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The *bipectinata* Complex: A Study in Interspecific Hybridization in the Genus *Drosophila* (Insecta : Diptera)

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Abstract

Although there is no evidence of hybridization in nature, interspecific crosses in all six possible pairwise combinations may be effected in the laboratory amongst the four species of the *Drosophila bipectinata* complex (*D. bipectinata*, *D. parabipectinata*, *D. malerkotliana* and *D. pseudoananassae*) of the Oriental–Australian biogeographic zones. Results range from a high degree of crossability in one direction with production of large numbers of sterile male and fertile female offspring in a 1 : 1 ratio (*bipectinata* × *parabipectinata*) to almost complete isolation in both directions; in the latter case (*malerkotliana* × *pseudoananassae*) the very small proportion of successful crosses yielded only a few females of low viability. These results combined with those previously obtained in polytene chromosomal and electrophoretic investigations suggest very close phylogenetic relationships among *bipectinata*, *parabipectinata* and *malerkotliana*, with a greater degree of divergence between these species and *pseudoananassae*.

Introduction

Interspecific hybridization in animal species and the genetic phenomena associated with it have been studied experimentally for many years. As early as 1922, J. B. S. Haldane reviewed the results of hybridization experiments in a variety of taxa and offered the empirical generalization (which has since become known as 'Haldane's Law') that when amongst the offspring of an interracial or interspecific cross one sex is absent, rare or sterile, that sex is the heterogamic one.

At the time of Haldane's review, only one case of interspecific hybridization had been reported in the genus *Drosophila*, that of *melanogaster* × *simulans*; hybridization experiments between these two species were first attempted by Sturtevant (1920), but considerable subsequent work has been done (Agnew 1971; Barker 1962, 1967; Parsons 1972).

Following Haldane (1922) and the discovery and description of increasingly large numbers of *Drosophila* species, many further interspecific hybridization experiments were attempted. By 1952, Patterson and Stone were able to list 101 different cases of hybridizations effected in the laboratory amongst *Drosophila* species in eight different species groups. Much of the latter work was stimulated by the discovery of complexes of very similar species, and indeed certain forms were only recognized as 'species' on the basis of laboratory findings of their crossability, or lack of it, with similar forms.

Since the comprehensive review by Patterson and Stone (1952), considerable further work has been performed on interspecific hybridizations in *Drosophila*.

In particular, the enormous Hawaiian fauna, substantially unknown at the time of Patterson and Stone's writing, has been investigated in some detail (Craddock 1974). The Neotropical *willistoni* group has also attracted considerable attention (Dobzhansky and Powell 1975). In almost all of the cases of interspecific hybridizations reported in the genus, Haldane's Law is clearly manifest.

One of the most acclaimed virtues of the genus *Drosophila* is that it provides numerous groups or clusters of very similar species which are particularly rewarding subjects for experimental investigations, and in spite of the 150 or more cases of interspecific hybridizations that have now been reported in *Drosophila* species, many groups have been very little studied. The large *melanogaster* group contains

