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Male tarsal sex-comb teeth pattern in the *Drosophila bipectinata* complex

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Summary. Species within the *D. bipectinata* species complex pose problems experimentally because of their morphological similarity. By measuring the number of teeth in each row of the male tarsal sex-comb, an unknown specimen can be allocated to a hybrid or parental group by means of a discriminant function. This paper describes which rows of the sex-comb are the best discriminators for particular comparisons.
Key words. Sex-comb pattern; discriminant analysis; *Drosophila* sibling species; hybrids.

The *Drosophila bipectinata* complex is classified in the *ananassae*-subgroup of the *D. melanogaster* species group[1]. The complex includes four species, *D. bipectinata, D. malerkotliana, D. parabipectinata,* and *D. pseudoananassae*. The four species and a majority of their hybrids can be distinguished from one another by the number of teeth in the male tarsal sex-comb[2]. The only other differences reported between the species are in the coloration of the abdominal tergites in males; in *bipectinata* and some populations of *pseudoananassae* and *malerkotliana,* male abdomens are light, while in *parabipectinata* and other populations of *pseudoananassae* and *malerkotliana,* male abdomens are black. Male genitalia are similar in all four species[3,4]. Females, of the four species, are similar in appearance to one another.

Teeth in the sex-comb are arranged in rows, and the presence or absence of teeth in rows, as well as the total number of teeth in a row, vary between right and left legs of the same individual, between individuals, and between species. This study examines the pattern of the sex-combs and uses discriminant analysis to test which rows best distinguish the species and their hybrids. The question is whether sex-comb pattern or total sex-comb teeth number is the best character to use in situations where studies involving the species and their hybrids require some means of distinguishing them.

20 male flies from each of the four species and 12 different hybrids (six crosses and six reciprocal crosses) were measured. Hybrid males were either obtained from vials which housed five virgin females of one species and five virgin males of another species, or from virgin females which had been observed to mate with a foreign species in another study concerned with behavior. The location and number of teeth making up the sex-combs were recorded for the right foretarsus for all males. In addition, the pattern of teeth on the left foretarsus was recorded for the *bipectinata, malerkotliana,* and *parabipectinata* males. Magnification used for examination was ×100.

Figure 1 illustrates the sex-comb pattern characteristic of each species. Teeth which make up the sex-comb occur on the first and second tarsal segment of each front leg in all species. In addition, the front foretarsi of *malerkotliana* also bear teeth on tarsal segment three. In *bipectinata* and *parabipectinata* teeth in rows 1 and 2 are comb-like and project horizontally. In *malerkotliana* and *pseudoananassae,* teeth in the same row may overlap one another. Teeth arrangement is, therefore, less comb-like in these two species than in *bipectinata* and *parabipectinata.*

There were no significant differences in row teeth number between sex-combs on the right and left sides of the body (table 1). Individual differences between right and left sides rarely exceeded one.

Table 1 gives the number of teeth per row in the sex-combs of all the species and species hybrids together with the standard errors. Linear discriminant-function analyses were used to classify the species and their hybrids. The discriminating variables were the teeth number in each of the rows 1–5. Any row for which no teeth were present for both of the groups compared was omitted. Linear discriminant procedures require equality of covariance matrices. In our study, these matrices were equal except in comparisons which included *malerkotliana.* However, Colgan[5] argues that this requirement does not influence results when sample sizes are, as in our case, equal and large. We assume, therefore, that the above analysis is appropriate for all the groups compared.

Figure 2 shows the distribution of the discriminant scores for the six species comparisons. 100% correct classification was achieved for all pairs of species compared except for *bipectinata* and *parabipectinata* which were classified with an accuracy of 95%.

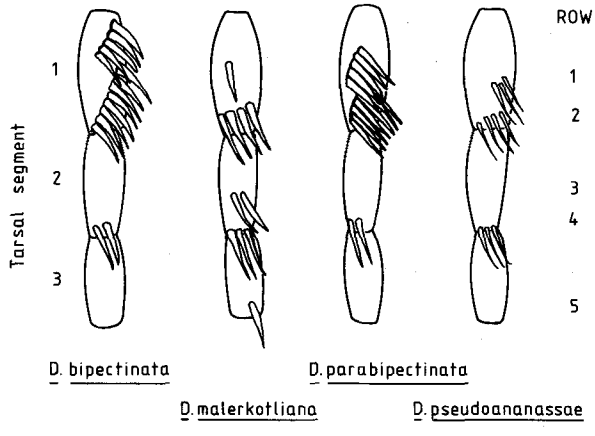

Figure 1. Male tarsal segments 1, 2 and 3 bearing sex-combs.

