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Polymorphism among anterior and posterior sets of teeth in the periphallic organ of three species of *Drosophila*

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Abstract: For the first time, morphological polymorphism among the anterior and posterior sets of primary teeth of the periphallic organ was observed among natural populations of *Drosophila*. Natural and laboratory populations of 3 different species (*D. ananassae*, *D. bipectinata*, and *D. malerkotliana*) belonging to the *ananassae* subgroup were used to study the intra- and interspecific variations in the number of teeth of the anterior and posterior sets of the periphallic organ. There are about 22, 20, and 20 possible combinations of sets of teeth recorded in *D. ananassae*, *D. bipectinata*, and *D. malerkotliana*, respectively. The maximum frequency of anterior and posterior sets of teeth was found to be (5, 3) in *D. ananassae* and (2, 3) in the case of *D. bipectinata* and *D. malerkotliana*. The percentage of perfect bilateral symmetry was lower than that of asymmetry in all 3 species. A significant difference was found in anterior and posterior sets of teeth between different populations. These results indicate that the primary teeth of the anterior and posterior sets of the periphallic organ in *Drosophila* exhibit morphological polymorphism.

Key words: Bilateral symmetry, *Drosophila ananassae*, *Drosophila bipectinata*, *Drosophila malerkotliana*, morphological variation, periphallic organ

1. Introduction

The genital organ in *Drosophila* plays a vital role in differentiation of species, as in many species of insects. Very closely related species such as *D. melanogaster* and *D. simulans* have been differentiated based on genital plate characters (Sturtevant, 1919, 1920). Hsu (1949) gave a detailed description and characters of male genital apparatus of more than 170 drosophilids in relation to systematics. The *ananassae* subgroup species comprises pale to dark flies; male abdomens are black distally in some species, whereas females are lighter in color (Hsu, 1949). There are 22 species belonging to the *ananassae* subgroup recorded thus far (Matsuda et al., 2009). Sex combs are arranged in transverse rows on the first 2 or 3 tarsal segments of the male foreleg, while the genital plate consists of 2 claspers; the primary clasper has 2 sets of teeth, anterior and posterior (Bock and Wheeler, 1972). The genital plate of *D. ananassae* consists of primary teeth in 2 sets, i.e. anterior and posterior. The anterior set has 4–5 teeth and the posterior set has 2–4 (Hsu, 1949), whereas *D. bipectinata* and *D. malerkotliana* have an anterior set with 2 teeth and a posterior set with 3 teeth on the primary clasper region (Parshad and Paika, 1964; Bock and Wheeler, 1972).

Polymorphism is a natural phenomenon occurring in most living organisms. The genus *Drosophila* has been used extensively in many morphological polymorphic studies, such as evolution and variation of sex combs and their patterns (Mishra and Singh, 2006; Ahuja and Singh, 2008), and color polymorphism (Hatadani et al., 2004). Differences in morphological traits such as thorax length, sternopleural bristles, wing length, wing-to-thorax ratio, sex comb tooth number, and ovariole number have been studied in *D. ananassae* and *D. pallidosa* (Vishalakshi and Singh, 2008a). Variation of morphological traits under inbreeding and nutritional and environmental stress on fluctuating asymmetry has been extensively studied (Imasheva et al., 1999; Vishalakshi and Singh, 2008b, 2008c, 2009; Sisodia and Singh, 2009). There is no information on variations in the number of teeth in anterior and posterior sets of teeth in the periphallic organs of natural *Drosophila* populations; a literature review also reveals that there is no information on polymorphism of anterior and posterior sets of teeth in periphallic organs of any species of *Drosophila*. Hence, the present study was undertaken to analyze the variations of teeth on the periphallic organ in natural populations of *D. ananassae*, *D. bipectinata*, and *D. malerkotliana.*

