XIV. Cytogenetic Relations in the *Drosophila nasuta* Subgroup of the *immigrans* Group of Species[1,2]

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INTRODUCTION

The group of species discussed here belongs to the *Drosophila nasuta* subgroup of the *Drosophila immigrans* species group (superspecies). Since the description of *Drosophila nasuta* (Lamb, 1914) from the Seychelles Islands near Africa, a number of morphologically similar species have been found in the Pacific-Australasian area. Most of these have been considered synonyms of the original *nasuta* (see, for example, Wheeler and Takada, 1964). The evidence from this study demonstrates the divergence of a number of these populations of different continental areas and Pacific area islands into what are now separate sibling species.

Unlike *D. immigrans*, males of the *nasuta* subgroup have white to silvery markings on the *frons* of all but one of the species. The largest geographical species complex, *D. sulfurigaster*, which consists of three subspecies or races, has pronounced whitish bands along the frontal orbits of the males. *D. pulaua* has bands of the same type but much fainter. *D. nixifrons*, which we have not seen, is said to have such bands also. Three of the species, *D. albomicans*, *D. kohkoa* and *D. kepulauana*, are characterized by a whitish to silvery sheen over the entire *frons*; this is also true of the original species, *nasuta*, according to the published notes. *D. pallidifrons*, from Ponape in the Caroline Islands, has no

[1] These field and laboratory studies have been supported in part by a U. S. Public Health Service Research Grant, No. GM-11609 and earlier by GM-15769 from the National Institute of General Medical Sciences; some of the earlier field work was also supported by a contract with AEC [AT-(40-1)-1323 and 2952] to the late Prof. W. S. Stone, and by grant GB-711 from the National Science Foundation.

[2] This paper is dedicated to Professor Wilson S. Stone, our great friend, colleague and mentor before his untimely death; he was largely responsible for beginninig this project and others in our laboratory, as part of a major emphasis on island evolution.

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silvery-white frontal markings at all. The *nasuta* subgroup is like all other members of the *immigrans* superspecies thus far studied in having a double-length rod chromosome, the result of a fusion of two of the six primitive elements of the basic *Drosophila* chromosome complement.

Systematics and Distribution

Widespread collections from islands of the South Pacific and from Southeast Asia have provided the material for this genetic and cytological investigation (Table 1). The points of origin of the stocks of the various species are also shown

Table 1

Summary distribution of species of the *Drosophila nasuta* subgroup.

D. nasuta Lamb*	**D. sulfurigaster** Duda
Seychelles Islands	*D. s. sulfurigaster* Duda
D. albomicans Duda	Australia
Ryukyu Islands	New Guinea-Papua
Taiwan [Formosa]	New Ireland
Pescadores Islands	*D. s. bilimbata* Bezzi
Thailand	Hawaii
D. kohkoa, n. sp.	Palmyra I.
Palawan, Philippines	Samoa
Sarawak, Malaysia	Niue
Brunei, Borneo	Tonga
Cambodia	Fiji
Thailand	Guam
D. kepulauana, n. sp.	*D. s. albostrigata, n. subsp.*
Palawan, Philippines	Philippine Islands
Brunei, Borneo	Sarawak, Malaysia
D. pulaua, n. sp.	Brunei, Borneo
Sarawak, Malaysia	Continental Malaysia
D. pallidifrons, n. sp.	Cambodia
Ponape, Caroline Is.	Thailand
	D. nixifrons Tan, Hsu & Sheng*
	China (mainland)

* Not seen in the present study.

on Figures 1 and 2. It is clear that these flies are almost entirely confined to the tropics of the Pacific-Asian area, and are only rarely found beyond 30° North or 30° South Latitude.

We have not had a sample of the Seychelles form, *D. nasuta* Lamb. However, due to its great isolation by water and by distance from the populations which we have studied, together with the marked divergent evolution that characterizes this group of species, we regard *nasuta sensu strictu* as most probably a different species.

The original University of Texas *D. spinofemora* stock was lost before this study began. Spieth (personal communication) found that all of the later lines established from Hawaii differed somewhat in mating behavior from that of the earlier stock which he observed in 1952. The significance of this is not known.

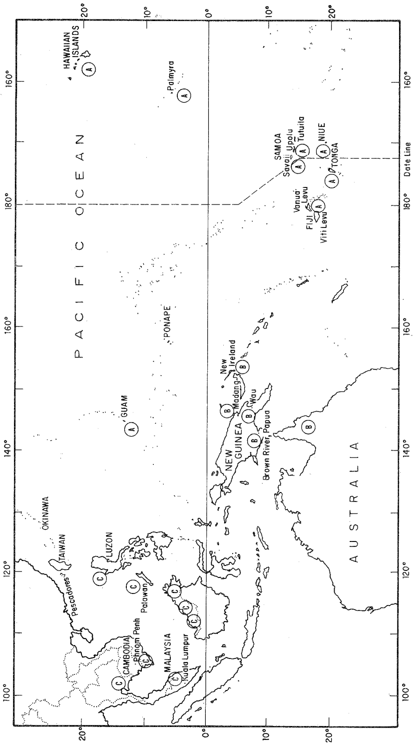


Fig. 1. Distribution of the three types of *Drosophila sulfurigaster*. A. *D. s. bilimbata*; B. *D. s. sulfurigaster*; C. *D. s. albostrigata*, n. subsp.

