

Evolutionary genetics of the *Drosophila montium* subgroup. I. Reproductive isolations and the phylogeny

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ABSTRACT

Seventeen species of the *Drosophila montium* subgroup, originated from the Southeast Asia, were genetically examined to clarify the phylogenetic relationships. Among 272 interspecific crosses, 61 combinations were successful in mating and 39 combinations produced hybrid flies. These results enabled us to classify the subgroup into three species complexes: the *kikkawai* complex (6 species), the *jambulina* complex (4 species) and the *auraria* complex (7 species), which were very similar to that obtained by the electrophoretic classification (Ohnishi and Watanabe, 1984). Asymmetrical mating preference between species was found in the present experiment. They were applied to estimate the relative age of species according to the hypothesis proposed by Watanabe and Kawanishi (1979). The evolving order was as follows: *kikkawai*, *leontia*, *pen-nae*, *lini*-like, *lini*, *bocki* in the *kikkawai* complex, *punjabiensis*, *punjabiensis*-like, *jambulina*, *barbarae* in the *jambulina* complex, and *quadraria*, *yuwanaensis*, *rufa*, *subauraria*, *biauraria*, *triauraria*, *auraria* in the *auraria* complex. Hybrid flies, if produced, were mostly fertile or partially fertile in both sexes (32/39) within species complex crosses. Therefore premating isolation played a more important role than postmating isolation in speciation of the *D. montium* subgroup.

1. INTRODUCTION

The *Drosophila montium* subgroup is the largest subgroup (79 species) among the *D. melanogaster* species group (Lemeunier et al., 1986). Distribution is wide from the Oriental to Afrotropical regions, but the center of the primary radiation seems to have been in the Southeast Asia (Bock and Wheeler, 1972). The interrelationships among members of this subgroup have been biochemically studied by Ohnishi and Watanabe (1984). They classified 29 species into several complexes using the two-dimensional electrophoresis technique. Watanabe and Kawanishi (1979, 1981) proposed a model constructing the phylogenetic tree based on asymmetrical mating preferences between closely related species. In short, (1) females of older species mate well with males of younger species, but females of younger species do not mate well with males of older species, (2) the immediate ancestor of a younger species is the most crossable species among older species, so

that phylogenetic branches are incorporated.

Two kinds of reproductive isolation mechanisms, premating and postmating, have been concluded in many pairs of sibling species of *Drosophila*. The degree of the both isolating mechanisms variously develops in the process of species formation (see Debzhansky, 1970). We present a case that the postmating isolation did not develop well in the *Drosophila montium* subgroup, although the premating isolation developed fairly well.

The primary purpose of the present paper is to compare the biochemical distance with the genetic one, examining the degrees of premating and postmating isolations using the same materials. The secondary aim is to construct a phylogenetic tree based on mating preference.

2. MATERIALS AND METHODS

Flies

Table 1 shows 17 species of the *D. montium* subgroup used for the experiment. Sixteen species except *D. yuwanensis* (Kim and Okada, in press) are the same materials as used by Ohnishi and Watanabe (1984) for the biochemical studies.

Table 1. Flies of the *D. montium* species subgroup used in this study

Species	Source
<i>D. pennae</i>	Texas stock no. 3028.1, New Guinea
<i>D. bocki</i>	AO-1, Thailand
<i>D. kikkawai</i>	Okinawa, Japan
<i>D. leontia</i>	AO-2, Thailand
<i>D. lini</i>	Texas stock no. 3146.1, Taiwan
<i>D. lini-like</i>	MMY326, Maymyo, Burma, 1981
<i>D. barbarae</i>	Texas stock no. 3033.1, Malaysia
<i>D. jambulina</i>	TMU, India, 1979
<i>D. punjabiensis-like</i>	Texas stock no. 3116.1, Thailand
<i>D. punjabiensis</i>	TMU, India, 1979
<i>D. quadraria</i>	Texas stock no. 3075.1, Taiwan
<i>D. triauraria</i>	Tsukuba, Japan, 1976
<i>D. auraria</i>	Mishima, Japan, 1978
<i>D. biauraria</i>	Tsukuba, Japan, 1976
<i>D. subauraria</i>	KT-4, Kitagami, Japan 1982
<i>D. rufa</i>	Mishima, Japan, 1978
<i>D. yuwanensis*</i>	Amamioshima, Japan, 1978

*Kim and Okada (in press).

