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Morphological Comparison among *Drosophila lini* and Its Two New Sibling Species (Diptera: Drosophilidae)

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ABSTRACT—Morphological differences are investigated using several culture strains of three sibling species collected from Taiwan and Guangdong in China and Pyinoolwin and Yangon in Myanmar. Careful examination of male terminalia reveals distinguishable differences in the paramere and the aedeagal basal process among the three species. In addition, a number of quantitative characters are compared. Kruskal-Wallis tests with Bonferroni correction, which are carried out separately for each sex, detect significant differences in 15 characters, of which two are male-specific, among the three species. Canonical discriminant analysis using these characters reveals that the three species can be distinguished from each other with high confidence for both sexes. The results clearly show the presence of three good species, *Drosophila* (*Sophophora*) *lini* Bock & Wheeler, 1972 and its two new siblings. The new species are described as *Drosophila* (*Sophophora*) *ohnishii* sp. nov. from Pyinoolwin and *Drosophila* (*Sophophora*) *ogumai* sp. nov. from Yangon. The morphological differentiation among the three sibling species does not coincide with the degree of reproductive isolation (based on a previous study). The premating isolation pattern suggests two possibilities that premating isolation has been evolved or reinforced in sympatric populations between *D. ohnishii* and *D. lini* and between *D. ohnishii* and *D. ogumai* or that it has evolved in a very restricted local population of *D. ohnishii*, possibly by a few mutations.

Key words: discriminant analysis, *kikkawai* species-complex, *montium* species-subgroup, Oriental Region, quantitative character

INTRODUCTION

The *Drosophila* (*Sophophora*) *montium* species-subgroup is the largest in the *D. melanogaster* species-group, comprising a total of 85 species from Asia and Africa (Lemeunier *et al.,* 1986; Toda, unpublished data). This subgroup includes a variety of species at different stages of speciation process and provides very useful materials for studies of evolutionary genetics. The relationships among members of this subgroup have been investigated by various methods; hybridization (cross experiment) tests (David *et al*., 1978; Kim *et al*., 1989), two dimensional electrophoresis and conventional starch gel electrophoresis (Ohnishi *et al.,* 1983a, b; Ohnishi and Watanabe, 1984), chromosomal (metaphase karyotype) analysis (Baimai and Chumchong, 1980; Baimai *et al.,* 1986), and eco-physiological comparison (Kimura, 1987). Through these studies several groups of very closely related species have been recognized as species-complexes: the *D. auraria,* the *D. bakoue*, the *D. bocqueti,* the *D. jambulina*, the *D. kikkawai*, the *D. nikananu* and the *D. serrata* complex. The members of each species-complex are hardly distinguishable in morphology and show various incipient stages in differentiation of characters such as genes, proteins, physiology, behavior and morphology. Those are very good materials for studying the speciation mechanisms.

A few genetic investigators have suggested the presence of two new species very closely related to *Drosophila* (*Sophophora*) *lini* Bock & Wheeler, 1972. *Drosophila lini* was described as a new species of the *montium* subgroup on the basis of a strain (Texas 3146.1) established from a single female collected from Taiwan in 1968 (Bock and Wheeler, 1972). When Tsacas and David (1977) established the *kikkawai* complex, they included *D. lini* in this species-complex.

Ohnishi and Watanabe (1984) was the first to recognize the presence of a sibling species of *D. lini* based on the results of two electrophoretic analyses. They analyzed protein difference by two-dimensional electrophoresis and allozyme variation by starch gel electrophoresis, using a strain (MMY326) originating from a single female collected

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Table 1. Isofemale strains investigated

Species	Strains	Source
D. lini	Bowling Green 14028-0581.0 (Texas 3146.1)	Yun-Shui, Taiwan, 1968
	DHS401	Dinghushan, Guangdong, southern China, 1992
	DHS501	ditto
	NKS9212	Nankunshan, Guangdong, southern China, 1992
	NKS9231	ditto
D. ohnishii	MMY307	Pynoolwin, central Myanmar, 1981
	MMY326	ditto
D. ogumai	RGN3	Yangon, southern Myanmar, 1981
	RGN206	ditto

at Pyinoolwin, central Myanmar in 1981, along with such strains of other 28 species of the *montium* subgroup. They placed MMY326 as a member of the *kikkawai* complex and tentatively named it as *Drosophila lini*-like. Then, Kim *et al.* (1989) examined the crossability between *D. lini* and *D. lini*-like (MMY326), and revealed that the premating isolation between them was almost complete. Furthermore, Kim *et al.* (1993) examined restriction enzyme patterns of mitochondrial DNA in 18 species of the *montium* subgroup, and placed *D. lini*-like (MMY326) closest to *D. lini*. More recently, Oguma *et al.* (1995) examined the reproductive isolation and courtship behavior between nine isofemale strains from Taiwan (Bowling Green stock no. 14028-0581.0=Texas old stock no. 3146.1=*D. lini*), mainland China (DHS315, DHS401, DHS501, NKS9231; all from Guangdong Province) and Myanmar (MMY326=*D. lini*-like, MMY307 from Pyinoolwin; RGN3, RGN206 from Yangon). They suggested the existence of at least three genetically distinct sibling species in them: *D. lini* (BG14028-0581.0, DHS315, DHS401, DHS501 and NKS9231) distributed from Taiwan to southern China, *D. lini*-like (MMY326 and MMY307) in central Myanmar and the other species (RGN3 and RGN206) in southern Myanmar. However, those species can be hardly distinguished from each other morphologically, and the two new sibling species have not been formally described yet.

In this study we compare the morphology precisely for both qualitative and quantitative characters among the three sibling species, using nine isofemale strains of which eight are the same as in Oguma *et al.* (1995), and describe them, two of them as new species.

MATERIALS AND METHODS

Specimens examined

Ten male and 10 female specimens were examined for each of the nine isofemale strains (Table 1). The strains have been maintained on a standard *Drosophila* culture medium for long periods since the establishment each from a single wild-caught female.

External morphology was observed under a stereoscopic microscope and metric characters were measured with an ocular micrometer. The detailed structure of head, male foreleg and male and female terminalia were observed in a droplet of glycerol under a compound light microscope, after detaching the organs from the body and cleaning them by warming in 10% KOH solution at about 100°C for several minutes. Drawings were made on the basis of microscope photographs taken by a computer-interfaced digital camera.

All type specimens were deposited in Systematic Entomology, The Hokkaido University Museum, Hokkaido University, Sapporo, Japan (SEHU).

Terminology and quantitative characters

We followed McAlpine (1981) for morphological terminology and Zhang and Toda (1992) for the definitions of measurements and indices. A total of 31 quantitative characters were measured and/or calculated for both sexes, 10 characters for male, and one character for female (Table 2).

