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A NEW SPECIES OF THE *DROSOPHILA KIKKAWAI* COMPLEX FROM THAILAND (DIPTERA: DROSOPHILIDAE)

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Abstract. *Drosophila bocki,* n. sp., a sibling species of *D. kikkawai* and *D. leontia,* is described from Thailand. All 3 species are morphologically very similar but are reproductively isolated from one another. The distributions of the species overlap and, in Thailand, *D. kikkawai* occurs sympatrically either with *D. bocki* or with *D. leontia.* The latter 2 species are also sympatric in certain populations in Thailand.

The *Drosophila melanogaster* species group consists of over 70 species divided into a number of subgroups (Bock & Wheeler 1972). *Drosophila kikkawai* Burla is one of the most widespread and cytologically the best known species of the *montium* subgroup (Ward 1949; Baimai 1969, 1970, identified in error as *Drosophila montium*). In particular, it has been shown that the *D. kikkawai* populations on the islands of Borneo and New Guinea exhibit variations in metaphase karyotypes as well as sexual isolation to some degree. Recently, Tsacas & David (1977) described *Drosophila leontia,* a new sibling species of *D. kikkawai* from Singapore. These results seem to suggest that "*D. kikkawai*" could be a cluster of closely related species which are extremely similar in general morphology.

To investigate the complex status of "*D. kikkawai,*" many strains of different geographic origins were obtained and maintained in the laboratory at 25 °C. Systematic hybridization tests revealed 3 sibling species which are morphologically indistinguishable; even the detailed structures of the male genitalia are very similar (FIG. 2–4). This paper presents a description of the new sibling species, *Drosophila bocki,* from Thailand.

MATERIALS AND METHODS

Samples of the *D. kikkawai* complex were collected by the author at several localities in Thailand. Culture stocks derived from individual isofemale lines have been maintained in the laboratory at approximately 25 °C. The culture stocks employed in this study are shown in TABLE 1. Songkla-A and Songkla-B stocks were established from the same collection; Khao Yai National Park and Lamtakong are some 60 km apart.

Each strain was examined cytologically for metaphase chromosome configurations in the usual manner (Baimai 1977).

A series of hybridization experiments was carried out between a standard strain of *D. kikkawai* from Bangkok (stock no. A03 B7) and the different strains from other parts of Thailand. Mass matings involved 4–5 pairs per vial, with 5–6 replicates in

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TABLE 1. Data of the culture stocks of the 3 species in this study.

SPECIES	STOCK NO.	LOCALITY	NO. OF ISOFE-MALES	COLLECTION DATE	METAPHASE CHROMOSOME 4
D. kikkawai	A03B7	Bangkok	10	June 1971	V-shaped
	A08B3	Songkla-A, 900 km S of Bangkok	4	January 1976	V-shaped
D. leontia	A02B11	Chiang Mai, 700 km N of Bangkok	12	May 1971	dot
	A08B9	Songkla-B, 900 km S of Bangkok	14	January 1976	dot
D. bocki	A01B3	Khao Yai National Park, 200 km NE of Bangkok	5	March 1971	dot
	A10B2	Lamtakong, 200 km NE of Bangkok	3	August 1977	dot

each case, kept at 25 °C for 3–4 weeks. For those unsuccessful crosses in the first experiments, further mass matings involved 20–25 pairs per half-pint culture bottle, with 4–5 replicates performed in each case. For those crosses producing F_1 progeny, the F_1 were allowed to interbreed to check for fertility. In the case of unsuccessful crosses, a sample of females involved in each of the crosses was then dissected to check for sperm storage in the spermathecae and seminal receptacles.

RESULTS AND DISCUSSION

All "strains" under investigation generally showed similar metaphase chromosome configurations, except for the 4th chromosomes, which differ in size and shape due mainly to the amount for extra heterochromatin (TABLE 1).

The results of all combinations of crosses between "strains" are shown in FIG. 1. Three types of crossability among these strains were obtained. Fully fertile crosses were found between Khao Yai and Lamtakong, Bangkok and Songkla-A, and Chiangmai and Songkla-B. Partially fertile crosses were encountered among the strains from Bangkok, Chiangmai, Songkla-A and Songkla-B (designated as PF in FIG. 1). The latter 2 strains are sympatric. However, natural hybridization has not been observed among the 18 wild-caught females from the Songkla population examined. All other combinations of crosses among these strains were completely sterile (designated as S in FIG. 1).

