

## Unique phenotypes and variation in the sex comb patterns and their evolutionary implications in the *Drosophila bipectinata* species complex (Diptera: Drosophilidae)

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**Abstract.** Understanding the genetic mechanisms of morphological evolution is one of the greatest challenges in evolutionary biology and for such studies sexually dimorphic traits in closely related species are of prime interest. In the *Drosophila bipectinata* species complex, which consists of four closely related species, namely *D. bipectinata*, *D. parabiepectinata*, *D. malerkotliana* and *D. pseudoananassae*, the pattern of sex combs (a sexually dimorphic trait) is found to be highly diversified. The present investigation documents some unique and new sex comb phenotypes and demarcates intra- and interspecific variations in the sex comb pattern among the four species and their hybrids. There is remarkable similarity in sex comb pattern of *D. bipectinata* and *D. parabiepectinata* but it differs from that of *D. malerkotliana* and *D. pseudoananassae*, which is in consistent with the phylogenetic relationships among the four species traced out by cytological, biochemical and molecular studies. The genetic basis of inheritance of sex comb patterns, its plausible implication with biogeographical distribution of species and the relationship between degree of hybridization and phylogenetic proximity have been addressed.

### INTRODUCTION

Sex combs are one of the most striking morphological traits of taxonomic importance that is confined to the subgenus *Sophophora* under the genus *Drosophila*. They consist of a row of stout, rounded black bristles (teeth) on the foretarsi (first and second tarsomeres) of males (Lachaise & Chassagnard, 2000) and exhibit great divergence among the species of *Drosophila*. Sex comb patterns are a species-specific trait that show considerable numerical variation in teeth among the individuals of the same species. It is a very important morphological feature in differentiating especially those species where other taxonomic characters are either absent or less prominent. In the *D. bipectinata* complex that belongs to the *ananassae* subgroup of the large and diversified *melanogaster* species group (subgenus *Sophophora*, genus *Drosophila*), there are four closely related species, namely *D. bipectinata* Duda, 1923, *D. parabiepectinata* Bock, 1971, *D. malerkotliana* Parshad & Paika, 1964 and *D. pseudoananassae* Bock, 1971 (Bock & Wheeler, 1972; Mishra & Singh, 2005a). The most obvious phenotypic features for distinguishing these species are sex comb patterns and abdominal tip pigmentation in males (Kopp & Barmina, 2005). Recently, Matsuda et al. (2005) have described three subspecies of *D. bipectinata* based on their reproductive isolation, namely *D. b. bipectinata* from Southeast Asia and Okinawa, *D. b. szentivanyii* from Papua New Guinea and *D. b. pacificiae* from South Pacific Ocean. Bock (1971a) described two subspecies of

*D. malerkotliana* (*D. m. malerkotliana* and *D. m. pallidaler* later on named as *D. m. pallens* by Bock & Wheeler, 1972) and *D. pseudoananassae* (*D. p. pseudoananassae* and *D. p. nigrens*) on the basis of differences in the male abdominal coloration. In both species, western populations have black abdominal tips while eastern populations have pale brown abdominal tips (Tomimura et al., 2005 and the reference map there in). Males of *D. m. malerkotliana* have a black abdominal tip and the species is distributed in India, Thailand, Malaya, Java, Sumatra and Africa, while males of *D. m. pallens* have a brown abdominal tip and the species is distributed in Borneo, the Philippines, and Celebes, east of Wallace's line, but not of Weber's line (Okada, 1981; Tomimura et al., 2005). *D. p. pseudoananassae* is distributed in Australia, New Guinea, the Philippines and Lombok, and the abdominal tip of males is brown, while *D. p. nigrens* is distributed in Sri Lanka, India, Java, Borneo and Malaya, and the abdominal tip of males is black (Okada, 1981; Tomimura et al., 2005). Thus, *D. parabiepectinata*, *D. m. malerkotliana* and *D. p. pseudoananassae* are pigmented in a sexually dimorphic pattern where the last three abdominal segments are completely melanized in males but not in females (Kopp & Barmina, 2005). Hence, distinguishing males on the basis of melanization of the abdominal tip may lead to committing mistakes, as there may be very little difference in the density of pigmentation. On the other hand, sex comb morphology is a very prominent and easily distinguishable trait even for a non-

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taxonomist. Therefore, the study of sex comb phenotypes becomes indispensable to identify correctly and distinguish these four species. However, females of all species and subspecies of this complex are extremely similar and cannot be differentiated morphologically (Bock, 1971a; 1978; Singh & Singh, 2001; Mishra & Singh, 2006).

Members of the *D. bipectinata* complex are distributed from east Africa, through Southeast Asia, Australia, India, New Guinea, and the South Pacific Islands, to South America (Tomimura et al., 2005; Kopp & Barmina, 2005, for details see distribution maps there in). The most widespread among them is *D. bipectinata* (Bock, 1978; Singh & Singh, 2001), which is supposed to increasing its distribution range rapidly (Yang et al., 1972).

The four species of this complex are intercrossable in the laboratory but not in natural condition. There is only one report of natural hybridization between *D. bipectinata* and *D. malerkotliana*, although the frequency of hybridization was very low (Gupta et al., 1980). Hybrid males are sterile while females are fertile, which conforms to most interspecific hybrids in the genus *Drosophila* (Mishra & Singh, 2005b). The causes of hybrid male sterility have been documented in different interspecific crosses of the *D. bipectinata* complex (Mishra & Singh, 2005a, 2006). Further, this complex is emerging as a promising group for investigating phenotypic evolution and speciation (Kopp & Barmina, 2005). The extensive morphological variation within and among species with respect to the sex comb teeth number and abdominal tip pigmentation, together with the ease of interspecific hybridization and extremely low (283000–385000 years ago) genetic divergence time (Kopp & Barmina, 2005), makes the *D. bipectinata* species complex an excellent model for investigating the genetic basis of morphological evolution.

Since there is considerable numerical variation in sex comb teeth among the four species, in this communication, we endeavor to make a lucid demarcation of sex comb patterns in each species and their hybrids. Further, the degree of hybridization among the species plausibly reflects their phylogenetic proximity. The other questions addressed are: (1) what is the genetic basis of sex comb inheritance, (2) how sex comb pattern is concerned with the phylogenetic relationship of the four species, and (3) how the variation in the sex comb pattern is correlated with the geographical distribution of these species.