The contribution of each of the discriminative variables varied between pairs of species (fig. 2). Row 2, for example, was most important in discriminating capacity in the *bipectinata* and *parabipectinata* comparison. The distribution of the discriminant scores around the group mean (centroid) indicates within species variability. It may be seen (fig. 2) that the most to the least variable species, in order, are *parabipectinata, bipectinata, malerkotliana* and *pseudoananassae*. This analysis has shown that the numbers of teeth per row of the sex-comb are good discriminators and as such are useful for assigning unknown specimens to their correct group.

The same discriminant variables were used to classify each kind of hybrid and its parent species. Each analysis involved the two parents and one of two reciprocal hybrids. The percentage accuracy for correctly classifying hybrids was less than for species comparisons, in most cases (table 2). Nevertheless, better than chance classification was achieved in all cases. This means that the discriminators chosen are useful for assigning unknown specimens to hybrid groups.

Table 1. The number of teeth in each row of the male sex-comb. t, t-test (paired comparison) between rows in the right and left sex-combs of the same individual, $p > 0.05$. *D. bipectinata* (bip), *D. parabipectinata* (par), *D. malerkotliana* (mal) and *D. pseudoananassae* (psn)

		Right male tarsal sex-comb Male parent			
Female parent	Row	bip	par	mal	psn
bip	1	5.75 (0.18)	4.80 (0.21)	2.65 (0.24)	5.50 (0.14)
	2	8.90 (0.19)	8.60 (0.17)	5.40 (0.22)	7.70 (0.23)
	3			1.10 (0.10)	
	4	2.05 (0.09)	1.65 (0.11)	2.80 (0.09)	1.50 (0.11)
	5			0.20 (0.09)	
par	1	4.95 (0.17)	4.70 (0.16)	3.50 (0.17)	5.20 (0.30)
	2	8.20 (0.27)	6.80 (0.21)	5.20 (0.20)	6.90 (0.20)
	3		0.05 (0.05)	1.05 (0.05)	
	4	1.45 (0.11)	1.35 (0.11)	2.45 (0.11)	1.50 (0.11)
	5				
mal	1	3.30 (0.16)	1.90 (0.29)	1.20 (0.12)	2.00 (0.21)
	2	5.65 (0.20)	4.65 (0.23)	3.90 (0.10)	4.25 (0.18)
	3	1.05 (0.05)	1.65 (0.18)	1.95 0.09)	1.35 (0.20)
	4	2.75 (0.12)	2.75 (0.14)	3.05 (0.09)	2.80 (0.16)
	5		0.45 (0.11)	0.65 (0.11)	0.50 (0.11)
psn	1	4.05 (0.29)	2.70 (0.19)	2.65 (0.17)	2.40 (0.13)
	2	5.95 (0.43)	4.95 (0.18)	4.95 (0.17)	4.30 (0.13)
	3			0.20 (0.14)	
	4	2.30 (0.24)	3.05 (0.11)	3.40 (0.11)	3.05 (0.09)
	5			0.10 (0.07)	

Row	Left male tarsal sex-comb					
	bip × bip	t	par × par	t	mal × mal	t
1	5.80 (0.17)	0.2	4.75 (0.16)	0.2	1.05 (0.09)	0.8
2	8.60 (0.27)	1.0	7.08 (0.17)	1.2	4.10 (0.12)	1.4
3	0.05 (0.05)	0.0	0.10 (0.07)	0.0	1.85 (0.11)	1.7
4	1.95 (0.14)	0.5	1.55 (0.14)	1.7	3.05 (0.14)	0.0
5					0.50 (0.11)	1.0

