylogenetically closer to each other than either is to *D. malerkotliana* or to *D. pseudoananassae*. *D. pseudoananassae* appears to be distinct from other three species of this complex.

In a study by Watanabe and Kawanishi, the proposed direction of evolution in the *bipectinata* complex is: *pseudoananassae* → *malerkotliana* → *bipectinata* → *parabipectinata*, and it was concluded that the species groups of *Drosophila* evolved near Borneo which is the centre of many *Drosophila* species and have expanded radially either by chance or according to the adaptive potential of each species. A detailed evolutionary study in the *D. bipectinata* species complex will certainly throw light on the mechanism of speciation in this species complex, which is an important aspect in the study of evolution.

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