## MATERIAL AND METHODS

The details of the strains used of four members of the *D. bipectinata* complex are given in Table 1. All stocks were maintained in the laboratory on simple yeast-agar culture medium at approximately 24°C. For scoring sex comb teeth number, 100 males were randomly taken from each stock and the teeth number on right foreleg in each male was scored under a microscope at 100× magnification after dissecting the forelegs on a slide, mounted with few drops of 0.67% saline. For hybridization, reciprocal crosses were made among four species by employing one strain of each species (*D. b. bipectinata* PN, *D. parabiptectina* MY, *D. m. malerkotliana* RC and *D. p. nigrens* CM). In total, twelve crosses were made by confining 3–4 virgin females with 3–4 virgin males in a food vial (1" × 3") to produce

TABLE 1. Details of different strains of all the four species of the *D. bipectinata* complex.

Species	Strains	
	Abbrev.	Biogeographic origins
<i>D. b. bipectinata</i>	TD	Thiruvananthapuram, Kerala, India
	NG	Niligiri, Tamilnadu, India
	AD	Alipurduar, West Bengal, India
	AR	Arumanai, Tamilnadu, India
	MY	Mysore, Karnataka, India
	SL	Siliguri, West Bengal, India
<i>D. parabiptectinata</i>	PN	Pune, Maharashtra, India
	MY	Mysore, Karnataka, India
<i>D. m. malerkotliana</i>	CL	Celebes, Indonesia
	RC	Raichur, Karnataka, India
	BP	Baripada, Orissa, India
	MY	Mysore, Karnataka, India
<i>D. p. nigrens</i>	BHU	Varanasi, Uttar Pradesh, India
	CM	Chiang Mai, Thailand

F<sub>1</sub> hybrids. The teeth number was scored in hundred males in each cross using the same method as mentioned above.

The range of teeth number in each row of first and second tarsomeres of foretarsus, their total number and mean number are calculated in all the four species and their hybrids. To analyse the data one-way analysis of variance (ANOVA), Duncan's multiple-range test (DMRT), t-test and Homogeneity of coefficient of variation have been employed (Zar, 2005).

## RESULTS

Each row of teeth on tarsomeres is called a sex comb. The details of number of teeth, their range of variation in each comb and mean number of teeth for all the strains of four species are given in Table 2. There are two combs on the first tarsomere and one comb on the second tarsomere in *D. b. bipectinata* and they show extreme similarity in pattern with that of *D. parabiptectinata* in all the above measurements.

There is large variation in sex comb patterns within *D. b. bipectinata* (Figs 1a–i) in comparison with *D. parabiptectinata* (Table 2B). We found the following new sex comb phenotypes in *D. bipectinata*: 1. presence of three sex combs on the first tarsomere (Fig. 1e), 2. some unique phenotypes of sex comb (Figs 1d, f, g, h, i), 3. presence of three teeth in the comb on second tarsomere (Fig. 1c), and 4. absence of sex comb on second tarsomere (Fig. 1b). In addition, another interesting observation documented for the first time is that, instead of a single set of proximal and distal combs on the first and second tarsomeres, there can be two sets, each showing variation in the number of teeth. However, their frequency is less than 15%. For example, in *D. bipectinata*, instead of a single proximal comb set in the TD strain, there are two sets with a number of teeth 6/3, 4/4, 5/2, 3/3, 2/5 and 4/3 (upper set/lower set). Similarly, in the AR strain, it is 1/5, 1/6, 1/7 and 7/4; in the MY strain, it is 3/1, 3/3, 1/7 and 5/1; in the NG strain, it is 1/5, 1/6, 1/7, 2/4, 3/5, 4/1, 4/3, 5/3, 6/2 and 7/2; in the AD strain, it is 1/4, 1/5, 1/6, 2/5, 4/1, 5/2 and 5/3; and in the SL strain, it is 5/2 and 1/5. In

TABLE 2A. Details of sex comb teeth arrangements in different strains of four species of the *D. bipectinata* complex.

Species	Strains	First tarsomere teeth no.(range)		Second tarsomere teeth no. (range)		Mean $\pm$ SE (range)
		Proximal comb	Distal comb	Proximal comb	Distal comb	
<i>D. b. bipectinata</i>	TD	5(4–7)	7(6–10)	2(1–2)	0	15.93 $\pm$ 0.15 (12–20)
	NG	5(4–7)	8(6–10)	2(1–3)	0	15.73 $\pm$ 0.15 (13–19)
	AD	5(4–7)	7(5–9)	1(1–2)	0	13.54 $\pm$ 0.14 (11–17)
	AR	6(5–8)	8(6–10)	1(1–2)	0	15.69 $\pm$ 0.14 (13–19)
	MY	5(4–7)	7(6–10)	1(1–2)	0	14.24 $\pm$ 0.14 (11–18)
	SL	5(4–8)	7(6–10)	1(1–2)	0	14.19 $\pm$ 0.18 (11–19)
	PN	5(4–7)	7(6–10)	2(0–2)	0	14.02 $\pm$ 0.18 (10–18)
					Mean	14.75 $\pm$ 0.06
<i>D. parabipectinata</i>	MY	5(4–7)	6(4–10)	2(0–2)	0	14.19 $\pm$ 0.19 (10–18)
	CL	6(4–8)	8(7–10)	1(0–2)	0	15.11 $\pm$ 0.16 (11–18)
					Mean	14.65 $\pm$ 0.12
<i>D. m. malerkotliana</i>	RC	1(0–1)	3(2–4)	1 (1–2)	2(1–3)	6.49 $\pm$ 0.12 (3–9)
	BP	1(0–2)	3(1–4)	1(1–2)	2(1–3)	6.32 $\pm$ 0.11 (4–10)
	MY	1(0–2)	3(2–4)	1(1–2)	2(1–3)	7.33 $\pm$ 0.12 (5–10)
	BHU	1(0–1)	2(1–4)	1(0–1)	2(1–3)	5.29 $\pm$ 0.12 (3–10)
			Mean			6.35 $\pm$ 0.06
<i>D. p. nigrens</i>	CM	2(1–3)	3(3–4)	2(1–4)	0	7.88 $\pm$ 0.13 (4–10)
					Mean	7.88 $\pm$ 0.13

the PN strain no such variation was found. There is also variation in the number of teeth in the two sets of the distal comb: for the TD strain, 1/6, 1/7, 1/9, 2/8, 2/9, 3/5, 3/6, 3/7, 3/9, 4/5, 4/6, 4/7, 5/1 (upper set /lower set) and in only one male three sets were observed, where the number of teeth is 1/2/8 (upper set /lower set /lowest set); for the AR strain, 1/7 and 1/10; for the NG strain, 1/7, 1/8, 1/9 and 2/9; for the AD strain, 1/7 and 2/4; for the PN strain, 1/7; for the SL strain 1/5 and 5/2; and for the MY strain, no such variation was observed. In the second tarsomere, there is much less variation in teeth number in