Intra-and interspecific crossability tests

Mating experiments were carried out using the 'no-choice' method. Virgin

females and males from each species were separately aged for two days, and ten females and ten males were put together in a vial (3×10 cm) containing a standard medium. After two days of mating under a constant light, all females were dissected to determine whether or not they had been inseminated. The experiment was repeated from three (30 females) to ten (100 females) times.

Phylogeny

A phylogeny was constructed according to the method proposed by Watanabe and Kawanishi (1981). In the first step, species were arranged in order of age by assuming that older species females mate more often with newer species males than the reciprocal mating. Next, newer species was each examined to determine which older species was more crossable to the given newer species. The most crossable old species was assumed as the immediate ancestor of the new species.

Tests for viability and fertility of interspecific hybrids

Ten virgin females and ten males, 3–4 day old, were crossed in a vial for 5 days. They were transferred to a new vial and maintained for another 5 days. The experiment was replicated ten times for each cross. When F₁ hybrid flies appeared, they were counted and sib-crossed to examine the F₁ fertility. If the sib-cross did not produce F₂ larvae, the F₁ males or females were backcrossed to parental females or males, respectively, to see whether or not they produced larvae. Development of post-mating isolating mechanisms was judged from the crosses which had been successful but not produced larvae.

3. RESULTS

Interspecific crossability

Table 2 shows intra- and interspecific crossabilities of 289 combinations using 17 species of the *D. montium* subgroup. The intraspecific crossability ranged from 78.3% to 100%, whereas the interspecific crossability varied from 0% to 93.8%. From these, 17 species can be divided into 3 groups, namely the *kikkawai* complex (6 species), the *jambulina* complex (4 species) and the *auraria* complex (7 species). The *kikkawai* complex and the *jambulina* complex seemed to be much closer than to the *auraria* complex.

The frequency of fertile combination in interspecific matings was about 60% within (49/84) and about 6% between (12/188) species complexes. Five combinations in the *kikkawai* complex and one combination in the *auraria* complex, which were marked with asterisks, were unsuccessful in mating in this experiment, but these combinations produced F₁ progeny in the next experiment (Table 4), where the parental flies were kept for 10 days in a vial. They needed more than two days to meet with success in mating.

Table 2. Intra- and interspecific crossability (%) among 17 species of the *D. montium* subgroup

Female	Male																
	<i>Kikkawai</i> complex					<i>jambulina</i> complex					<i>auraria</i> complex						
	<i>pen</i>	<i>boc</i>	<i>kik</i>	<i>leo</i>	<i>lin</i>	<i>lin-li</i>	<i>bar</i>	<i>jam</i>	<i>pun-li</i>	<i>pun</i>	<i>qua</i>	<i>tri</i>	<i>aur</i>	<i>bia</i>	<i>sub</i>	<i>ruf</i>	<i>yuw</i>
<i>pennae</i>	93.3	4.1	26.5	0*	8.0	30.0	0	0	0	0	0	0	0	0	0	0	0
<i>bocki</i>	0	98.0	0	0	0	0*	0	0	0	0	0	0	0	0	0	0	0
<i>kikkawai</i>	28.6	22.9	100	4.0	0	10.0	0	0	0	0	0	0	0	0	0	0	0
<i>leontia</i>	3.4	18.4	1.4	92.0	2.0	0*	0	0	0	0	0	0	0	0	0	0	0
<i>lini</i>	0	3.4	8.2	0	98.3	0*	0	2.0	3.3	0	0	0	0	0	0	0	0
<i>lini-like</i>	0*	2.0	4.1	0	1.3	97.9	0	0	3.3	0	0	0	0	0	0	0	0
<i>barbarae</i>	0	0	0	0	0	0	90.0	0	0	0	0	3.3	0	0	0	0	0
<i>jambulina</i>	0	0	0	0	0	2.0	3.3	100	15.0	0	0	0	0	0	0	0	0
<i>pungabiensis-like</i>	0	0	0	0	2.5	0	0	45.8	100	1.4	0	0	0	0	0	0	0
<i>pungabiensis</i>	0	0	0	0	0	0	4.1	1.7	20.3	96.0	0	0	3.4	0	0	0	0
<i>quadraria</i>	0	3.3	2.5	0	0	0	0	2.5	0	0	95.9	93.8	70.8	92.9	8.3	10.0	5.0
<i>triauraria</i>	0	0	0	0	0	0	0	0	0	0	78.6	88.0	58.0	83.7	0	0	2.0
<i>auraria</i>	0	0	0	0	0	0	0	0	0	0	0	2.0	84.0	13.3	6.0	0	0
<i>biauraria</i>	0	0	3.3	0	0	0	0	2.6	0	0	86.0	88.0	34.0	95.8	0	0	0
<i>subauraria</i>	0	0	0	0	0	0	0	0	0	0	0	0*	4.1	2.1	82.0	0	6.0
<i>rufa</i>	0	0	0	0	0	0	0	0	0	0	0	6.1	2.0	0	2.0	78.3	2.0
<i>yuwaniensis</i>	0	0	0	0	0	0	0	0	0	0	2.0	0	0	0	0	6.0	93.3