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2. Materials and methods

Drosophila flies were collected during the monsoon season from different spots in and around the Dharwad district. *D. ananassae* was collected from Dharwad market (DM), Hubli market (HM), the Karnatak University campus (KUC), and Navanagar (NAVN). *D. bipectinata* and *D. malerkotliana* were collected from Sangatigopp (SGP), Yalakki Shettar Colony (YSC), and Kundagol (KUN) by the net-sweeping method. The study area includes different habitats such as domestic places, fruit markets, agriculture fields, forests, and laboratory populations. The laboratory populations were obtained from the *Drosophila* Stock Center, Department of Zoology, University of Mysore, Mysore, India: stock no. 11.001 for *D. ananassae*, stock no. 12.001 for *D. malerkotliana*, and stock no. 13.001 for *D. bipectinata*. The laboratory populations were cultured in standard wheat cream agar medium and maintained at 22 ± 1 °C. The wild males collected from natural populations were brought directly to the laboratory and segregated by their respective species based on their taxonomic characters as mentioned by Hsu (1949), Parshad and Paika (1964), and Bock and Wheeler (1972). The flies were anesthetized, and posterior portions of the abdomen were dissected from 30 males from each location, following the procedure of Parshad and Paika (1964). The tips of the abdomens were boiled in 10% KOH for about 20 min and then washed in tap water. The dissection was carried out in a drop of glycerin. The genital plates were later dehydrated in 90% ethanol (absolute alcohol) and finally mounted in a mounting medium. The slides were later observed under a microscope, and the numbers of anterior and posterior teeth were recorded. The prepared slides were also photographed using a ProgRes C3 camera (ProgRes Image Capture Software) fixed to the microscope at 40× optical magnification.

The possible numbers of teeth combinations (anterior + posterior + extra teeth, if present) were recorded for all 3 species collected from different spots. The frequency of possible combinations of anterior and posterior sets of teeth, along with perfect bilateral symmetry, was recorded separately for right and left sides (A + P + E). For the convenience of statistical analysis, the right and left sides of anterior teeth [Ant (R + L)] and posterior teeth [Post (R + L)] were combined separately.

The data were tested for the normal distribution by using a one-sample Kolmogorov–Smirnov test. They were found to be normally distributed. The data were subjected to factorial analysis of variance (ANOVA) to analyze whether significant differences existed among anterior and posterior sets of teeth in specimens from different localities and between different species. The software used for statistical analysis was SPSS 16.0. In order to study the presence of fluctuating asymmetry (FA), the data were tested for directional asymmetry (DA) and antisymmetry, as mentioned by Palmer (1994). The mean of the absolute value of the difference in total number of teeth between the right and left sides, |R – L|, of anterior and posterior sets of teeth of the periphallic organ was calculated. The difference between |R – L| was found to be significant between sides, as the distribution was not normal. Hence, calculations of FA were not considered for further results.

3. Results

D. ananassae has 22 and *D. bipectinata* and *D. malerkotliana* have 20 possible combinations of anterior, posterior, and extra sets of teeth (Tables 1–3). The maximum frequency of combinations in *D. ananassae* is (5, 3), whereas *D. bipectinata* and *D. malerkotliana* have a maximum frequency of (2, 3) combinations (Figures 1a–1c). There is intraspecific variation among the anterior and posterior sets of teeth in some of the genital plates showing the presence of extra teeth/an extra set of teeth (Figures 1d–1f). It was found that the possibility of perfect bilateral symmetry of anterior, posterior, and extra teeth was 38%, 32.6%, and 37.3% in *D. ananassae*, *D. bipectinata*, and *D. malerkotliana*, respectively, which is less than half of the total number. The percentage of frequency of asymmetry between right and left sides of the anterior and posterior sets of teeth was 62%, 67.4%, and 62.7% in *D. ananassae*, *D. bipectinata*, and *D. malerkotliana*, respectively, which is more than half of the total number analyzed (Figures 2–4).

Results of factorial ANOVA revealed no significant difference among anterior sets of teeth for species × population ($F = 1.642$, $P = 0.111$). For posterior sets of teeth, a significant difference was found between species × population ($F = 6.189$, $P < 0.001$).

4. Discussion

In the present study, polymorphism was observed for the first time in primary teeth of the genital plates among natural populations of 3 species belonging to the *ananassae* subgroup of genus *Drosophila*. *D. bipectinata* and *D. malerkotliana* have shown similar patterns, whereas *D. ananassae* has a different pattern of arrangement of the anterior and posterior sets of teeth.

