Jpn. J. Genet. (1982) 57, pp. 113-141

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Genetic studies of the *Drosophila nasuta* subgroup, with notes on distribution and morphology

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(*Received July* 28, 1981)

ABSTRACT

The evolutionary genetic studies of more than ten species or subspecies belonging to the *D. nasuta* subgroup lead the following conclusions:

Morphological differentiation has been observed qualitatively as well as quantitatively, but closely related species could not be identified by means of the techniques of external morphology.

By the hybridization tests, *D. nasuta*, Indian *D. nasuta*, *D. albomicans*, and *D. kepulauana* are closely related species, and four subspecies of *D. sulfurigaster* are close to *D. pulaua*. *D. kohkoa*, *D. pallidifrons* and Taxon-F are related species to *D. sulfurigaster*, but are differentiated from each other.

Judging from the allozyme analyses, the local populations of *D. nasuta* were genetically similar in two *Esterase* loci. The Chiangmai population was differentiated from other allopatric populations of *D. sulfurigaster albostrigata*.

The pattern of the speciation process in the *D. nasuta* subgroup was discussed.

1. INTRODUCTION

The *Drosophila nasuta* subgroup of the *Drosophila immigrans* species group consists of more than ten species or subspecies in various levels of the speciation process. Since the description of *D. nasuta* by Lamb (1914) from the Seychelles Islands, little attention was paid to this cluster of species until the extensive studies of Professor M. R. Wheeler and his colleagues (Wilson *et al.* 1969) who reported several new species and subspecies, and discussed the fertility of hybrids and their cytogenetic similarities and differences. Their studies showed that the subgroup consists of several species complexes, involving species, subspecies and semispecies.

During the last decade there have been reported of additional collections as well as the results from a variety of laboratory studies since this subgroup was recognized as providing excellent material for the study of speciation in *Dro-*

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sophila (Spieth 1969; Kanapi and Wheeler 1970; Nirmala and Krishnamurthy 1973, 1974; Ranganath and Krishnamurthy 1976, 1978, 1981; Lin *et al.* 1977; Thongmeearkom *et al.* 1977).

During this period, however, there was virtually no information regarding the "true" *D. nasuta*, since it had not been re-collected in the Seychelles, and its identification with samples from other areas was open to doubt. Fortunately, we have been able to make two well organized collecting trips into the critical areas, in 1971 and 1979, mostly in southeast Asia, the Indian Ocean area, as far as Kenya, Africa. Our first report (Wakahama and Kitagawa 1972) showed the karyotype of *D. nasuta* from Mahé, Seychelles, collected in 1971. The species has the typical karyotype of this subgroup as presented by Wilson *et al.* (1969). As we have accumulated more data on the members of this subgroup, using the newly collected material, we have found that the subgroup contains several cryptic or incipient species.

The purpose of this paper is to present the results of our studies on the genetic differences among the species and subspecies of the *D. nasuta* subgroup, and to clarify the relationship between two very closely related species, *D. nasuta* and *D. albomicans*.

2. DISTRIBUTION

The *D. nasuta* subgroup has a wide distribution area from Hawaii to Africa. In the Pacific area and in southeast Asia, the distributions of eight species or subspecies have been reported by Wilson *et al.* (1969). Since 1971, two of the present authors (K. I. W. and O. K.) and their colleagues collected flies belonging to this subgroup at many new localities including Seychelles, Kenya, Madagascar, Sri Lanka, and also Japan. Krishnamurthy and his colleagues collected *D. nasuta* and *D. sulfurigaster neonasuta* at Mysore and other localities in India (Ranganath and Krishnamurthy 1976, 1978). All the collection data on the distribution of the *D. nasuta* subgroup of flies are summarized in Fig. 1 and Table 1 (Wilson *et al.* 1969; Mather *et al.* 1974; Ranganath and Krishnamurthy 1978; Siddaveere Gowda *et al.* 1977; Lambert 1977; Mather and Thongmeearkom 1978; Clyde 1980 and our collection data).

Flies captured at Mahé, Seychelles in 1971 are believed to be the real *D. nasuta* as described by Lamb in 1914 (Wakahama and Kitagawa 1972). In 1979, a large number of *D. nasuta* were again captured in Mahé. The distribution of *D. nasuta* is widespread, from Mombasa, Kenya; Tananarive, Madagascar; Mahé, Seychelles; Mauritius Island; Reunion Island; Kandy, Sri Lanka; Mysore and other localities in India. Recently, David and Tsacas (1980) reported *D. nasuta* collections at Congo, Cameroun, and Dahomey on the west coast of Africa, and they have suggested that these flies have invaded that region quite recently by passive transportation. *D. nasuta* was also collected at

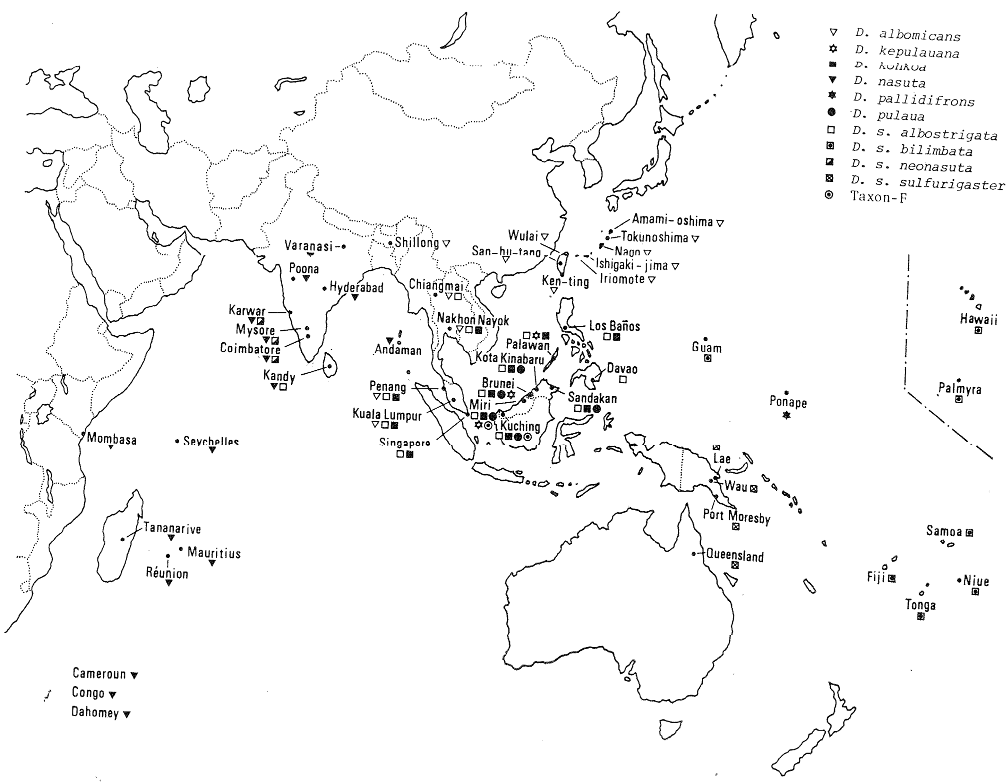

Fig. 1. Distribution of species and subspecies belonging to the *D. nasuta* subgroup. Symbols of species are shown in the figure.

Andaman Island, India (N. B. Krishnamurthy, personal communication).

Of *D. albomicans*, the north-most population is at Amami-oshima, Japan. On several Japanese islands of the Ryukyu Group, including Okinawa, *D. albomicans* was one of the dominant species. *D. albomicans* was also a dominant species in large areas of Taiwan (Lin *et al.* 1977 and our collection). Furthermore, this species coexists with *D. s. albostrigata* and also *D. kohkoa* in Thailand. Recently, Singh (1977) collected *D. albomicans* at Shillong, India. However, no one has reported *D. nasuta* and *D. albomicans* sympatrically.

Among the four subspecies of *D. sulfurigaster*, *D. s. albostrigata* was collected sympatrically with other members of this subgroup in their distribution, and was dominant in southeast Asia. In Kandy, Sri Lanka, *D. s. albostrigata* was found with *D. nasuta*. In Chiangmai and Nakhon Nayok, Thailand, *D. s. albostrigata* was collected with *D. albomicans*, and in Nakhon Nayok and in continental Malaysia, it occurs with *D. kohkoa*. *D. s. sulfurigaster* is one of the most common species in Papua, New Guinea, and *D. s. bilimbata* is widely scattered in many islands in the Pacific Ocean. *D. s. neonasuta* is the newly

Table 1. *Strains used in morphological and genetical analyses, and the main collection data of the D. nasuta subgroup*

Species	Symbols and TMU stock numbers	Locality collected	Date collected and collectors
D. nasuta	(1) KDY-105	Kandy, Sri Lanka	1971 a
	(2) KDY-154	Kandy, Sri Lanka	1971 a
	(3) SEZ-4	Mahé, Seychelles	1971 b
	(4) SEZ-11	Mahé, Seychelles	1971 b
	(5) MBA-31	Mombasa, Kenya	1971 b
	(6) MBA-32	Mombasa, Kenya	1971 b
	(7) TNR-34	Tananarive, Madagascar	1971 b
	(8) TNR-35	Tananarive, Madagascar	1971 b
		Mahé, Seychelles	1979 m
		Reunion Island	1979 m
		Mombasa, Kenya	1979 m
Indian *D. nasuta*	(9) 3253,1.1	Mysore, India	1971 c
	(10) 3253,1.4	Mysore, India	1971 c
		13 localities of Peninsular India	1971-1972 c
		Coimbatore, India	1979 m
		Mysore, India	1979 m
		Varanasi, India	1979 m
D. albomicans	(11) CNX-89	Chiangmai, Thailand	1971 b
	(12) CNX-105	Chiangmai, Thailand	1971 b
	(13) NGO-63	Nago, Japan	1973 d
	(14) NGO-43.28	Nago, Japan	1973 d
	(15) ISG-2	Ishigaki-jima, Japan	1973 e
	(16) ISG-7	Ishigaki-jima, Japan	1973 e
	(17) AMM-3	Amami-oshima, Japan	1973 f
	(18) AMM-5	Amami-oshima, Japan	1973 f
	(19) SHT-17	San-hu-tan, Taiwan	1971 g
	(20) SHT-18	San-hu-tan, Taiwan	1971 g
		Many localities of Taiwan	1971-1980 g
		Iriomote, Japan	1973 d
		Tokunoshima, Japan	1973 f
		Nakhon Nayok, Thailand	1977 d; 1979 n
		Chiangmai, Thailand	1977 d
		Shillong, India	1977 B. K. Singh
		Penang, Malaysia	1979 n
		Kuala Lumpur, Malaysia	1979 n

