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REPRODUCTIVE ISOLATION AMONG THREE SPECIES BELONGING TO THE *DROSOPHILA HYPOCAUSTA* SUBGROUP OF THE *IMMIGRANS* SPECIES GROUP, WITH A DESCRIPTION OF A NEW SPECIES

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INTRODUCTION

The *Drosophila hypocausta* subgroup is one of subgroups of the *D. immigrans* species group. This subgroup has been knownto include seven species, including *D. hypocausta* Osten Sacken 1882, from Phillipine and *D. neohypocausta* Lin and Wheeler 1973, from Taiwan. We recently collected many specimens of a drosophilid fly in Malaysia and Thailand, which was thought to be a new species belonging to this subgroup.

In the present paper, we will describe a new species on the basis of examinations on morphology. Studies on reproductive isolation among the three species belonging to the *D. hypocausta* subgroup provided evidence to support the description of a new species.

MATERIALS AND METHODS

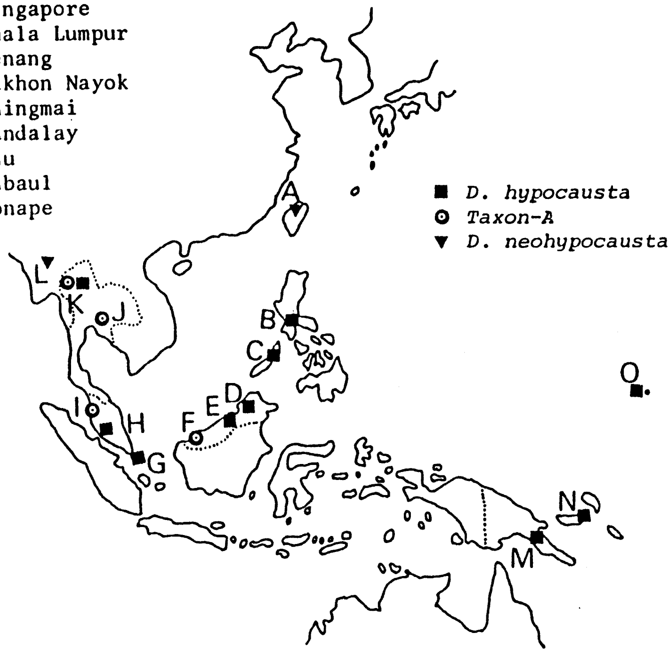
All strains used are summarized as follows:

Species & Strains	Locality	Year
D. neohypocausta		
TW	Chung-tou, Taiwan	1968
D. hypocausta		
R164	Palawan, Phillipine	1979
W103	Singapore	1979
Taxon-A		
Y110, Y115	Penang, Malaysia	1979
Z17, Z28	Nakon Noyok, Thailand	1979

Distributions of the three species are shown in Fig. 1, which was made on the basis of a list of stocks having been kept in our laboratory. Stocks of *D. hypocausta* and a new species, Taxon-A, were derived from single females collected in 1979 and 1981 by research groups which were supported by the funds of Overseas Scientific Expedition of the Ministry of Education, Science and Culture of Japan (Nos. 404149 and 504344). *D. neohypocausta*, TW, was started by one wild caught female in 1968 by F. J. Lin.

Fig. 1. Distributions of three species belonging to the *D. hypocausta* subgroup

A: Taiwan
B: Los Banos
C: Palawan
D: Kota Kinabaru
E: Miri
F: Kuching
G: Singapore
H: Kuala Lumpur
I: Penang
J: Nakhon Nayok
K: Chingmai
L: Mandalay
M: Wau
N: Rabaul
O: Ponape



Flies used were reared and aged in the medium (sucrose-dry yeast-cornmeal-agar) seeded with live yeast, at 25 ± 1°C in artificial light and dark cycle (LD = 12 : 12, the light period being between 8:00 a.m. and 8:00 p.m.). Throughout the experiments, the observation of matings and the recording of courtship sounds were carried out in the morning hours of the light period at 8 days of age.

Procedures for detecting sound were basically the same as those described by Ewing and Bennet-Clark (1968) (see Ikeda *et al.*, 1980). The intrapulse frequency was analyzed by the sonograph (Kay Elemetrics Co., 7030C).

To test mating propensity within and between species, 5 females and 8 males were allowed to mate in a food vial for 48 hours. Thereafter, the females were dissected to see whether they had sperm in their spermathecae and seminal receptacles. 20 - 22 replicates were performed, so that 100 - 110 females were examined for each combination.