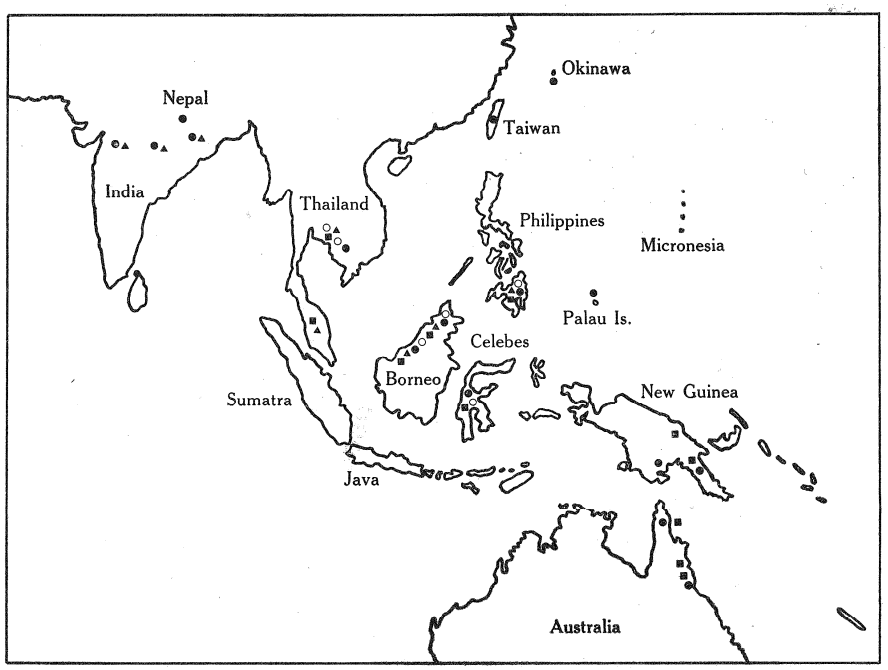

Fig. 1. Distributions of the species of the *bipectinata* complex [excluding Fiji and Samoa for *D. bipectinata*; after Bock and Wheeler (1972) and Bock (1977)]. ● *D. bipectinata*. ○ *D. parabipectinata*. ▲ *D. malerkotliana*. ■ *D. pseudoananassae*.

in excess of 70 described species (Bock and Wheeler 1972), and although *melanogaster* and *simulans*, and several other species of the group, have been studied in detail, many complexes remain untouched. One such complex within the *melanogaster* group found to be a rewarding subject for experimental investigation is the *bipectinata* complex.

The *bipectinata* complex consists of four morphologically very similar species distributed in the Australian and/or Oriental biogeographic zones. The most widespread of the species (Fig. 1) is *D. bipectinata* Duda, which ranges from India to Fiji and Samoa; the other species, *D. parabipectinata* Bock, *D. malerkotliana* Parshad & Paika, and *D. pseudoananassae* Bock, are less widespread, but in parts of south-east Asia all four species are sympatric. Females of the four species are for all practical

purposes indistinguishable. Males are distinguished principally by reference to the structure of the sex-comb (Fig. 2), but the abdominal coloration may also be useful, especially in separating *bipectinata* (pale tan) and *parabipectinata* (shiny black). The male genitalia of all four species are extremely similar, an unusual situation within the genus *Drosophila* where the male genitalia of species even very similar in external morphology are usually quite distinct.

Preliminary attempts at intercrossing the species of the *bipectinata* complex revealed that successful crosses could be effected in the laboratory, and analysis of the polytene chromosomal rearrangements in the hybrid larvae yielded some phylogenetic information concerning the species (Bock 1971). This paper reports the results of detailed systematic crosses and discusses the additional phylogenetic information that may be inferred from their results.

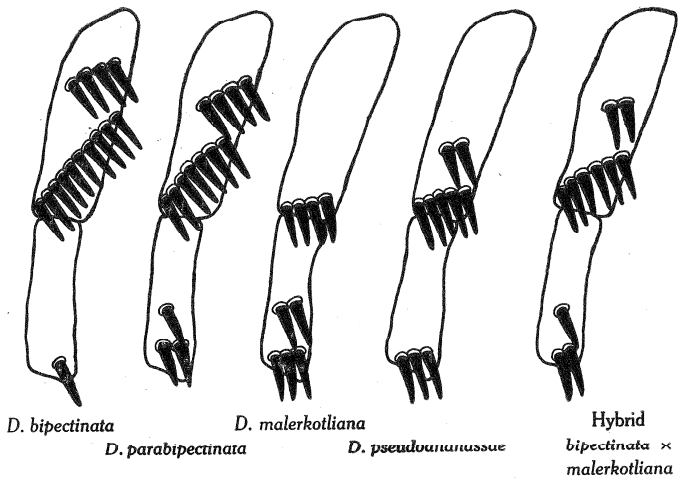

Fig. 2. Sex-combs in the *bipectinata* species complex.

Materials and Methods

The procedure followed in testing interspecific sexual isolations was the confinement of a single male of one species with a single female of the other in a 10 by 2·5 cm tube of standard culture medium. Males and virgin females were collected and aged for 7 days in small batches before individual crosses were commenced (this procedure also served as a check on virginity); crosses were then prepared after light etherization of the parents. All tubes of attempted crosses were retained for 12–14 days, after which time each tube was examined. Tubes in which both flies were not still alive were discarded unless evidence of larval activity was noted in the culture medium. If no evidence of larval activity was noted in the medium amongst any of the remaining tubes, the females were dissected to determine whether they had been inseminated. Results for each set of attempted interspecific crosses were thus collected as the total number of inseminations (tubes in which larval activity was noted, or in which the female was inseminated) out of the total number of successfully attempted crosses (both parents still alive after the 12–14-day period if no evidence of larval activity). All flies were kept at 24°C.