(to be continued)

Table 1. (*Continued*)

Species	Symbols and TMU stock numbers	Locality collected	Date collected and collectors
D. kepulauana	(21) 3056,8	Palawan, Philippines	1967 h
	(22) 3122,3	Brunei, Borneo	1968 i
		Kota Tinggi, Malaysia	1973 q
		Palawan, Philippines	1979 n
		Miri, Sarawak, Malaysia	1979 n
D. kohkoa	(23) KCH-179	Kuching, Sarawak, Malaysia	1971 b
	(24) KCH-225	Kuching, Sarawak, Malaysia	1971 b
	(25) SIN-86	Singapore, Singapore	1971 b
	(26) SIN-95	Singapore, Singapore	1971 b
	(27) KUL-190	Kuala Lumpur, Malaysia	1971 b
	(28) KUL-211	Kuala Lumpur, Malaysia	1971 b
		Brunei, Borneo	1971 b
		Kota Kinabalu, Sabah, Malaysia	1971 b; 1979 n
		Nakhon Nayok, Thailand	1977 d
		Los Baños, Luzon, Philippines	1979 n
		Sandakan, Sabah, Malaysia	1979 n
		Miri, Sarawak, Malaysia	1979 n
		Singapore, Singapore	1979 n
		Kuching, Sarawak, Malaysia	1979 n
		Kuala Lumpur, Malaysia	1979 n
		Penang, Malaysia	1979 n
D. pulaua	(29) BKI-24	Kota Kinabalu, Sabah, Malaysia	1971 b
	(30) BKI-28	Kota Kinabalu, Sabah, Malaysia	1971 b
	(31) BTN-111	Brunei, Borneo	1971 b
	(32) BTN-130	Brunei, Borneo	1971 b
		Sandakan, Sabah, Malaysia	1979 n
		Kota Kinabalu, Sarawak, Malaysia	1979 n
		Miri, Sarawak, Malasia	1979 n
		Kuching, Sarawak, Malaysia	1979 n

(to be continued)

Table 1. (*Continued*)

Species	Symbols and TMU stock numbers	Locality collected	Date collected and collectors
D. sulfurigaster albostrigata	(33) BTN-102	Brunei, Borneo	1971 b
	(34) BTN-168	Brunei, Borneo	1971 b
	(35) KCH-235	Kuching, Sarawak, Malaysia	1971 b
	(36) KCH-241	Kuching, Sarawak, Malaysia	1971 b
	(37) KDY-128	Kandy, Sri Lanka	1971 a
	(38) KDY-149	Kandy, Sri Lanka	1971 a
	(39) CNX-115	Chiangmai, Thailand	1971 b
	(40) CNX-147	Chiangmai, Thailand	1971 b
	(41) KUL-152	Kuala Lumpur, Malaysia	1971 b
	(42) KUL-209	Kuala Lumpur, Malaysia	1971 b
	(43) TAG	Tagaytay, Luzon, Philippines	1966 j
		Palawan, Philippines	1967 h; 1979 n
		Davao, Philippines	1968 p
		Kota Kinabalu, Sabah, Malaysia	1971 b; 1979 n
		Singapore, Singapore	1971 b; 1979 n
		Penang, Malaysia	1971 a; 1979 n
		Chiangmai, Thailand	1977 d
		Nakhon Nayok, Thailand	1977 d
		Kuala Lumpur, Malaysia	1979 n
		Kandy, Sri Lanka	1979 m
		Los Baños, Luzon, Philippines	1979 n
		Sandakan, Sabah, Malaysia	1979 n
		Miri, Sarawak, Malaysia	1979 n
		Kuching, Sarawak, Malaysia	1979 n
D. sulfurigaster bilimbata	(44) PAL	Palmyra, Line Island	1962 k
	(45) HAW	Hawaii	1966 k
		Tutuila, American Samoa	1962 k; 1965 r
		Tongatapu, Tonga	1963 k
		Savaii, Samoa	1965 r; 1967 k
		Upolu, Samoa	1965 r
		Niue Island	1965 k
		Oahu, Hawaii	1966 W. S. Stone and D. E. Hardy; J. Grossfield 1967; S. Rockwood; o
		Maui, Hawaii	1966 W. S. Stone and H. T. Spieth; 1967 H. T. Spieth
		Viti Levu, Fiji	1966 s; 1967 k; 1968 s
		Guam, Marianas Island	1968 H. L. Carson

(to be continued)

Table 1. (*Continued*)

Species	Symbols and TMU stock numbers	Locality collected	Date collected and collectors
D. sulfurigaster neonasuta		Mysore, India	1971 c
		6 localities of Peninsular India	1976 c
		Coimbatore, India	1979 m
D. sulfurigaster sulfurigaster	(46) POM-22	Port Moresby, Papua, New Guinea	1968 p
	(47) POM-101	Port Moresby, Papua, New Guinea	1977 l
	(48) WAU-152	Wau, Papua, New Guinea	1977 l
	(49) WAU-164	Wau, Papua, New Guinea	1977 l
		Queensland, Australia	1955 q
		Wau, Papua, New Guinea	1979 n
		Port Moresby, Papua, New Guinea	1979 n
		Lae, Papua, New Guinea	1979 n
D. pallidifrons	(50) 2535,4	Ponape, Caroline Island	1959 k; 1966 H. T. Spieth
Taxon-F	(51) KCH-208	Kuching, Sarawak, Malaysia	1971 b
	(52) KCH-223	Kuching, Sarawak, Malaysia	1971 b
		Miri, Sarawak, Malaysia	1979 n

The numbers in parentheses are corresponded to the numbers in Figs. 2 and 3.

Symbols of collectors: a: T. Okada, H. Kurokawa and H. Ikeda; b: K. I. Wakahama, T. Watanabe and O. Kitagawa; c: H. A. Ranganath and N. B. Krishnamurthy, L. Siddaveere Gowda *et al.*; d: O. Kitagawa and H. Ikeda; e: O. Kitagawa and Y. Fuyama; f: O. Kitagawa and K. I. Wakahama; g: F. J. Lin; h: M. Delfinado; i: D. E. Hardy and M. Delfinado; j: L. H. Throckmorton and F. J. Lin; k: W. S. Stone and M. R. Wheeler; l: T. Okada; m: O. Kitagawa, H. Ikeda and A. Fukatami; n: T. K. Watanabe, F. Hihara and Y. Fuyama; o: C. Kanapi; p: K. Moriwaki; q: W. B. Mather; r: W. S. Stone and C. Oliver; s: M. R. Wheeler.

The flies, which are Nos. 9, 10, 21, 22, 43, 44, 45, and 50, were kindly supplied by Professors W. S. Stone and M. R. Wheeler, the University of Texas, to O. Kitagawa.

found species at Mysore and its vicinity in India (Ranganath and Krishnamurthy 1976). These four subspecies of *sulfurigaster* have only been found allopatrically.

According to our collection data, in Miri, Sarawak, Malaysia, five species, i.e., *D. s. albostrigata*, *D. kohkoa*, *D. pulaua*, *D. kepulauana*, and Taxon-F, of this subgroup have been collected. This seems to be a reason to suggest that Borneo is near the place of the origin of this subgroup, and that there has since been dispersal eastward to Hawaii and westward to Africa, with further speciation occurring along the way. *D. pulaua* was only collected in Borneo, and *D. kepulauana* was found at Palawan, Philippines and Brunei and Miri in Borneo. *D. kohkoa* was collected in this area, and also occurs in continental Malaysia and in Philippines. *D. pallidifrons* is only known from Ponape, Caroline Islands (Wilson *et al.* 1969).

Taxon-F is the tentative name of a new species which was collected only at Kuching and Miri (Sarawak, Malaysia) in 1971 and 1979, respectively, but the description of the species has not been published yet. In Lae (Papua, New Guinea) and in Kuala Lumpur and Penang (continental Malaysia), some additional new species belonging to this subgroup were collected in 1979. The descriptions of these new species will be published in a later article.

3. MATERIALS AND METHODS

I) *Measurement of metric characters and the principal component analysis*: Fifty-two lines from ten species or subspecies were analyzed for their metric characters (Table 2). With few exceptions, two iso-female lines per locality were used. These lines were maintained with the usual cornmeal-molasses-agar medium under uncrowded conditions. For each line, twenty five females and 25 males were randomly sampled and their metric characters were investigated. First, bristles were counted, then the left wing and the left fore leg of a given fly were picked off to make preparations by Eukitt. Fifteen characters measured in this experiment were femur length, tibia length, first tarsus length, wing length, wing width, various wing indices (Costal index, 4C index, 4th-vein index, 5X index and C3 fringe), the wing length/wing width ratio, the number of bristles on the 4th and 5th abdominal segments, the number of spinules, and the number of sternopleurals (Fig. 2).

For both sexes, means and standard errors of each line were calculated and analysis of variance was made for each character. Then the principal component analysis was carried out based on the means of 52 lines for males and females separately. All computation was done by using a computer, FACOM 230-0S2.