*Crossable when examined for 10 days (see Table 4).

Table 3. The average percentages of unsuccessful mating (below the diagonal) and the genetic distances obtained by two dimensional electrophoresis (above the diagonal)

	Kikkawai complex										jambulina complex						auraria complex					
	pen	boc	kik	leo	lin	lin-li	bar	jam	pun-li	pun	qua	tri	aur	bia	sub	ruf	yuu					
Female																						
<i>pennae</i>		.030	.042	.073	.066	.134	.079	.133	.146	.141	—	.205	—	—	—	.198	—					
<i>bocki</i>	97.9		.060	.067	.060	.129	.110	.115	.141	.146	—	.212	—	—	—	.205	—					
<i>kikkawai</i>	72.4	88.5		.079	.119	.152	.133	.150	.152	.157	—	.210	—	.245	—	.227	—					
<i>leontia</i>	98.3	90.8	97.3		.115	.173	.153	.171	.148	.141	—	.183	—	—	—	.212	—					
<i>lini</i>	96.0	98.3	95.9	99.0		.115	.157	.198	.212	.217	—	.293	—	—	—	.264	—					
<i>lini-like</i>	85.0	93.0	92.9	100	99.3		.202	.280	.247	.264	—	.305	—	—	—	.275	—					
<i>barbarae</i>	100	100	100	100	100	100		.070	.190	.195	—	.248	—	—	—	.230	—					
<i>jambulina</i>	100	100	100	100	99.0	99.0	98.3		.134	.188	—	.265	—	—	—	.247	—					
<i>punejabiensis-like</i>	100	100	100	100	97.1	98.3	100	69.6		.092	—	.232	—	—	—	.225	—					
<i>punejabiensis</i>	100	100	100	100	100	100	97.9	99.1	89.1		—	.200	—	—	—	.217	—					
<i>quadraria</i>	100	98.3	98.7	100	100	100	100	98.7	100	100		.019	.048	.163	.178	.198	—					
<i>tricararia</i>	100	100	100	100	100	100	98.3	100	100	100	13.8		.034	.153	.178	.198	—					
<i>auraria</i>	100	100	100	100	100	100	100	100	100	98.3	64.6	70.0		.180	.194	.198	—					
<i>biauraria</i>	100	100	98.3	100	100	100	100	98.7	100	100	10.5	14.1	76.3		.148	.198	—					
<i>subauraria</i>	100	100	100	100	100	100	100	100	100	100	95.8	100	94.9	98.9		.198	—					
<i>rufa</i>	100	100	100	100	100	100	100	100	100	100	95.0	96.9	99.0	100	99.0		—					
<i>yamanensis</i>	100	100	100	100	100	100	100	100	100	100	96.5	99.0	100	100	100	97.0	96.0					

Average percentages of unsuccessful mating were calculated from Table 2. Genetic distances are from original data of Ohmishi and Watanabe (1984).

D. yuwanensis clearly belonged to the *auraria* complex, since this species mated with *D. quadraria*, *D. triauraria*, *D. subauraria* and *D. rufa* of the *auraria* complex, but not with any species of the *kikkawai* and of *jambulina* complexes.