The F_1 progeny from the successful crosses were fully fertile and produced large numbers of F_2 offspring which were completely fertile. The partially fertile crosses yielded considerable numbers of F_1 progeny of both sexes. Of these, the F_1 male hybrids were completely sterile, whereas the F_1 female hybrids were fertile when they were backcrossed to parental strains. These results are in accordance with the recent findings reported by David et al. (1978). In the case of unsuccessful crosses, females had apparently not mated at all, since there were no sperm present in either sper-

Female \ Male		D. bocki		D. kikkawai		D. leontia	
		Khao Yai	Lamtakong	Bangkok	Songkla-A	Songkla-B	Chiangmai
D. bocki	Khao Yai		F	S	S	S	S
	Lamtakong	F		S	S	S	S
D. kikkawai	Bangkok	S	S		F	PF	PF
	Songkla-A	S	S	F		PF	PF
D. leontia	Songkla-B	S	S	PF	PF		F
	Chiangmai	S	S	PF	PF	F	

FIG. 1. Results of reciprocal crosses among different strains of the 3 sibling species. F=fertile; S=sterile (i.e., no insemination); PF=partially fertile.

mathecae or seminal receptacles. These results clearly show reproductive isolation among the 3 crossing types, which may therefore be regarded as 3 sibling species. Thus Bangkok and Songkla-A strains are *D. kikkawai* (which is distinguished by its metaphase karyotype as noted below), while the Chiangmai and Songkla-B strains represent *D. leontia* (which is characterized by the dot-shaped chromosome 4) recently described by Tsacas & David (1977). The strains from Khao Yai National Park and Lamtakong represent a new sibling species, *Drosophila bocki*. Generally, these 3 sibling species are morphologically very similar. In addition, *D. leontia* and *D. bocki* manifest similar metaphase karyotypes. However, certain external diagnostic characters may be helpful in distinguishing the 3 species (TABLE 2).

Drosophila bocki Baimai, **new species** FIG. 2–4

Body length. ♂, 1.8–1.9 mm; ♀, 2.0–2.1 mm.

Head (♂♀). Arista with 9 branches (including terminal fork). Antennae and front brown. Face, cheeks, and proboscis yellowish brown, bristles black. Orbital bristles in ratio 3:1:3. Carina rounded. Greatest width of cheek 0.1× greatest diameter of eye. Eyes bright red. *Thorax* (♂♀). Brown; legs light brown; bristles black. Acrostical hairs in 6 rows. Prescutellars absent. All scutellars convergent. Ratio anterior/posterior dorsocentrals 0.5. Sterno-index about 0.6. Sex-combs on first 2 joints of fore-tarsi in ♂, consisting of 2 sections; proximal (metatarsal) comb with about 22 teeth, shorter and smaller above, lowermost 2 slightly displaced from axis of remaining teeth; distal comb on 2nd tarsal segment consisting of about 18 uniform teeth. *Wing* (♂♀). Transparent. Approximate indices: costal index 1.8; 4V index 3.1; M-index (Bock 1976) 1.1; 5X index 3.7. Third costal section with heavy setation on basal 0.6. Wing lengths: ♂, 1.5–1.6 mm; ♀, 1.6–1.7 mm. *Abdomen.* ♀. Tergum 1 brownish yellow. Terga 2–5 with dark brown to black