Data analyses

First, to find meaningful characters for discriminating the three sibling species, we intensively examined three strains (BG14028-0581.0, MMY326 and RGN206), one from each species, for the above 42 quantitative characters. As some metric characters would have been affected by the body size, *i.e.* culture conditions, correlation with the thorax length (ThL) was examined for all characters except for the body length, the wing length and the wing width, separately for each sex. Sexual difference was examined by unpaired *t*-test for each character and interspecific difference was examined by Kruskal-Wallis test separately for each sex, except for the four characters representing the body size. Bonferroni correction (Rice, 1989) was applied for coping with probability errors caused by multiple comparisons. For the characters selected by Kruskal-Wallis tests with Bonferroni correction, 10 males and 10 females were measured in each of the remaining six strains. Combining such data of the nine strains, we performed the canonical discriminant analysis, using the computer software STATISTICA (StatSoft, 2000), for the three species, separately for each sex.

RESULTS

Description

The following three species are very similar in general morphology. External qualitative characters commonly seen in all the three species are first referred to in the redescription of *D. lini* but not repeated in the description of two new species. Quantitative characters that vary among the three species are analyzed in the subsequent section.

Drosophila (*Sophophora*) *lini* Bock & Wheeler, 1972

(Figs. 1A, 2A-C)

Drosophila (*Sophophora*) *lini* Bock & Wheeler, 1972: 59.

Table 2. Definitions of quantitative characters examined

Both sexes	
BL	straight distance from anterior edge of pedicel to tip of abdomen
ThL	distance from anterior notal margin to apex of scutellum
WL	distance from humeral cross vein to wing apex
WW	maximum wing width
dorsal	number of dorsal branches of arista
ventral	number of ventral branches of arista
spcs	number of supraservical setae
pocls	number of post ocular setae
ocps	number of occipital setae
ms	number of medial cibarial sensilla
ps	number of posterior cibarial sensilla
FW/HW	frontal width / head width
ch/o	maximum width of gena / maximum diameter of eye
prob	proclinate orbital seta / posterior reclinate orbital seta in length
rcorb	anterior reclinate orbital seta / posterior reclinate orbital seta in length
vb	subvibrissal seta / vibrissa in length
dcl	anterior dorsocentral seta / posterior dorsocentral seta in length
sctl	basal scutellar seta / apical scutellar seta in length
sterno	anterior katepisternal seta / posterior katepisternal seta in length
sterno2	mid katepisternal seta / posterior katepisternal seta in length
orbito	distance between proclinate and posterior reclinate orbital setae / distance between inner vertical and posterior reclinate orbital setae
dcp	length distance between ipsilateral dorsocentral setae / cross distance between anterior dorsocentral setae
sctlp	distance between ipsilateral scutellar setae / cross distance between apical scutellar setae
WW/WL	maximum wing width / distance from humeral cross vein to wing apex
C	second costal section between subcostal break and R_{2+3} / third costal section between R_{2+3} and R_{4+5}
4c	third costal section between R_{2+3} and R_{4+5} / M_1 between r-m and dm-cu
4v	M_1 between dm-cu and wing margin / M_1 between r-m and dm-cu
5x	CuA_1 between dm-cu and wing margin / dm-cu between M_1 and CuA_1
ac	third costal section between R_{2+3} and R_{4+5} / distance between distal ends of R_{4+5} and M_1
M	CuA_1 between dm-cu and wing margin / M_1 between r-m and dm-cu
C3F	length of heavy setation in third costal section / (length of heavy setation in third costal section + length of light setation in third costal section)
Male	
sc1	number of teeth in sex-comb of 1st tarsomere
sc2	number of teeth in sex-comb of 2nd tarsomere
epands	number of latero-dorsal setae on epandrium
vlbs	number of ventral lobe setae on epandrium
surpg	number of pegs on surstylus
sursp	number of spines on surstylus
cers	number of setae on cercus
cersp	number of spines on cercus
pg2	number of pegs on secondary clasper
st2	number of setae on secondary clasper
Female	
ovms	number of marginal ovisensilla on oviscapt

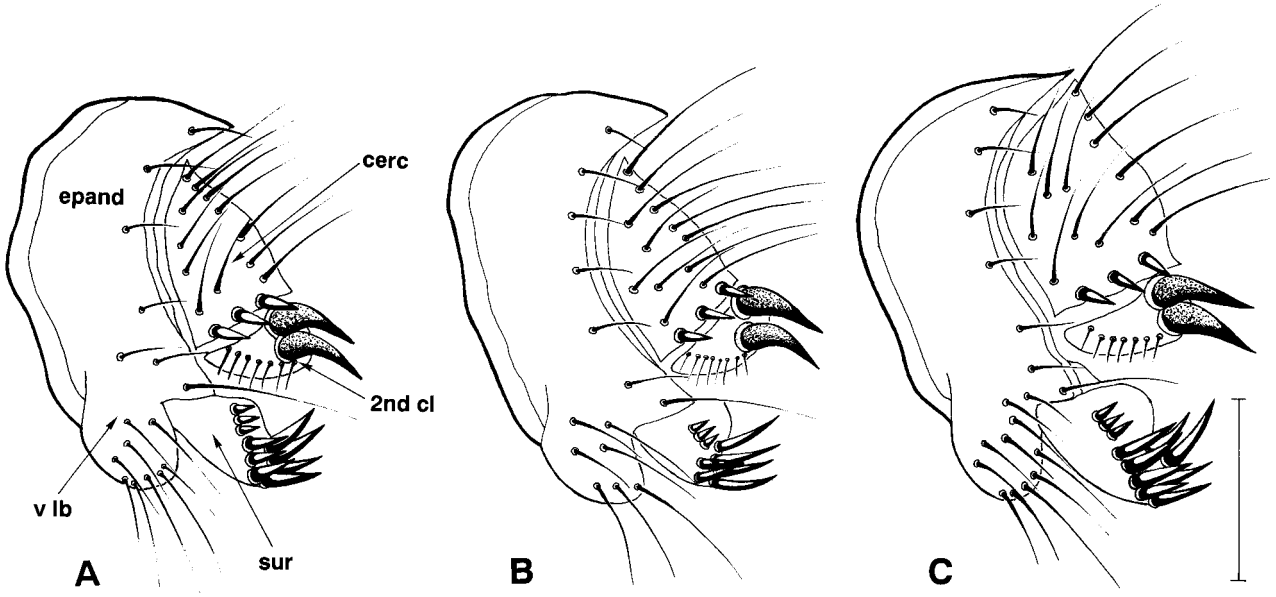


Fig. 1. Periphallic organs. A, *Drosophila* (*Sophophora*) *lini* Bock & Wheeler, 1972; B, *Drosophila* (*Sophophora*) *ohnishii* sp. nov.; C, *Drosophila* (*Sophophora*) *ogumai* sp. nov. epand=epandrium, v lb=ventral lobe of epandrium, sur=surstylus (primary clasper), cerc=cercus, 2nd cl=secondary clasper. (Scale-line=0.1 mm).