II) *Hybridization test*: A series of reciprocal crosses was carried out, when

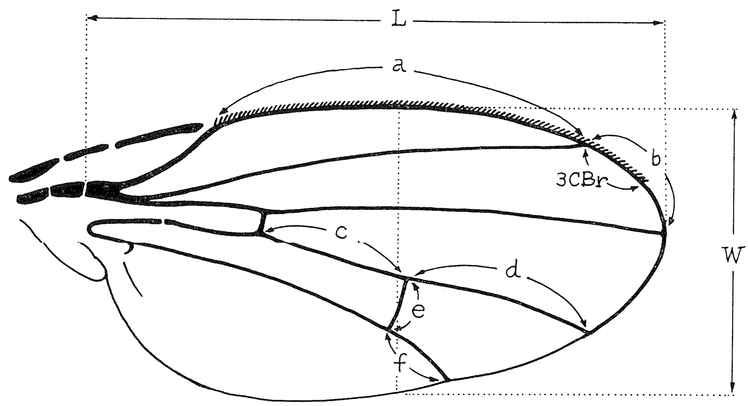

Fig. 2. Wing indices measured for metric characters.

possible, among the species and subspecies belonging to this subgroup. New materials collected in 1977 and 1979 were mainly used for this test. Virgin females and males were sorted within three hours of eclosion and aged for five to seven days before mating. Several virgin females and males were put together in a standard shell vial (3 cm diameter, 11 cm long); where the first crosses were sterile for 5 days, flies were transferred into fresh vials twice in every five days. After fifteen days in each mating test where no larvae appeared, the results were designated as sterile crosses. When F_1 flies were obtained, F_1 ♀♀ × F_1 ♂♂ crosses were made to test for F_1 fertility. If the above crosses sterile, four kinds of backcrosses were made. In these crosses, the same procedure was followed as for the parental crosses unless 10 females and 10 males were used for one vial.

III) *Allozyme analysis in Esterase loci*: The method of electrophoresis used in this experiment was the thin layer (0.7 mm thick) agar gel electrophoresis mentioned by Ohba and Sasaki (1968). A mixed substrate (2% α- and 0.5% β-naphtyl acetate aceton solution) was used, then allozymes of both *Est-*α and *Est-*β loci were simultaneously investigated on a agar plate. All materials used for the allozyme analysis were laboratory stocks kept by small mass culture for two to eight generations at 25°C constant temperature rooms. At most 10 individuals of each isofemale line were investigated.

IV) *Sexual isolation and insemination test*: Four *D. nasuta* strains (the Mahé, Mombasa, Tananarive, and Kandy populations), four Japanese *albomicans* strains (Amami-oshima, Tokunoshima, Nago and Ishigaki-jima), one Taiwanese *albomicans* and one Thai *albomicans* were used for estimating the sexual isolation. Each strain was established by mixing 4–10 isofemale lines. All

Table 2. *Metric characters for*

			Isofemale lines	Femur*	Tibia*	First tarsus*	Wing length*	Wing width*
D. nasuta	Kandy, Sri Lanka	(1)	KDY-105	6.28	5.70	2.90	21.61	9.85
		(2)	KDY-154	6.68	6.01	3.07	22.01	9.87
	Mahé, Seychelles	(3)	SEZ-4	6.57	5.97	3.07	21.56	9.87
		(4)	SEZ-11	6.58	5.89	3.07	21.29	9.72
	Mombasa, Kenya	(5)	MBA-31	6.70	5.80	3.28	22.34	10.08
		(6)	MBA-32	6.55	5.90	3.12	22.53	10.15
	Tananarive, Madagascar	(7)	TNR-34	6.63	5.94	3.17	21.84	10.10
		(8)	TNR-35	6.27	5.88	3.03	20.78	9.34
	Mysore, India	(9)	3253,1.1	6.63	6.08	3.19	21.80	9.96
		(10)	3253,1.4	6.50	5.95	3.09	21.31	9.70
D. albomicans	Chiangmai, Thailand	(11)	CNX-89	6.62	5.98	3.20	22.32	9.81
		(12)	CNX-105	6.42	5.94	3.14	21.84	9.52
	Nago, Japan	(13)	NGO-63	6.69	5.93	3.31	22.64	10.28
		(14)	NGO-43.28	6.69	6.14	3.29	22.99	10.10
	Ishigaki-jima, Japan	(15)	ISG-2	6.31	5.72	2.98	21.78	9.75
		(16)	ISG-7	6.42	5.74	2.90	22.02	9.83
	Amami-oshima, Japan	(17)	AMM-3	6.33	5.72	3.07	21.82	9.82
		(18)	AMM-5	6.31	5.72	3.11	21.58	9.52
	San-hu-tang, Taiwan	(19)	SHT-17	6.60	5.95	3.34	22.52	10.12
		(20)	SHT-13	6.58	5.99	3.18	22.45	10.35
D. kepulauana	Palawan, Philippines	(21)	3056,8	6.06	5.53	2.89	20.13	9.08
	Brunei, Borneo	(22)	3122,3	6.42	5.86	3.09	21.05	9.10
D. kohkoa	Kuching, Malaysia	(23)	KCH-179	6.04	5.27	2.92	20.33	8.79
		(24)	KCH-225	6.26	5.63	3.10	22.44	9.91
	Singapore	(25)	SIN-86	6.44	5.57	3.10	21.63	9.75
		(26)	SIN-95	6.28	5.66	3.06	22.06	9.31
	Kuala Lumpur, Malaysia	(27)	KUL-190	6.35	5.66	3.14	21.60	9.49
		(28)	KUL-211	6.08	5.59	3.04	20.22	9.01
D. pulaua	Kota Kinabaru, Malaysia	(29)	BKI-24	6.33	5.77	3.11	21.37	9.64
		(30)	BKI-28	6.39	5.76	3.09	21.85	9.66
	Brunei Town, Brunei	(31)	BTN-111	6.27	5.72	2.90	20.21	8.91
		(32)	BTN-130	6.50	5.87	2.94	22.10	9.61
D. s.albostrigata	Brunei Town, Brunei	(33)	BTN-102	6.44	6.00	2.97	22.26	9.54
		(34)	BTN-168	6.12	5.73	3.01	21.27	9.55
	Kuching, Malaysia	(35)	KCH-235	6.04	5.52	2.82	21.03	8.98
		(36)	KCH-241	6.45	6.04	2.99	21.44	9.57
	Kandy, Sri Lanka	(37)	KDY-128	6.61	6.03	3.08	21.81	9.43
		(38)	KDY-149	6.46	5.83	3.03	21.20	9.59
	Chiangmai, Thailand	(39)	CNX-115	6.55	5.91	3.08	22.39	10.49
		(40)	CNX-147	6.47	5.83	3.07	22.40	9.78
	Kuala Lumpur, Malaysia	(41)	KUL-152	6.17	5.57	2.71	20.26	9.00
		(42)	KUL-209	6.43	5.73	2.88	21.44	9.59
	Luzon, Philippines	(43)	TAG	6.20	5.72	2.92	21.25	9.21
D. s.bilimbata	Palmyra, Line Is.	(44)	PAL	6.36	5.62	2.83	21.00	9.61
	Hawaii, U.S.A.	(45)	HAW	6.43	5.81	2.90	20.95	10.04
D. s.sulfurigaster	Port Moresby, Papua New Guinea	(46)	POM-22	6.20	5.77	2.96	21.60	9.74
		(47)	POM-101	6.62	6.05	3.00	22.59	10.10
	Wau, Papua New Guinea	(48)	WAU-152	6.62	6.13	3.17	22.73	10.37
		(49)	WAU-164	6.59	6.15	3.11	22.25	10.04
D. pallidifrons	Ponape, Caroline Is.	(50)	2535,4	6.12	5.52	2.80	20.99	9.16
Taxon-F	Kuching, Malaysia	(51)	KCH-208	6.33	5.57	2.99	20.75	9.62
		(52)	KCH-223	6.01	5.36	2.90	21.07	9.30

* 1unit=100μ

males of the D. nasuta subgroup

A/D	B/C	D/C	F/E	3CBr/B	WL/WW	No. of abd bristle 4th	No. of abd bristle 5th	No of spin.	No of stern.
3.15	0.77	1.41	1.07	0.59	2.19	32.08	47.64	7.36	10.12
3.43	0.72	1.36	1.07	0.53	2.23	30.60	45.44	7.72	10.08
3.20	0.76	1.46	1.21	0.52	2.18	27.44	44.44	8.08	10.92
3.15	0.78	1.39	1.03	0.46	2.19	32.24	41.72	8.72	10.12
3.51	0.74	1.52	1.13	0.53	2.22	32.96	49.20	8.52	11.84
3.58	0.71	1.50	1.10	0.52	2.22	27.40	42.28	8.08	12.20
3.54	0.72	1.47	1.08	0.50	2.16	29.08	41.04	7.56	10.04
3.41	0.71	1.41	1.15	0.53	2.23	31.32	49.76	9.60	9.92
3.20	0.83	1.51	1.11	0.52	2.19	27.96	38.44	9.64	8.20
3.19	0.83	1.53	1.15	0.53	2.20	26.48	40.04	10.20	10.36
3.10	0.80	1.50	1.24	0.54	2.28	30.76	47.28	10.08	10.76
3.45	0.79	1.60	1.23	0.50	2.29	25.56	35.72	9.56	10.52
3.39	0.76	1.43	1.13	0.52	2.20	30.76	43.56	9.52	11.12
3.69	0.68	1.38	1.21	0.51	2.28	29.28	38.72	9.04	10.52
3.34	0.77	1.41	1.10	0.50	2.24	30.36	44.96	8.44	10.60
3.35	0.76	1.41	1.11	0.47	2.24	28.68	42.44	8.64	10.76
3.58	0.68	1.36	1.09	0.49	2.22	29.16	41.60	9.04	11.20
3.34	0.75	1.40	1.15	0.50	2.27	27.48	39.80	8.64	10.40
3.33	0.77	1.50	1.15	0.55	2.23	32.72	49.48	8.60	11.56
3.30	0.80	1.54	1.21	0.54	2.17	31.96	49.08	9.48	11.76
3.09	0.88	1.66	1.30	0.63	2.22	33.04	47.00	8.84	9.64
3.28	0.77	1.41	1.14	0.54	2.31	27.32	46.00	10.68	10.20
3.61	0.73	1.57	1.30	0.59	2.31	26.56	38.40	10.92	11.60
3.28	0.79	1.60	1.08	0.54	2.26	25.64	37.72	8.88	9.88
3.59	0.74	1.52	1.22	0.48	2.22	23.76	34.68	8.68	8.76
3.10	0.81	1.46	1.10	0.51	2.37	24.84	34.80	8.92	9.24
3.53	0.74	1.45	1.10	0.54	2.28	25.64	37.04	9.36	10.52
3.24	0.78	1.50	1.17	0.57	2.24	26.00	37.36	9.12	11.48
3.20	0.88	1.57	1.32	0.52	2.22	24.44	35.12	9.16	11.16
3.08	0.84	1.59	1.26	0.54	2.26	23.00	36.08	9.44	9.48
3.46	0.78	1.56	1.23	0.55	2.27	24.08	33.84	9.16	9.84
3.01	0.81	1.41	1.21	0.53	2.30	27.92	37.84	9.16	11.12
3.51	0.77	1.53	1.41	0.50	2.33	28.92	43.44	9.64	11.72
3.64	0.72	1.40	1.15	0.47	2.23	26.08	39.76	8.84	10.72
3.42	0.71	1.31	1.02	0.48	2.34	19.92	30.52	8.60	8.52
3.29	0.79	1.47	1.35	0.54	2.24	26.72	39.16	9.88	9.68
3.24	0.77	1.36	1.30	0.47	2.31	28.08	42.96	9.60	12.96
3.32	0.74	1.33	1.09	0.44	2.21	30.68	50.00	7.48	12.56
3.25	0.82	1.47	1.16	0.49	2.13	29.96	44.44	9.40	11.12
3.63	0.76	1.48	1.26	0.51	2.29	30.40	39.12	8.08	10.56
3.52	0.70	1.28	1.17	0.46	2.25	24.76	38.40	9.48	8.32
3.25	0.80	1.46	1.16	0.43	2.24	26.04	40.32	9.08	10.80
3.38	0.72	1.36	1.24	0.48	2.31	27.48	43.88	9.88	10.92
2.68	0.93	1.51	1.14	0.52	2.19	27.16	42.04	8.76	11.84
2.79	0.91	1.51	1.13	0.49	2.09	27.68	39.24	9.08	10.32
3.04	0.83	1.44	1.20	0.49	2.22	26.36	36.64	9.84	10.56
3.27	0.75	1.40	1.20	0.50	2.24	29.12	44.16	10.88	11.92
3.10	0.79	1.42	1.11	0.50	2.19	26.56	39.40	10.52	11.20
3.20	0.77	1.44	1.20	0.51	2.22	26.60	38.60	10.00	11.00
3.37	0.74	1.45	1.32	0.49	2.29	29.80	36.32	10.08	9.56
2.89	0.87	1.54	1.10	0.51	2.16	29.28	28.84	8.24	12.20
2.97	0.80	1.43	1.27	0.52	2.27	32.28	42.04	8.64	12.60