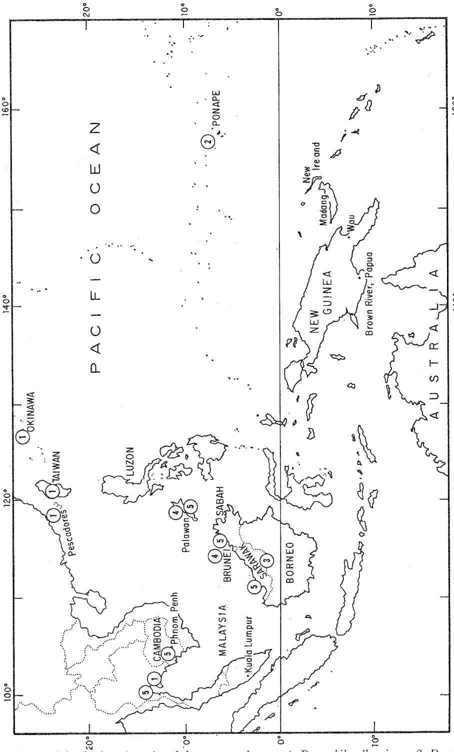


FIG. 2. Distribution of species of the *nasuta* subgroup. 1. *Drosophila albomicans*; 2. *D. pallidifrons*, n.sp.; 3. *D. pulaua*; n.sp.; 4. *D. kepulauana*, n.sp.; 5. *D. kohkoa*, n.sp.

From each of the repeated collections made in the Hawaiian Islands from 1966–1968, iso-female lines have been checked for mating behavior and taxonomic identity; and several sorts of cytological and genetic tests have been made, showing all such lines to be the same. This Hawaiian form, in which no inversions have been observed, has been used as our "standard" for genetic tests as well as cytological comparisons.

The **immigrans** group

The *immigrans* group, or complex, contains an estimated 70 nominal species, without including the 25 species of African *Zaprionus* and 7 species of *Samoaia*, which are fairly certainly related to the complex. Duda (1923) established the subgenus *Spinulophila* for some of these species, and later (1925) changed the name to *Acanthophila* (which is preoccupied by *Acanthophila* Hein, 1870, in Lepidoptera). Other species have been placed in *Chaetodrosophilella* Duda (at times considered a genus, at others a subgenus of *Drosophila*) or in *Phorticella* Duda (sometimes considered a genus, at other times a subgenus of *Zaprionus*). Judging from the original descriptions, and from published keys, the 70± species seem to be assignable to five discrete subgroups, with a few species being so imperfectly known or so aberrant that they cannot be assigned as yet even on a tentative basis. As a "first approximation," then, the following subgroups may be distinguished:

Subgroup I. The "typical" *immigrans* types, the males with some degree of modification of the fore tarsi. The nominal species which are presumed to belong here[1] are:

1. *brouni* Hutton 1901
2. *cilifemur* Villeneuve 1923
3. *curviceps* Okada & Kurokawa
4. *flexipilosa* Pipkin 1964
5. *formosana* Duda 1926
6. *formosana* Sturtevant 1927
7. *immigrans* Sturtevant 1921
8. *metallescens* Malloch 1934
9. *monochaeta* Sturtevant 1927
10. *ruberrima* de Meijere 1911
11. *rubra* Sturtevant 1927
12. *signata* Duda 1923
13. *subfasciata* de Meijere 1914
14. *synpanishi* Okada 1964
15. *unicolor* de Meijere 1914
16. *ustulata* de Meijere 1908

Subgroup II. The "*nasuta*" subgroup; males lack tarsal ornamentation, but usually have the frons silvery-whitish, all or in part. This section of the *immigrans* group is discussed in greater detail in the following pages.

1. *albomicans* Duda 1924
2. *albovittata* Duda 1926
3. *bilimbata* Bezzi 1928
4. *kepulauana* Wheeler, n. sp.
5. *kohkoa* Wheeler, n. sp.
6. *komaii* Kikkawa & Peng 1938
7. *nasuta* Lamb 1914
8. *nixifrons* Tan, Hsu & Sheng 1949
9. *pallidifrons* Wheeler, n. sp.
10. *pulaua* Wheeler, n. sp.
11. *setifemur* Malloch 1924
12. *spinofemora* Patterson & Wheeler 1942
13. *sulfurigaster* Duda 1923
14. *willowsi* Curran 1936

[1] No attempt is being made to indicate synonyms, homonyms, etc. at this time.