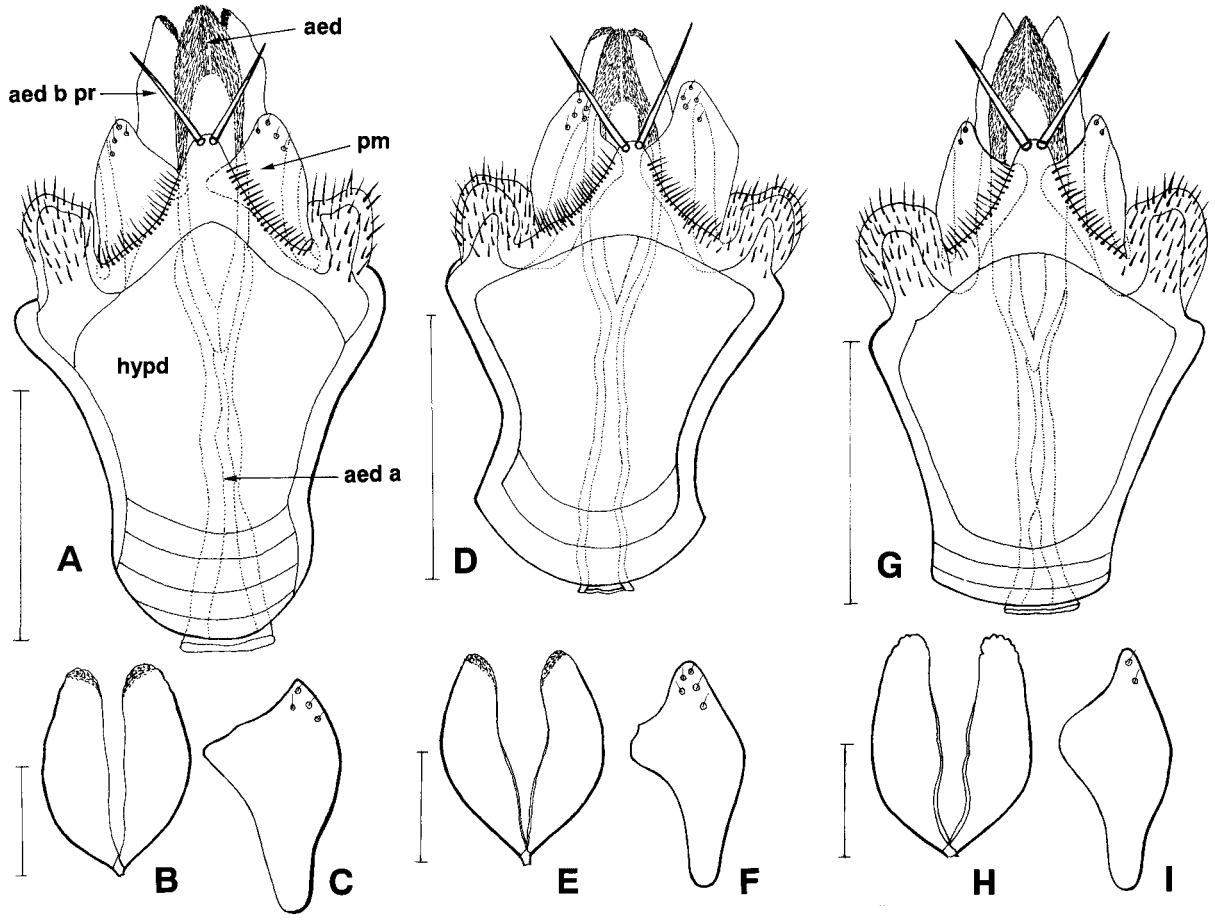


Fig. 2. Phallic organs (A,D,G: whole in ventral view; B,E,H: aedeagal basal processes in dorsal view; C,F,I: paramere). A–C, *Drosophila* (*Sophophora*) *lini* Bock & Wheeler, 1972; D–F, *Drosophila* (*Sophophora*) *ohnishii* sp. nov.; G–I, *Drosophila* (*Sophophora*) *ogumai* sp. nov. hypd=hypandrium, pm=paramere, aed=aedeagus, aed a=aedeagal apodeme, aed b pr=aedeagal basal process. (Scale-line=0.1 mm).

Diagnosis. Paramere apically round and with 3-5 minute sensilla, submedially with strong, triangular, inward expansion of which posterior margin only slightly concave (Fig. 2A,C). Aedeagal basal process with fine, irregular serrations on slightly wider, apical margin (Fig. 2B).

Description ♂ and ♀ . Head: Eyes bright red. Postocellar setae convergent. Supracervical setae tapered, thin, apically slightly curved and sharp. Frons including ocellar triangle pale brown; ocelli orange. Anterior reclinate orbital seta much closer to proclinate seta than to posterior reclinate seta. Antennal pedicel usually yellowish brown. Face and gena yellowish brown. Carina convex, somewhat broader in ♀ . Palpus and clypeus brownish yellow. Palpus with 1 prominent terminal and another subprominent, lateromedian setae. Cibarial posterior setae long, tapered, thin, curved and apically sharp; medial and anterior setae shorter than