Correlation between the genetic distance values estimated by electrophoresis (Ohnishi and Watanabe 1984) and the unsuccessful mating values were examined. The former is shown above the diagonal and the latter below in Table 3. The interspecific crossability data (Table 2) were converted to the average percentages of unsuccessful mating, subtracting the average crossability of reciprocal crosses from 100. Eighty-one interspecific combinations were available for the comparison. The correlation coefficient (r) was 0.580 as a whole (d.f.=79) which was statistically significant. However, the correlation coefficient within each species complex became insignificant; 0.293 for the *kikkawai* complex (d.f.=13), 0.275 for the *jambulina* complex (d.f.=4), and 0.511 for the *auraria* complex (d.f.=13). This result suggests that the biochemical genetic distances are roughly correlated with the mating values in the *D. montium* subgroup, but the comparison within species complexes did not make any sense.

Direction of species evolution within complexes

For the construction of precise phylogeny within species complexes, the differences in the results of reciprocal matings for each pair of the interspecific combination were read from Table 2. Fig. 1 shows the relation of species within the *kikkawai* complex. The six species in the figure were arranged as follows. If species A females mated more often with species B males than its reciprocal mating, the A species was put to the left of the B species. The most parsimonious order was *kikkawai*, *leontia*, *pennae*, *lini*-like, *lini* and *bocki*. The interpretation of this order is that females of a left side species (e.g., *pennae*) readily accepted males of its right side species (e.g., *lini*-like) while females of the right species often rejected males of the left species. Among the 15 pairwise combinations of species, 14 showed difference in the reciprocal mating, in which 6 were statistically significant. Although one combination, *kikkawai* and *lini*, was incompatible with the order, *kikkawai* was regarded as the oldest species and *bocki* as the youngest species in the *kikkawai* complex.

Fig. 1 also shows the phylogentic relationship of the six species among the *kikkawai* complex. The vertical lines represent the relation between ancestors and descendants, being higher possibilities in solid lines and lower possibilities in dashed line. The length of horizontal lines represent the age of species. For example, *bocki* is the youngest species following *lini*. The average crossability between them was 1.7% (Table 2, $[3.4\% + 0\%]/2$). However, the ancestral species showing the highest crossability with *bocki* was *kikkawai* ($[(22.9\% + 0\%)]/2 = 11.5\%$) rather than *lini*. Thus, the *bocki* was assumed to be derived from *kikkawai*, not from *lini*. This figure could be read as follows; *D. kikkawai* is the oldest species in the complex, from which *leontia*, *pennae* and *bocki* were derived. So, these

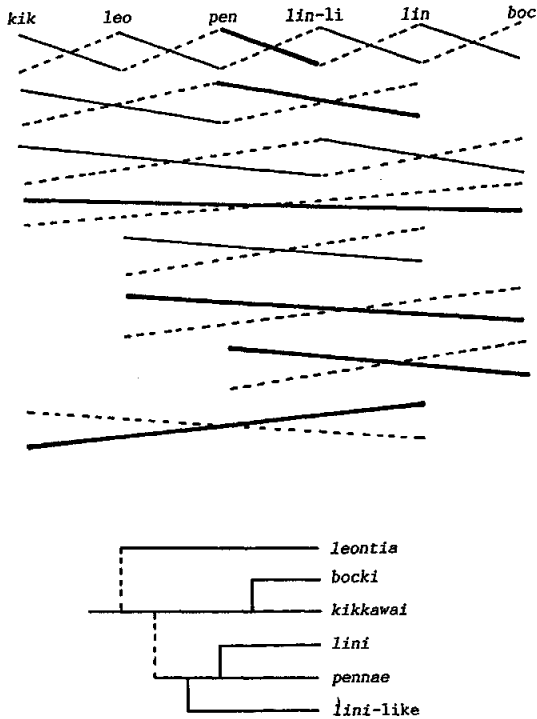


Fig. 1. Asymmetrical mating success and the phylogeny of the *kikkawai* complex. A 'cross' with one solid and one broken line each indicates a pair showing difference in the reciprocal matings. Females are put above and males below. A heavy solid line means statistically significant difference. The phylogenetic tree is explained in the text.