RESULTS

1. A description of a new species

A description of a new species is given in the appendix. This will be formally published shortly in a proper journal. Till then, a new species is tentatively referred to as Taxon-A.

2. Reproductive isolation among three species

Proportions of females inseminated are shown in Tab. 1. The three species showed the high rate of insemination, about 85% and more on the average in the intraspecific crosses. No female was inseminated in the crosses between *D. neohypocausta* and the others, showing the existence of complete ethological isolation between them. Taxon-A females were highly receptive to *D. hypocausta* males, giving the average rate of insemination of 67.1%. On the contrary, the reciprocal crosses gave the low rate of insemination, 13.4% of *D. hypocausta* females being inseminated.

Insemination tests were carried out also in the complete dark using *D. hypocausta* (R164) and Taxon-A (Y110). None of 100 females tested was found to be inseminated for 48 hours in intraspecific crosses respectively. Thus those two species are considered to require light in order to mate.

D. hypocausta females produced fertile F_1 females and sterile males when crossed to Taxon-A males. No the reciprocal cross produced viable F_1 offspring, whereas a few first instar larvae were found in cultures.

3. Duration of copulation

The mean duration of copulation was obtained on 20 - 25 pairs (N) each of the three species. The results are shown below. No significant

Species	Strain	N	Duration
D. neohypocausta	TW	25	9.7 ± 0.33 min.
D. hypocausta	R164	20	8.8 ± 0.51
Taxon-A	Y110	20	14.9 ± 0.70

difference in the mean value was found between *D. neohypocausta* and *D. hypocausta*, although the value of Taxon-A was significantly larger than those of the others ($P < 0.001$).

4. Courtship behaviors of three species

Schematic representations of courtship behaviors of the three species are shown in Fig. 2.

D. neohypocausta: The male sighted a female, approached, repeatedly tapped and moved behind her. He placed his head under the female wings, and drummed the middle dorsal surface of the female's abdomen with his forelegs persistetly for a few seconds. At the same time, the male continuously licked or tried to lick the female's genitalia. The receptive female gradually spread both wings to about 100° at its maximum, and finally permitted the male to mount. When the female was unreceptive, the male moved in front of the female with a crab-like sideway motion so that the male always sighted the female. While moving, the male always tried to tap her body. The male positioning himself in front of the female frequently thrusted at the female with his head. Thereafter, the male moved back behind the female. The male never displayed wing motion during courtship.

Tab. 1. Proportions of females inseminated for combinations among three species belonging to the *D. hypocausta* subgroup

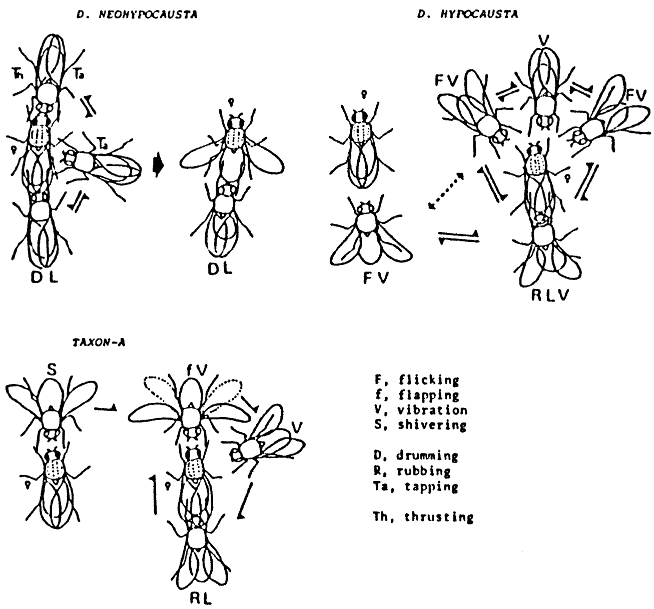
N, the number of females dissected; %In, the percentage of females inseminated.