Table 3. Mean±SD and range of 42 quantitative characters in three species

Character	*D. lini*								*D. ohnishii*								*D. ogumai*							
	Male				Female				Male				Female				Male				Female			
	Mean±SD	Min.	Max.	(*N*)	Mean±SD	Min.	Max.	(*N*)	Mean±SD	Min.	Max.	(*N*)	Mean±SD	Min.	Max.	(*N*)	Mean±SD	Min.	Max.	(*N*)	Mean±SD	Min.	Max.	(*N*)
BL	2.06±0.07	1.95	2.15	(10)	2.27±0.12	2.03	2.39	(10)	1.94±0.10	1.74	2.09	(10)	2.22±0.10	1.74	2.09	(10)	2.03±0.07	1.96	2.17	(10)	2.52±0.11	2.39	2.71	(10)
ThL	0.85±0.03	0.80	0.89	(10)	0.92±0.02	0.88	0.95	(10)	0.80±0.02	0.77	0.82	(10)	0.93±0.02	0.90	0.95	(10)	0.81±0.02	0.78	0.84	(10)	0.91±0.02	0.88	0.94	(10)
WL	1.55±0.07	1.45	1.64	(10)	1.65±0.05	1.58	1.71	(10)	1.57±0.03	1.51	1.64	(10)	1.76±0.07	1.58	1.81	(10)	1.52±0.04	1.43	1.58	(10)	1.73±0.05	1.67	1.82	(10)
WW	0.73±0.03	0.69	0.78	(10)	0.79±0.02	0.76	0.82	(10)	0.72±0.02	0.69	0.75	(10)	0.79±0.03	0.76	0.85	(10)	0.71±0.03	0.66	0.76	(10)	0.81±0.02	0.77	0.83	(10)
dorsal	4.0±0.0	4	4	(10)	4.2±0.4	4	5	(10)	4.0±0.0	4	4	(10)	4.0±0.0	4	4	(10)	4.0±0.0	4	4	(10)	4.2±0.4	4	5	(10)
ventral	3.0±0.0	3	3	(10)	2.9±0.3	2	3	(10)	3.0±0.0	3	3	(10)	3.0±0.0	3	3	(10)	2.8±0.4	2	3	(10)	3.0±0.0	3	3	(10)
spcs	14.0±1.7	11	20	(50)	16.8±1.5	13	21	(50)	14.5±0.9	12	17	(20)	15.2±2.0	10	20	(20)	15.9±1.8	14	20	(20)	18.0±1.4	15	22	(20)
pocls	13.3±1.2	11	15	(10)	15.0±1.4	13	18	(10)	14.1± 10.7	13	15	(10)	14.8±1.2	12	16	(10)	14.4±1.3	11	17	(10)	14.4±1.8	11	17	(10)
ocps	11.7±1.6	8	15	(50)	13.9±2.0	10	21	(50)	12.2±1.7	9	16	(20)	14.7±2.4	10	20	(20)	15.9±1.9	12	21	(20)	16.1±1.7	13	25	(20)
ms	7.3±0.8	6	9	(10)	8.0±0.6	7	9	(10)	7.9±0.7	8	9	(10)	8.2±0.4	8	9	(10)	7.5±0.9	6	9	(10)	7.5±0.9	6	9	(10)
ps	20.0±2.0	12	26	(50)	20.9±1.7	14	26	(50)	19.2±1.6	16	24	(20)	19.2±2.1	12	24	(20)	21.5±2.0	18	25	(20)	22.9±1.6	19	26	(20)
FW/HW	0.45±0.02	0.41	0.48	(50)	0.45±0.01	0.42	0.47	(50)	0.47±0.01	0.45	0.49	(20)	0.47±0.01	0.45	0.49	(20)	0.46±0.02	0.44	0.48	(20)	0.47±0.01	0.45	0.48	(20)
ch/o	0.09±0.00	0.07	0.09	(10)	0.09±0.01	0.08	0.11	(10)	0.09±0.01	0.08	0.10	(10)	0.08±0.00	0.08	0.09	(10)	0.08±0.01	0.07	0.10	(10)	0.08±0.00	0.07	0.09	(10)
prob	1.00±0.05	0.91	1.12	(10)	0.87±0.08	0.74	1.03	(10)	1.01±0.07	0.88	1.14	(10)	0.86±0.07	0.78	1.00	(10)	1.10±0.08	1.00	1.25	(10)	0.94±0.10	0.82	1.15	(10)
rcorb	0.43±0.06	0.34	0.53	(50)	0.46±0.06	0.37	0.57	(50)	0.45±0.04	0.38	0.51	(20)	0.48±0.03	0.42	0.55	(20)	0.49±0.03	0.42	0.53	(20)	0.55±0.06	0.45	0.65	(20)
vb	0.76±0.10	0.60	0.96	(10)	0.78±0.09	0.67	0.93	(10)	0.78±0.06	0.67	0.86	(10)	0.76±0.13	0.63	0.95	(10)	0.84±0.11	0.69	1.00	(10)	0.68±0.06	0.78	0.98	(10)
dcl	0.58±0.04	0.48	0.63	(10)	0.60±0.05	0.49	0.67	(10)	0.57±0.06	0.44	0.67	(10)	0.58±0.03	0.53	0.63	(10)	0.61±0.04	0.56	0.68	(10)	0.60±0.01	0.59	0.63	(10)
sctl	0.87±0.08	0.76	1.08	(10)	0.91±0.04	0.82	0.96	(10)	0.84±0.09	0.70	1.00	(10)	0.85±0.07	0.77	1.01	(10)	0.82±0.05	0.74	0.90	(10)	0.83±0.07	0.73	0.99	(10)
sterno	0.52±0.04	0.44	0.59	(10)	0.57±0.04	0.49	0.65	(10)	0.55±0.04	0.50	0.63	(10)	0.58±0.04	0.49	0.63	(10)	0.59±0.05	0.53	0.70	(10)	0.61±0.05	0.47	0.67	(10)
sterno2	0.24±0.04	0.15	0.29	(10)	0.29±0.07	0.18	0.41	(10)	0.27±0.03	0.20	0.32	(10)	0.30±0.08	0.24	0.50	(10)	0.26±0.05	0.14	0.30	(10)	0.31±0.05	0.24	0.38	(10)
orbito	0.69±0.11	0.56	0.95	(50)	0.59±0.06	0.48	0.67	(50)	0.70±0.06	0.64	0.79	(20)	0.64±0.04	0.55	0.69	(20)	0.62±0.05	0.51	0.86	(20)	0.61±0.04	0.51	0.66	(20)
dcp	0.62±0.04	0.35	0.50	(10)	0.41±0.02	0.39	0.43	(10)	0.47±0.03	0.40	0.50	(10)	0.43±0.02	0.39	0.46	(10)	0.46±0.05	0.32	0.52	(10)	0.46±0.04	0.40	0.51	(10)
sctlp	1.00±0.05	0.86	1.16	(50)	1.05±0.07	0.94	1.18	(50)	1.09±0.14	0.63	1.43	(20)	1.05±0.07	0.90	1.13	(20)	1.13±0.07	0.99	1.20	(20)	1.21±0.08	1.05	1.37	(20)
WW/WL	0.47±0.02	0.44	0.49	(10)	0.48±0.02	0.45	0.51	(10)	0.46±0.01	0.44	0.48	(10)	0.45±0.03	0.42	0.54	(10)	0.47±0.01	0.44	0.50	(10)	0.47±0.02	0.44	0.49	(10)
C	1.83±0.08	1.67	1.93	(10)	1.95±0.06	1.83	2.03	(10)	1.71±0.07	1.62	1.82	(10)	1.95±0.15	1.56	2.15	(10)	1.69±0.12	1.53	1.95	(10)	1.82±0.10	1.59	1.92	(10)
4c	1.62±0.18	1.41	2.07	(50)	1.46±0.06	1.36	1.57	(50)	2.10±0.19	1.88	2.59	(20)	1.45±0.08	1.32	1.58	(20)	1.75±0.14	1.57	2.02	(20)	1.61±0.09	1.41	1.73	(20)
4v	2.74±0.23	2.44	3.15	(50)	2.41±0.07	2.43	2.54	(50)	2.62±0.27	2.34	3.33	(20)	2.55±0.17	2.27	2.78	(20)	2.84±0.19	2.58	3.14	(20)	2.78±0.16	2.51	3.07	(20)
5x	2.82±0.39	1.96	3.35	(50)	2.87±0.21	2.35	3.82	(50)	2.59±0.24	2.35	3.09	(20)	2.54±0.27	2.09	2.96	(20)	3.17±0.39	2.68	3.91	(20)	2.69±0.25	2.31	3.13	(20)
ac	2.93±0.29	2.58	3.57	(50)	2.79±0.16	2.41	3.07	(50)	2.91±0.15	2.74	3.25	(20)	2.86±0.16	2.58	3.19	(20)	2.79±0.17	2.48	3.08	(20)	2.83±0.18	2.64	3.18	(20)
M	0.89±0.10	0.85	1.17	(50)	1.47±0.12	0.87	1.28	(50)	0.95±0.12	0.82	1.25	(20)	0.90±0.09	0.74	1.02	(20)	1.11±0.09	1.01	1.28	(20)	1.03±0.08	0.92	1.19	(20)
C3F	0.53±0.03	0.47	0.56	(50)	0.53±0.03	0.47	0.59	(50)	0.52±0.04	0.46	0.60	(20)	0.51±0.03	0.45	0.56	(20)	0.58±0.02	0.55	0.61	(20)	0.58±0.04	0.52	0.63	(20)
sc1	21.9±1.6	16	18	(10)					20.3±1.3	19	24	(10)					19.9±1.8	17	23	(10)				
sc2	16.7±1.0	13	20	(50)					15.3±1.4	12	18	(20)					15.6±1.0	13	17	(20)				
epands	5.6±0.5	5	6	(10)					6.1±0.7	5	8	(10)					6.9±0.7	6	8	(10)				
vlbs	9.2±1.0	7	12	(50)					9.1±1.0	8	12	(20)					10.1±1.1	7	12	(20)				
surpg	3.5±0.5	3	4	(10)					3.8±0.4	3	4	(10)					3.7±0.5	3	4	(10)				
sursp	7.0±0.8	6	8	(10)					8.3±0.6	8	10	(10)					8.5±0.7	8	10	(10)				
cers	13.1±0.5	12	14	(10)					13.1±0.9	12	15	(10)					15.1±1.7	12	17	(10)				
cersp	3.0±0.0	3	3	(10)					3.0±0.0	3	3	(10)					3.0±0.0	3	3	(10)				
pg2	2.0±0.0	2	2	(10)					2.0±0.0	2	2	(10)					2.0±0.0	2	2	(10)				
st2	7.2±0.7	6	8	(10)					7.0±0.6	6	8	(10)					7.2±0.6	6	9	(10)				
ovms					13.7±1.4	11	15	(10)					13.8±1.2	12	16	(10)					14.8±0.6	14	16	(10)