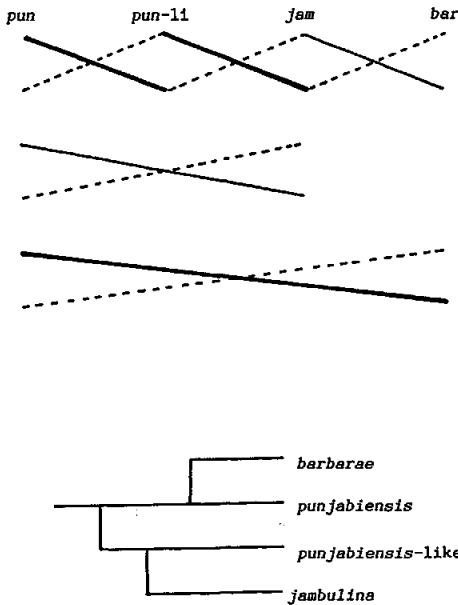


Fig. 2. Asymmetrical mating success and the phylogeny of the *jambulina* complex.

three species are in a relation of sistership sharing the same mother of *kikkawai*. And *lini* and *lini*-like are sisters sharing the mother of *pennae*.

As to the *jambulina* complex, 4 species were arranged as the same manner of the *kikkawai* complex (Fig. 2). The direction of species evolution was *punjabiensis*, *punjabiensis*-like, *jambulina* and *barbarae*. The ancestor of *barbarae* seemed to be *punjabiensis* which was the oldest species of this complex.

Fig. 3 shows asymmetrical mating success and the phylogeny of the *auraria* complex. Females of *quadraria* easily mated with males of the most species of this complex. The evolutionary sequence was expected as follows; *quadraria*, *yuwanensis*, *rufa*, *subauraria*, *biauraria*, *triauraria* and *auraria*. Every species had directly been derived from *quadraria*, although discrepancies of the sequence were observed in three species pairs; *yuwanensis* vs. *subauraria*, *yuwanensis* vs. *triauraria* and *subauraria* vs. *auraria*.

Postmating isolation

The degrees of postmating isolation among 17 species of the *D. montium* subgroup were summarized in Table 4. Among 272 interspecific crosses, 46 combinations produced F₁ larvae, in which 45 were observed within complex combinations. When Table 4 is compared with Table 2, it is clear that more than 90% (205/226) of no hybrid larvae combinations were ascribed to no mating occurred. Thus, premating isolation played an important role for reproductive

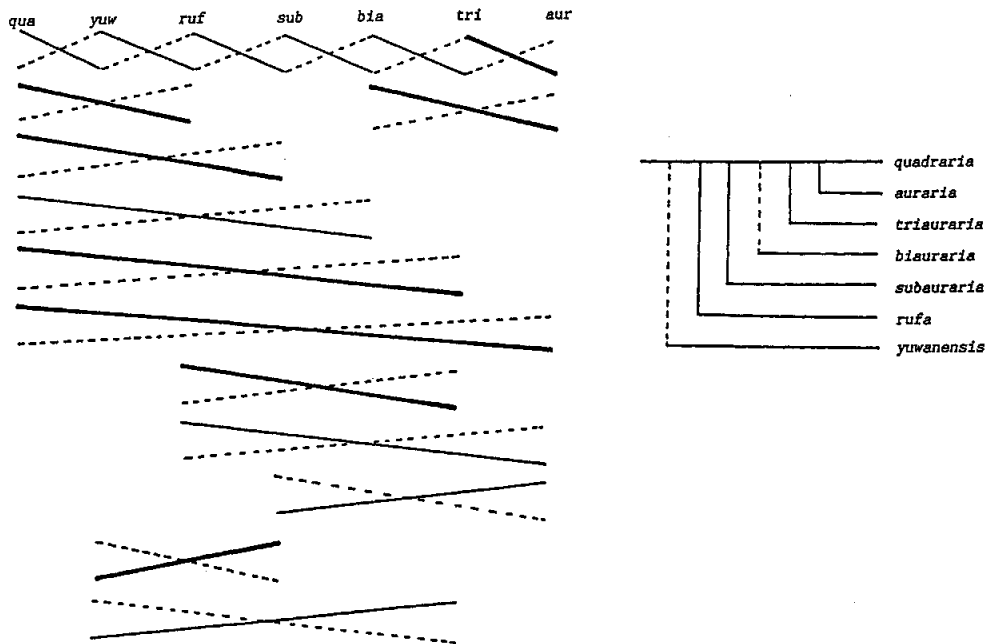


Fig. 3. Asymmetrical mating success and the phylogeny of the *auraria* complex.