Where larval activity was noted in tubes after the period of confinement, the parents were discarded and the tubes retained for examination and testing of any F_1 progeny that emerged.

The number of possible interspecific crosses is six. All possible combinations were attempted, and reciprocal crosses were set up in each case. Two strains of each species were used (Borneo and Thailand strains of *bipectinata*, *parabipectinata* and *malerkotliana*; Borneo and Cairns strains of

pseudoananassae), giving a total of 48 different combinations including reciprocals (Table 1). Control crosses for each species were also performed by the same method as that used to test interspecific isolation.

Sexual isolation between the strains of each species (i.e. sexual isolation within species) was also tested by the 'male-choice' method which enables calculation of an isolation index I (Stalker 1942) defined by the formula

$$I = \frac{\%\text{ homogamic inseminations} - \%\text{ heterogamic inseminations}}{\%\text{ homogamic inseminations} + \%\text{ heterogamic inseminations}}.$$

In the male-choice test, a male of one strain is confined with two females, one of the same strain (the homogamic female), the other of a different strain (the heterogamic female) for a period of 24 h; both females are then dissected to determine whether they have been inseminated. The procedure is repeated a number of times to accumulate sufficient data for a meaningful calculation of the isolation index, which in theory may range from -1 to $+1$. Whether positive or negative the index is significant if a χ^2 test reveals a significant difference between the numbers of homogamic and heterogamic inseminations. Since it was not possible to distinguish between females of each pair of strains tested in the *bipectinata* complex, females of one strain were marked with a dot of black ink on the dorsum of the thorax before the tests were commenced; in case the marking produced any effect on the outcome of the tests, a second batch of crosses was run in each case in which the females of the other strain were marked. All flies used in the intraspecific tests were, as before, collected and aged for 7 days before testing was commenced.

Table 1. Interspecific and control crosses in the *bipectinata* complex

bip, *D. bipectinata*; *par*, *D. parabipectinata*; *mal*, *D. malerkotliana*; *psn*, *D. pseudoananassae*; B, Borneo strain; T, Thailand strain; C, Cairns strain. Control results are shown in bold type. For further explanation see text

♀ \ ♂	bip_B	bip_T	par_B	par_T	mal_B	mal_T	psn_B	psn_C
bip_B	**60/60**		51*/60	54*/60	11/59 ↔	1*/56	0/60	3*/60
bip_T		**60/60**	41*/59	38*/56	0*/58	0*/57	0/57	0*/56
par_B	6/60	1/55	**54/60**		1*/60	1*/60	0*/60	0*/60
par_T	10/59 ↔	2/56		**57/60**	6/60 ↔	0*/60	0*/60	1*/60
mal_B	13/51	23/56	11/59	5/57	**56/60**		2/161	5*/154
mal_T	13/53	8/58	17/56	22/56		**57/60**	0/57	0/56
psn_B	1/56	4/57	24/60	26/59	1/162	0/54	**53/60**	
psn_C	33/57	28/56	37/60	15/56	23/154	2/55		**57/60**

Results

Interspecific Sexual Isolation Tests

Table 1 gives details of the results of all interspecific and control sexual isolation tests, showing in each case the total number of inseminations out of the number of successfully attempted (as defined above) crosses. Control results are given in the diagonal from top left to bottom right of the table. Numbers of inseminations in the interspecific crosses in the upper right-hand portion of the table significantly different at the 5% level from corresponding numbers in the reciprocal crosses (lower

left-hand portion of the table) are marked with an asterisk. Further significance tests revealed that the numbers of inseminations obtained in the interspecific crosses are significantly smaller (at the 5% level) from the control numbers in all except two cases: *parabipectinata* Borneo males × *bipectinata* Borneo females (51 inseminations in 60 crosses) does not differ significantly from *parabipectinata* Borneo control, and *parabipectinata* Thailand males × *bipectinata* Borneo females (54 inseminations in 60 crosses) does not differ significantly from either *parabipectinata* Borneo or Thailand controls.