posterior setae.

Thorax: Scutum and thoracic pleura brownish yellow. Acrostichal setulae in 6 rows in front of anterior dorsocentral setae, 4 rows between dorsocentral setae. Basal scutellar setae parallel; apical setae cruciate at right angle.

Legs: Preapical dorsal seta present on tibia of all legs; apical seta on tibia of fore- and midlegs. Longitudinal sex-combs present along entire lengths of 1st and 2nd tarsomeres of ♂ foreleg.

Wing transparent, slightly yellowish. Veins brownish yellow. Basal medial-cubital (bm-cu) crossvein absent.

Abdomen: Second to 6th tergites yellow, each with very distinct apical black band, except for ♂ 6th black dorsally.

Male terminalia: Epandrium pale brown, broad, not pubescent, with triangular expansion covering base of surstylus; ventral lobe apically round. Cercus separated from epandrium, not pubescent, triangular; lower, oval part separated, differentiated as secondary clasper. Hypandrium pubescent on caudolateral lobes and caudal margin, caudomedially with strong protrusion apically bearing a pair of stout paramedian setae as long as paramere. Paramere large, longer than wide. Aedeagus slender, apically finely hirsute, basally with a pair of lobate processes as long as aedeagus, fused to apodeme; apodeme rod-like, as long as aedeagus.

Table 4. Quantitative characters showing significant correlations with the thorax length in the three sibling species

Species:	*D. lini*	*D. ohnishii*	*D. ogumai*
Strain:	BG14028-0581.0	MMY326	RGN206
	(*P* value)	(*P* value)	(*P* value)
Male			
spcs	0.0460	ns	ns
ps	0.0292	0.0421	ns
FW/HW	0.0009*	ns	ns
vb	ns	ns	0.0249
sctl	ns	ns	0.0353
M	0.0482	ns	ns
ch/o	ns	0.0471	ns
Female			
ocps	0.0388	ns	ns
prorb	ns	0.0076	ns
dcp	ns	0.0261	ns
sterno2	0.0432	ns	ns
ovms	0.0251	ns	ns

* Significant after Bonferroni correction.

Table 5. Quantitative characters with significant sexual difference (by unpaired *t*-test) in the three sibling species

Species:	*D. lini*	*D. ohnishii*	*D. ogumai*
Strain:	BG14028-0581.0	MMY326	RGN206
	(*P* value)	(*P* value)	(*P* value)
spcs	0.0285	0.0030	0.0027
ocps	0.0010*	0.0026	0.0378
ps	ns	ns	0.0186
ms	ns	ns	0.0047
FW/HW	0.0095	ns	0.0100
prorb	0.0010*	0.0002*	0.0015*
rcorb	ns	0.0490	ns
sterno	0.0287	ns	ns
sterno2	ns	ns	0.0313
orbito	0.0007*	<0.0001*	ns
dcp	ns	0.0265	ns
C	0.0021	0.0005*	0.0230
4c	0.0438	0.0041	ns
ac	ns	0.0122	ns
5x	ns	ns	0.0222
C3F	0.0190	ns	ns

* Significant after Bonferroni correction.

Table 6. Quantitative characters with significant difference (by Kruskal-Wallis test) among the three sibling species

Character	Male	Female
	(*P* value)	(*P* value)
spcs	0.0083	0.0003*
ocps	<0.0001*	0.0001*
ps	<0.0001*	<0.0001*
FW/HW	0.0010*	ns
ch/O	ns	0.0064
prorb	0.0042	ns
rcrob	0.0003*	0.0002*
dcl	0.0458	ns
sctl	ns	0.0156
sterno	0.0104	0.0482
orbito	<0.0001*	<0.0001*
sctlp	0.0098	<0.0001*
WW/WL	ns	0.0121
c	0.0085	0.0093
4c	0.0026	0.0002*
4v	0.0065	0.0003*
5x	0.0006*	0.0066
ac	<0.0001*	<0.0001*
M	0.0011*	<0.0001*
C3F	0.0020*	0.0003*
sc1	0.0147	–
sc2	<0.0001*	–
epands	0.0041	–
vlbs	<0.0001*	–
sursp	0.0021	–
cers	0.0167	–

* Significant after Bonferroni correction.

Specimens examined. Ten ♂ and 10 ♀ each from the following isofemale strains: BG14028-0581.0, DHS401, DHS501, NKS9212 and NKS9231.

Distribution. China (Taiwan, Guangdong).