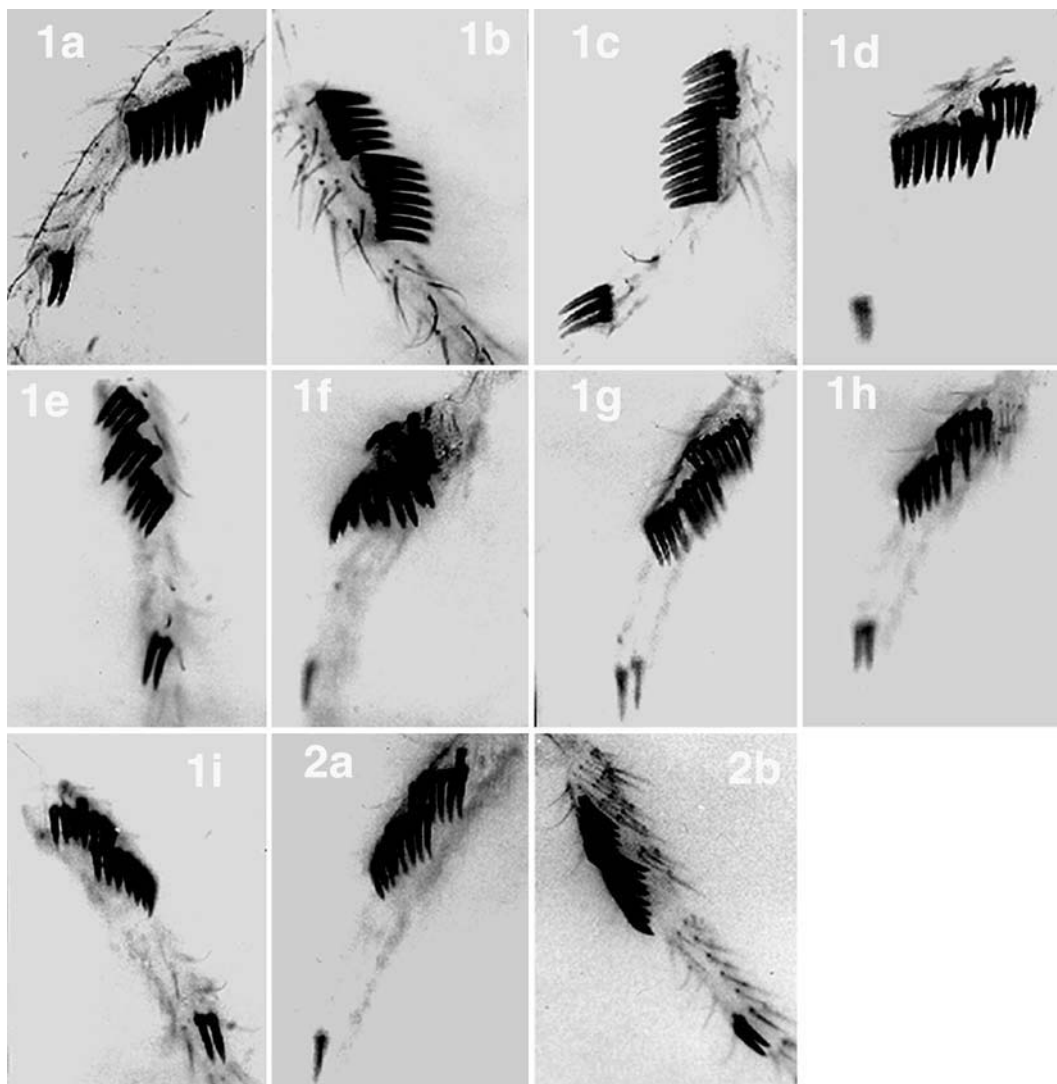
comparison to the first tarsomere and only in some males of TD, NG and PN strains, two sets were observed instead of a single sex comb set. In the TD strain, the variation in the number of teeth on the second tarsomere is 1/1, 1/2, 1/3 (upper set / lower set) while in the NG and PN strains, they are 1/1, 1/2 (upper set / lower set) and 1/1(upper set / lower set), respectively.

*D. parabipectinata* exhibits minimum variation in sex comb patterns (Figs 2a–b, Table 2B). There are two sets instead of a single set in the proximal comb of first tarsomere in some males as in the case of *D. b. bipectinata*.

TABLE 2B. Intraspecific variability in sex comb teeth number in different species of the *D. bipectinata* complex.

Species	Strains	Mean $\pm$ SD	CV	$\chi^2$ <sup>#</sup>	P-value
<i>D. b. bipectinata</i>	TD	15.93 $\pm$ 1.506	0.095		
	AR	15.69 $\pm$ 1.433	0.091		
	MY	14.24 $\pm$ 1.372	0.096		
	NG	15.73 $\pm$ 1.510	0.096	18.2	< 0.01**
	AD	13.54 $\pm$ 1.459	0.108		
	PN	14.02 $\pm$ 1.670	0.119		
	SL	14.19 $\pm$ 1.760	0.119		
<i>D. parabipectinata</i>	CL	15.06 $\pm$ 1.613	0.107		
	MY	14.16 $\pm$ 1.916	0.135	5.198	< 0.05*
<i>D. m. malerkotliana</i>	BP	6.32 $\pm$ 1.081	0.171		
	BHU	5.29 $\pm$ 1.192	0.225		
	MY	7.33 $\pm$ 1.164	0.159	13.74	< 0.01**
	RC	6.48 $\pm$ 1.590	0.179		
<i>D. p. nigrens</i>	CM	7.88 $\pm$ 1.372	0.174		

<sup>#</sup>Evaluating test for homogeneity for intraspecific co-efficient of variation (CV); \*, \*\* Significant.