Subgroup III. The "*quadrilineata*" subgroup. These species all have a pale mesonotum with a prominent series of darker stripes.

1. *annulipes* Duda 1924
2. *circumdata* Duda 1926
3. *crockeri* Curran 1936
4. *hexastriata* Tan, Hsu & Sheng 1949
5. *lineata* van der Wulp 1881
6. *lineolata* de Meijere 1914
7. *nigrilineata* Angus 1967
8. *notostriata* Okada 1966
9. *pentastriata* Okada 1966
10. *pseudotetrachaeta* Angus 1967
11. *quadrilineata* de Meijere 1911
12. *solennis* Walker 1860
13. *tetrachaeta* Angus 1964
14. *virgata* Tan, Hsu & Sheng 1949

Subgroup IV. The "*lineosa*" subgroup. These species are similar to *Zaprionus*, *s.s.*, in having prominent silvery to chalky-white longitudinal stripes on the mesonotum.

1. *albicornis* (Enderlein) 1922 (*Zaprionus*)
2. *albostriata* Malloch 1924
3. *argentostriata* Bock 1966
4. *bakeri* (Sturtevant) 1927 (*Zaprionus*)
5. *bistriata* de Meijere 1911
6. *fenestrata* (Duda) 1923 (*Phorticella*)
7. *lineata* (de Meijere) 1911 (*Stegana*)
8. *lineosa* (Walker) 1860 (*Notiphila*)
9. *multistriata* Duda 1923
10. *multistriata* (Sturtevant) 1927 (*Zaprionus*)
11. *obscuricornis* (de Meijere) 1915 (*Stegana*)
12. *silvistriata* Bock & Baimai 1967

Subgroup V. The "*hypocausta*" subgroup. In this subgroup there is usually a strong sexual dimorphism in body color, males being much darker; further, the comb-like bristle row on the inner side of the first femur is absent or poorly developed.

1. *calceolata* Duda 1926
2. *hypocausta* Osten Sacken 1882
3. *hypopygialis* Malloch 1934
4. *nasutoides* Okada 1964
5. *pararubida* Mather 1961
6. *rubida* Mather 1960
7. *xanthogaster* Duda 1924

Subgroup VI. The following species are listed in this strictly artificial group since their true affinities are unknown or they seem to be quite aberrant with respect to groups I–V.

1. *balneorum* Sturtevant 1927
2. *coei* (Okada) 1966 (*Chaetodrosophilella*)
3. *cubivicittata* Okada 1966
4. *fuscicostata* Okada 1966
5. *trichaeta* Angus 1967
6. *trilimbata* Bezzi 1928

The **nasuta** subgroup

In addition to discussing each of the ten "names" which have been used in this subgroup, we are also naming four new species and one new subspecies which were discovered in the course of the cytological and hybridization studies.

In all, we are recognizing eight valid species, as follows: *nasuta* (*sensu strictu*) and *nixifrons*, neither of which has been available in laboratory culture; *sulfurigaster* and *pulaua*, with silvery-white frontal orbits on males; *albomicans*, *kepulauana* and *kohkoa*, in which the entire male frons is silvery to whitish; and *pallidifrons*, the only member lacking frontal pollinosity.

Table 2 and Figures 1 and 2 show the localities from which we have had laboratory cultures. Other reported distributions are not shown since we have had no way to verify the species identifications. This problem is especially acute since the species of this complex are so remarkably similar in appearance and, as a result, nearly all of them have been placed at one time or another as synonyms of *nasuta* (see, for example, Wheeler and Takada, 1964: 180).

Type specimens of the new forms described here are located in the *Drosophila Type and Reference Collection*, University of Texas, Austin.

1. *Drosophila albomicans* Duda 1923: 43, 47, 48; 1924a: 209; 1924b: 245, Fig. 70; 1926: 83, 88–89; 1940: 23. *D. komaii* is a probable synonym.

Duda (1923: 47) stated that this new species was being described in another publication from specimens in the Berlin Entomological Museum from Paroe, Formosa. He also reported numerous specimens in the Budapest Museum from Formosa and New Guinea: The other publication never appeared, however, and the name is a *nomen nudum* here. Hennig (1941) reported that the Berlin museum had 8 "Typen" plus 9 other specimens from Paroe, and 47 additional specimens from three other localities on Formosa.

Duda (1923: 48), however, in a brief discussion of *sulfurigaster*, compared *albomicans* with it, mentioning several traits, so that it is necessary to cite the name as valid at this date and page.

The males of *albomicans*, when viewed "head on," show a silvery to chalky white coloration over the entire frons[2]. The face, especially the carina, and the third antennal segments are a little darkened, and there is a noticeable dark longitudinal band on the pleura, reaching back to the wing base.

Distribution: Okinawa, Taiwan (Formosa), Pescadores Islands, Thailand. See Table 2 and Figure 2.

2. *Drosophila albovittata* Duda 1926: 87.