***Drosophila* (*Sophophora*) *ohnishii* sp. nov.**

(Figs. 1B, 2D-F)

Drosophila (*Sophophora*) *lini*-like, Ohnishi & Watanabe, 1984: 802; Kim *et al*., 1989: 178; 1993: 992; Oguma *et al*., 1995: 312.

Diagnosis. Paramere apically round and with 3-5 minute sensilla, submedially with moderate, inward expansion of which posterior margin distinctly concave (Fig. 2D,F). Aedeagal basal process with fine, irregular serrations on narrower, apical margin than in *D. lini* (Fig. 2E).

Holotype ♂ from MMY326.

Paratypes. Nine ♂ and 10 ♀ from MMY326; 10 ♂ and 10 ♀ from MMY307.

Distribution. Myanmar (Pyinoolwin).

Relationships. This species is very closely related to *D. lini*: reciprocal crosses between these two species can produce F_1 hybrids, of which females are fertile but males sterile (Oguma *et al*., 1995). Morphologically, these two species can hardly be distinguished from each other even by the characters of male terminalia which are given in the diagnosis. A combination of some quantitative characters can be used, but not absolutely, to discriminate these two species (see below).

Etymology. Patronym, in honor of Dr. S. Ohnishi who found this species first.

***Drosophila* (*Sophophora*) *ogumai* sp. nov.**

(Figs. 1C, 2G-I)

Diagnosis. Paramere apically narrow and with 2-3 minute sensilla, submedially with moderate, inward expansion of which posterior margin distinctly concave (Fig. 2G,I). Aedeagal basal process irregular on apical margin but without fine serrations (Fig. 2H).

Holotype ♂ from RGN206.

Paratypes. Nine ♂ and 10 ♀ from RGN206; 10 ♂ and 10 ♀ from RGN3.

Distribution. Myanmar (Yangon).

Relationships. This species is also closely related to the foregoing two species: crosses with them produce F_1 fertile females but sterile males, but crosses between *ohnishii* females and *ogumai* males produce no F_1 hybrids (Oguma *et al*., 1995). Morphologically, this species can be distinguished from the other two species by the diagnostic characters, but only for males. Combinations of some quantitative characters can be used, not only for males but also for females, to discriminate this species from the others (see below).

Etymology. Patronym, in honor of Dr. Y. Oguma who detected the presence of postmating isolation between this species and the other two species for the first time.

Comparison of quantitative characters

The mean±SD and the range are shown for 42 quantitative characters, separately for each sex and for each species, in Table 3. In interspecific comparison for each sex, there is no character with nonoverlapping ranges between species, which can be used as the specific diagnosis.

Using the data for the three strains, BG14028-0581.0 (*D. lini*), MMY326 (*D. ohnishii*) and RGN206 (*D. ogumai*), which were measured for all the 42 quantitative characters, correlations with the body size (ThL: thorax length) were examined for 38 characters. Seven characters for male and five characters for female showed significant correlations with ThL in any of the three species (Table 4). However,

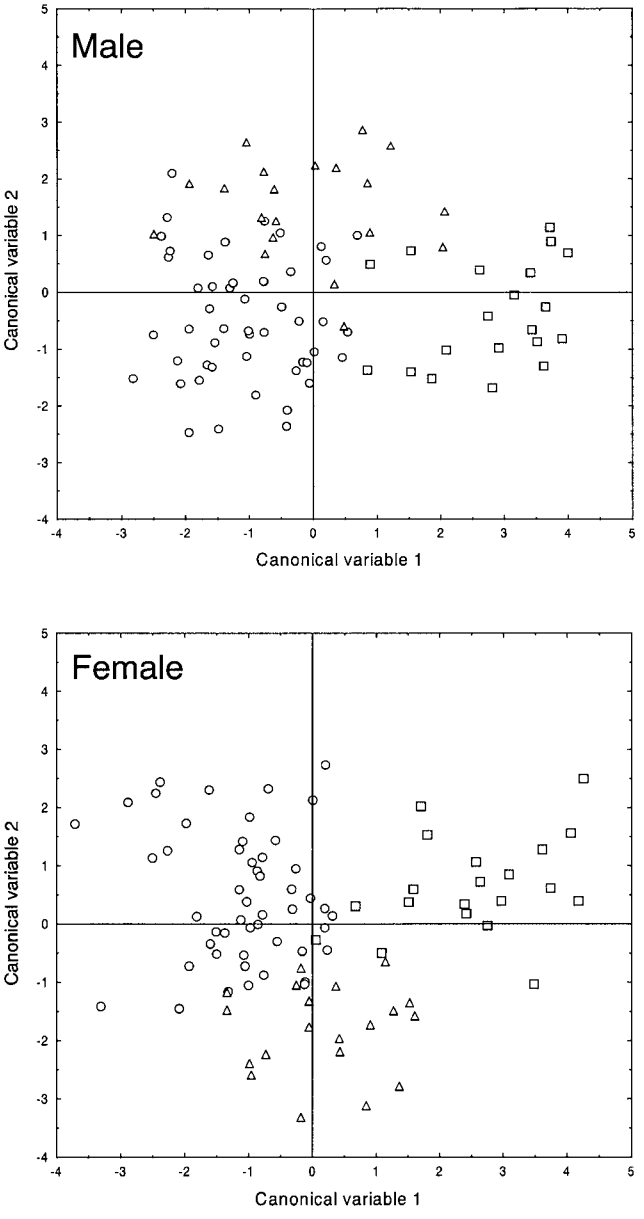


Fig. 3. Scatterplots of canonical scores for 90 specimens: 50 of *D. lini* (circle) and 20 each of *D. ohnishii* (triangle) and *D. ogumai* (square) in each sex.

only FW/HW in male of BG14028-0581.0 held a significant correlation after Bonferroni correction. Since no character was thus regarded as being affected constantly by the body size, *i.e.* culture conditions, all the characters except for those representing the body size were subjected to the following analyses.

Significant sexual difference was detected by unpaired *t*-test for 16 characters in any of the three species (Table 5). Among a total of 27 such cases were seven cases still significant after Bonferroni correction; especially, prorb (relative length of the proclinate orbital seta to the posterior reclinate orbital seta) was significantly different between male and female in all the three species. Therefore, the following analyses were carried out separately for each sex.

Kruskal-Wallis tests detected significant interspecific difference in 23 characters for male and 17 characters for female (Table 6). Of 26 characters that differed significantly among the species in either sex, 15 characters showed still significant interspecific difference after Bonferroni correction. These 15 characters were used in the canonical discriminant analysis.