Earlier studies by Hsu (1949) and Bock and Wheeler (1972) on *D. ananassae* and *D. bipectinata*, and by Parshad and Paika (1964) on *D. malerkotliana*, revealed that the number of teeth ranges from 4 to 5 (anterior) and 2 to 4 (posterior) in *D. ananassae*, whereas in D. *bipectinata* and *D. malerkotliana* there are 2 (anterior) and 3 (posterior) teeth on either side of the primary clasper (Figures 1a–1c). In the present study, the primary teeth of genital plates ranges from 2 to 6 (anterior) and 1 to 5 (posterior) in *D. ananassae*. *D. bipectinata* and *D. malerkotliana* showed similarity in their number of teeth, which were found to

Table 1. Possible combinations of anterior, posterior, and extra teeth of the genital organ in *D. ananassae* recorded from different localities.

Serial no.	Possible combinations of teeth (anterior, posterior, extra)	DM		HM		KUC		NAVN		LAB	
		Rs	Ls	Rs	Ls	Rs	Ls	Rs	Ls	Rs	Ls
1	2, 4, 1	-	-	-	-	-	-	-	-	1	-
2	3, 3	-	1	-	-	-	-	-	-	-	-
3	3, 4	-	-	-	-	1	-	-	1	-	-
4	4, 2	2	1	1	1	2	3	-	2	-	-
5	4, 2, 1	-	-	-	-	-	-	1	-	-	-
6	4, 2, 2	-	-	-	-	1	-	-	-	-	-
7	4, 3	3	1	3	-	8	12	5	2	2	2
8	4, 3, 1	-	-	1	3	1	-	-	2	-	2
9	4, 3, 2	-	-	1	-	-	-	-	-	-	-
10	4, 4	2	-	1	4	3	2	1	1	3	5
11	5, 1	-	-	-	-	-	1	-	1	-	-
12	5, 1, 1	-	1	-	-	-	-	-	-	-	-
13	5, 2	6	1	-	1	3	2	2	3	-	1
14	5, 2, 1	-	1	-	-	-	-	1	-	-	-
15	5, 3	13	16	15	15	10	9	16	15	12	13
16	5, 3, 1	-	1	-	-	-	-	-	-	-	-
17	5, 3, 2	-	-	-	-	-	-	-	-	1	-
18	5, 4	1	3	4	3	-	-	2	1	7	4
19	5, 5	-	-	1	-	-	-	-	-	2	-
20	6, 2	-	1	1	-	-	1	1	-	-	1
21	6, 3	2	4	1	3	1	-	-	2	1	1
22	6, 4	1	-	1	-	-	-	-	-	1	1
Total		30	30	30	30	30	30	30	30	30	30

DM – Dharwad market, HM – Hubli market, KUC – Karnatak University campus, NAVN – Navanagar, LAB – laboratory population, Rs – right side, Ls – left side.

Table 2. Possible combinations of anterior, posterior, and extra teeth of the genital organ in *D. bipectinata* recorded from different localities.

Serial no.	Possible combinations of teeth (anterior, posterior, extra)	SGP		YSC		KUC		KUN		LAB	
		Rs	Ls	Rs	Ls	Rs	Ls	Rs	Ls	Rs	Ls
1	1, 1	2	-	-	-	-	-	-	-	-	-
2	1, 3	-	-	-	-	-	-	-	1	-	-
3	1, 3, 1	-	-	-	-	-	2	-	-	-	-
4	1, 4	-	1	-	-	-	-	-	-	-	-
5	2, 2	2	-	2	3	4	1	2	4	1	-
6	2, 2, 1	3	3	1	1	2	-	-	2	-	2
7	2, 1, 1	1	-	-	-	-	-	-	-	-	-
8	2, 2, 2	-	1	1	1	-	-	1	-	2	-
9	2, 3	10	11	16	17	15	10	20	12	4	7
10	2, 3, 1	-	-	2	-	-	1	-	1	6	5
11	2, 3, 3	-	2	-	-	-	-	-	-	-	-
12	2, 4	5	4	6	4	7	10	4	6	9	13
13	2, 4, 1	-	-	-	-	-	-	-	-	-	1
14	3, 1, 1	1	-	-	-	-	-	-	-	-	-
15	3, 2	-	1	1	1	-	-	3	-	-	-
16	3, 2, 1	-	1	-	-	-	-	-	-	-	-
17	3, 2, 2	-	-	-	-	-	-	-	-	-	1
18	3, 3	5	3	1	2	2	5	-	4	7	1
19	3, 4			-	1	-	1	-	-	-	-
20	6, 0	-	1	-	-	-	-	-	-	-	-
Total		30	30	30	30	30	30	30	30	30	30

SGP – Sangatigopp forest, YSC – Yalakki Shettar Colony, KUC – Karnatak University campus, KUN – Kundagol, LAB – laboratory populations, Rs – right side, Ls – left side.