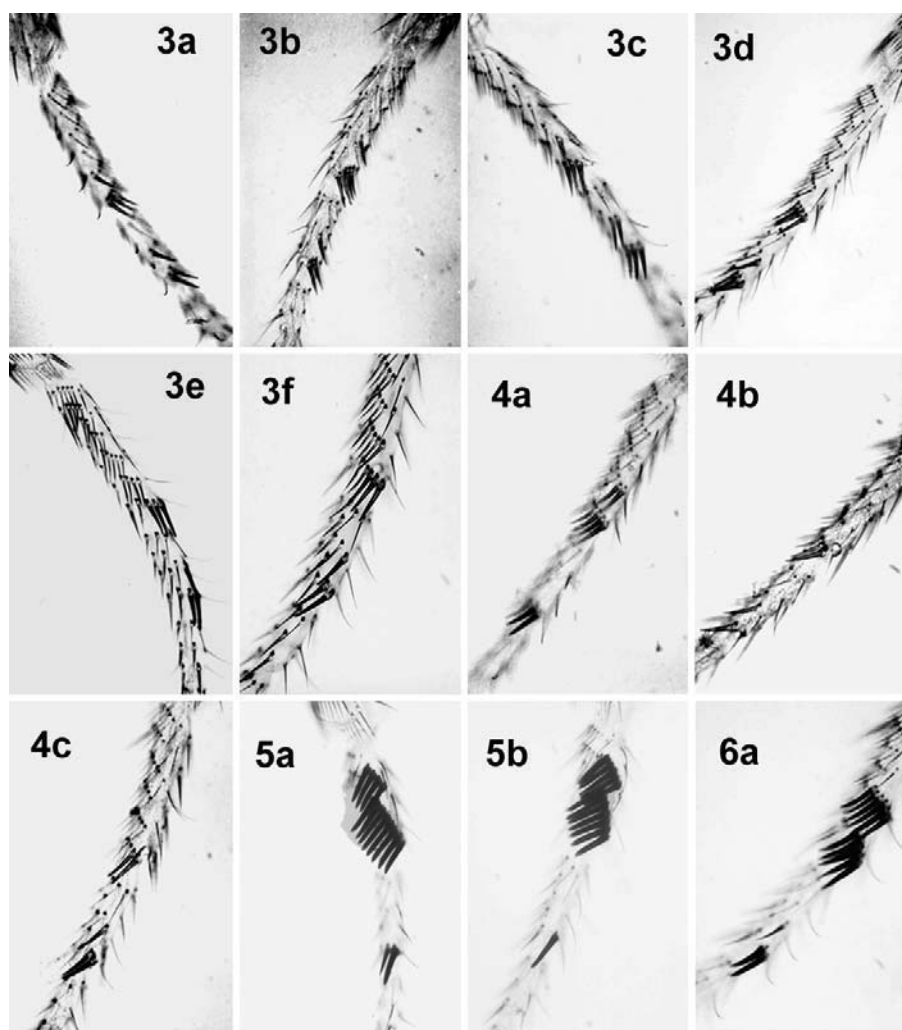
Figs 1–2: Sex comb patterns. 1a–i – *D. b. bipectinata*; 2a–b – *D. parabiptinata*.

For example, in the CL strain, the number of teeth is 1/4, 1/5, 1/6, 1/7, 2/5, 2/6, 2/7, 3/7 and 6/2 (upper set / lower set), while in the MY strain, it is 1/3, 1/4, 1/5, 4/2, 5/1 and 5/2 (upper set / lower set). However, the variation in teeth number on the second tarsomere is low. Here also, two sets are found instead of a single set in the distal comb of the first tarsomere where only one variant is observed both in the CL (6/1 – upper set / lower set) and the MY (1/7 – upper set / lower set) strains. No such variation was observed for the second tarsomere of both strains. The sex combs on first tarsomere in both species (*bipectinata* and *parabiptinata*) are arranged in longitudinal rows (Bock, 1971a).

Among the four investigated species, *D. malerkotliana* is unique in exhibiting four sex combs: two combs on both the first and second tarsomeres. The total teeth number of *D. m. malerkotliana* is lower (almost half) than that of *D. b. bipectinata* and *D. parabiptinata* (Table 2A). It exhibits higher variation in sex comb patterns (Figs 3a–f) than *D. parabiptinata* (Figs 2a–b, Table 2B) and *D. p. nigrens* (Figs 4a–c). As in *D. b. bipectinata* and *D. parabiptinata*, *D. m. malerkotliana* also has two sets

instead of a single set in the proximal comb of the second tarsomere in the MY strain, having teeth number 1/1 and 1/2 (upper set / lower set), but no such variation is recorded in other combs. In other strains, no such variation is observed in any comb. The total number of teeth in *D. p. nigrens* is higher than that of *D. m. malerkotliana* but lower than that of *D. b. bipectinata* and *D. parabiptinata* (Table 2A). However, the variation in sex comb phenotypes in this taxon (Figs 4a–c) is higher than that of *D. parabiptinata* but lower than that of *D. b. bipectinata* and *D. m. malerkotliana*. There was no record of two sets instead of a single sex comb set in *D. p. nigrens*. However, their presence cannot be ruled out in other strains of *D. p. nigrens*. Sex combs on the first tarsomere are arranged transversely both in *D. m. malerkotliana* and *D. p. nigrens* (Bock, 1971a).

The four species have been compared for variability in teeth number within the strain and also between the strains that revealed higher variability in *D. b. bipectinata* and *D. m. malerkotliana* than *D. parabiptinata* (Table 2B). Intraspecific variation in teeth number of seven *D. b. bipectinata* strains and four *D. m. malerkot-*



Figs 3–6a: Sex comb patterns. 3a–f – *D. m. malerkotliana*; 4a–c – *D. p. nigrens*; 5a–b – hybrid of female *D. b. bipectinata* × male *D. parabiptinata*; 6a – hybrid of female *D. b. bipectinata* × male *D. m. malerkotliana*.