Table 2. Summed data for interspecific sexual isolation tests

Abbreviations of species names as in Table 1

Cross	Inseminations	Percentage
(1) *bipectinata* × *parabipectinata*		
bip ♂ × *par* ♀	19/230	8·3
bip ♀ × *par* ♂	184/235	78·3
Total, both directions	203/465	43·7
(2) *bipectinata* × *malerkotliana*		
bip ♂ × *mal* ♀	57/218	26·1
bip ♀ × *mal* ♂	12/230	5·2
Total, both directions	69/448	15·4
(3) *parabipectinata* × *malerkotliana*		
par ♂ × *mal* ♀	55/228	24·1
par ♀ × *mal* ♂	8/240	3·3
Total, both directions	63/468	13·5
(4) *bipectinata* × *pseudoananassae*		
bip ♂ × *psn* ♀	66/226	29·2
bip ♀ × *psn* ♂	3/233	1·3
Total, both directions	69/459	15·0
(5) *parabipectinata* × *pseudoananassae*		
par ♂ × *psn* ♀	102/235	43·4
par ♀ × *psn* ♂	1/240	0·4
Total, both directions	103/475	21·7
(6) *malerkotliana* × *pseudoananassae*		
mal ♂ × *psn* ♀	26/425	6·1
mal ♀ × *psn* ♂	8/428	1·9
Total, both directions	34/853	4·1

The data presented in Table 1 may be considered from a further aspect, that of sympatry *v.* allopatry in the various combinations of strains used in the crosses. (For example, *bipectinata* Borneo × *parabipectinata* Borneo is a cross between two sympatric strains; *bipectinata* Borneo × *parabipectinata* Thailand is a cross between allopatric strains.) There are 42 possible such comparisons, including reciprocals, in the data of Table 1. Those in which there is a significant difference (at the 5% level) between numbers of sympatric and allopatric inseminations are indicated in Table 1 by double-headed arrows.

If the differences between the crossabilities of strains dependent on their sympatry or allopatry of origin are disregarded, the data presented in Table 1 may be consolidated under the six headings representing all possible interspecific crosses. Table 2 presents

the summed results for the six crosses; the distinctions between reciprocal crosses are retained since they are mostly substantial, but the average is also given in each case to facilitate rough comparison.

Progeny of Interspecific Crosses

(1) *D. bipectinata* × *D. parabipectinata*

In those crosses which were obtained, abundant male and female offspring were produced in a 1 : 1 ratio (Table 3). Examination of the testes of the F_1 males revealed that (irrespective of whether *bipectinata* was the male or the female parent) the testes were of normal size and structure, i.e. yellow, with 3–4 large outer coils. Numerous spermatozoa were produced which were, however, immotile, and joined together

Table 3. Numbers of males and females in samples of progeny from interspecific crosses and controls

A significant excess of females is indicated with an asterisk

Interspecific crosses	Males	Females
(1) *bipectinata* ♂ × *parabipectinata* ♀	59	64
bipectinata ♀ × *parabipectinata* ♂	82	87
(2) *bectinata* ♂ × *malerkotliana* ♀	65	64
bipectinata ♀ × *malerkotliana* ♂	47	56
(3) *parabipectinata* ♂ × *malerkotliana* ♀	58	69
parabipectinata ♀ × *malerkotliana* ♂	48	43
(4) *bipectinata* ♂ × *pseudoananassae* ♀	1	> 100*
(5) *parabipectinata* ♂ × *pseudoananassae* ♀	10	132*
(6) *malerkotliana* ♂ × *pseudoananassae* ♀	0	> 100*
Controls		
bipectinata	60	71
parabipectinata	48	61
malerkotliana	62	54
pseudoananassae	51	42

in small bundles. The sterility of the F_1 males was further confirmed by attempted crosses; in no cases were spermatozoa found in the reproductive tracts of the females subsequently dissected, although in several instances males had been observed in copulatory positions with females immediately prior to dissection of the latter.