Table 3. Possible combinations of anterior, posterior, and extra teeth of the genital organ in *D. malerkotliana* recorded from different localities.

Serial no.	Possible combination of teeth (anterior, posterior, extra)	SGP		YSC		KUC		KUN		LAB	
		Rs	Ls	Rs	Ls	Rs	Ls	Rs	Ls	Rs	Ls
1	1, 2	-	-	-	-	-	-	-	1	-	-
2	1, 2, 1	-	-	-	-	-	-	-	-	1	-
3	1, 3	-	1	-	-	1	-	1	1	-	-
4	1, 3, 1	-	-	-	-	-	-	-	-	-	-
5	2, 1	-	-	1	-	-	2	1	-	-	-
6	2, 1, 1	-	1	-	-	1	-	-	-	-	3
7	2, 1, 1, 1	-	-	-	-	-	-	-	-	2	4
8	2, 2	2	1	3	3	3	-	7	3	2	1
9	2, 2, 1	3	2	2	3	4	4	2	1	12	12
10	2, 2, 1, 1	-	-	-	-	-	-	-	-	-	1
11	2, 2, 2	2	2	-	1	2	1	2	3	1	-
12	2, 3	15	18	18	12	16	16	15	13	7	4
13	2, 3, 1	1	-	2	1	1	1	2	2	2	3
14	2, 4	6	5	2	7	1	4	-	5	-	1
15	3, 1, 1	-	-	-	-	-	-	-	-	-	1
16	3, 2	1	-	-	2	-	2	-	-	1	-
17	3, 2, 1	-	-	-	-	1	-	-	-	1	-
18	3, 2, 1, 1	-	-	-	-	-	-	-	-	1	-
19	3, 3	-	-	2	1	-	-	-	-	-	-
20	3, 4	-	-	-	-	-	-	-	-	-	-
Total		30	30	30	30	30	30	30	30	30	30

SGP – Sangatigopp forest, YSC – Yalakki Shettar Colony, KUC – Karnatak University campus, KUN – Kundagol, LAB – laboratory populations, Rs – right side, Ls – left side.