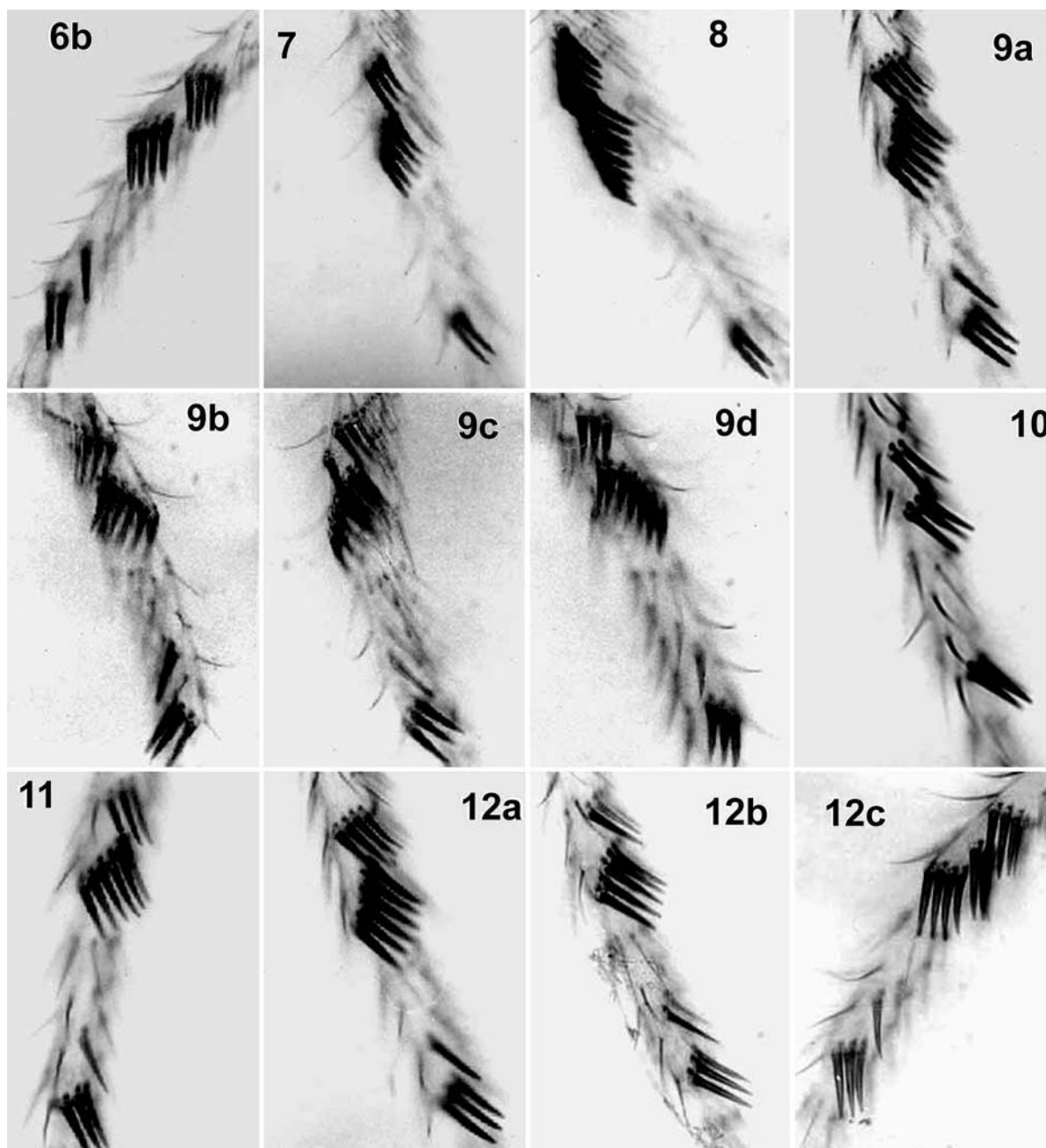
*liana* strains shows highly significant differences (Tables 3A and B). A t-test of intraspecific difference of mean number of teeth between two strains of *D. parabiptinata* shows a highly significant difference ( $t = 3.7$ ,  $df = 198$ ,  $p < 0.001$ ). Further, the interspecific variation in mean number of teeth among the four species using one strain (the same strain which was used for hybridization

experiment) of each species has been calculated by employing ANOVA. Interestingly, here also we get a statistically highly significant difference (Table 3C). The pairwise comparisons among the four species using the same strains as above have been performed using DMRT statistical tool and, as expected, there is significant differ-

TABLE 3. Analysis of variance (ANOVA) for interspecific and intraspecific variation in the mean number of sex comb teeth.

	Source	df	SS	MS	F	P
	Total	699	2214.63			
A <i>D. b. bipectinata</i> strains	Between strains	6	580.60	96.77	41.04	< 0.001*
	Within strains	693	1634.00	2.36		
	Total	399	733.88			
B <i>D. m. malerkotliana</i> strains	Between strains	3	210.43	70.14	53.06	< 0.001*
	Within strains	396	523.45	1.32		
	Total	399	6259.39			
C <i>D. bipectinata</i> complex	Between strains	3	5866.11	1955.37	1968.87	< 0.001*
	Within strains	396	393.29	0.99		

\* Significant.