Table 3. *Metric characters for*

		Isofemale lines		Femur*	Tibia*	First tarsus*	Wing length*	Wing width*
D. nasuta	Kandy, Sri Lanka	(1)	KDY-105	6.83	6.07	3.19	24.90	11.33
		(2)	KDY-154	6.98	6.36	3.30	25.29	11.28
	Mahé, Seychelles	(3)	SEZ-4	6.78	6.16	3.24	24.75	11.14
		(4)	SEZ-11	6.90	6.26	3.29	24.36	10.99
	Mombasa, Kenya	(5)	MBA-31	7.11	6.30	3.47	25.69	11.52
		(6)	MBA-32	6.86	6.09	3.38	25.76	11.54
	Tananarive, Madagascar	(7)	TNR-34	6.88	6.17	3.45	24.86	11.40
		(8)	TNR-35	6.24	5.64	3.07	23.13	10.29
	Mysore, India	(9)	3253,1.1	6.77	6.23	3.35	25.14	11.31
		(10)	3253,1.4	6.78	6.26	3.32	24.24	10.99
D. albomicans	Chiangmai, Thailand	(11)	CNX-89	7.00	6.37	3.38	25.33	11.25
		(12)	CNX-105	6.52	6.00	3.28	23.92	10.85
	Nago, Japan	(13)	NGO-63	6.95	6.32	3.64	25.84	11.76
		(14)	NGO-43.28	6.91	6.28	3.39	25.27	11.35
	Ishigaki-jima, Japan	(15)	ISG-2	6.90	6.14	3.34	24.65	11.28
		(16)	ISG-7	6.82	6.06	3.07	24.75	11.08
	Amami-oshima, Japan	(17)	AMM-3	6.63	5.88	3.32	24.45	11.07
		(18)	AMM-5	6.54	5.93	3.30	24.35	11.04
	San-hu-tang, Taiwan	(19)	SHT-17	6.99	6.25	3.50	25.90	11.60
		(20)	SHT-18	7.10	6.39	3.47	25.89	11.89
D. kepulauana	Palawan, Philippines	(21)	3056,8	6.48	5.97	3.23	23.17	10.44
	Brunei, Borneo	(22)	3122,3	6.89	6.22	3.30	24.41	10.73
D. kohkoa	Kuching, Malaysia	(23)	KCH-179	6.42	5.61	3.20	22.75	9.90
		(24)	KCH-225	6.70	6.17	3.25	24.67	11.46
	Singapore	(25)	SIN-86	6.96	5.91	3.25	24.08	11.05
		(26)	SIN-95	6.56	5.96	3.28	24.25	10.45
	Kuala Lumpur, Malaysia	(27)	KUL-190	6.80	6.05	3.43	24.21	10.85
		(28)	KUL-211	6.39	5.66	3.26	22.30	10.03
D. pulaua	Kota Kinabaru, Malaysia	(29)	BKI-24	6.83	6.25	3.45	24.94	11.07
		(30)	BKI-28	6.89	6.20	3.41	25.67	11.23
	Brunei Town, Brunei	(31)	BTN-111	6.86	6.22	3.26	23.65	10.28
		(32)	BTN-130	6.82	6.23	3.23	25.27	10.90
D. s.albostrigata	Brunei Town, Brunei	(33)	BTN-102	6.90	6.28	3.24	25.12	10.78
		(34)	BTN-160	6.41	5.88	3.33	24.03	10.70
	Kuching, Malaysia	(35)	KCH-235	6.38	5.84	3.13	24.32	10.49
		(36)	KCH-241	6.78	6.24	3.17	24.05	10.72
	Kandy, Sri Lanka	(37)	KDY-128	6.64	6.17	3.30	24.78	10.70
		(38)	KDY-149	6.82	6.15	3.32	24.61	10.86
	Chiangmai, Thailand	(39)	CNX-115	6.70	6.08	3.28	25.12	11.68
		(40)	CNX-147	6.75	6.15	3.26	25.53	11.19
	Kuala Lumpur, Malaysia	(41)	KUL-152	6.50	5.78	3.12	22.95	10.17
		(42)	KUL-209	6.53	5.90	3.07	24.31	10.74
	Luzon, Philippines	(43)	TAG	6.80	6.16	3.20	24.48	10.48
D. s.bilimbata	Palmyra, Line Is.	(44)	PAL	6.79	6.06	3.16	24.54	11.06
	Hawaii, U.S.A.	(45)	HAW	6.89	6.21	3.25	24.66	11.63
D. s.sulfurigaster	Port Moresby, Papua New Guinea	(46)	POM-22	6.61	6.04	3.21	24.70	10.93
		(47)	POM-101	7.08	6.50	3.34	26.09	11.48
	Wau, Papua New Guinea	(48)	WAU-152	6.94	6.43	3.43	26.55	11.72
		(49)	WAU-164	7.02	6.43	3.36	25.82	11.41
D. pallidifrons	Ponape, Caroline Is.	(50)	2535,4	6.69	6.00	2.95	24.54	11.01
Taxon-F	Kuching, Malaysia	(51)	KCH-208	6.62	5.92	3.12	22.76	10.53
		(52)	KCH-223	6.33	5.69	3.08	23.16	10.58