Female \ Male		*D.hypocausta*						*Taxon-A*										*D.neohypo.*	
		R164		W103		Total		Y110		Y115		Z17		Z28		Total			
		N	%In.	N	%In.	N	%In.	N	%In.	N	%In.	N	%In.	N	%In.	N	%In.	N	%In.
D.hypo.	R164	110	98.2	100	75.0	210	87.1	100	14.0	100	12.0	100	22.0	100	0.0	400	12.0	50	0.0
	W103	100	80.0	100	85.0	200	82.5	100	24.0	100	14.0	100	17.0	100	4.0	400	14.8	50	0.0
	Total	210	89.5	200	80.0	410	84.9	200	19.0	200	13.0	200	19.5	200	2.0	800	13.4	100	0.0
Taxon-A	Y110	100	94.0	100	69.0	200	81.5	140	97.9	100	98.0	100	100.0	100	97.0	440	98.2	50	0.0
	Y115	100	71.0	100	54.0	200	62.5	100	69.0	145	77.2	100	65.0	100	77.0	445	72.6	50	0.0
	Z17	100	55.0	100	57.0	200	56.0	100	98.0	115	98.3	155	97.4	100	98.0	470	97.9	50	0.0
	Z28	100	76.0	100	61.0	200	68.5	110	93.6	120	98.3	100	91.0	175	89.7	505	92.9	50	0.0
	Total	400	74.0	400	60.3	800	67.1	450	90.4	480	91.9	455	89.5	475	90.3	1860	90.5	200	0.0
D.neohypo.		50	0.0	50	0.0	100	0.0	50	0.0	50	0.0	50	0.0	50	0.0	200	0.0	125	84.8

D. hypocausta: The male sighted a moving female, approached, repeatedly tapped, and frequently flicked both wings to about 80° and vibrated at the same time. The pulse sound emitted by flicking-vibration is referred to as the FVp sound. Then the male approached one female much more closely behind, placed his head under the female's wings, extended one wing to about 20 - 40°, vibrated both wings (the sine sound produced being referred to as the LVs), and finally attemp ed to mount. During wing vibration, the male continuously licked or tried to lick the female's genitalia, and rubbed the anterior lateral surface of the female's abdomen with his forelegs. Thus the female may receive the three, at least, different kinds of stimuli at the sa time from the courting male.

When the female was unreceptive and stayed stationarily, the mal moved in front of the female in a crab-like manner. Then he brought h body diagonally in front of the female's head and flicked one wing to about 40° and simultaneously vibrated both wings; the sine sound emit is referred to as the FVs sound. The male moved from one side (the l in Fig. 2) to the other (the right), and then behaved in the same man Between the bilateral FVs sounds, the hVs sound was emitted by vibrat of both wings without spreading. After repeating the frontal movemen several times, the male rapidly moved back behind the female. No apparent receptive behavior of the female was observed.

Fig. 2. Courtship behaviors of three species belonging to the *D. hypocausta* subgroup



Taxon-A: The male sighted the female, placed himself directly in front of the female's head. He extended his hind legs so that the body was inclined. The male raised and spread both wings to about 90°

and slightly shivered for several seconds in the inclined posture; the pulse sound emitted was hardly detected. Thereafter the male quickly flapped both wings to about 180° at its maximum three to four times, emitting the pulse sound, fVp. Just after several wing-flappings, the male spread one wing to about 20 - 40° and vibrated for a moment, producing the pulse sound, Vp, which was followed by the irregular sine sound, Vs. Then the male quickly moved behind the female in the crab-like manner, and displayed behaviors emitting either the Vs sound or the fVp + Vp + Vs sounds. The male tried to lick the female's genitalia and to rub the lateral surface of the female's abdomen with his forelegs in the head-under-wings posture, but these were less frequent compared with the male of *D. hypocausta*.

5. The nature of courtship sounds examined by means of the oscillograph and sonograph techniques

D. hypocausta: The male emitted four kinds of courtship sounds, FVs, hVs, FVp and LVs, which were clearly distinguished from each other and from those of Taxon-A males by oscilloscope patterns and sonograms (Fig. 3). The FVs sound was a sine sound lasting less than 50 msec, with the dominant frequency of about 400 Hz. The hVs sound was a sine sound with a low amplitude and with the dominant frequency of about 400 Hz which was slightly decreased as the sound proceeded. The FVp sound was a burst consisting of a train of pulses comprising 2 - 4 cycles. The average interpulse interval was 7.8 ± 0.2 msec and the average number of pulses per burst (PPB) was 15.2 ± 1.3 for R164. This sound clearly defined harmonic, with the fundamental frequency of about 80 Hz and with the dominant of about 500 Hz.