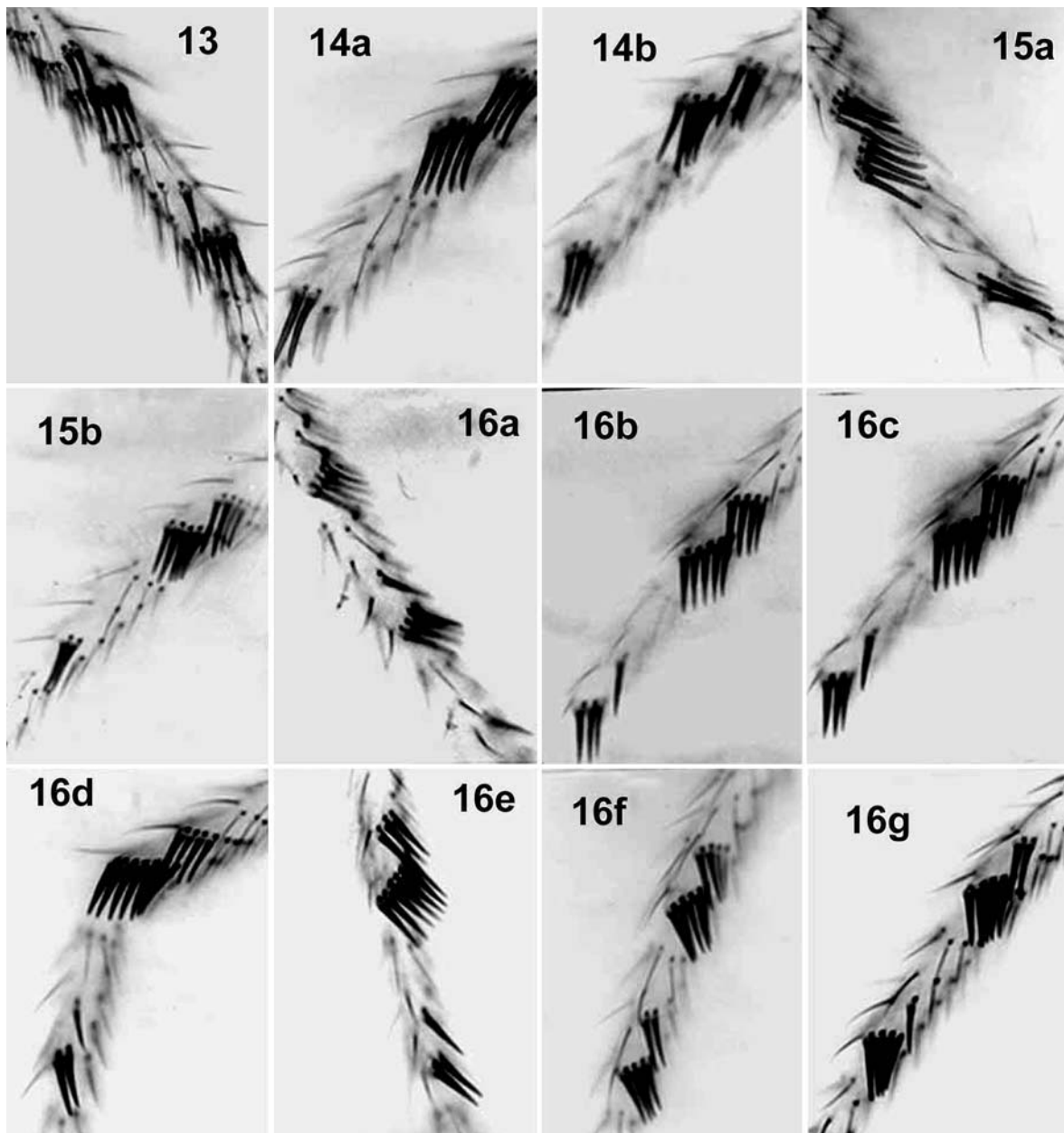


Figs 6b–12: Sex comb patterns. 6b – hybrid of female *D. b. bipectinata* × male *D. m. malerkotliana*; 7 – hybrid of female *D. b. bipectinata* × male *D. p. nigrens*; 8 – hybrid of female *D. parabiepectinata* × male *D. b. bipectinata*; 9a–d – hybrid of female *D. parabiepectinata* × male *D. m. malerkotliana*; 10 – hybrid of female *D. parabiepectinata* × male *D. p. nigrens*; 11 – hybrid of female *D. m. malerkotliana* × male *D. b. bipectinata*; 12a–c – hybrid of female *D. m. malerkotliana* × male *D. parabiepectinata*.

ence in all species except between *D. b. bipectinata* and *D. parabiepectinata* (Table 4).

The degree of hybridization among the four species varied. It is easier to obtain hybrids between *D. b. bipectinata* and *D. parabiepectinata* and when *D. b. bipectinata* was the female parent, the degree of hybridization was comparatively higher. When these two species are crossed with *D. m. malerkotliana*, the hybridization is a bit more difficult. Finally, in the crosses where *D. p. nigrens* is one of the parental species, obtaining hybrids is difficult (for degree of crossability, see Mishra & Singh, 2006).

The range and mean teeth number and number of teeth in each comb of both tarsomeres for the hybrids are given in Table 5, and their variations are shown in Figs 5a–16g. The pattern of sex comb teeth in hybrids is intermediate of their parental species. In some hybrids, there are two sets instead of a single comb set on the first and second tarsomeres. However, their frequency is less than 10%. For example, in the hybrids between *D. parabiepectinata* females and *D. b. bipectinata* males, instead of a single set of proximal comb, there are two sets with teeth number 1/5 (upper set /lower set). Similarly, instead of a single distal comb set, there are two sets with teeth



Figs 13–16: Sex comb patterns. 13 – hybrid of female *D. m. malerkotliana* × male *D. p. nigrens*; 14a–b – hybrid of female *D. p. nigrens* × male *D. b. bipectinata*; 15a–b – hybrid of female *D. p. nigrens* × male *D. parabipectinata*; 16a–g – hybrid of female *D. p. nigrens* × male *D. m. malerkotliana*.

number 7/1 (upper set /lower set). The same pattern is found in the hybrids between male *D. b. bipectinata* and female *D. p. nigrens*, where two sets having teeth numbers 1/3 and 1/5 (upper set /lower set) are present instead of a single proximal comb set, and 1/5 and 2/5 teeth are present instead of a single distal comb set. The hybrids between *D. parabipectinata* and *D. m. malerkotliana* have four sex combs, two combs both on the first and second tarsomeres. In this case, the variability in comb pattern is higher when *D. parabipectinata* is the female parent. The number of teeth in the two proximal comb sets on first tarsomere is 1/4, 2/1, 3/2 (upper set /lower set) and in the distal comb is 6/1, 7/1 (upper set /lower set). Further, in the proximal comb of second tarsomere,

there is only one variant where the two sets instead of a single set have 1/1 (upper set /lower set) teeth. In the reciprocal cross (female parent is *D. m. malerkotliana*), there is only one variant for each comb on both tarsomeres; for proximal and distal combs of first tarsomere, it is 3/2 and 3/4 (upper set /lower set), respectively, while for proximal and distal combs of second tarsomere, it is 1/1 and 2/1 (upper set /lower set), respectively. In the hybrids between female *D. m. malerkotliana* and male *D. p. nigrens*, only one variant is present where the single distal comb set of the second tarsomere is replaced with two sets having teeth number 2/1 (upper set /lower set). Similarly, in the hybrids between female *D. p. nigrens* and male *D. parabipectinata*, instead of a single distal

TABLE 4. DMRT for pairwise comparison among four species of the *D. bipectinata* complex.

Comparisons	Difference of means	Critical difference	P
bip vs parab	0.10	2	> 0.05
bip vs maler	8.40	4	< 0.05*
parab vs maler	8.30	3	< 0.05*
bip vs pseu	6.87	3	< 0.05*
parab vs pseu	6.77	2	< 0.05*
maler vs pseu	1.53	2	< 0.05*

bip = *D. b. bipectinata*; parab = *D. parabipectinata*; maler = *D. m. malerkotliana*; pseu = *D. p. nigrens*; \* Significant.

comb set on the second tarsomere, there are two sets with teeth number 1/1 and 1/2 (upper set /lower set).

Although, in most crosses, the teeth number in hybrids differs from their midparent value (average of teeth number of parental species involved in a cross), they are always intermediate of their parental species (Table 6). Further, the teeth number of hybrids differs from maternal and paternal species in all crosses except few crosses where they are similar with either the maternal or the paternal parents (Table 6). Reciprocal crosses show similar teeth number with only two exceptions (Table 6).