* 1unit=100μ

females of the D. nasuta subgroup

A/D	D/C	D/C	Γ/C	3CDr/D	WL/WW	No. of abd. bristle 4th	5th	No. of spin.	No. of stern.
3.20	0.79	1.47	1.09	0.54	2.20	27.16	24.72	7.24	11.12
3.48	0.71	1.41	1.05	0.50	2.24	20.64	18.00	7.84	11.48
3.36	0.73	1.46	1.19	0.52	2.22	26.40	23.04	7.88	10.76
3.41	0.75	1.43	0.99	0.45	2.22	27.36	26.36	8.76	11.20
3.74	0.73	1.57	1.17	0.48	2.23	24.76	21.92	7.84	13.48
3.76	0.68	1.52	1.15	0.54	2.23	22.24	21.04	7.96	13.52
3.84	0.67	1.53	1.06	0.52	2.18	26.60	23.00	7.76	10.64
3.41	0.71	1.40	1.09	0.53	2.25	23.72	20.52	9.00	10.80
3.37	0.78	1.54	1.09	0.53	2.22	20.76	19.20	9.00	8.52
3.31	0.80	1.59	1.12	0.51	2.21	21.92	19.44	10.20	11.08
3.44	0.71	1.42	1.14	0.54	2.25	19.80	16.84	9.36	11.16
3.37	0.82	1.56	1.12	0.46	2.20	19.92	16.88	9.44	11.04
3.49	0.74	1.49	1.06	0.56	2.20	28.00	24.52	9.28	11.36
3.84	0.70	1.45	1.17	0.53	2.23	23.80	22.84	9.28	11.04
3.36	0.80	1.48	1.00	0.52	2.19	26.24	22.84	9.64	12.76
3.51	0.75	1.44	1.16	0.51	2.24	22.00	18.80	8.84	11.80
3.55	0.74	1.49	1.10	0.45	2.21	22.00	20.52	9.12	12.16
3.37	0.76	1.50	1.21	0.50	2.21	22.00	21.20	8.80	11.56
3.55	0.74	1.52	1.12	0.54	2.23	26.56	23.76	8.76	12.16
3.59	0.77	1.56	1.18	0.50	2.18	30.64	26.88	9.44	13.40
3.09	0.90	1.70	1.29	0.57	2.22	18.16	17.04	8.88	10.72
3.29	0.70	1.46	1.12	0.51	2.27	21.04	18.04	10.80	19.92
3.88	0.69	1.60	1.28	0.59	2.28	19.36	17.16	11.04	13.20
3.31	0.84	1.64	1.07	0.49	2.15	20.96	17.36	9.28	10.60
3.78	0.69	1.55	1.20	0.53	2.18	18.56	16.88	8.60	9.92
3.26	0.80	1.51	1.08	0.51	2.32	19.96	17.12	8.28	9.36
3.76	0.71	1.49	1.09	0.53	2.23	17.92	16.44	9.52	12.32
3.30	0.78	1.53	1.15	0.56	2.22	17.52	16.08	9.24	12.40
3.36	0.78	1.57	1.29	0.50	2.25	18.48	16.80	9.96	12.20
3.27	0.81	1.62	1.23	0.54	2.29	19.72	17.32	9.08	10.60
3.89	0.71	1.56	1.22	0.54	2.30	19.76	17.20	8.92	9.80
3.32	0.75	1.43	1.18	0.54	2.32	19.12	16.72	9.00	11.60
3.77	0.75	1.56	1.38	0.51	2.33	21.48	18.96	9.36	12.96
3.82	0.69	1.40	1.15	0.48	2.25	17.60	16.96	8.40	10.24
3.64	0.69	1.36	1.04	0.44	2.32	16.56	16.12	9.12	10.16
3.60	0.74	1.47	1.33	0.54	2.25	21.56	20.00	9.96	9.28
3.49	0.73	1.41	1.24	0.47	2.32	19.84	18.92	8.40	12.72
3.47	0.72	1.35	1.07	0.45	2.27	22.44	21.28	7.48	13.16
3.32	0.80	1.52	1.18	0.48	2.15	23.30	21.24	9.00	10.96
3.82	0.74	1.56	1.28	0.48	2.28	18.72	17.88	7.84	11.64
3.58	0.67	1.30	1.15	0.47	2.26	19.04	18.88	9.24	9.12
3.39	0.77	1.49	1.14	0.44	2.26	22.48	21.36	8.68	10.76
3.58	0.69	1.37	1.29	0.44	2.34	21.96	19.64	10.08	10.84
2.97	0.88	1.53	1.10	0.49	2.22	20.64	19.04	9.12	12.88
2.86	0.91	1.53	1.09	0.48	2.12	21.04	18.68	8.92	10.56
3.37	0.78	1.52	1.23	0.48	2.26	20.24	17.64	9.36	11.64
3.54	0.72	1.45	1.18	0.51	2.27	26.16	23.04	11.08	11.84
3.50	0.72	1.45	1.11	0.49	2.27	22.60	19.96	10.12	11.24
3.43	0.74	1.50	1.18	0.52	2.26	24.88	22.44	9.72	11.00
3.54	0.73	1.46	1.37	0.46	2.23	25.60	17.72	10.60	11.24
3.05	0.83	1.54	1.12	0.50	2.16	20.88	17.56	8.40	12.84
3.13	0.76	1.43	1.31	0.49	2.19	22.24	18.56	9.24	13.60

possible combinations were investigated by means of direct observation of the so-called male-choice method. Ten females of one strain, each with a small artificial mark on the mesonotum, and ten females of another strain, unmarked, were put together with fifteen males of one of these strains in a shell vial. Whenever matings occurred, the mating pairs were removed by an aspirator and the mating combination was checked immediately. Released females were reserved for the insemination test if needed. All flies had earlier been anesthetized by CO_2 gas for sexing within three hours after their emergence and the next day the females were marked during light etherization or cold temperature techniques. The females without marking were also etherized in the same manner. Observations were continued until ten matings occurred or for one hour, maximum. The released females after mating were dissected for the insemination test.

4. RESULTS

Comparisons of metric characters by analysis of variance and the principal component analysis: Analysis of variance showed that the differences between species were significant in the following characters in both sexes; femur length, tibia length, wing length, wing width, Costal index, 4C index, 5X index, C3 fringe, wing length/wing width ratio and the number of abdominal bristles. Differences between localities within a species were not significant in all characters except the first tarsus length and the number of sternopleurals in both sexes. Differences between species as well as between lines within locality were significant in all characters in both sexes. Means of each character in each line are shown separately in Tables 2 and 3, for males and females, respectively.

For the principal component analysis, eigen values of the principal components were larger than 1 in the first 5 components in both sexes, and the cumulative percentage of the first 5 components to the total variance shared 77.1% in females and 79.6% in males. The cumulative percentage of the first 3 components to the total variance became 60.5% in females and 59.9% in males and then these components were used for the analysis.

Table 4 shows factor loadings for each principal component. In the first component, wing length, wing width, and the number of abdominal bristles showed high positive correlation to the component. This components should be a size factor, and these characters seem to increase with the increase of their body size. In the second component, Costal index and wing length/wing width ratio were positively correlated and that of 4C index was negatively correlated to the component. It is suggested that the basal parts of wings, a and c in Fig. 2, increase when wing length increases, and thus the second component is the shape factor of the wing. In the third component, factor

Table 4. *Factor loadings of the principal components*

	Component I		Component II		Component III	
	Female	Male	Female	Male	Female	Male
Femur	0.857	0.892	0.113	0.062	0.218	0.184
Tibia	0.823	0.782	0.112	0.114	0.256	0.311
First tarsus	0.715	0.749	0.086	0.075	0.261	0.361
Wing length	0.857	0.796	0.198	0.225	0.130	0.302
Wing width	0.904	0.904	−0.187	−0.090	−0.007	−0.007
Costal index (a/b)	0.114	0.047	0.806	0.764	0.149	0.190
4C index (b/c)	−0.044	−0.069	−0.907	−0.886	0.163	0.035
4th vein index (d/c)	0.094	−0.066	−0.554	−0.672	0.656	0.468
5X index (f/e)	−0.283	−0.251	0.225	−0.206	0.566	0.625
C3 fringe (3CBr/b)	0.075	−0.096	−0.109	−0.513	0.571	0.286
WL/WW	−0.252	−0.461	0.716	0.454	0.225	0.422
No. of abd. bristle (4th)	0.723	0.542	−0.073	−0.274	−0.335	−0.333
No. of abd. bristle (5th)	0.725	0.561	−0.007	−0.195	−0.417	−0.337
No. of spinules	−0.115	−0.166	0.088	−0.051	0.434	0.657
No. of sternopleurals	0.186	0.364	−0.063	−0.228	0.045	−0.080

loadings of the 4th vein index, 5X index, and C3 fringe were positively correlated to the component. It is suggested that the distal ends of wings, d and f in Fig. 2, increase when wing length increases, and so the third component concerns also a kind of shape factor of wings. For the number of sternopleurals, the cumulative percentage to the total variance of the first 3 components was the lowest among 15 characters, 4.1% in females and 19.1% in males.

Figs. 3 and 4 show distribution of scores combining the first and second components for males and females, respectively. In Fig. 3, for the first component, the size factor, most lines of *D. nasuta* and *D. albomicans* were positive and the other species were negative, that is, the body sizes of these two species were relatively larger than the others. In these two species, lines of *D. albomicans* were distributed around *D. nasuta* lines, divided into roughly the Nago-Wulai group and the Chiangmai, Amami-oshima and Ishigaki-jima group. In three subspecies of *D. sulfurigaster*, lines of *D. s. albostrigata* and *D. s. sulfurigaster* were positive for the second component but *D. s. bilimbata* were negative, which indicates that the wing shapes of *D. s. albostrigata* and *D. s. sulfurigaster* are similar, and different from that of *D. s. bilimbata*. Of four lines of *D. s. sulfurigaster*, Nos. 47, 48 and 49 were sampled from the F_1 progeny of wild-caught females. It is clear that these new stocks are larger than the old one, No. 46. Distribution of scores in females were similar as were those in males.

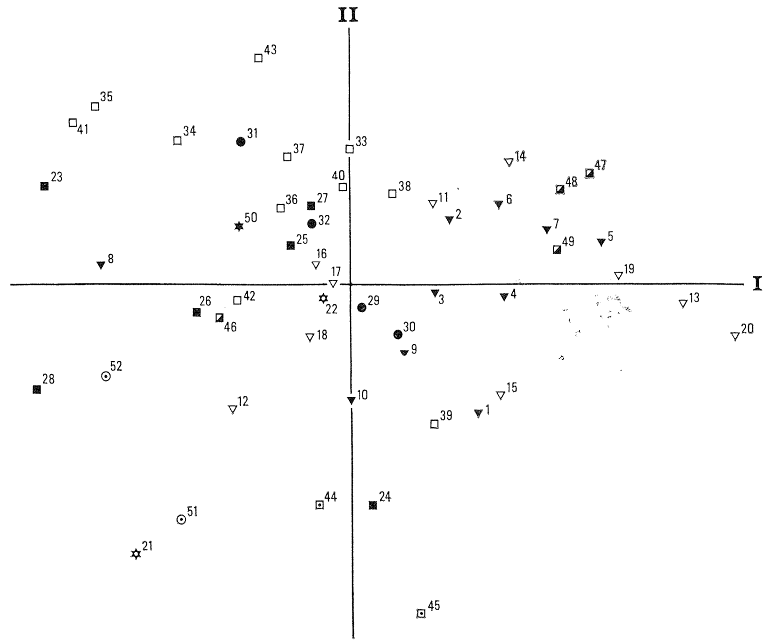

Fig. 3. Scores of PCA for the I and II components in males of the *D. nasuta* subgroup.