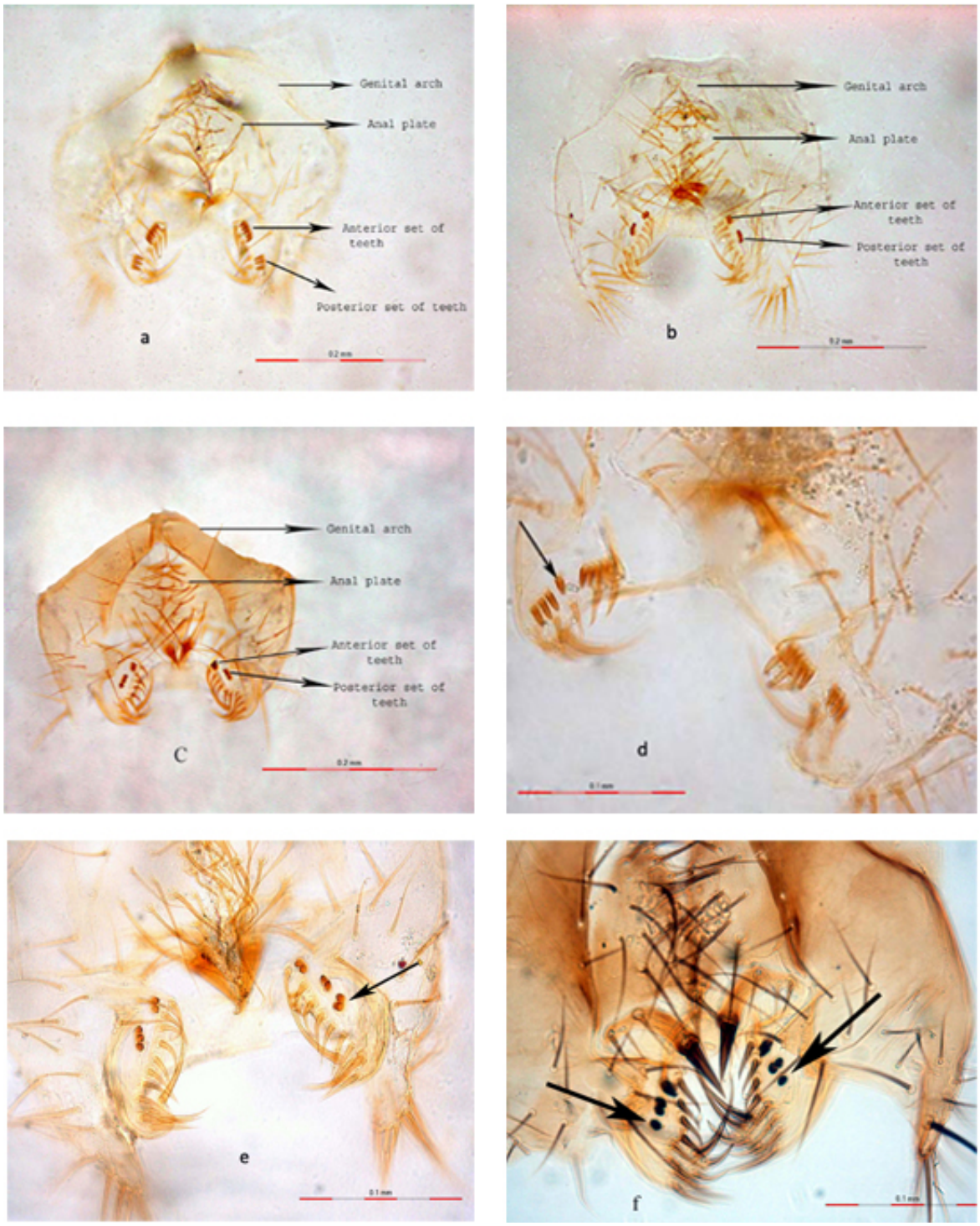

Figure 1. Arrangement of anterior and posterior sets of teeth in periphallic organs of different species of *Drosophila*: **a)** *D. ananassae*, bilateral symmetry; **b)** *D. bipectinata*, bilateral symmetry; **c)** *D. malerkotliana,* bilateral symmetry; **d)** *D. ananassae*, altered symmetry; **e)** *D. bipectinata*, altered symmetry; **f)** *D. malerkotliana*, altered symmetry. Arrows indicate presence of extra teeth.

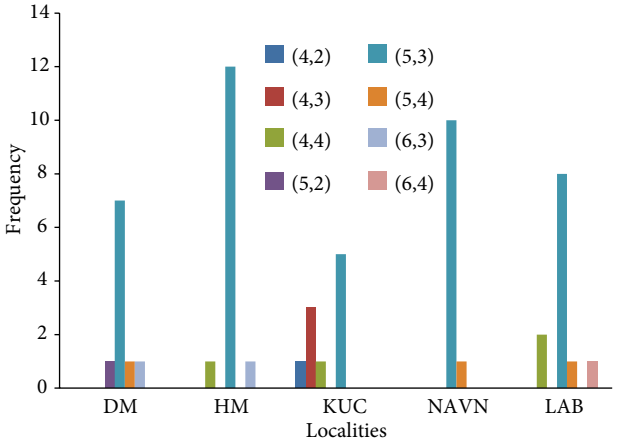

Figure 2. Frequency of anterior, posterior, and extra teeth showing perfect bilateral symmetry in *D. ananassae*.
DM – Dharwad market, HM – Hubli market, KUC – Karnatak University campus, NAVN – Navanagar, LAB – laboratory populations.

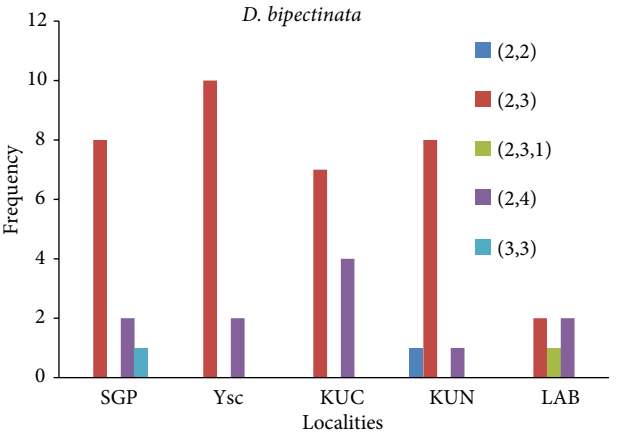

Figure 3. Frequency of anterior, posterior, and extra teeth showing perfect bilateral symmetry in *D. bipectinata*.
SGP – Sangatigopp forest, YSC – Yalakki Shettar Colony, KUC – Karnatak University campus, KUN – Kundagol, LAB – laboratory populations.