#### DISCUSSION AND CONCLUSION

*D. b. bipectinata* and *D. parabipectinata* have a similar sex comb pattern (Table 2), so it is very difficult to distinguish the two species based on this character. However, they can be easily distinguished by the sharp contrast in the abdominal tip pigmentation between males of the two species: *D. bipectinata* has a pale brown abdominal tip while *D. parabipectinata* has a melanized abdominal tip. Nevertheless, *D. b. bipectinata* and *D. parabipectinata* can be easily distinguished from *D. m. malerkotliana* and *D. p. nigrens* on the basis of sex comb patterns. As in *D. b. bipectinata* and *D. parabipectinata*, the number of teeth in *D. p. nigrens* is lesser in the proximal comb than

in the distal comb of the first tarsomere and the number of teeth on the second tarsomere is similar to that on the proximal comb of the first tarsomere (Table 2). In the above three species there are only three combs: two combs on the first tarsomere and one comb on the second, but in *D. malerkotliana* there are four combs: two combs on both the first and the second tarsomeres. Further, the number of teeth is lesser in the proximal than in the distal combs on both tarsomeres (Table 2). The sex comb teeth of both *D. bipectinata* and *D. parabipectinata* are thicker and more prominent than in *D. pseudoananassae* and *D. malerkotliana*, where teeth are slightly tougher and thicker than the bristles (Figs 1a–4c). Species similarities in sex comb patterns are consistent with the phylogenetic relationship derived from cytological (Bock, 1971b), biochemical (Yang et al., 1972; Hegde & Krishnamurthy, 1976), morphological (Mishra & Singh, 2006) and molecular studies (Kopp & Barmina, 2005). Further, the sex comb patterns of these four species is well in line with the hypothesis that “as far as phylogeny may be reconstructed, *malerkotliana*, *pseudoananassae* and a population ancestral to *bipectinata* and *parabipectinata* are derived directly from a common ancestral population” (Bock, 1971b). Therefore, similarity in sex comb patterns may be correlated with the phylogenetic proximity of these species.

From the results, it is obvious that the variation in sex comb patterns is highest in *D. b. bipectinata* (Figs 1a–i). The interstrain variability in teeth number is also higher (Table 2B), and some unique sex comb phenotypes are also observed in this species. Further, *D. b. bipectinata* also shows a tendency to expend its geographical distribution (Yang et al., 1972). Notably, there is more variation in teeth patterns in the proximal comb of the first tarsomere in *D. b. bipectinata*. This might be the result of higher sexual selection of these teeth during evolution (Polak et al., 2004). In *D. parabipectinata*, although the sex comb pattern is similar to that of *D. bipectinata*, it has

TABLE 5. Details of sex comb teeth arrangements in interspecific hybrids of the *D. bipectinata* species complex.

Crosses	Range of total teeth no.	Mean teeth no.	First tarsomere		Second tarsomere	
			Proximal comb teeth no. (range)	Distal comb teeth no. (range)	Proximal comb teeth no. (range)	Distal comb teeth no. (range)
bip × parab	12–16	14.38 ± 0.11	5(4–7)	8(5–9)	2(1–3)	0
parab × bip	12–16	14.47 ± 0.10	5(4–7)	7(6–9)	2(1–2)	0
bip × maler	10–15	12.94 ± 0.14	4(2–6)	5(4–7)	1(0–1)	3
maler × bip	10–14	11.77 ± 0.10	4(2–5)	5(4–7)	1(0–2)	2
bip × pseu	9–14	11.98 ± 0.15	4(2–5)	6(5–7)	3(1–4)	0
pseu × bip	9–14	11.91 ± 0.14	4(1–6)	6(4–7)	2(1–4)	0
parab × maler	10–15	12.86 ± 0.13	3(2–4)	6(4–7)	1(0–2)	2(2–4)
maler × parab	9–15	12.13 ± 0.12	3(2–4)	5(4–7)	1(0–2)	2(2–3)
parab × pseu	8–13	11.03 ± 0.13	4(2–6)	6(3–7)	2(1–4)	0
pseu × parab	8–13	11.11 ± 0.13	4(1–5)	6(3–7)	2(1–3)	0
maler × pseu	5–9	7.56 ± 0.09	0(0–1)	4(3–5)	1(1–2)	2(2–3)
pseu × maler	6–9	7.71 ± 0.09	1(0–2)	3(3–4)	1(0–1)	3(2–4)

bip = *D. b. bipectinata*; parab = *D. parabipectinata*; maler = *D. m. malerkotliana*; pseu = *D. p. nigrens*.



TABLE 6. Mean number of sex comb teeth in the interspecific hybrids and their comparison with parental species and midparent value (comparison between reciprocal crosses is also given).

Female × Male	Mean ± SE	Midparent value	t <sub>1</sub>	t <sub>2</sub>	t <sub>3</sub>	t <sub>4</sub>
bip × parab	14.38 ± 0.11	14.10	1.61	1.81	0.88	0.60
parab × bip	14.47 ± 0.10		2.19*	1.31	2.30*	
bip × maler	12.94 ± 0.14	10.35	13.74***	5.01***	36.07***	6.87***
maler × bip	11.77 ± 0.10		8.35***	34.24***	11.49***	
bip × pseu	11.98 ± 0.15	10.94	5.65***	8.99***	20.28***	0.34
pseu × bip	11.91 ± 0.14		5.67***	21.17***	9.74***	
parab × maler	12.86 ± 0.13	10.34	14.24***	5.85***	36.77***	4.12***
maler × parab	12.12 ± 0.12		10.22***	33.03***	9.19***	
pseu × parab	11.11 ± 0.13	11.03	0.49	13.45***	17.37***	0.43
parab × pseu	11.03 ± 0.13		0.03	16.96***	13.81***	
maler × pseu	7.56 ± 0.09	7.18	2.81**	7.09***	1.96	1.02
pseu × maler	7.71 ± 0.09		3.84***	1.09	7.97***	

bip = *D. b. bipectinata*; parab = *D. parabipectinata*; maler = *D. m. malerkotliana*; pseu = *D. p. nigrens*. Midparent value = average of teeth number of parental species involved in a cross. t<sub>1</sub>, difference between hybrid and midparent value; t<sub>2</sub>, difference between hybrid and maternal species; t<sub>3</sub>, difference between hybrid and paternal species and t<sub>4</sub>, difference between reciprocal hybrids. \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

less variation in sex comb phenotypes. It also has a restricted geographical distribution. The diversity of teeth patterns in *D. m. malerkotliana* is greater than in *D. p. nigrens* and its geographical distribution is also wider. These findings elicit that species having wider biogeographical distributions endow with higher diversity, while species with restricted distributions have comparatively less diversity of sex comb patterns.