Duda clearly stated that he was proposing this as a new name for *sulfurigaster* (based on a single male from Madang, New Guinea) since he now had better material (from Sumatra) that showed a more distinctive characteristic. Such name changes are not allowable, however, as stated in Article 18a of the International Rules of Zoological Nomenclature (. . . *a name, once established, cannot afterwards be rejected, even by its own author, because of inappropriateness*), and therefore, as an unjustified new name, *albovittata* is an absolute synonym of *sulfurigaster*. The taxon represented by Duda's Sumatra material is, according

[2] The morphological terms to be used here are those currently used by most dipterists, the *frons* or *front* being the area between the compound eyes and above the antennae; the *face* being that part below the antennal baseline. Ferris (1950), however, stated that the entire area from the ocelli to the ventral margin of the cranium consitutes the *frons*; the term *postfrons* was applied to the area between the ptilinal suture and the ocelli, and the term *prefrons* was applied to the area anterior to, that is, below, the ptilinal suture.

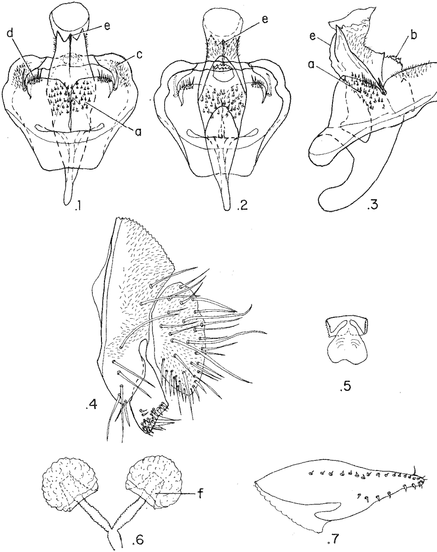


FIG. 3. Drawings of male and female genitalia of *D. pallidifrons*. 3.1 ventral view, .2 dorsal view and .3 lateral view of the ♂ internal genitalia; .4 lateral view of the left half of the ♂ external genitalia; .5 bridge which connects the claspers; .6 spermatheca; .7 lateral view of ovipositor. Labels on this and other figures are: a) bristled area of ventral side of penis; b) dorsal cylindrical process of penis; c) setae on hypandrium; d) setae on novasternum; e) medioventral fan-shaped flap; f) basal introvert of spermatheca; g) apical indentation of spermatheca.

to our present studies, not the same as that from New Guinea, and thus requires description and naming. We are proposing *albostrigata*, as *Drosophila sulfurigaster albostrigata* Wheeler, new subspecies (see later).

3. *Drosophila bilimbata* Bezzi 1928:159.

This was described from the Fiji Islands, the localities being listed as follows:

from the Lautoka Mountains; at Loloti, "bred from the fruits of Kawika, *Eugenia malaccensis*"; and "in thousands on decaying pomelos, *Citrus decumana*, and on decaying fruits of *Spondias dulcis*."

In our own collections, we have found the species to be common and widespread in Fiji. It is clearly the same species as that present on most islands of Polynesia, and, judging from the cytological and hybridization data, represents one of three "races" or "subspecies" of a very widespread species, *sulfurigaster*. Accordingly, we are using the combination, *sulfurigaster bilimbata* for this Polynesian type; *spinofemora* Patterson and Wheeler, which came originally from Hawaii, is a synonym.

The known distributions of the three types of *sulfurigaster* are shown on Figure 1 and listed in Table 2.

✓ 4. *Drosophila komaii* Kikkawa and Peng 1938:525.

The types were originally placed in the Zoological Institute of Kyoto Imperial University, but have now been moved to Tokyo (Dr. T. Okada, personal communication); they came from Amami-Osima and Isiga Kizima, of the Ryukyu Island group, and from Taihoku [Taipei[3]] and Sintiku [Hsin-chu], Taiwan. In most respects it seems to agree with *albomicans* Duda, also known principally from Okinawa and Taiwan. A major discrepancy is cytological—*komaii* was described as having chromosomes of the "D" type, *i.e.*, three pairs of rods and one pair of V's; we have not found this configuration in any stocks from Okinawa or Taiwan and feel fairly confident that the earlier report was in error. Accordingly, we are placing *komaii* as a synonym of *albomicans*.

5. *Drosophila nasuta* Lamb 1914:346; Fig. 30; Pl. 20, Fig. 32.

The specimens came from the Seychelles Islands, from several localities varying from 800 to 2000 feet. Males have the entire frons silvery; this was not only recorded by Lamb but was also seen by Duda (1940) who wrote: I received from Mr. Lamb a ♂ and ♀ from the Seychelles Expedition which match exactly my species [i.e., *albomicans*]. His remarks should mean only that the two were much alike, and in view of the great similarities between the species of this complex and the remarkable degree of speciation which has taken place in southeast Asia, it seems more reasonable to assume that the true *nasuta*, more than 3000 miles from the closest known *albomicans*, is still another species.