Fig. 3 shows plots of canonical scores for measured

Table 7. Factor structure coefficients (correlations between the characters and the discriminant functions) and classification functions for the three sibling species, resulting from canonical discriminant analysis in which classifications were based on equal *a priori* probability for each species

	Factor structure coefficients		Classification functions		
	Function 1	Function 2	*D. lini*	*D. ohnishii*	*D. ogumai*
A priori probability:			*p*=.33333	*p*=.33333	*p*=.33333
Male					
spcs	0.266227	–0.000475	2.393	2.475	2.311
ocps	0.550960	–0.104288	0.887	1.226	2.466
ps	0.174051	–0.149233	–1.729	–1.665	–1.655
FW/HW	0.140366	0.429669	1282.870	1332.124	1326.998
rcorb	0.203348	0.016609	104.630	119.611	135.538
orbito	–0.137896	0.094232	69.357	67.346	61.627
sctlp	0.243104	0.207933	25.251	33.425	29.349
4c	0.153670	–0.162753	29.403	30.957	41.292
4v	0.111083	–0.296968	51.253	46.618	46.572
5x	0.208429	–0.374703	0.255	–0.272	3.056
ac	–0.096502	0.017271	–7.206	–7.671	–13.344
M	0.195389	–0.358703	34.881	35.268	30.307
C3F	0.311355	–0.356125	182.338	164.375	197.085
sc2	–0.188396	–0.397294	17.412	16.226	16.264
vlbs	0.168478	–0.130057	7.261	6.767	7.661
Constant			–679.703	–677.275	–721.889
Female					
spcs	0.153029	0.435593	–1.106	–2.088	–1.520
ocps	0.363089	0.076757	–0.834	–0.318	0.040
ps	0.161460	0.249951	–2.734	–2.890	–2.540
FW/HW	0.297272	–0.326475	2527.289	2634.603	2582.174
rcorb	0.313515	0.039127	54.713	68.337	80.395
orbito	0.077671	–0.241863	124.013	139.055	129.650
sctlp	0.499406	0.258703	158.399	163.628	183.733
4c	0.317850	0.229277	–31.282	–26.295	–4.801
4v	0.151690	0.263008	123.466	119.749	114.503
5x	–0.117790	0.238431	50.220	46.883	48.991
ac	0.041117	–0.077814	95.241	98.971	95.038
M	0.021024	0.261844	–157.073	–151.888	–160.064
C3F	0.295669	0.391708	338.958	313.277	343.224
Constant			–1012.122	–1061.614	–1104.296

Table 8. Summary of *posterior* classifications. Rows: observed classifications, columns: predicted classifications

	Correct (%)	*D. lini*	*D. ohnishii*	*D. ogumai*
Male				
D. lini	88.0	44	6	0
D. ohnishii	80.0	2	16	2
D. ogumai	90.0	1	1	18
Total	86.7	47	23	20
Female				
D. lini	90.0	45	5	0
D. ohnishii	100.0	0	20	0
D. ogumai	85.0	2	1	17
Total	91.1	47	26	17

specimens, resulting from the canonical discriminant analyses, separately for each sex. A total of 90 individuals, 10 each from the nine strains (five strains of *D. lini* and two each of *D. ohnishii* and *D. ogumai*), were measured in each sex, and the 15 characters were used in male and 13 of them, excluding two male-specific characters, in female for the analyses. At a glance of the figure, the first canonical function can be regarded as discriminating *D. ogumai* from the other species, and the second function as discriminating *D. ohnishii* from the others, in both sexes. The first function means large ocps (the number of occipital setae) and C3F (the relative length of heavy setation in the third costal section) in both sexes, and large sctlp (the distance between ipsilateral scutellar setae / the cross distance between apical scutellar setae), 4c (the relative length of the third costal section to M_1 between r-m and dm-cu), rcorb (the relative length of the anterior reclinate orbital seta to the posterior reclinate orbital seta) and FW/HW (the relative width of frons to head width) in female (Table 7). The second function seems to be reverse in the direction between male and female plots, meaning large FW/HW, small sc2 (the number of teeth in sex-comb of foreleg 2nd tarsomere), 5x (the relative length of CuA_1 between dm-cu and wing margin to dm-cu between M_1 and CuA_1), M (the relative length of CuA_1

Table 9. Degrees of premating and postmating isolation based on the data of Oguma *et al.* (1995)

Female Species		Male								
		D. lini					*D. ogumai*		*D. ohnishii*	
	Strain	TWN*	DHS315	DHS401	DHS501	NKS9231	RGN3	RGN206	MMY307	MMY326
A) Copulation rate calculated from the data of pair-mating experiment										
D. lini										
	TWN	0.1	–	0.1	0.3	0.4	0.2	0.2	0.0	–
	DHS401	0.4	–	0.2	0.2	0.2	0.5	0.3	0.2	–
	DHS501	0.0	–	0.4	3.0	0.3	0.1	0.0	0.1	–
	NKS9231	0.1	–	0.2	0.0	0.2	0.3	0.2	0.0	–
D. ogumai										
	RGN3	0.3	–	0.2	0.2	0.0	0.3	0.3	0.0	–
	RGN206	0.1	–	0.3	0.2	0.3	0.4	0.4	0.0	–
D. ohnishii										
	MMY307	0.0	–	0.0	0.0	0.0	0.0	0.0	0.4	–
B) Strength of postmating isolation										
D. lini										
	TWN	–	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5
	DHS315	0.0	–	0.0	0.0	0.0	0.5	0.5	0.5	0.5
	DHS401	0.0	0.0	–	0.0	0.0	0.5	0.5	0.5	0.5
	DHS501	0.0	0.0	0.0	–	0.0	0.5	0.5	0.5	0.5
	NKS9231	0.0	0.0	0.0	0.0	–	0.5	0.5	0.5	0.5
D. ogumai										
	RGN3	0.5	0.5	0.5	0.5	0.5	–	0.0	0.5	0.5
	RGN206	0.5	0.5	0.5	0.5	0.5	0.0	–	0.5	0.5
D. ohnishii										
	MMY307	0.5	0.5	0.5	0.5	0.5	1.0	0.5	–	0.0
	MMY326	0.5	0.5	0.5	0.5	0.5	1.0	1.0	0.0	–

* TWN: BG14028-0581.0

between dm-cu and wing margin to M_1 between r-m and dm-cu), C3F and 4v (the relative length of M_1 between dm-cu and wing margin to M_1 between r-m and dm-cu) in male, but large C3F and small FW/HW in female (Table 7). Classification functions for the three species, based on equal *a priori* probability for each species, are shown in Table 7, and the results of *posterior* classifications of the 90 cases (specimens) in each sex are shown in Table 8. The percentage of correct classifications was rather high in both sexes, 86.7% in male and 91.1% in female. Thus, the present results suggest that the 15 (for male) or 13 (for female) quantitative characters in combination are effective to discriminate the three sibling species, even their females, from each other with considerable confidence.