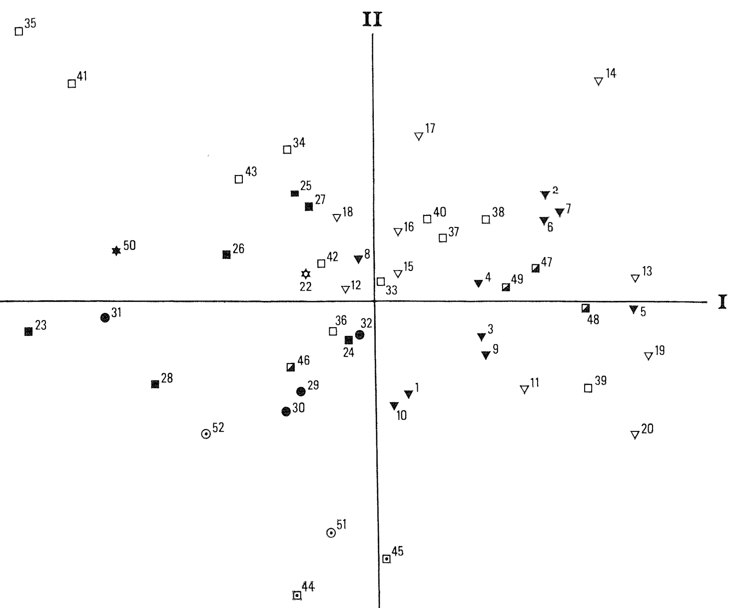

Fig. 4. Scores of PCA for the I and II components in females of the *D. nasuta* subgroup.

♀ \ ♂	*kohkoa*	*Taxon-F*	*kepulauana*	*albomicans*	*nasuta*	*(Indian) nasuta*	*s. neonasuta*	*s. albostrigata*	*s. bilimbata*	*s. sulfurigaster*	*pulaua*	*pallidifrons*
kohkoa	\	F [3]	f [1]	F [1]	0 [3]	0 [3]	0 [3]	f [1]	0 [1]	f [1]	f [1]	0 [1]
Taxon-F	F [3]	\	F [3]	0 [3]	0 [3]	0 [3]	0 [3]	0 [3]	0 [3]	0 [3]	0 [3]	0 [3]
kepulauana	f [1]	0 [3]	\	**F** [1] [3]	**F** [3]	**F** [3]	0 [3]	f [1]	0 [1]	f [1]	0 [1]	0 [1]
albomicans	f [1]	f [3]	**F** [1] [3]	\	**F** [3]	**F** [3]	[2] 0	f [1]	f [1]	f [1]	f [1]	0 [1]
nasuta	0 [3]	0 [3]	**F** [3]	**F** [3]	\	**F** [3]	0 [3]	0 [3]	0 [3]	f [3]	0 [3]	0 [3]
(Indian) nasuta	0 [3]	0 [3]	**F** [3]	**F** [3]	**F** [3]	\	[2] 0	0 [3]	F [3]	F [3]	0 [3]	0 [3]
s. neonasuta	0 [3]	0 [3]	0 [3]	0 [3]	0 [3]	[2] 0	\	[2] **F** [3]	[2] F [3]	f [3]	[2] F	[2] 0
s. albostrigata	f [1]	0 [3]	f [1]	F [1]	0 [3]	0 [3]	[2] **F** [3]	\	F [1] [3]	**F** [1] [3]	**F** [1] [3]	0 [1]
s. bilimbata	0 [1]	F [3]	0 [1]	F [1]	0 [3]	0 [3]	[2] F	**F** [1] [3]	\	**F** [1] [3]	**F** [1] [3]	f [1]
s. sulfurigaster	o [1]	f [3]	f [1] [3]	r [1]	o [3]	o [3]	f [3]	**F** [1] [3]	**F** [1] [3]	\	**F** [1] [3]	f [1]
pulaua	f [1]	0 [3]	0 [1]	f [1]	0 [3]	0 [3]	[2] F [3]	0 [1] [3]	0 [1] [3]	0 [1] [3]	\	f [1]
pallidifrons	0 [1]	0 [3]	0 [1]	0 [1]	0 [3]	0 [3]	[2] 0	0 [1]	f [1]	f [1]	f [1]	\

Fig. 5. Result of all possible combinations of interspecific and intersubspecific crosses in the *D. nasuta* subgroup. The figure was compiled from Wilson *et al.* (1969)[1], Nirmala and Krishnamurthy (1973–1974)[2], and present data[3].

F: F_1 ♀ × F_1 ♂ fertile	1: Wilson *et al.* (1969)
F: F_1 ♀ fertile, F_1 ♂ sterile	2: Nirmala and Krishnamurthy (1973–74)
f : P×P fertile, F_1 ♀ ♂ sterile	3: Present data.
o : P×P sterile	

Hybridization experiments: The results of the hybridization experiments are shown in Fig. 5, which was synthesized from Wilson *et al.* (1969), Nirmala and Krishnamurthy (1973) and present data. *D. nasuta* females produced fertile F_1 offspring, males and females, with Indian *nasuta, D. albomicans* and *D. kepulauana* males and produced a few sterile offspring with *D. s. sulfurigaster* males. These results are strikingly different from those of *D. albomicans*. *D. albomicans* females accepted males of all species of this subgroup used in this experiment, except *D. s. neonasuta* and *D. pallidifrons*. Three subspecies of *D. sulfurigaster*, except *D. neonasuta*, produced fertile offspring by crossing among them. When *D. pulaua* males crossed with females of the three subspecies of *D. sulfurigaster* mentioned above, completely fertile F_1 flies were obtained. However, in the reciprocal crosses, no hybrid larvae were detected. *D. kohkoa* and Taxon-F produced fertile F_1 females but not males in some combinations of matings, such as the reciprocal crosses between *D. kohkoa* and Taxon-F, Taxon-F ♀×*D. kepulauana* ♂, *D. kohkoa* ♀×*D. albomicans* ♂, *D. albomicans* ♀×Taxon-F ♂, and *D. s. bilimbata* ♀×Taxon-F ♂.

Allozyme variations in Esterase loci: *Esterase* loci which were detected using α- and β-naphthyl acetates as the substrate were highly polymorphic in all species belonging to this subgroup. Each *Est*-α and *Est*-β locus consists of 6 or more different alleles including the null.

Allelic frequencies of local populations of *D. nasuta*, *D. kohkoa*, and *D. s. albostrigata* are shown in Table 5. Genetic differentiation among populations of *D. nasuta* was relatively small in spite of their widespread distribution, even in such small samples. For *Est*-α, the most common allele was *Est*-α^2 and it was fixed in the Kandy population. The *Est*-β locus was highly polymorphic in all geographic populations, and *Est*-β^F was the most common allele. The mean heterozygosity was 0.2090 in the *Est*-α and 0.5268 in the *Est*-β.

For *D. s. albostrigata*, the Chiangmai population, Thailand, was the only unique one at the *Est*-β locus. At the *Est*-α locus, *Est*-α^1 allele was commonest in all populations, reaching about 80% on the average. The Kota Kinabalu, Malaysia, population contained 35% of the null allele. At the *Est*-β locus in this species, *Est*-β^F was about 50% and *Est*-β^M was 37% on the average. However, *Est*-β^F was 70% and β^M was 28% in the Chiangmai population. The average heterozygosity was 0.3165 and 0.5768 in the *Est*-α and -β, respectively.

The genetic differentiation among the Kuching, Singapore, and Kuala Lumpur populations of *D. kohkoa* was relatively small. In this species, the *Est*-α locus contained a lot of variants represented by 7 different alleles. *Est*-α^3 allele was the most common one. The heterozygosity in the *Est*-α locus of this species was 0.6298, 0.6403, and 0.6404 in the Kuching, Singapore and Kuala Lumpur populations, respectively. The *Est*-β locus was also highly polymorphic. Three alleles of this locus were maintained at more than 10% frequencies in all populations. Heterozygosity for this locus was 0.6367, 0.7410, and 0.6811 for above three populations, respectively.

Allelic frequencies of *Est*-α and -β loci in *D. albomicans* are presented in Table 6. At the *Est*-α locus of the Japanese *D. albomicans*, *Est*-α^3 was the commonest and the genetic constitution of the Japanese populations were strikingly different from the Chiangmai, Thailand, and the Wulai, Taiwan, populations in allelic frequencies at both α and β loci. In the Chiangmai population, lack of the α^2 allele was observed and α^3 and α^1 were in high frequencies. The Wulai population was similar to the Chiangmai, showing higher α^1 and lower α^2. The average heterozygosity for the *Est*-α locus is 0.6424.

At the *Est*-β locus, the differences in allelic frequencies among countries were also apparent. The characteristic of the Japanese populations was the higher frequency of the β^0 (null) allele, being the most common allele in the

Table 5. *Allelic frequencies at the α- and β-Esterase loci for different geographical strains of D. nasuta, D. kohkoa, D. sulfurigaster albostrigata (All samples were collected in 1971.)*

		D. nasuta			*D. kohkoa*			*D. sulfurigaster albostrigata*						
Locality		SEZ	MBA	KDY	KCH	SIN	KUL	BKI	BTN	SIN	KUL	PEN	CNX	KDY
No. of flies tested		200	30	50	150	290	260	80	70	110	270	50	390	520
Alleles	α: *4*	—	—	—	6.60	6.78	16.65	—	—	—	—	—	—	—
	3	0.77	11.67	0	56.88	54.89	55.74	—	—	—	—	—	—	—
	2	73.84	88.33	100.00	10.68	12.59	10.78	3.18	2.14	2.73	2.46	10.75	12.91	0
	1	9.73	0	0	9.84	6.20	6.54	61.33	86.75	97.27	81.45	87.41	69.37	74.38
	1^-	—	—	—	1.50	1.10	2.06	0	0.71	0	1.50	0	2.61	1.88
	1^S	—	—	—	0	0	1.43	0	0	0	0	0	0.51	5.66
	0	15.66	0	0	14.50	18.44	6.80	35.49	10.41	0	14.59	1.83	14.60	18.10
	β: F^F	—	—	—	0	0	1.01	0	0	0	6.06	0	0.28	0
	F^f	0	0	5.00	3.81	3.61	4.76	0	0	0	0.89	0	0	0
	F	64.50	70.00	60.00	55.05	40.51	50.54	41.47	38.22	42.38	39.99	47.42	79.44	52.79
	F^-	0	0	4.00	—	—	—	—	—	—	—	—	—	—
	M	13.50	20.00	5.00	0	7.36	11.76	49.55	50.98	41.56	39.68	28.34	15.68	35.14
	S	17.00	0	13.00	17.89	22.57	8.19	1.31	3.12	4.11	2.16	0	0	6.48
	S^-	0	10.00	0	5.37	5.97	3.49	3.31	0	0	0.54	0	1.87	1.91
	S^S	0	0	13.00	2.65	1.70	0.41	—	—	—	—	—	—	—
	0	—	—	—	15.23	18.28	19.84	4.36	7.68	11.95	10.66	24.25	2.73	3.67