✓ 6. *Drosophila nixifrons* Tan, Hsu and Sheng 1949:202.

The culture which was used to describe this species came from Meitan, China, a village south of Chungking, about 800 miles from the nearest seacoast. We cannot recognize the species among the stocks which are now available; the chromosome constitution, especially, is not at all like any we have seen: one pair of V's, three pairs of rods (both X and Y are rods, the Y being longer), and one pair of dots. We are considering *nixifrons* to be a valid, but unrecognized species with silvery orbits.

✓ 7. *Drosophila setifemur* Malloch 1924:351; redescription, Clark 1957:221.

This Australian species is a member of the *sulfurigaster* complex; it also

[3] Modern place-names for Taiwan were furnished by Mr. Fei-Jann Lin.

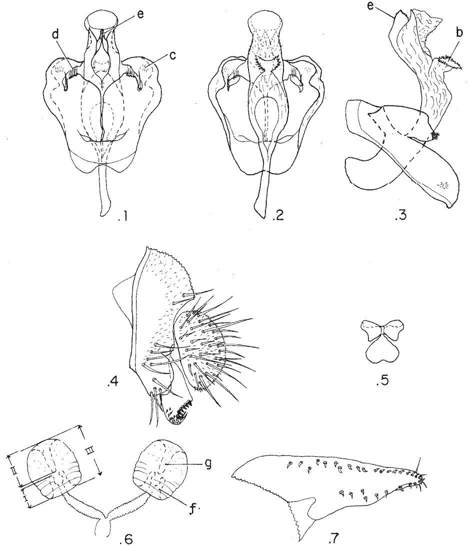


FIG. 4. Drawings of male and female genitalia of *D. sulfurigaster bilimbata*. The sequence of drawings is as in Fig. 3.

occurs on New Guinea (see Figure 1, and Table 2), and forms one of the three "subspecies" of *sulfurigaster*. Nomenclaturally we are placing it as a synonym of *D. s. sulfurigaster* (type from New Guinea).

8. *Drosophila spinofemora* Patterson and Wheeler 1942:104.

The type-culture came from Honolulu, Hawaii; this fly is not only very common and widespread in the Hawaiian Islands but is the same as those found on nearly all Polynesian islands, as shown on Figure 1 and in Table 2. *D. sulfurigaster bilimbata* appears to be the best name for these populations, and *spinofemora* is a synonym.

9. *Drosophila sulfurigaster* Duda 1923:48; 1926:83, 87–89.

Duda's original material was one male from the Budapest Museum, labelled "N. Guinea, 1896, Friedrich-Wilhemshafen"; this locality is now known as Madang. He characterized it as having only the frontal orbits whitish rather than the entire front as in *albomicans*. He later (1926) reported on numerous specimens from Sumatra which he considered to be the same species, and since the whitish orbits of the males seemed to be so characteristic, he changed the name to *albovittata*, and gave a redescription (see remarks under *albovittata*).

Our work on comparative cytology and hybridization indicates that the New Guinea–Australian type forms a race or subspecies of *sulfurigaster*, the Malaysian, Indonesian, Philippine populations belong to a different subspecies, while the Polynesian group represents a third type.

Wakahama (personal communication) has good evidence that some form of *sulfurigaster* occurs on Taiwan; we have not seen cultures of it from this area, however, and cannot say which of the three subtypes it represents.

Figure 1 and Table 2 show the distributions of the three subspecies of *sulfurigaster*, based on the present study. *D. sulfurigaster sulfurigaster* (with synonyms, *setifemur* and *willowsi*) occurs only in the Australia–New Guinea region. *D. sulfurigaster bilimbata* (synonym: *spinofemora*) is widespread, ranging from Hawaii and Polynesia to Guam. The third form is as follows:

10. **Drosophila sulfurigaster albostrigata** Wheeler, n. subsp.

Type locality: Semongok Forest Reserve, 12.5 miles south of Kuching, Sarawak, Borneo, Malaysia. *Type culture*: U. T. No. 3121.2–♀33, collected March 17, 1968 by Drs. D. E. Hardy and M. Delfinado from bananas and fruits of *Lanzium domesticum*. Additional collections, all from southeastern Asia, are shown on Figure 1 and in Table 2 and include the Philippine Islands, continental Malaysia, Cambodia and Thailand.

We cannot find any satisfactory distinguishing morphological characters to separate this form from the other subspecies of *sulfurigaster*. It differs greatly, however, in the number and distribution of chromosomal inversions (Table 4) and in its behavior in crosses to the other subspecies (Table 9).

11. *Drosophila willowsi* Curran 1936:42.

This species was based on two males from the Santa Cruz Islands, about midway between the New Hebrides and the Solomon Islands. Since only the orbits have a whitish pollinosity, this is probably the same form as *sulfurigaster* on New Guinea and New Ireland: *D. s. sulfurigaster*.