The LVs sound was a burst consisting of a train of sine sounds each of which (referring to as a unit) lasted 321.07 msec on the average, ranging between 227.34 and 454.26 msec for 10 males of R164. The average number of units per burst was 19.4 for 10 males of R164, giving that the total length of a burst was about 6 seconds. The frequency was changed within a unit of the sine sound as the sound proceeded, which started with a minimum frequency, reached a maximum and then returned to the minimum. The dominant frequencies were about 400 Hz and 800 Hz on the average, although the exact numerical analysis has not been completed.

Taxon-A: The male emitted three kinds of courtship sounds, fVp, Vp and Vs (Fig. 4). The fVp was always followed by the Vp sound with the average interval of 68.3 msec (for Y110). The Vs sound always followed immediately after the Vp. Thus a set of the three sounds was produced by the serial courtship behaviors including wing flapping, spreading and vibration, however it was not able to distinguish between behaviors emitting Vp and Vs. The sine sound with a low amplitude was detected when the male extended one wing and vibrated both wings in the head-under-wings posture behind the female. According to the oscilloscope pattern and sonograms, the nature of this sound was essentially the same as the Vs sound, but the former lasted longer than the latter. The fVp was consisted of 2 - 4 pulses (one pulse being emitted one wing flapping) each of 4 - 5 cycles, with the average interpulse inter-

Fig. 3. Oscilloscope patterns and sonograms of courtship sounds emitted by *D. hypocausta* male

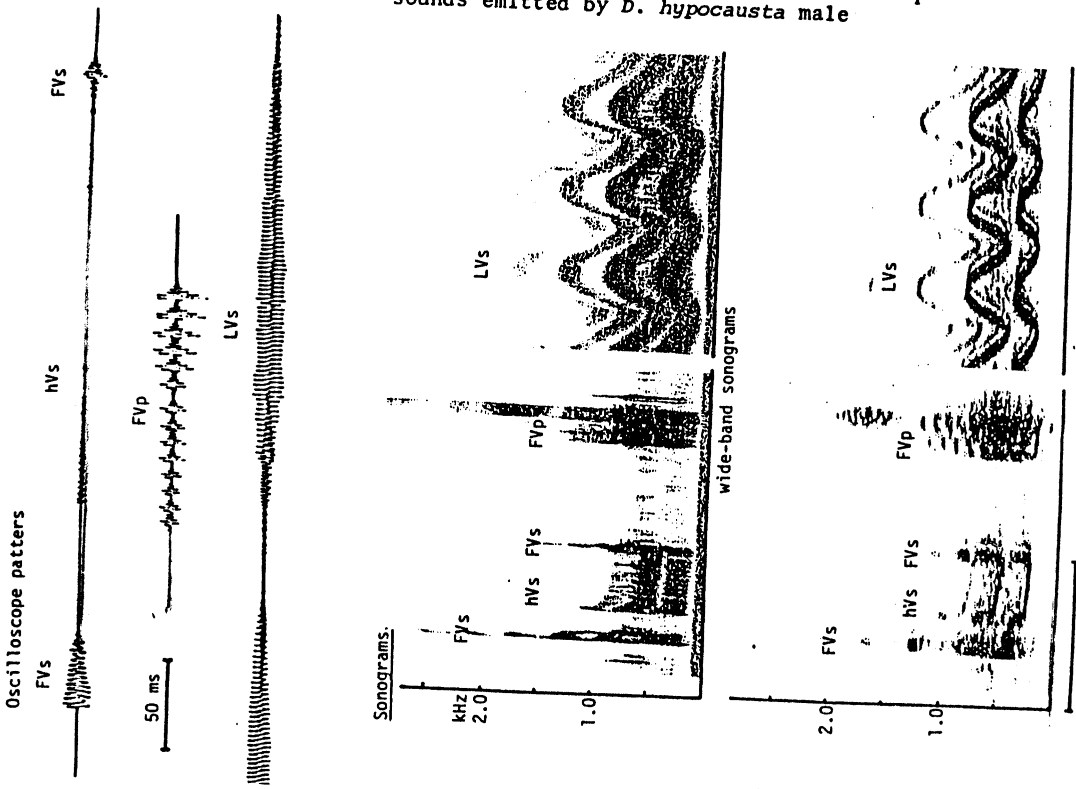
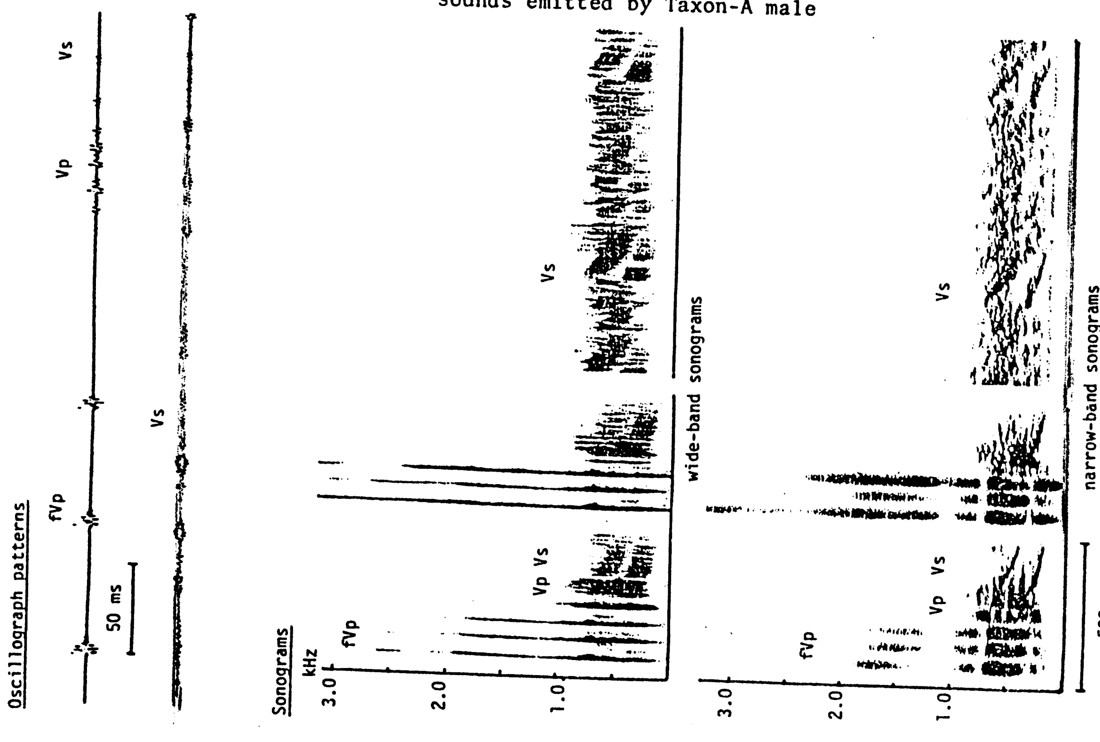