As noted above, *bipectinata* and *parabipectinata* males differ in abdominal coloration; the hybrid male abdominal coloration in all cases was found to be intermediate between that of the parental species, i.e. weakly black.

Hybrid females were also tested for fertility and found to cross readily with males of either parental species (Table 4); numerous male and female adults were obtained from each set of crosses. In most cases examination of the testes of the male progeny revealed that the spermatozoa were immotile, but in a few males motile spermatozoa were observed.

(2) *D. bipectinata* × *D. malerkotliana*

Offspring of this cross were obtained in a 1 : 1 sex ratio (Table 3) irrespective of whether *bipectinata* was the male or the female parent; testes of the F_1 males

were well developed but smaller than normal testes. The hybrid testes were found to contain bundles of immotile spermatozoa.

Males of *bipectinata* and *malerkotliana* differ considerably in the structure of the sex-comb; hybrid males were found to possess sex-combs intermediate in structure between those of the parental species (Fig. 2).

F_1 females proved to be fertile when back-crossed to males of either parental species (Table 4); adult males and females were obtained from the successful crosses, but examination of the testes revealed that the males were sterile.

Table 4. Sexual isolation tests on interspecific hybrid females

A significant difference between the numbers of inseminations in each row is indicated by an asterisk. Abbreviations are as in Table 1. (Hybrid females in each case are derived from the more productive of the two possible parental crosses, i.e. *bip* ♀×*par* ♂, etc.)

♀ \ ♂:	*bip*	*par*	*mal*	*psn*
(*bip*×*par*)	56/60	58/60		
(*bip*×*mal*)	52/60		48/59	
(*par*×*mal*)		32/60	3*/60	
(*bip*×*psn*)	48/60			41/60
(*par*×*psn*)		57/60		0*/59
(*mal*×*psn*)			36/52	11*/52

(3) *D. parabipectinata* × *D. malerkotliana*

Offspring of successful crosses were obtained in a 1 : 1 ratio (Table 3); the F_1 male testes were similar to those obtained in the preceding cross, and sex-combs were intermediate between those of the parental species. F_1 females were found to be of reduced fertility (Table 4), but where *parabipectinata* was the male parent in the cross, both male and female progeny were obtained (the former possessing testes without spermatozoa).

(4) *D. bipectinata* × *D. pseudoananassae*

Examination of the offspring from those tubes which produced larvae revealed only one male, the testes of which were very small, poorly developed, and devoid of spermatozoa at any stage of development. F_1 females were, however, fertile in the majority of attempted crosses with males of each parental species (Table 4), and male and female progeny were obtained in each case; examination of the testes revealed that the males were sterile.

(5) *D. parabipectinata* × *D. pseudoananassae*

A highly significant excess of females was obtained amongst the progeny of successful crosses (Table 3); testes of the F_1 males were poorly developed, darkly pigmented and devoid of spermatozoa. F_1 male sex-combs were (as in crosses 2 and 3) intermediate between those of the parental species. The F_1 females were found to cross with *parabipectinata* males only (Table 4), but very few offspring were produced, the males with reduced testes.

(6) *D. malerkotliana* × *D. pseudoananassae*

Over 100 adult females were obtained from the few successful crosses. No viable adult males were obtained; however, a few males were observed to emerge from pupae, dying within an hour of eclosion without expansion of the wings. Examination of the testes of these males revealed that the testes were of grossly abnormal structure, very small, darkly pigmented and devoid of spermatozoa.

F_1 females tested against males of each parental species were inseminated in some cases (Table 4), but a total of only eight adult flies was obtained from all crosses in which *malerkotliana* was the male parent; all flies died within a few days of eclosion. Only one adult female was obtained from all tubes which produced larvae in the crosses in which *pseudoananassae* was the male parent; the fly died soon after eclosion.