12. **Drosophila kohkoa** Wheeler, n. sp.

Type locality: Ari Ksatr, a village across the Mekong River from Phnom Penh, Cambodia. *Type culture*: U. T. No. 3057.3, collected May 1967 by M: Delfinado from fallen mango fruits and banana bait. Additional material from: Semongok Forest Reserve, Sarawak, Malaysia; Bon Chakkrarat, northeast of Bangkok, Thailand; Balsahan River, Iwahig, Palwan, Philippine Is.; Manilos, Belait Dist., Brunei, Borneo.

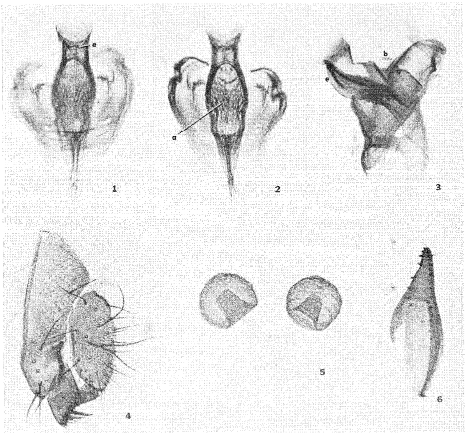


FIG. 5. Photographs of male and female genitalia of *D. pallidifrons*.

The name is compounded from the Cambodian *Koh*, meaning island, and from Thai *Ko*, also meaning island. The chromosome complement and polytene chromosome patterns are described in the following pages; the courtship behavior is discussed by Spieth in the next article. The wing is shown in Figure 8 and some details of the male genitalia are shown in Figure 9. In this species the face, and especially the carina and third antennal segment, is rather darker than in the other species.

13. **Drosophila pulaua** Wheeler, n. sp.

Type locality: Semongok Forest Reserve, 12.5 miles south of Kuching, Sarawak, Malaysia. *Type culture*: U. T. No. 3121.5, descended from a single female from the above locality, collected in March 1968 by D. E. Hardy and M. Delfinado. There are no additional records. The name is from Malaysian, meaning island.

The chromosome complement and polytene chromosome patterns are described in the following pages; the courtship behavior is discussed by Spieth in the next article; the wing is shown in Figure 8 and some details of the male genitalia are shown in Figure 9.

✓ 14. **Drosophila kepulauana** Wheeler, n. sp.

Type locality: Manilos, Belait Dist., about 65 miles southwest of Brunei Town, Brunei, Borneo. *Type culture*: U. T. No. 3122.3, collected March 1968 by D. E. Hardy and M. Delfinado. Additional material: a culture from Panitian, Gungnan, southwestern Palawan, Philippine Is. The name is Indonesian, meaning archipelago.

The chromosome complement and polytene chromosome patterns are described in the following pages; the courtship behavior is discussed by Spieth in the next article. The wing is shown in Figure 8 and some details of the male genitalia are shown in Figure 9.

✓15. **Drosophila pallidifrons** Wheeler, n. sp.

Type locality: Kolonia, Ponape Island, eastern Caroline Islands.

Type culture: U. T. No. 2535.4 collected in July and August, 1959, by M. R. Wheeler, W. S. Stone and M. Wasserman, mostly from fallen breadfruit.

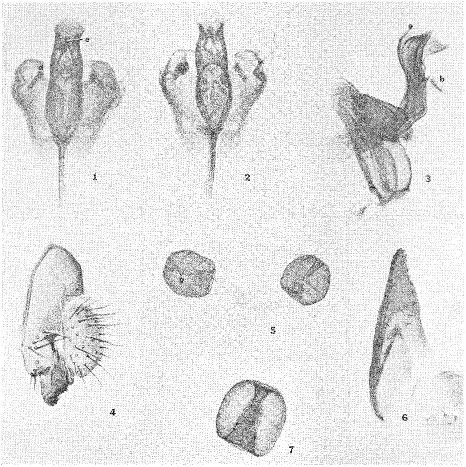


FIG. 6. Photographs of male and female genitalia of *D. sulfurigaster bilimbata* (.1 — .6);.7 spermatheca of *D. albomicans*.

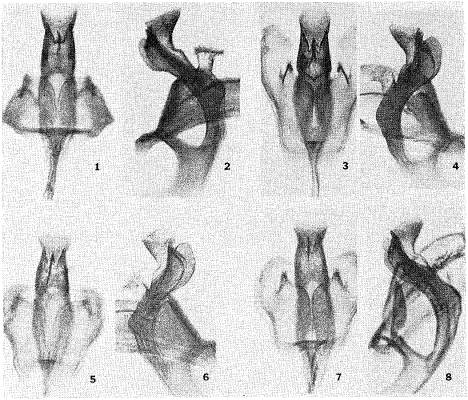


FIG. 7. Photographs of internal male genitalia of: .1–.2 *D. albomicans* (from Taiwan); .3–.4 *D. sulfurigaster sulfurigaster* (from Madang, New Guinea); .5–.6 *D. sulfurigaster bilimbata* (from Samoa); .7–.8 *D. sulfurigaster albostrigata* (from Kuala Lumpur, Malaysia).