Table 6. *Allelic frequencies at the α- and β-Esterase loci in D. albomicans*

Locality		Japan					Taiwan	Thailand
		Amami-oshima	Tokuno-shima	Nago	Ishigaki-jima	Iriomote	Wulai	Chiangmai
Collection date		1973 k	1972 i	1973 j	1973 j	1973 j	1971 f	1971 f
No. of flies tested		310	470	1088	120	220	170	37
Alleles	α^3	42.17	41.13	50.14	30.86	34.93	37.50	57.88
	α^{3-}	0	0	0	6.72	11.83	0	0
	α^2	40.79	48.75	41.50	32.05	25.97	16.43	0
	α^1	6.21	3.72	2.63	13.05	16.99	33.86	26.14
	α^0	10.82	6.21	5.72	17.28	9.83	12.20	14.96
	β^{F+}	1.63	0	2.56	0	6.66	0	0
	β^F	36.78	46.35	47.20	48.97	25.83	58.48	5.85
	β^M	16.54	11.52	16.25	2.61	22.29	16.70	59.46
	β^S	0.97	0	1.99	12.81	6.42	20.07	18.73
	β^{S-}	0	0	0	0	0	0	10.48
	β^0	44.08	42.12	32.00	35.55	38.75	4.63	5.35

Amami-oshima and Iriomote populations. In the other Japanese populations, the β^F allele was most common. The β^S allele was rare in northern populations and common in southern ones among Japanese populations. In the Wulai population, the *Est*-β^F was the commonest allele (58%) and the *Est*-β^S was next. This suggests that some clinal variations exist in allelic frequencies at the *Est*-β locus in *D. albomicans*. In the Chiangmai population, the *Est*-β^M allele was highest (about 60%) and the *Est*-β^S was next. The average heterozygosity was 0.6191, which is similar to that of the *Est*-α locus.

Sexual isolation and insemination tests between D. nasuta and D. albomicans: Joint isolation indices are shown in Table 7. Even between *D. nasuta* and *D. albomicans*, no sexual isolation was observed. The average isolation index was 0.002, −0.009, and 0.069 in crosses between *D. nasuta* and *D. albomicans*, among *D. nasuta*, and among *D. albomicans*, respectively.

The insemination test shows the observed frequency of the copulated females which contained sperm. The values shown in Table 8 were relatively low compared with the general tendency of other *Drosophila* species. Between the crosses of *D. albomicans* females and males the lowest value (60.46%) was observed and between *D. albomicans* females × *D. nasuta* males was maximum (73.55%).

Table 7. *Joint isolation indices among intra- and interspecific populations of D. nasuta and D. albomicans*

	Drosophila albomicans						*Drosophila nasuta*			
	Amami-oshima	Tokuno-shima	Nago	Ishigaki-jima	Wulai	Chiangmai	Kandy	Mombasa	Seychelles	Tananarive
Amami-oshima	**0.017 ±0.029**	−0.042	0.122	0.014	0.069	0.096	−0.048	−0.169	0.023	0.089
Tokunoshima		**0.031 ±0.019**	0.073	0.122	0.090	0.030	0.049	−0.037	0.034	−0.037
Nago			**0.048 ±0.016**	0.075	0.073	0.067	−0.024	−0.029	0.007	0.068
Ishigaki-jima				**0.035 ±0.024**	0.163	−0.099	0.013	0.024	0.000	0.007
Wulai	*albomicans × albomicans* 0.069±0.016				**0.042 ±0.027**	0.063	0.083	−0.111	0.032	−0.083
Chiangmai						**0.034 ±0.017**	0.056	0.034	0.055	0.007
Kandy							**0.004 ±0.016**	−0.067	0.008	−0.033
Mombasa					*albomicans × nasuta* 0.002±0.012			**−0.032 ±0.024**	0.069	0.001
Seychelles									**0.022 ±0.010**	−0.034
Tananarive							*nasuta × nasuta* −0.009±0.018			**−0.002 ±0.017**

Table 8. *Insemination rates in crosses between D. albomicans and D. nasuta*

Females		Males	No. of females tested	% of insemination	Mean % of insemination	
D. albomicans	×	*D. albomicans*				
Amami-oshima		Nago	65	58.46	60.46	
Nago		Amami-oshima	56	46.43		
Taiwan		Ishigaki-jima	62	93.55		
Ishigaki-jima		Taiwan	50	52.00		
Amami-oshima		Tokunoshima	27	51.85		
D. nasuta	×	*D. nasuta*				
Mombasa		Tananarive	42	38.10	63.19	
Tananarive		Mombasa	157	86.62		
Mombasa		Kandy	37	64.86		
D. albomicans	×	*D. nasuta*				
Amami-oshima		Tananarive	36	75.00	73.55	71.48
Nago		Mombasa	86	65.12		
Ishigaki-jima		Seychelles	43	69.77		
Tokunoshima		Kandy	51	84.31		
D. nasuta	×	*D. albomicans*				
Tananarive		Amami-oshima	52	94.23	69.41	
Mombasa		Nago	44	54.55		
Seychelles		Ishigaki-jima	42	66.67		
Kandy		Tokunoshima	42	69.05		
Kandy		Nago	24	70.83		
Seychelles		Nago	18	61.11		

5. DISCUSSION

Morphological characters: The frons, orbits, and sternopleural dark bands in males. Several investigators have already reported that species belonging to the *D. nasuta* subgroup could be classified into three categories as to the male frons; those with an entire silvery whitish frons, those with white orbits, and those without whitish patterns (Wilson *et al.* 1969; Thongmeearkom *et al.* 1977). The first group consists of *D. nasuta*, Indian *D. nasuta*, *D. albomicans*, *D. kepulauana*, and *D. kohkoa*. The former three have the bright square type of silvery whitish frons, but *D. kepulauana* and *D. kohkoa* have H-shaped, lighter ones. The darkness of the bands on the mesopleuron (or episternum and pteropleurite of the thorax) of males is clearly correlated with the coloration of the frons, flies with more bright areas on the frons show more dark bands on the thorax. Thus, *D. nasuta*, Indian *D. nasuta* and *D. albomicans* have more darkish bands than *D. kepulauana* and *D. kohkoa*.

The members of the second group have prominent whitish orbits; this group includes *D. s. albostrigata*, *D. s. bilimbata*, *D. s. neonasuta*, *D. s. sulfurigaster* and *D. pulaua*. *D. pulaua* has very pale white bands along the edges of the compound eyes, but the four subspecies of *D. sulfurigaster* have very obvious whitish orbits. Among these four, *D. s. albostrigata* and *D. s. neonasuta* have the most strikingly whitish ones, *D. s. bilimbata* is next, and *D. s. sulfurigaster* has narrower and lighter belts of white bands. On the dark bands on the male thorax, those of the second group are smaller in size and lighter in color compared with *D. nasuta* and *D. albomicans*. Among species of this group, *D. s. albostrigata* and *D.* s. *neonasuta* have a darker stripe than the other three, corresponding to the brightness of the white orbit.

D. pallidifrons and Taxon-F have neither white orbits nor dark bands on male thorax, and constitute the third group.

Quantitative characters: Fifteen characters were investigated for 52 lines of ten species or subspecies of the *D. nasuta* subgroup. The analysis of variance showed that all populations contain great variations in the quantitative characters measured in this subgroup. Variations between lines within a locality were significant for all characters. From these results, one could safely conclude that the *D. nasuta* subgroup has diverged morphologically, as shown by the significant differences between species which were detected in ten characters. Two of the authors (E. T. and O. K.) have clearly classified *D. melanogaster* populations into the Japanese and French ones using the principal component analysis (see D. I. S. 52, 1977), showing that the principal component analysis can be a useful tool in the field of numerical taxonomy in *Drosophila*.

Judging from the distribution of scores obtained in the present investigation (Figs. 3 and 4), scores belonging to the same species tended to aggregate with each other, but the distribution ranges of species largely overlapped. *D. nasuta* and *D. albomicans* were relatively large flies in both sexes. Scores of *D. nasuta* were more aggregated than those of *D. albomicans*. Lack of clear genetic divergence in *D. nasuta* populations was evident here as well as in the smaller values of genetic distance obtained from allozyme variations. On the contrary, scores of the *D. albomicans* populations were distributed over a wider range than those of *D. nasuta*.

Of the three subspecies of *D. sulfurigaster*, *D. s. bilimbata* was somewhat differentiated from the other two subspecies, being negative in the second component. The number 46 line of *D. s. sulfurigaster* was captured in 1968, and has been kept for nine years under laboratory conditions. On the other hand, the other three were investigated using the F_1 flies of the wild-caught inseminated females. The scores of these three were tightly aggregated. Except for one of the Chiangmai populations (No. 39), scores of *D. s. albostrigata* covered a relatively small range. Although *D. pulaua* is a closely related species to *D. s. albostrigata* especially in males, Generally speaking, differentiation in quantitative characters of the *D. nasuta* subgroup is not so clearly evident as the shape of the silvery frons and the thoracic bands in males, but no overlapping was observed in some of the paired species, for instance, *D. albomicans* and *D. pulaua*, *D. nasuta* and *D. pulaua*, and *D. nasuta* and *D. kohkoa* in males. From the data presented here it would seem that the distribution of scores reflected their genetic divergence, although they were not adequate for species identification and the phyletic analysis of this subgroup.

Fertility of hybrids: The data presented here were synthesized from those of Wilson *et al.* (1969), Nirmala and Krishnamurthy (1973–74) and ours. The results of the experiments were very complicated. *D. nasuta*, Indian *D. nasuta*, *D. albomicans* and *D. kepulauana* composed a species complex in which each species produced fertile F_1 offspring in the interspecific crosses with various degrees of difficulties, and again did so in successive generations, although they are completely allopatric species. *D. albomicans* females could produce F_1 offspring with the all the members of this subgroup except with *D. pallidifrons* and *D. s. neonasuta* males, and *D. albomicans* males produced viable F_1 offspring with the three subspecies of *D. sulfurigaster* and also with *D. kohkoa*, and *D. pulaua*.