Table 5. Intraspecific sexual isolation data

Hom., number of homogamic females inseminated out of 60; Het., number of heterogamic females inseminated out of 60; *I*, isolation index. Other abbreviations are as in Table 1. ♀, Marked females. The significant isolation index is indicated by an asterisk

	Hom.	Het.	*I*
D. bipectinata			
B♂×(B♀+T♀)	59	57	0·01
(B♀+T♀)	53	52	
T♂×(B♀+T♀)	56	55	0·02
(B♀+T♀)	46	44	
D. parabipectinata			
B♂×(B♀+T♀)	57	56	0·02
(B♀+T♀)	57	53	
T♂×(B♀+T♀)	55	53	0·03
(B♀+T♀)	58	54	
D. malerkotliana			
B♂×(B♀+T♀)	51	52	0·03
(B♀+T♀)	50	43	
T♂×(B♀+T♀)	57	56	0·02
(B♀+T♀)	53	49	
D. pseudoananassae			
B♂×(B♀+C♀)	22	42	−0·36*
(B♀+C♀)	17	41	
C♂×(B♀+C♀)	58	56	0·02
(B♀+C♀)	57	54	

Intraspecific Sexual Isolation Tests

Results of the intraspecific tests are summarized in Table 5. χ^2 tests were applied to each pair of results to check the effect of marking one of the females; in no case was there found to be a significant association between marking and insemination preference, i.e. there is no indication that the results were affected by one of the females having been marked. Isolation indices were therefore calculated on the summed results for each pair of crosses, and were further tested for significance of difference

between numbers of homogamic and numbers of heterogamic inseminations. Only one isolation index, as indicated in Table 5, was found to be significant.

Discussion

The results of the intraspecific sexual isolation tests are, with one exception, of little interest, merely indicating that there is no evidence of incipient speciation among any of the strains of the four species tested; the single significant index obtained was negative. Where the value of the isolation index does not differ significantly from zero, no reproductive isolation between the strains tested is indicated. A significant positive index indicates preference by the male for the homogamic female, or rejection of the male by the heterogamic female, i.e. a greater or lesser degree of reproductive isolation between the strains, which have presumably undergone some genetic (and concomitantly behavioural) divergence since their separation. A significant negative index may be interpreted as preference by the male for the heterogamic female, or rejection of the male by the homogamic female (and acceptance by the heterogamic). A negative index does not allow as simple an interpretation in evolutionary context as do the other two categories since it implies a greater preference between allopatric than between sympatric conspecific males and females; in all probability the males and females tested would never have the opportunity to mate in nature. Males of the Borneo strain of *pseudoananassae* certainly will mate with their own females (Table 1): the population would otherwise face rapid extinction, and the Borneo culture was maintained in the laboratory for the duration of the present investigation. A detailed comparative ethological study of the strains involved could help to provide an explanation of the negative index.

The interspecific tests have yielded considerably more information, and it is, indeed, tempting to speculate on the degrees of relationship of the four species to one another on the basis of the information derived from the hybridization studies.

It is evident (Table 2) that *bipectinata* and *parabipectinata* are almost freely crossable at least in one direction; at the opposite extreme, *malerkotliana* and *pseudoananassae* are barely crossable in either direction. Between these extremes, the four other combinations have yielded intermediate results. It is likely that closely related species will be more crossable than distantly related ones; reproductive isolation in *Drosophila*, as in most animals, occurs at the behavioural level, and more similar mating behaviours are likely to be found in closely related species. Thus, to the extent that degrees of crossability are an indication of phylogenetic affinity, *bipectinata* and *parabipectinata* are the most closely related species of the complex while *malerkotliana* and *pseudoananassae* are the most distantly related.

The F_1 data from the successful interspecific crosses reinforce the above conclusions: through the range of crosses there is a progressive increase in the degree of testis malformation in the F_1 males and a reduction of fertility of the F_1 females, coupled with gross aberrations of the sex ratios in crosses in which *pseudoananassae* was one of the parents. As noted above, Haldane's Law is very generally followed in interspecific crosses in the genus *Drosophila*, and although exceptions are known in some groups of animals (White 1973), in the case of the *bipectinata* complex it is manifest in all interspecific crosses, where the F_1 males range from sterile to absent. The smallest departure from the normal is evident in the *bipectinata* × *parabipectinata* crosses, where the progeny sex ratio is 1 : 1, F_1 male testes are of normal size, but

spermatozoa are immotile; the greatest departure is evident in the *malerkotliana* × *pseudoananassae* crosses where no viable adult males were obtained; between these extremes, intermediate degrees of sex ratio aberration and F_1 male testis reduction are apparent but it appears that *malerkotliana* is more closely related to *bipectinata* than to *pseudoananassae*.