Table 2. Classification accuracy of hybrids and their parents. * a best discriminating variable which is different in hybrid/hybrid and species/species comparisons. *D. bipectinata* (bip), *D. parabipectinata* (par), *D. malerkotliana* (mal) and *D. pseudoananassae* (psn)

Groups Species	Hybrid Female	Male	% Group cases correctly classified	First and second best discriminating rows
bip, par	bip	par	78	2, 1*
	par	bip	68	4, 2
bip, psn,	bip	psn	86	2, 1
	psn	bip	85	2, 1
bip, mal,	bip	mal	91	1, 3
	mal	bip	100	1, 3
mal, par,	mal	par	80	4*, 1
	par	mal	100	3, 1
mal, psn	mal	psn	83	3, 1
	psn	mal	78	3, 1
psn, par,	psn	par	78	4, 2
	par	psn	77	4, 2

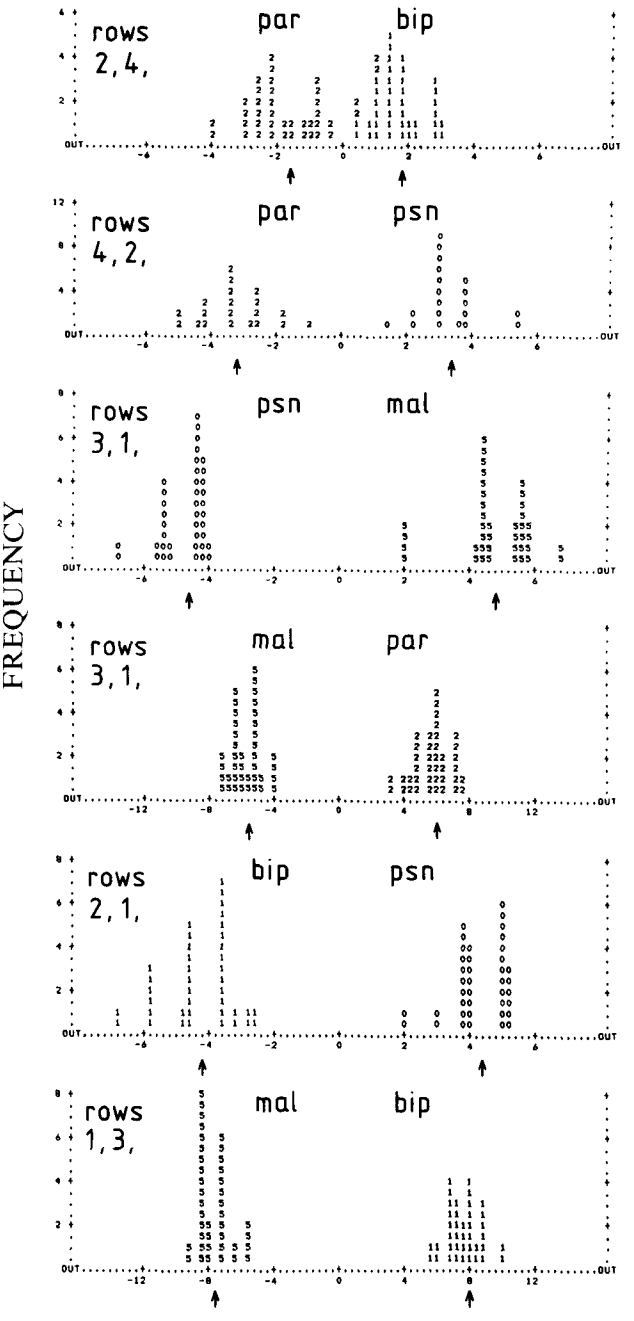

Figure 2. Distribution of discriminant scores for *D. bipectinata* (bip), *D. parabipectinata* (par), *D. malerkotliana* (mal) and *D. pseudoananassae* (psn) sex-combs. Scores are based on linear discriminant function analysis using teeth number per row of the sex-comb as variables. The number of variables used ranged from three to five depending on the number of rows present in the sex-combs of the species compared. The arrows beneath each histogram denote the group centroids.

The hybrid male sex-comb patterns are intermediate between those of either parental species, with some maternal bias except for hybrids of *malerkotliana* with *bipectinata* and with *parabipectinata*, which are either intermediate or like *malerkotliana*. This indicates some dominance of *malerkotliana* genes.