The degree of hybridization among the four species indicates their genetic compatibility, which in turn reflects their phylogenetic proximity. The degree of crossability is higher between *D. bipectinata* and *D. parabipectinata* than that of *D. bipectinata* or *D. parabipectinata* and *D. malerkotliana*, and it is lowest between *D. malerkotliana* and *D. pseudoananassae* (Mishra & Singh, 2006). These observations reinforce that *D. bipectinata* and *D. parabipectinata* are very closely related species. These species are closer to *D. malerkotliana* but distantly related with *D. pseudoananassae*.

In the hybrids of these four species, the number of teeth is intermediate of their parental species. However, when the difference of teeth number of the hybrids and the midparent value of their corresponding parental species are statistically tested, no fixed pattern is found. In some hybrids (*D. b. bipectinata* × *D. m. malerkotliana*, *D. parabipectinata* × *D. m. malerkotliana*, *D. b. bipectinata* × *D. p. nigrens*), the difference is highly significant (p < 0.001), while in others it is less significant as observed in the hybrids of female *D. m. malerkotliana* and male *D. p. nigrens* (p < 0.01) and female *D. parabipectinata* and male *D. b. bipectinata* (p < 0.05), or not significant (p > 0.05) as in the case of hybrids of female *D. b. bipectinata* and male *D. parabipectinata*, and reciprocal crosses of *D. p. nigrens* and *D. parabipectinata* (Table 6). These observations support a polygenic mode of inheritance of sex combs. However, our results slightly differ from Crossley & Taylor (1985) who, although reported intermediate sex comb patterns in hybrids, found no significant difference

between teeth number of the hybrids and their corresponding midparent values. The reason for this difference may be attributed to the following factors: (1) Quantitative Trait Loci (genetic factors underlying quantitative traits) at different regions are responsible for higher and lower number of sex comb teeth (Nuzhdin & Reitwich, 2000). The expression of higher QTL number may increase the teeth number in hybrids compared to their midparent value and (2) the mutations in genes in the bristle formation pathway could perhaps also modify teeth number (Mackay, 1995). Further, there is a significant difference in teeth number in different strains of the same species, and even among different individuals of the same strain (Table 2). These findings corroborate the genetic heterogeneity in teeth number and its additive effect.

Sex comb in males of *Drosophila* (*Sophophora*) is a diversifying secondary sexual character, which is sexually selected for size and symmetry (Polak et al., 2004). In most *Sophophora* species, there are two sex combs, one on the first tarsomere and another on the second. However, variation in comb patterns is found in different species of the subgenus *Sophophora*. *D. melanogaster*, the most extensively studied species, has only one sex comb (Santamaria, 1998). However, *Drosophila* species other than those in the subgenus *Sophophora* entirely lack sex combs as it is the case of the *saltans* and *willistoni* groups. Considering these variability, Stern (1954) speculated that the evolutionary process which diversified sex comb phenotypes in different species began in response to mutation to preexisting developmental prepatterns. An interesting query regarding the genetic basis of inheritability of teeth number is whether the same gene is responsible for both intra- and interspecific variability of this trait. Nuzhdin & Reiwitich (2000) tried to unravel this mystery. They found that the same chromosome region has contributed both to intraspecific variability in *D.*

*melanogaster* and interspecific difference between *D. mauritiana* and *D. simulans*.

The functional significance of sex comb is also a matter of discussion. Different workers empirically assigned different functions to sex combs, all functions pertaining to the sexual behaviour of males. Spieth (1952) found a distinct correlation between the behaviour used by males during mating and the presence of sex combs. Males with sex combs attempt intromission before mounting is complete, but males without sex combs do not. Based on this, he speculated that sex combs are necessary in precopulatory maneuvering by males. Narda (1968) observed elimination of tapping by removing first and second tarsomeres that decreases considerably the sexual isolation between *D. bipectinata* and *D. malerkotliana*. It is also speculated that sex combs are scent receptors and teeth number reflects evolutionary divergence in response to species-specific scents (Crossley & Taylor, 1985). In *D. silvestris*, it has been demonstrated that cilia on the first and the second tarsomeres of male foretarsi are used as a brush to stimulate the female during courtship (Carson & Tremoto, 1984). Sex combs are used during both courtship and copulation in *D. melanogaster* and the copulatory success of males decreases when sex combs are surgically removed (Speith, 1952; Coyne, 1985). Males lacking the foreleg tarsomere with the sex comb have great difficulty in grasping the female genitalia resulting into substantial decrease in mating ability (Cook, 1977; Coyne, 1985). Moreover, the role of sex combs as precise grasping organ is reinforced by the presence of small cuticular projections at the base of each tooth (Coyne, 1985) that may provide mechanosensory information to the males. A row of bristles was found near the tip of the female ovipositor (Coyne, 1985), which may act as an anchor to the male sex comb bristles during copulation. Therefore, it can be suggested that sex combs are primarily structures adapted for grasping the female securely during the act of intromission. Further, it has been documented that the number of teeth and their positioning are perhaps under sexual selection that causes rapid changes in sex comb morphology and correlated changes in mating behaviour (Carson & Lander, 1984). Obeying this selection process, sex combs strongly differ in the number of rows, and in their position and orientation among races and species. These variations are manifested in *D. bipectinata* (Figs 1b–i).

In summary, (1) sex comb patterns are correlated with the phylogenetic relationship in closely related species, at least in the four members of the *D. bipectinata* complex, (2) the degree of hybridization is higher in phylogenetically closely related species and lower in distantly related species, (3) species with wider geographical distribution have higher divergence in sex comb phenotypes than those with restricted geographical distribution, (4) interspecific hybrids show intermediate sex comb patterns and no fixed relationship was found between the midparent values and sex comb teeth number of hybrids, which corroborates a polygenic mode of inheritance, and (5) it is documented that sex combs are a secondary sexual trait

primarily used by males for grasping females during intromission.

Understanding the genetic basis of inheritance of sex combs and their divergence during evolution is only the first step to catch the historical context of the evolution of this trait. To understand the pattern of this trait in ancestral population and the sequence of genetic changes involved during change of its phenotypes, further studies are required.

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