The results of the hybridization experiments using *D. nasuta* were different from Indian *nasuta* in the crosses with two subspecies of *D. sulfurigaster*. Indian *nasuta* was closer to these subspecies, producing fertile F_1 females in the crosses with *D. s. bilimbata* and *D. s. sulfurigaster* males, whereas *D. nasuta* gave only sterile progeny with *D. s. sulfurigaster* males. However, we believe that *D. nasuta*, Indian *D. nasuta*, and *D. albomicans* are best considered as semispecies of the *nasuta* complex. *D. kepulauana* exists as very closely related allopatric populations to the *nasuta* complex, and produced fertile offspring but did not mate freely even in laboratory conditions. Levene's isolation indices (Z_I) between *D. kepulauana* and three closely related species, *D. nasuta*, Indian *D. nasuta* and *D. albomicans*, were 2.58 ± 0.29, 1.86 ± 0.23, and 1.71 ± 0.21, respectively.

D. s. albostrigata was captured sympatrically with *D. albomicans* in Thailand, but natural hybrids have not found, as determined by the check of isozyme variations in the F_1 progeny from the wild-caught females.

The most interesting result, with respect to the three subspecies of *sulfurigaster*, was the relationship with *D. pulaua*. All females of the *sulfurigaster* subspecies produced fertile F_1 progeny in crosses with *D. pulaua*, whereas *D. pulaua* females could mate with *sulfurigaster* males but no offspring were obtained. In many *Drosophila* species groups the insemination reaction in interspecific, even in intraspecific crosses in some cases, has been well documented (Patterson and Stone 1952). In dissections of *D. pulaua* females two days after mating with *sulfurigaster* males, there was clear evidence of an insemination reaction; the hard mass in the female's uterus probably prevented the production of interspecific hybrids. In the reciprocal crosses, some of the *sulfurigaster* females showed an insemination reaction, but the degree of its development was not strong and disappeared gradually. The insemination reaction has developed as a one-sided reproductive isolating mechanism in the case of the *sulfurigaster* and *D. pulaua*. However, *D. pulaua* is undoubtedly the closest species to the four subspecies of *D. sulfurigaster*.

D. pallidifrons produced sterile F_1 progeny only with *D. pulaua*, *D. s. bilimbata* and *D. s. sulfurigaster*. *D. s. albostrigata* and *D. s. neonasuta* did not produce F_1 offspring with *D. pallidifrons* in either of the reciprocal crosses. Here one can see the genetic divergence among the four subspecies of *D. sulfurigaster*. *D. kohkoa* and Taxon-F are related but clearly different species and both species show genetic divergence from other members of this subgroup.

Isozyme variations at Esterase loci: Many investigators have made an attempt to investigate isozyme variations as a measure of genetic divergence between populations, species, and even higher taxa (Ayala, Prakash, and Selander and their coworkers). In the *D. nasuta* subgroup, Kanapi and Wheeler (1970) analysed 8 enzyme systems using three subspecies of *D. sulfurigaster*, plus *D. albomicans*, and *D. pallidifrons*.

At seven out of 8 systems (Esterase, Leucine aminopeptidase, Alcohol dehydrogenase, Octanol dehydrogenase, Alkaline phosphatase, Acid phosphatase and Catalase) they found variants. Only in Xanthine dehydrogenase, no variants were detected. At the *Esterase-C* and *Esterase-F* loci, numerous variations were observed including the null alleles. Judging from relative mobilities and zymogram characteristics, the *Est-C* and *-F* loci in Kanapi and Wheeler (1970) correspond to the *Est-α* and *-β* of the present study.

In spite of the widespread distribution, from India to Africa, the genetic divergence among local populations of *D. nasuta* was relatively small. *D. nasuta* is one of the marginal species of this subgroup and therefore it is reasonable that there is no clear evidence of genetic divergence among local populations.

Although *D. s. albostrigata* is the most common species in southeast Asia, only the Chiangmai population had a greatly different genetic constitution from other local populations, and the next most extreme was the Kota Kinabalu population.

In *D. kohkoa*, genetic divergence among three local populations, Kuching, Singapore and Kuala Lumpur, was small. Heterozygosity for *Est-α* of this species was higher as twice as those of *D. nasuta* and *D. s. albostrigata* and was almost the same as *D. albomicans*.

D. albomicans is the species among which remarkable genetic divergence has occurred. The five Japanese, the Wulai, Taiwan, and the Chiangmai, Thailand, populations were investigated for their allozyme variations. These three groups belonging to different countries, were clearly distinguished by the genetic constitution of the *Esterase* loci. The Japanese populations, from Amami-oshima, Tokunoshima, Nago, Ishigaki-jima, and Iriomote, represent an area where the longest geographical distance was 740 km (between Amami-oshima and Iriomote). The difference in allelic frequencies between the Iriomote and the Wulai populations was clearly seen in *Est*-α^2 and -α^1, and also in all of *Est*-β alleles, though the geographical distance is only 250 km. This suggests that man-made passive transport is effective for the gene flow between these populations. The Chiangmai population showed the biggest difference with all other *D. albomicans* populations. Moreover for the karyotype configuration, the fourth chromosomes of the Thai populations were shorter in size than those of the Japanese and Taiwanese populations (Wilson *et al.* 1969 and our observation). The Thai populations of *D. albomicans* have diverged more, genetically, from the Japanese ones as compared with the Taiwanese populations. In Taiwanese and Thai populations, we can see much lower frequencies in the null allele of *Est*-β loci.

The genetic divergence among the Japanese populations of *D. albomicans* was small, although the information comes from only two *Esterase* loci; also at some localities the small number of originally captured females were used. The Japanese, Taiwanese, and Thai *albomicans* have genetically differentiated at the level of the subspecies or even of the semispecies. This assumption was clearly supported by the analysis of pre-adult viability in which remarkable hybrid breakdown among them has been obtained (Inoue and Kitagawa, 1975).

Regarding the evolutionary status between *D. nasuta* and *D. albomicans*, the genetic constitution of the *Esterase* loci were quite different. Inoue and Kitagawa (1975) have also observed extreme hybrid breakdown and striking sex-ratio distortion in some interspecific crosses between them. The karyotypes of these two species are different (Wilson *et al.* 1969; Wakahama and Kitagawa, 1972); the differences resulted in abnormal chromosomal segregation in meiosis of the F_1 hybrid males, with the result that fifty percent of the males in the F_2 generation were completely sterile (Kitagawa *et al.* unpublished data). Thus it seems clear that *D. nasuta* and *D. albomicans* should not be considered subspecies but biologically different species, in spite of the lack of pre-mating isolation.

The development of pre-mating isolation preceding post-mating barriers has been reported in Hawaiian *Drosophila* (Ahearn *et al.* 1974). It seems to be generally accepted, however, that speciation in *Drosophila*, and perhaps in most living organisms, usually begins allopatrically and post-matings reproductive isolating barriers, that is, the decrease of hybrid performance, develop earlier than do pre-mating barriers. Thus, detailed and careful investigation of hybrids' performance gives us fundamental information on the first stages of allopatric speciation. The fact that genetic divergence has been detected in the form of hybrid breakdown without accompanying pre-mating barriers between *D. nasuta* and *D. albomicans*, illustrates one of the typical genetic processes of an early stage of allopatric speciation in *Drosophila*.

In spite of the wide range of distribution, the local populations of *D. nasuta* have not genetically differentiated, even at the genic (isozyme) level. On the other hand, the *D. albomicans* populations have already advanced to the first step of the speciation process in three major localities, i.e., Japan, Taiwan, and Thailand. If the geographical isolation continues in the same manner as at present, these three populations of *D. albomicans* might be expected to develop into full biological species in the future.

Judging from the geographical distribution of the *D. nasuta* subgroup, it may be assumed that the origin of this subgroup was in southeast Asia. The fact that genetic differentiation among *D. nasuta* populations is relatively small, could be explained from the marginality of this species, and that *D. nasuta* has moved into its present distribution relatively late. On the contrary, *D. albomicans* occurs from the temperate to subtropical climatic regions, from southern Japan to India, even though the mean temperature and the climatic conditions at these localities were quite different. Such diverse climatic conditions could be effective in furthering genetic differentiation in this species.

In the *D. nasuta* subgroup, the differentiation demonstrated by the genetic tests is almost parallel with the morphological divergence, although there are some exceptions, e.g., the species *D. kohkoa* is similar to *D. nasuta* in external morphology but is closer to. *D. sulfurigaster* on the basis of the hybridization tests. Even in *Drosophila* as well as in other species of insects, the speciation processes are versatile and diversified phenomena, and each process needs to be explained in detail, case by case. However, allopatric speciation and the observed decrease in hybrid performance in the *D. nasuta* subgroup is a typical sample of the speciation process in living organisms (White 1977). The detailed analysis of allozyme variation currently being conducted at Prof. F. Ayala's laboratory at the University of California, Davis, will provide more precise data on differentiation at the genic level.

The authors wish to express many thanks for collecting and supplying living materials for this study to Professors M. R. Wheeler, the University of Texas (U.S.A.), F. J. Lin, Academia Sinica (Taiwan), V. Baimai, Mahidol University (Thailand), N. B. Krishnamurthy, University of Mysore (India), J. P. Gupta, Banaras Hindu University (India), T. Okada, H. Kurokawa, H. Ikeda, F. Hihara, and Y. Fuyama (Japan). They are also indebted to Emeritus Professor D. Moriwaki and Professor S. Ohba, Tokyo Metropolitan University, for their encouragement throughout this study. They also wish to express their hearty thanks for reading the manuscript and giving invaluable criticism to Professors M. R. Wheeler, the University of Texas, F. J. Ayala, University of California, Davis, and H. L. Carson, University of Hawaii.

This work was mainly supported by the funds of the Overseas Scientific Expedition in 1971 (No. 7114), 1979 (No. 404149), and 1980 (No. 504344) of the Ministry of Education, Science and culture of Japan.

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