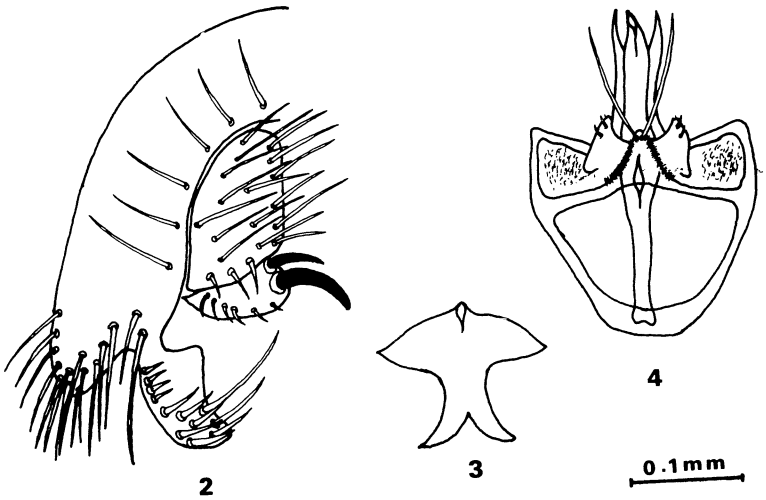

FIG. 2–4. Male genitalia of *D. bocki.*

bands on posterior margins, broader in middle. Terga 6 and 7 with lighter brown bands on posterior margins. Posterior margins of terga 3–5 usually with 4 strongly black bristles on either side, other bristles on these segments less prominent. ♂. Tergum 1 brownish yellow. Terga 2–4 with dark brown bands on posterior margins similar to those in ♀. Terga 5 and 6 diffusely brown on posterior margins. Tergum 7 lighter brown. Terga 3–5 with less strongly dark bristles than in ♀. Body color generally light brownish yellow. Frequently all parts of terga 6 and 7 of specimens of type culture are dark brown to black, presumably due to genetic variation. *Periphallic organ* (FIG. 2). General features similar to those of sibling species, light brown, epandrium (genital arch) with 6 bristles on posterior margin, and ventral margin with 1 long bristle and cluster of some 15 bristles of approximately same size; uniformly dark brown anal plate with very long bristles; surstyli (secondary clasper) sclerotized, bearing 1 small and 1 large curved tooth. Cercus (primary clasper) bears row of 4 pointed black teeth and cluster of 7 short and 1 long bristles at tip. Median lobe of decasternum bicornute (FIG. 3). *Phallic organ* (FIG. 4). Aedeagus slender, apically bifid and slightly curved. Anterior paramere large, apically curved with small sensilla. Posterior paramere long, slender, reaching tip of aedeagus. Hypandrium with pair of long submedian spines.

Egg guide. Dark brown with about 14 teeth.

Reproductive tract. ♂. Testes yellow, with 4 spiral coils and uncoiled arm. Accessory glands large. ♀. Ventral receptacle with about 5 tight coils, containing sperm in inseminated ♀. Spermathecae small, spherical, completely unsclerotized, containing no sperm in inseminated ♀.

Egg filaments. Two long slender filaments, not flattened apically.

Pupae. Anterior spiracles divergent, each with about 8 black branches.

Chromosomes. Larval ganglion metaphase chromosome complement consists of 2 pairs of V's, 1 pair of dots, and 1 pair of sex chromosomes. The X is a submetacentric chromosome (J-shaped in appearance) with a heterochromatic short arm. The Y is a small metacentric chromosome (V-shaped) composed totally of heterochromatin. Thus the metaphase figure generally conforms to a typical karyotype of the *montium* species subgroup. The salivary gland chromosome consists of 5 long arms and 1 short arm.

D. bocki is named after Dr I. R. Bock, a prominent *Drosophila* taxonomist in Australia.

Type culture source. Khao Yai National Park, northeastern Thailand.

Distribution. Thailand and Yun-shui, Chia-I, Taiwan.

Holotype ♂, THAILAND. Isofemale culture from Khao Yai National Park (14.5°N,

TABLE 2. Comparisons of differential characteristics of the 3 sibling species.

CHARACTERS	*D. leontia*	*D. bocki*	*D. kikkawai*
Basitarsi of foreleg (no. of teeth)	16–23*	17–22	22–26*
Hind tarsi of foreleg (no. of teeth)	14–18*	16–20	17–24*
Costal index ♂	1.75 ± 0.03*	1.74 ± 0.05	1.88 ± 0.03*
♀	1.91 ± 0.04*	1.86 ± 0.04	2.03 ± 0.02*
5X ♂	3.04 ± 0.07*	3.05 ± 0.05	2.35 ± 0.05*
♀	3.16 ± 0.17*	2.92 ± 0.06	2.20 ± 0.06*
Egg guide (no. of teeth)	12–13	13–14	14–15
Pupae spiracles (no. of horns)	7	8	9

* Data from Tsacas & David (1977).

101.5°E), 20.III.1971, net sweeping over banana baits, V. Baimai. Deposited in British Museum (Natural History), London. Paratypes: 5♂,5♀, same data and depository as holotype.

RELATIONSHIPS

D. bocki, D. kikkawai and *D. leontia* are morphologically very similar. It is very difficult, if not impossible, to separate these 3 sibling species on morphological criteria alone since only slight differences can be noticed in the sex-combs, wing indices (TABLE 2) and in the male genitalia (cf. Tsacas & David 1977: 680, fig. 2; Bock & Wheeler 1972: 58, fig. 93, 94), i.e., marginal bristles on the ventral margin of epandrium and teeth on the cercus. *D. bocki* and *D. leontia* exhibit similar metaphase karyotypes which differ from those of *D. kikkawai,* especially in the amount of extra heterochromatin in the 4th chromosome. A similar situation has been encountered among Hawaiian *Drosophila* where 2 closely related species differ only in metaphase chromosome configurations due to the amount of extra heterochromatin, but are morphologically indistinguishable (Baimai & Ahearn 1978, Hardy 1978). Clearly, genetic differentiation has preceded morphological divergence in these species complexes of *Drosophila.*

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