The best two discriminators in the analyses involving hybrids and their parents were the same as those which best discriminated the parental species from one another, except in two cases as indicated in table 2.

It is concluded that male sex-comb pattern, as defined by the numbers of teeth in each row of the sex-comb, is useful for distinguishing males within the *D. bipectinata* complex. It is preferred to the alternative of total sex-comb teeth number[2] because it classifies all possible hybrids with better than chance accuracy. Sex-comb patterns are relevant to a behavior genetic analysis of species-specific behaviors within this complex, which is in progress in this laboratory. Since the species occur sympatrically over parts of their ranges[4] and hybridization occurs in the wild[6], the use of sex-comb patterns for identifying species and hybrids is of general interest.

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Ecdysis inhibition in *Acrolepiopsis assectella* larvae by digitonin: antagonistic effects of cholesterol

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Summary. Digitonin has a toxic effect on *Acrolepiopsis assectella*, inhibiting development and molting of young larvae fed on a semi-synthetic diet. This effect depends on concentration. It does not occur when cholesterol is added to the diet.
Key words. Ecdysis; saponins; digitonin; cholesterol; lepidoptera; *Acrolepiopsis assectella.*

Studies of *Acrolepiopsis assectella* showed that larvae fed on leek flowers (*Allium porrum*) or on a diet containing dry leek flowers, were unable to molt after normal apolysis. They could not remove their head capsules and their trunk exuviae[1]. Preliminary experiments to investigate the biologically active principle led us to isolate a steroidal saponin close to digitonin. Structural studies of this saponin are being carried out, with Dr Harmatha in Prag[2]. Since saponins are known to produce a significant reduction in plasma cholesterol concentration in various animals[3,4], interference with insect development was tested. We report the toxic effect of digitonin on *A. assectella* and the antagonistic effects of cholesterol. Ecdysis inhibition will be compared with similar effects obtained with different compounds.

Materials and methods. The methods of breeding *A. assectella* Zell. and the preparation of artificial diets have been previously described[1]. In our experiments we used 25 mm OD, 60 mm long glass test tubes. Each tube contained 5 ml of the artificial medium, and was closed with carded cotton. After solidification and cooling, the nutrient medium in each tube was inoculated with 50 3-day-old eggs; the larvae hatched with 24 h; breeding temperature was 25 °C, photophase 14 h, relative humidity 60–65%.

Digitonin used in the test was a commercial compound purchased either from Merck (Rahway, New Jersey, USA), or from Nativelle (Paris, France). In fact this commercial compound contains only 80% of digitonin. In preliminary experiments digitonin was purified on TLC (silica plates, butanol/ethyl acetate/water: 4:1:5) and the biological activity of other saponins; tigonin, gitonin and minor saponis, was tested. These experiments have shown that only digitonin was biologically active. Further experiments were performed with commercial products but the values reported correspond to those of pure digitonin.

The graded amounts of digitonin were added to the diet in water

Table 1. Larvae of *A. assectella* killed at the first instar or during molting, in diets containing various concentrations of digitonin. L1 = number of newly hatched larvae

Digitonin (ppm)	Total L1 hatched	Dead larvae 1st instar	Molting number 1	2	3	4
0	336	3				
200	304	26		1		
400	306	47		11	1	
600	686*	329	7	80	6	2
800	193**	142	1	26	2	
1000	312	242	4	49	5	2
Total	2137	789	12	167	14	4
% of dead larvae during molting			6.1	84.8	7.1	2

*16 replicates, **6 replicates.

Table 2. Decreasing sensitivity of *A. assectella* to digitonin with increasing age of larvae: 30 larvae of each instar transferred from standard diet to standard diet (control), or to 1000 ppm digitonin-containing diet. Number of dead larvae

Number of instar transferred	Control	Digitonin-fed without symptom	Molting number 2	3	4	Total
1st	7	18	10	1	0	29 (97%)
2nd	2	12	1	9	5	27 (90%)
3rd	2	11		1	9	21 (70%)
4th	3	10			3	13 (43%)
5th	0	5				5 (17%)