Fig. 4. Oscilloscope patterns and sonograms of courtship sounds emitted by Taxon-A male



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val of 68.3 msec. The Vp sound was a burst consisting of a train of pulses with the average interpulse interval of 12.3 msec and the average PPB of 7.2 for Y110. The numerical analysis of sonograms has not been completed.

DISCUSSION

According to the present study, a new species, Taxon-A which will be designated as *Drosophila siamana* by Hihara and Lin shortly, was added to the *D. hypocausta* subgroup as the eighth member.

Taxon-A is the closely related species of *D. hypocausta*. The male of the two species are readily distinguishable from each other by the degree of coloration of the body; the aged male of *D. hypocausta* is characterized by the black abdomen, thorax and legs, while the male of Taxon-A is with the dark brown abdomen, thorax and the brown legs. It is hard to distinguish the females of the two species from each other on the basis of external morphology. Some degrees of ethological isolation was found between them. *D. hypocausta* females were much more discriminatory in acceptance of males than Taxon-A females. This may be closely associated with the facts that *D. hypocausta* males emitted simultaneously the three kinds of stimuli at least which were released through licking, rubbing and wing vibration behind the female, although the male of Taxon-A less frequently displayed these behaviors.

Among the three species examined here, there are considerable differences in the nature of stimuli transmitting from one sex to the other during courtship. These stimuli may act on mate recognition and female receptivity. That is, each species has a species specific stimulus-response system, which is termed as a Specific Mate Recognition System (SMRS) by Paterson (1978).

For the three species, it may be true that visual stimuli are important to find and/or to discriminate the partner, showing that these species are completely depedent on the presence of light for copulation. Because no copulation occurred for 48 hours in the dark for *D. hypocausta*, Taxon-A and possibly *D. neohypocausta*. The importance of visual stimuli is suggested by the fact that the males of the three species moved around the female in a crab-like manner, always facing the female, during courtship.