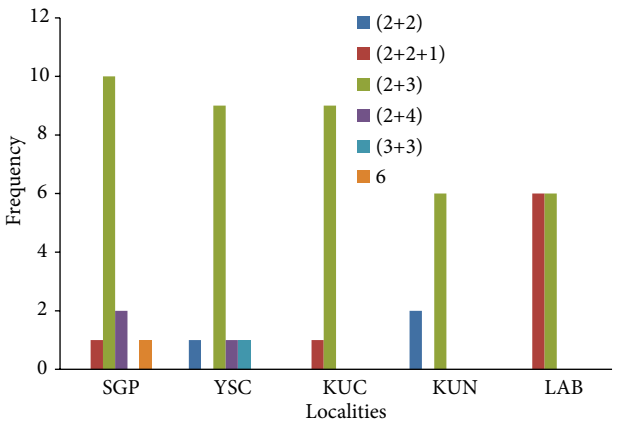

Figure 4. Frequency of anterior, posterior, and extra teeth showing perfect bilateral symmetry in *D. malerkotliana*.
SGP – Sangatigopp forest, YSC – Yalakki Shettar Colony, KUC – Karnatak University campus, KUN – Kundagol, LAB – laboratory populations.

be 1–3 in the anterior and 1–4 in the posterior region. The number of different sets of combinations of anterior and posterior teeth indicates the presence of morphological variations (Tables 1–3), with different combinations of teeth present on either side of the primary clasper.

Perfect bilateral symmetry (equal number of teeth in anterior and posterior sets on either side of primary clasper) is less frequent compared to asymmetry (deviation from perfect bilateral symmetry or unequal number of teeth in the anterior and posterior sets) in all 3 species. The extra teeth/set of teeth is present either in between or on the adjacent side of the anterior and posterior regions (Figures 1d–1f). Frequency of perfect bilateral symmetry varies among different natural populations and laboratory populations. The combination of (5, 3) appears to be dominant in *D. ananassae* populations, whereas the (2, 3) combination was dominant in *D. bipectinata* and *D. malerkotliana*. This implies that the abovementioned trait number/combination/pattern is more stable than other combinations. Another curious observation is that the laboratory strains of *D. bipectinata* and *D. malerkotliana* showed lower frequencies of perfect bilateral symmetry compared to their natural populations, whereas in the case of *D. ananassae*, the Karnatak University and Dharwad market localities showed lower frequency of perfect bilateral symmetry than the laboratory strain (Figures 2–4).

The lack of significant differences for anterior sets of teeth between species and among their populations suggests that this trait is rather less variable when compared to the posterior set of teeth. While there are significant differences among posterior sets for different populations in all 3 species, it is not clear if environment affects this trait. But if species collected from localities that have a greater geographical distance are compared, then there could be a more definite conclusion. Mishra and Singh (2006) reported the various sex comb patterns in the *bipectinata* species complex. They found some unique and new sex comb phenotypes and also found significant differences for intra- and interspecific populations.

The *ananassae* subgroup is considered to be a group characterized by several unusual genetic features and unique phenotypes, with various sex comb patterns (Singh, 2000; Mishra and Singh, 2006). The presence of different patterns of anterior and posterior sets of teeth is also unique in *ananassae* subgroup species. This kind of morphological variation has not been found in other subgroups of the genus *Drosophila* (personal observation). Consideration of this character as one of the keys in

taxonomic identification of species would be highly biased because of the different patterns found. Another important question that is raised here is why only certain selected patterns (of anterior and posterior teeth) are abundantly found in natural populations and others rarely. Such questions can only be answered by further studies on the heritability of this character and studies on the fitness of each character.

The studies of Polak and Rashed (2010) revealed that the spines present on the secondary clasper of the periphallic organ act like claws during copulation in *D. bipectinata*. The primary teeth of the periphallic organ have been found to be polymorphic in the 3 species studied. While the functions of these teeth are not precisely known, a study is in progress to analyze whether they involve holding the female ovipositor during copulation or help in copulation duration.

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