Additional material: U. T. Stock No. 3131.2, from the same general area on Ponape, April 1968, H. T. Spieth, coll.

This is the most distinctive species of the subgroup; not only do males lack the silvery pollinosity of the *frons* but there are also decided differences in both male and female genitalia, illustrated in Figures 3 and 5. The wing is shown in Figure 8. The chromosome complement and polytene chromosome patterns are described in the following pages, and Spieth discusses the courtship behavior in the next article.

Notes on the male and female genitalia of the nasuta subgroup

The members of the *nasuta* subgroup are remarkably similar in their systematic characters. For example, the egg and puparium of *albomicans*, figured by Okada (1968, as "*nasuta*?"), are essentially identical in all the species. The wings, shown in Figure 8, are similarly alike, with only a few minor differences being evident. The male and female genitalia, although showing the same gross morphology for all the species, reveal some detectable differences in most instances. *D. pallidifrons*, from Ponape, is the only highly distinct species. Figures 3 and 5 illustrate the special features: numerous short, strong bristles on

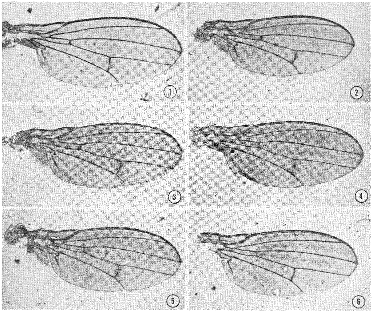


FIG. 8. Photographs of the wings of: .1 *sulfurigaster bilimbata*; .2 *pulaua*; .3 *albomicans*; .4 *kepulauana*; .5 *kohkoa*; .6 *pallidifrons*. All photographs were taken at the same magnification and developing and printing were identical; therefore size and pigmentation differences are real.

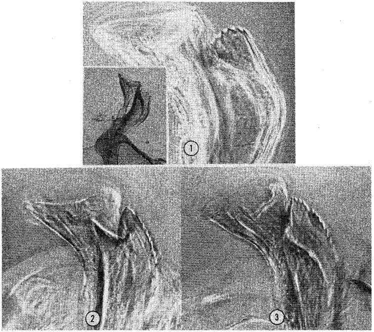


FIG. 9. Photographs of parts of the internal male genitalia of: .1 and inset, *pulaua*; .2 *kepulauana*; .3 *kohkoa*.

the ventral side of the penis; reduced size of the dorsal cylindrical expansion of the penis; two large sensilla and numerous setae on the novasternum; extremely small medioventral fan-shaped flap; numerous small setae on the hypandrium (see Okada, 1964, for terminology); a secondary row of stout bristles on the ovipositor; absence of an apical indentation on the spermatheca; and rougher surface structure of the spermatheca.

All other species are very similar, as is shown in Figures 4, 6 and 7. A careful study of the spermathecae might reveal statistically significant differences in proportions, such as width:length ratio, or in ratios of parts, as indicated in Figure 4.6. Here the ratio of length of basal introvert (I): length of apical indentation (II): overall length (III) was found to be 1:1:2.3 for *albomicans*, and 1:1.5:2.5 for *sulfurigaster* (Malaysian stock). These two also differ slightly in the width:length ratio—1:1 for *albomicans*, and 1:1.2 for Malaysian *sulfurigaster*.

The figures of the male genitalia of *sulfurigaster bilimbata* are representative of all the species except *pallidifrons*, and as shown in Figures 4, 6 and 7, these species are all remarkably similar. The medioventral fan-shaped flap appears to be the most useful genitalic character, but only in *pulaua* is it obviously different, as shown in Figure 9.1. A more detailed study of the male genitalia, including variation within populations, is underway and will be reported later.

Materials and Methods

The first series of tests in this investigation was made with flies from random stocks maintained at the University of Texas laboratory with the majority of them being subspecies of *D. sulfurigaster*. The *D. s. bilimbata* stocks were from collections made primarily by Stone and Wheeler in the South Pacific islands from 1955–1965 and included samples of populations from Hawaii, Palmyra, Tutuila, Savaii, Upolu, Niue, and Tonga. *D. s. sulfurigaster* material consisted of three probable iso-female lines from New Guinea and one from New Ireland collected by Marvin Wasserman in 1961, and one from Queensland, Australia received from Wharton B. Mather in 1955. The three *D. s. albostrigata* iso-female lines from continental Malaysia were received from Dr. Wasserman in 1962. Other laboratory stocks used include *D. pallidifrons* from Ponape collected by Wheeler, Stone, and Wasserman in 1959, and a stock of *albomicans* from Okinawa, which was given to us by Drs. Toyohi Okada and Osamu Kitagawa in 1966.