Auditory stimuli may not be included in the SMRS of *D. neohypocausta*, since the male never showed wing displays during courtship. The male of this species persistently tried to physically contact with the female through tapping, thrusting and drumming motions when he was courting. The findings suggested that the essential stimuli responsible for mating success may be the chemicals as well as the visual one. Thus the courtship behaviors of *D. neohypocausta* are evidently different from those of the other two species.

It is of interest that there are significant differences in the courtship behaviors between *D. hypocausta* and Taxon-A which are thought to be the closely related species on the basis of morphology and the interspecific hybridization test. Usually, differences both in the behavioral pattern and in the nature of the stimulus are not qualitative but quantitative between the closely related species. For example, Spieth (1952) found differences in only three elements out of 19 visible courtship behaviors tested between *D. melanogaster* and *D. simulans*. Ewing and Bennet-Calrk (1968) did not reveal the difference both in the oscilloscope pattern and in the intrapulse frequency of the courtship sound but only in the interpulse interval between the two species mentioned above.

The genetic basis of the courtship behaviors was preliminarily examined using F_1 hybrid males and backcrossed hybrid males occurring between *D. hypocausta* and Taxon-A. The result suggests that the *hypocausta* type behaviors such as those emitting FVp and LVs sounds may be determined by autosomal dominant genes. The Taxon-A type behaviors were found only in the backcross generation, suggesting that the genes are autosomal recessive. Thus it is likely to consider that the difference in the genetic basis determining the courtship behaviors may cause the significant difference in courtship behaviors between the above two species. Genetic processes occurred during incipient speciation will be discussed after completing the analysis of the genetic basis of courtship behaviors.

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APPENDIX

A DESCRIPTION OF A NEW SPECIES

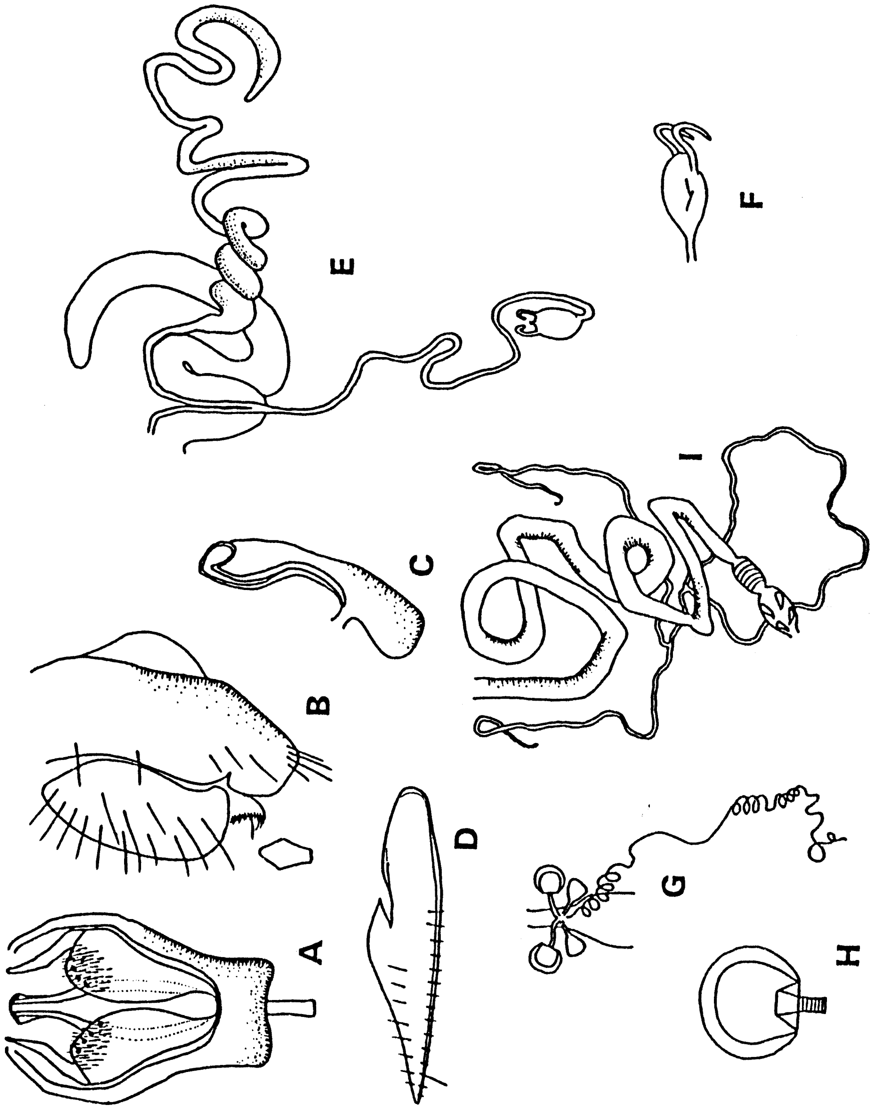
Drosophila siamana n. sp.