Table 4. Viability and fertility of interspecific hybrids among 17 species of the *D. montium* subgroup

Female	Male		Kikakwai complex							jambulina complex				auraria complex					
	pen	boc	kik	leo	tin	lin-li	bar	jam	pun-li	pun	qua	tri	aur	bia	sub	ruf	gaw		
<i>pennae</i>	—	10: 7	41:46	9:14*	10:6*	○	×	×	×	×	×	×	×	×	×	×	×		
<i>bocki</i>	×	—	×	×	×	○	×	×	×	×	×	×	×	×	×	×	×		
<i>kikakwai</i>	88:80	25:34	—	108:96	21:11	×	×	×	×	×	×	×	×	×	×	×	×		
<i>leonita</i>	14:11	92:90	125:111	—	12: 5	27:26	×	×	×	×	×	×	×	×	×	×	×		
<i>lini</i>	×	×	16:12	×	—	37:31	×	○	×	×	×	×	×	×	×	×	×		
<i>lini-like</i>	8:12	9:13	89:126	×	4: 2	—	×	×	×	×	×	×	×	×	×	×	×		
<i>barbarae</i>	×	×	×	×	×	×	—	×	×	×	×	×	×	×	×	×	×		
<i>jambulina</i>	×	×	×	×	×	×	×	—	×	×	×	×	×	×	×	×	×		
<i>pungabiensis-like</i>	×	×	×	×	×	×	×	×	7: 9	×	×	×	×	×	×	×	×		
<i>pungabiensis</i>	×	×	×	×	×	×	×	○	15:13	—	×	×	×	×	×	×	×		
<i>quadraria</i>	×	×	×	×	×	×	×	×	×	×	122:86*	112:80	64:44	34:29	×	×	×		
<i>triacuraria</i>	×	×	×	×	×	×	×	×	×	×	94:105	77:38	56:31	×	×	○	×		
<i>auraria</i>	×	×	×	×	×	×	×	×	×	×	×	—	38:26	×	×	×	×		
<i>biauraria</i>	×	×	×	×	×	×	×	×	×	×	71:43	86:75	81:54	×	×	×	×		
<i>subauraria</i>	×	×	×	×	×	×	×	×	×	×	×	7: 6*	13:12*	24:19	—	×	2: 1		
<i>rufa</i>	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	—	25:18*		
<i>guananensis</i>	×	×	×	×	×	×	×	×	×	×	20:17	×	×	×	×	×	17:12*		

The number indicates F₁ flies (♀:♂) being fertile. *: F₁ male sterile. ○: only F₁ larvae obtained. ×: no F₁ larvae.

Table 5. Summary of reproductive isolations

	No. of Mating		F ₁ Viability		
	Successful	Unsuccessful	Viable	Lethal	
				Embryonic	larval
<i>kikkawai</i> complex	22	8	19	1	2
<i>jambulina</i> complex	7	5	2	4	1
<i>auraria</i> complex	26	16	18	5	3
Between complexes	12	176	0	11	1
Total	67	205	39	21	7

isolation in the *D. montium* subgroup, especially between species complexes.

Hybrid lethality, including embryonic and larval lethality were often observed in crosses within and between species complexes. The proportion of no F₁ flies among successful mating combinations was 14% (3/22) for the *kikkawai* complex, whereas it was 100% (12/12) for the between species complexes. These values reflected that there was zygotically distant relationship between species. Three species complexes were strongly isolated zygotically as well as prezygotically. Four species of the *jambulina* complex were also fairly isolated even in the zygotic stages.

No sex-specific lethality was detected in the hybrids. F₁ males were viable as well as F₁ females. On the other hand, some sex-specific sterility were observed. Males of seven hybrid combinations were sterile but females of all viable hybrids were fertile. That is, the genetic isolation inducing hybrid sterility has not developed so much (7/39) compared with that inducing hybrid lethality (28/67).

Table 5 summarized the degree of reproductive isolation between 17 species of the *D. montium* subgroup. Species within each species complex were relatively easy to mate and produce fertile hybrids. If the mating was successful, a half of the interspecific combinations produced fertile hybrids in this subgroup. Species pairs between species complexes were perfectly isolated postzygotically.