DISCUSSION

In this study, we examined morphological differences among three sibling species that had proved to be biologically good species reproductively isolated from each other almost completely (Kim *et al*., 1989; Oguma *et al*., 1995). Of those three species, two were described as new species, *i.e*. *D. ohnishii* and *D. ogumai*, and the remaining known species, *D. lini*, was redescribed in the light of detailed examination of the male terminalia and many quantitative characters. However, they are very similar in morphology, hardly distinguishable from each other even by a few characters designated as the diagnosis, especially between *D. lini* and *D. ohnishii*, implying that they are still in the process of species differentiation, even though having reached almost to the level of good species. This is supported also by partial crossability and fertility of F_1 hybrids between them (Kim *et al*., 1989; Oguma *et al*., 1995).

It is conceived, but implicitly, that character differentiation does not always proceed in parallel among different biological properties in the process of speciation. Here, morphological differentiation among the three sibling species is compared with the degrees of premating and postmating

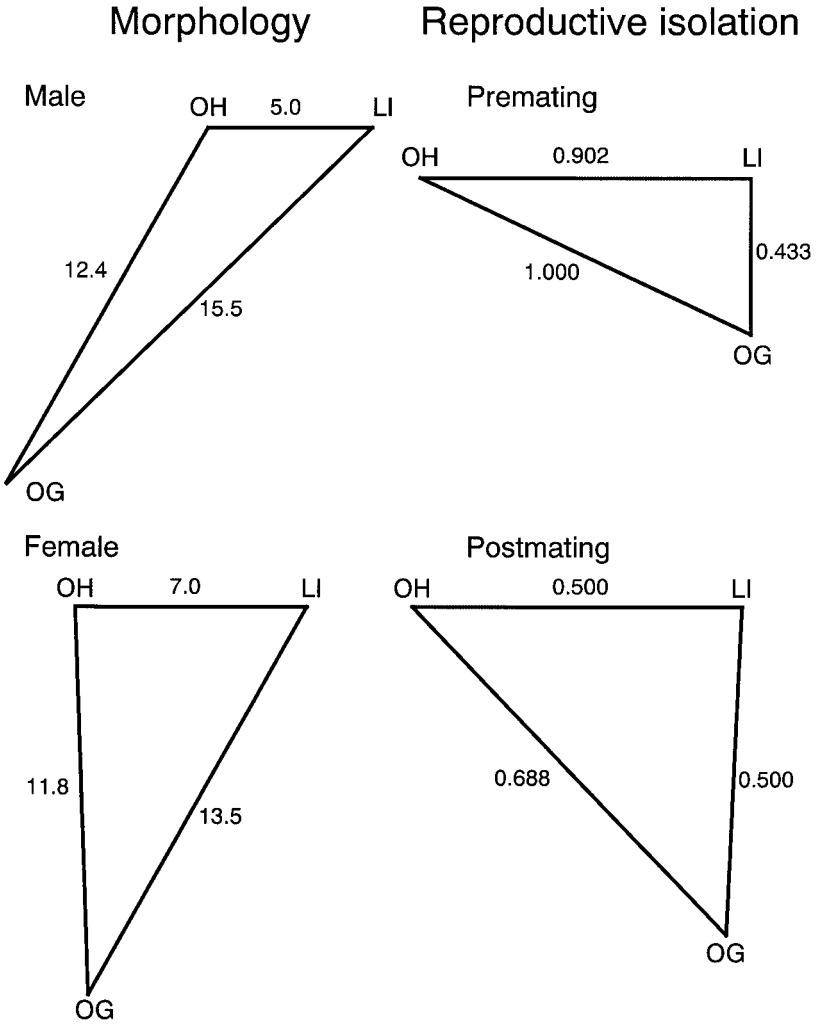


Fig. 4. Relationships among the three sibling species (LI: *D. lini*, OH: *D. ohnishii*, OG: *D. ogumai*) in morphology (measured by squared Mahalanobis distance), premating isolation (isolation index *I*, see text) and postmating isolation (see text), the last two based on data by Oguma *et al*. (1995).

isolation among them, based on the data presented by Oguma *et al*. (1995) for the reproductive isolation. Morphological difference was evaluated by the squared Mahalanobis distance based on 15 characters used in the canonical discriminant analysis for male and 13 such characters for female. Oguma *et al*. (1995) provided quantitative data for premating isolation based on their pair-mating experiment. They set up 10 replicates (*i.e.* pairs) for each cross and observed mating behavior of each pair until copulation occurred within five minutes. We converted their data of copulation frequency (the number of pairs having copulated within five min., Table 2 in Oguma *et al*., 1995) into those of copulation rate per pair (Table 9A), and calculated the following premating isolation index between different species:

$$I = (m_o - m_e)/ m_o,$$

where m_o and m_e = the mean copulation rate in homospecific and heterospecific matings, respectively, for concerned two species. As for the postmating isolation, Oguma *et al*. (1995) presented the data in qualitative categories representing different degrees of isolation (Fig. 4 in Oguma *et al*., 1995). We converted such categories into relative values: "F" (female and male F_1 hybrids fertile)=0, "f" (female F_1 hybrids fertile but male F_1 hybrids sterile)=0.5, and "(–)" (no F_1 hybrids produced) = 1 (Table 9B). Such values of all reciprocal crosses between different species were averaged for each pair of species. Relationships among the three species were represented by a triangle of which side lengths corresponded to the interspecific differences or degrees of isolation (Fig. 4).

At a glance of the figure, we notice that the interspecific differentiation patterns vary among the concerned biological properties. In morphology, *D. ogumai* is most remote from the other two species for both sexes. On the other hand, premating isolation is almost complete between *D. ohnishii* and the other two species, but moderate between *D. ogumai* and *D. lini*. Oguma *et al*. (1995) reported that females of *D. ohnishii* performed strong repelling behaviors, spreading and fluttering their wings or kicking males, against allospecific males and *D. ohnishii* males were strongly refused by allospecific females. Postmating isolation is more or less present between the three sibling species, heterospecific crosses usually producing F_1 fertile females but sterile males. However, some crosses between *D. ohnishii* females and *D. ogumai* males produce no F_1 hybrids. The pattern for premating isolation suggests two possible hypotheses. One possibility is that this property has been evolved or reinforced in sympatric populations between *D. ohnishii* and *D. lini* and between *D. ohnishii* and *D. ogumai*. Although no such sympatric populations have been found yet, it is probable that the range of *D. ohnishii* overlaps with that of *D. lini* in southwestern China to northern Myanmar and with that of *D. ogumai* somewhere between Pyinoolwin and Yangon, which are not so distant from each other. The other possibility is that the strong premating isolation between *D. ohnishii* and the other two species has evolved in a very restricted population at a locality of central Myanmar, perhaps by a few mutations.

Thus, the present study provides very interesting materials and information for studies of speciation mechanisms. To fully understand the evolution of the three sibling species, however, much more information is needed, especially about some gene sequences, premating isolation (*i.e*. mate discrimination) mechanisms, geographic distribution ranges and eco-physiological adaptations to environmental conditions in their ranges.

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