(Figure)

♂, ♀. Body about 3.0 mm in length. Eyes dark red, finely pilose. Frons reddish brown. Periorbit light brown. Carina light brown, flat above, narrow, lower portion wider, gradually shifting to buccal margin. Orbitals, anterior triangle dark brown, narrow. Antenna with 3rd joint reddish brown. Arista with branches long, 5 upper and 3 lower in addition to an irregularly branched folk. Orals 2, 2nd oral as long as vibrissa. Palpus brown, rounded at tip, with a few prominent and many shorter setae below. Cheek, 1/5 as broad as the greatest diameter of eye.

Mesoscutum and scutellum dull brown. Humerals 2. Acrostical hairs i 8 rows. Length distance of drosocentrals 1/3 cross distance. Sterno pleural bristles 3, sterno-index 0.7. Thoracic pleura of old male with dark brown patches, but they are absent in female and younger male.

Fig. *Drosophila siamana* n. sp.



A, phallic organs; B, periphallic organs; C, aedeagus; D, egg guide; E, male internal reproductive organs; F, ejaculatory bulb; G, female internal reproductive organs; H, spermathecae; I, digestive system.

Legs yellowish brown. Inner side of femur of foreleg with a row of about 9 stout bristles. Apical bristle on middle tibia, preapicals on all three tibiae; 2 prominent bristles on metatarsus of hind leg.

Wing slightly dusky, comparatively broad, veins light brown. Posterior crossvein clouded. R_{2+3} almost straight. R_{4+5} and M parallel. C-index about 4.0; 4V-index 1.2; 4C-index 1.9; 5X-index 1.0; Ac-index 1.5. C_1-bristle 1; C_3-fringe 1/2. Halter yellow.

Abdominal tergites dark brown in older male, dull light brown entirely in younger male, dull light brown with triangular brown caudal band on each anterior segment in female. Abdominal sternites quadrate, caudal tip of female 7th sternite separate into lateral flaps. Male genitalia as shown in Figs. A-C. Ovipositor (Fig. D): lobe reddish brown, narrowly pointed at tip, with about 13 marginal and 7 discal orange brown teeth. Testis (Fig. E) dark brown, with about 5 outer and 4 inner coils. Paragonia pale, thickened, folded about 1.5 times, basally fused to testicular duct; ejaculatory bulb (Fig. F) oval, with a pair of hook-shaped caeca. Spermatheca (Figs. G, H) brown and rounded; ventral receptacle with about 16 kinky coils. Mid intestine (Fig. I) with 2.5 -3.0 coils. Posterior branches of Malpighian tubules closely apposed with each other at tips, common stalks short.

Holotype ♂, in a stock, Y110, originated from a single fertilized female collected in Penang Island, Malaysia, 23. XI. 1979 (T. K. Watanabe, Y. Fuyama, F. Hihara). Type is deposited at Biological Institute, Ehime University.

Other specimens (in stocks) examined. Nakhon Nayok, Thailand, 3. XII. 1979 (Watanabe, Fuyama, Hihara), Kuchin, Malaysia, 13. XI. 1979 (Watanabe, Fuyama, Hihara), Chiang Mai, Thailand, 19. XII. 1981 (O. Kitagawa, F. Hihara).

Distribution. Malaysia (Mainland and Borneo), Thailand.

Relationships. This species closely resembles *D. hypocausta* in general, but differs from the latter in the shape of aedeagus and in having C_3-fringe larger and two prominent bristles on the metatarsus of the hind leg.

Remarks. One of the authors (Hihara) collected both the present species and *D. hypocausta* at the same dumping ground at the outskirts of Chiang Mai City of northern Thailand on Dec. 19, 1981. Thus these two species are regarded as sympatric in the geographical sense.