4. DISCUSSION

Biochemical phylogeny and mating success

In the present experiment seventeen species belonging to the *Drosophila montium* subgroup were divided into three species complexes by means of the degree of mating preference. This result was very compatible with the prediction done by the two-dimensional electrophoretic analysis (Ohnishi and Watanabe, 1984). Protein differences roughly correlated with the mating preference. In this respect, when we want to have some preliminary informations of a genetically unknown group, such a biochemical approach is a very useful tool for classification

between 17 species of the *D. montium* subgroup

F ₁ Fertility		Viable	Fertile
Fertile	Male sterile	Successful mating	Successful mating
17	2	0.86	0.77
2	0	0.29	0.29
13	5	0.69	0.50
0	0	0	0
32	7	0.58	0.48

and grouping of species. However, the biochemical tool is limited to such a classification as the level of between species complexes. For the more precise relationships within species complexes, in which fertile hybrids are often obtained, genetic analyses are needed.

The latent period for the successful mating must be considered for the further experiment. The present study has been done with 2 day old flies for the 2 days mating period. Some interspecific combinations (asterisks in Table 2) have succeeded in mating during the 3rd to 10th day after two species had encountered. *D. lini*-like often showed such a reluctant mating, although this species fully succeeded in intraspecific mating (97.9%) as well as the other species during the first 2 days.

Premating vs. postmating isolation

Among 272 interspecific crosses, 205 (75%) did not succeed in mating at all. The most (86%=176/205) came from between species complex crosses. Premating isolation was well developed for the pairs between species complexes. And even if they mated, all 12 combinations failed to produce F₁ flies. On the contrary, many hybrid crosses within species complexes produced viable and fertile hybrids. Conversely speaking, the 'species complex' is a species group producing viable and fertile hybrids.

Six species of the *kikkawai* complex were genetically very close to each other, because they can interspecifically produce fertile hybrids. Also 7 species of the *auraria* complex were close to each other in this respect. On the other hand, *barbarae* and *jambulina* were both slightly far either from *punjabiensis* or *punjabiensis*-like in the *jambulina* complex. The former two were isolated zygotically in the complex. As shown in Table 5, the degree of zygotic (postmating) isolations was lowest among the species in the *kikkawai*, middle in the *auraria* and high in the *jambulina* complexes.

At any rate, postmating isolations did not develop well in the *D. montium*

subgroup at the present. Therefore, premating isolation played an important role in the process of evolution.

Premating isolation and phylogeny

Kaneshiro (1976, 1980) and Watanabe and Kawanishi (1979, 1981) proposed models for determining the phylogenetic direction when asymmetric mating preference existed between reciprocal pairs of *Drosophila* species. These authors reached completely opposite conclusions. In short, females of derived species accept males of the ancestral species in the Kaneshiro model, but the derived females reject ancestral males in the Watanabe-Kawanishi model. At the present, neither model has conclusive evidence supporting the theory that the asymmetrical mating characteristics between ancestral and derived species indicate the direction of species evolution.

Recently, Lee and Watanabe (1987) applied the Watanabe-Kawanishi model to evolution of the *D. melanogaster* subgroup and obtained a plausible phylogenetic tree. The *D. montium* subgroup belongs to the same species group as the *D. melanogaster* subgroup both distributing widely from the Southeast Asia to the Afrotropical regions.

The oldest species predicted in the *kikkawai* complex was *D. kikkawai* which now distributes most widely in the world. The situation is very similar to the *D. melanogaster*, which is the oldest and the most widely distributed species among the *D. melanogaster* subgroup (Lee and Watanabe, 1987). The relation between species age and distribution has been discussed by Watanabe and Kawanishi (1983) using the *D. bipectinata* and the *D. takahashii* complex. A discrepancy observed in the pair of *D. kikkawai* and *D. lini* is unexplained at the present. More experiments are needed.

On the *jambulina* complex, the phylogenetic tree based on the mating preference was incompatible with the postmating isolation experiment. The old species pair, *punjabiensis* vs. *punjabiensis*-like, and *jambulina* vs. *punjabiensis*-like, were zygotically isolated. These results suggested that genetic systems controlling premating and postmating isolations were quite different.