Some evidence is available on other grounds in support of the relationships postulated above. Although each two of the species are distinguished by almost the same number of fixed interspecific inversions, pairing between homologous polytene chromosome arms in interspecific hybrid larvae is very good in *bipectinata–parabipectinata*, *bipectinata–malerkotliana* and *parabipectinata–malerkotliana* hybrids, but poor in the three hybrids involving *pseudoananassae* (Bock 1971), suggesting that the latter species is genetically somewhat removed from the other three. The same situation was indicated independently by the results of Yang *et al.* (1972), who studied isozyme variations within the complex. Analysis of numbers of isofemale lines for a total of 23 loci indicated least genetic similarity between *pseudoananassae* and the remaining three species, all of which were found to be very similar for the genetic patterns investigated.

One notable aspect of the results of the interspecific tests, apart from the phylogenetic inferences which may be drawn from them, is the difference in numbers of inseminations between reciprocal crosses in most cases. Where isolation is complete or almost so in both directions, the difference in numbers of inseminations is of course not significant, but in the majority of cases in which there is not a high degree of isolation in one direction, there is a significantly higher degree in the other direction. Patterson and Stone (1952) listed tables of numbers of inseminations in a variety of interspecific crosses in which differences between reciprocal crosses are mostly strongly apparent; the phenomenon indeed seems to be a general one in the genus *Drosophila*, although the same factors are not necessarily responsible in each group. Discrimination in mating may be exercised by either the male or (more usually) the female; Spieth (1966) showed that intensity of courtship by the male may be the significant factor in determining whether or not copulation occurs; in a species in which low intensity of courtship suffices to permit admission of the male to copulation by the female, alien males which normally court at higher intensity may also be admitted to copulation. Further elucidation of the precise factors responsible for the differences in numbers of inseminations between reciprocal crosses in the *bipectinata* complex must await a thorough comparative ethological study.

A final comment may be made on degrees of interspecific reproductive isolation between two species according to whether the strains involved are of sympatric or allopatric origin. Studies previously performed on several *Drosophila* species have revealed consistently higher degrees of isolation between sympatric than between allopatric strains of the same species [cf. Ehrman (1965) reporting on the *paulistorum* complex]. The comparisons listed in Table 1 indicate that in the majority of crosses in the *bipectinata* complex there was no significant difference in crossability when the results of sympatric *v.* allopatric crosses were compared; in the 10 cases where the difference was significant, the excess was of sympatric inseminations in three, i.e. there is evidence of increased interspecific isolation between the sympatric populations of the two species concerned in only 7 of the 42 possible comparisons. The phenomenon of increased reproductive isolation between sympatric strains is therefore far from a general one in the *bipectinata* complex.

Several potential avenues for future research are indicated by the results of the above study. A complete ethological investigation of the four species may contribute towards an understanding of the reason or reasons for the marked differences between reciprocal interspecific crosses. Further scope exists for studies in interspecific hybridization: preliminary results have indicated that F_1 female offspring of several crosses are fertile in matings with males of one of the remaining two species (i.e. with males of a third species). An investigation of the meiotic chromosomes in hybrid females may indicate whether interspecific recombination occurs in some or all cases. No evidence exists of hybridization among the species of the *bipectinata* complex in nature. The progeny of over 3000 wild-caught females established in the laboratory as isofemale lines were examined during the course of this investigation, and in no case did the offspring of any female consist largely or entirely of females (as in crosses in which *pseudoananassae* was one parent) or contain males showing the hybrid abdominal coloration or sex-combs. It may be inferred that where the species occur sympatrically in nature they are substantially if not entirely reproductively isolated (i.e. they meet the generally accepted criteria of 'species'). In summary, then, the *bipectinata* complex consists of four Oriental–Australian species related closely enough to one another to permit some degree of interspecific hybridization in the laboratory in all pairwise combinations. All lines of evidence suggest that *D. bipectinata*, *D. parabipectinata* and *D. malerkotliana* appear to be extremely closely related in spite of the morphological differentiation of the sex-comb of the last species from those of the former two. *D. pseudoananassae* appears to have undergone a greater degree of genetic divergence from the other three species than they have from one another.

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