Drosophila quadraria in the *auraria* complex was a very curious species. Females of *D. quadraria* mated not only with males of every species of the *auraria* complex but also with some males of other species complexes. Accordingly, every species in the *auraria* complex was expected to have been derived directly from *D. quadraria*. The distribution of the *auraria* complex is limited to the East Asia (China, Taiwan, Korea, Japan), and *D. quadraria* is only known from Taiwan. Recently, Kimura (1987) suggested that the original habitat of this complex was a warm forest where *D. rufa*, *D. asahiani*, *D. yuwanensis* and *D. biauraria* lived. Then habitat preference changed to the open grassland where *D. triauraria* and *D. subauraria* lived. Finally, domestic environments were occupied by *D. auraria*. On the other hand, Kurokawa (1967) had suggested that

D. auraria might be the ancestral form of the *auraria* complex from the studies of geographical, ecological and morphological analyses.

The speciation history predicted by Kimura (1987) was very compatible with the present phylogeny estimated by mating preference. *D. yuwanensis* and *D. rufa* were old species and *D. auraria* was the youngest species. Lee (1974) predicted that *D. quadraria* was the ancestral species of the *auraria* complex from the morphology, sexual isolations and isozyme analysis. However, it was still questionable that *D. quadraria* was the oldest species among the species complex, since this species was found only once in Taiwan. In the crosses between *D. quadraria* and *D. triauraria*, Kimura (1987) obtained fertile hybrids in both sexes and suspected that *D. quadraria* seemed to be the same species as *D. triauraria*. In the present experiment, we observed a clear difference in the results of reciprocal mating between these two species, and also they were zygotically isolated.

In conclusion, the relationship between three species complexes of the *D. montium* subgroup coincided with the results of three approaches; biochemical, pre-mating and post-mating studies, whereas the relationship within species complexes varied from the results in these three studies. This means that different genetic changes have been accumulating in the early stages of speciation such as species complex formation. Pleiotropic genes controlling the differences of species must be studied in the future.

REFERENCES

- BOCK, I. R. and WHEELER, M. R. (1972) The *Drosophila melanogaster* species group. *Univ. Texas Publ.* 7213, 1-102.
- DOBZHANSKY, TH. (1970) *Genetics of the evolutionary process*. Columbia Univ. Press, New York.
- KANESHIRO, K. Y. (1976) Ethological isolation and phylogeny in the *Planitibia* subgroup of Hawaiian *Drosophila*. *Evolution* 30, 740-745.
- KANESHIRO, K. Y. (1980) Sexual isolation, speciation and the direction of evolution. *Evolution* 34, 437-444.
- KIM, B. and OKADA, T. (1989) A new species of *Drosophila montium* species subgroup: *D. yuwanensis* (Diptera, Drosophilidae) from Amami-oshima Island, Japan. *Kontyu* (in press).
- KIMURA, M. T. (1987) Habitat differentiation and speciation in the *Drosophila auraria* species-complex (Diptera, Drosophilidae). *Kontyu* 55, 429-436.
- KUROKAWA, H. (1967) Population genetics on three races of *Drosophila auraria* Peng. III. Geographical and ecological distribution of the races A, B and C, with special regard to its speciation. *Jpn. J. Genet.* 42, 109-119.
- LEE, T. J. (1974) Speciation in the species complex *Drosophila auraria*. *Jpn. J. Genet.* 49, 305 (abstract in Japanese).
- LEE, W. H. and WATANABE, T. K. (1987) Evolutionary genetics of *Drosophila melanogaster* subgroup. I. Phylogenetic relationships based on matings, hybrids and proteins. *Jpn. J. Genet.* 62, 225-239.
- LEMEUNIER, F., DAVID, J. R., TSACAS, L. and ASHBURNER, M. (1986) The *Melanogaster* species group. In: *The genetics and biology of Drosophila*, Vol. 3e, pp. 147-256. Academic Press,

London.

- ONISHI, S. and WATANABE, T. K. (1984) Systematics of the *Drosophila montium* species subgroup: a biochemical approach. *Zool. Sci.* **1**, 801-807.
- WATANABE, T. K. and KAWANISHI, M. (1979) Mating preference and the direction of evolution in *Drosophila*. *Science* **205**, 906-907.
- WATANABE, T. K. and KAWANISHI, M. (1981) Asymmetrical mating success and the phylogeny of *Drosophila*. *Zool. Mag.* (Tokyo), **90**, 217-224.
- WATANABE, T. K. and KAWANISHI, M. (1983) Stasipatric speciation in *Drosophila*. *Jpn. J. Genet.* **58**, 264-274.