In 1966 and 1967 Stone and Wheeler collected more extensively in the islands of the South Pacific, providing additional strains from Hawaii, Palmyra, Tutuila, Savaii, Upolu, and Fiji. In 1967, *Drosophila* collections by Dr. Mercedes Delfinado yielded material from Palawan and Luzon, Philippine Islands, and from Cambodia; and Dr. L. H. Throckmorton sent us population samples from Taiwan and the Pescadores Islands collected by him and Mr. Fei-Jann Lin. In 1968 additional cultures were received from Drs. H. T. Spieth and H. L. Carson who collected on Ponape and Guam, from Throckmorton and Lin who made further collections in Taiwan and the Philippine Islands, and from Dr. D. Elmo Hardy and Dr. Delfinado who collected in Cambodia, Thailand, Sarawak, and Sabah in Malaysia, Brunei, Borneo and in the Philippine Islands. Additional flies from

Luzon, P. I., were received from Dr. Carmen Kanapi. See Table 2 for a complete listing of materials.

Females from the wild were isolated individually when they were received. Where possible males and females were separated upon collecting; therefore, the majority of iso-female lines established were from matings in the wild and not from matings in vials during shipment.

Larval brain squashes were made in the laboratory of each iso-female line and random stock to check the metaphase karyotype. Salivary gland preparations were made of each strain to determine heterozygosity for inversions; from thirty to fifty slides were made of random stocks (established from several to many iso-female lines) and at least ten slides were made from each iso-female line (although not necessarily of F_1 larvae). Representative lines were then selected from each locality for a complete series of crosses to detect sexual isolation and cytological variation.

Reciprocal crosses were made between flies from each of the different geographic locations. No attempt was made to ascertain percent fertility from pair matings at this time because of some degree of sexual isolation in many instances between members of the same species, and a high degree of sexual isolation in many interspecific crosses. Consequently, all matings were mass matings. They were carried out in large vials on cornmeal medium. Virgin females and males were collected twice each day and aged five to seven days before mating. In intraspecific crosses 15–20 pairs of flies per vial were used; in interspecific crosses 30 pairs per vial were used. Initially, five such mass matings were made for intraspecific crosses, and as many as ten to fifteen for interspecific crosses; where crosses were sterile, many of them were repeated in an attempt to get F_1 larvae for cytological analysis. Flies were transferred to fresh food every five days and at the end of thirty days were called sterile if no larvae were visible by that time. From sterile P_1 crosses the females were dissected in many cases to check for sperm in their spermathecae or seminal receptacles.

When F_1 flies were obtained, sex ratio counts were noted and F_1♀♀×F_1♂♂ crosses were made to test for fertility. If $F_1 \times F_1$ were sterile, a sample of both males and females was dissected to check for the presence of sperm in the testes or sperm storage organs. In addition, F_1 flies were sometimes backcrossed to both parent strains to see if either F_1 males or F_1 females were then fertile.

After analyzing the random stocks and iso-female lines as indicated above, salivary gland slides were prepared from the larvae of all fertile crosses to the standard Hawaiian strain and examined for fixed inversion differences, and photomicrographs were made. Also, a representative photographic record was kept of all inversion differences noted in subsequent P_1 crosses. A more extensive series of photographs will be published at a later date than are shown here.

Metaphase brain smears from each random or iso-female line were prepared by removing the ganglia of male larvae in physiological saline and allowing them to swell in a hypotonic solution of 1% sodium citrate for ten minutes. The ganglia were then transferred to aceto-orcein stain for five minutes and mounted in 45% acetic acid solution. Pressure was applied to the cover glass with a dissecting needle to spread the cells before squashing with thumb pressure; the cover glass was then sealed with clear nail polish.

TABLE 2

Strains of the *D. nasuta* subgroup species used in genetic tests and cytological investigations.

U. T. stock number	Locality collected	Type of stock; number of lines checked cytologically	Date collected and collector(s)	Line(s) used in crosses
D. sulfurigaster				
D. s. bilimbata				
3045.1	Tantalus, Oahu, Hawaii	random stock	1966; J. Grossfield	3045.1
3045.12	Hilo-Kona, Hawaii	random	1966; W. S. Stone & D. E. Hardy	....
3045.13	Maui, Hawaii	random	1966; W. S. Stone & H. T. Spieth	....
3074.1	Kipuka 9, Saddle Rd., Hawaii, Hawaii	16 iso-♀ lines	1967; M. Kambysellis	....
3074.2	Bonsey garden, Maui, Hawaii	20 iso-♀ lines	1967; H. T. Spieth	....
3074.3	Kipahulu, Maui, Hawaii	14 iso-♀ lines	1967; M. Kambysellis	....
3074.4	Bonsey garden, Maui, Hawaii	15 iso-♀ lines	1967; H. T. Spieth	....
3074.5	Kapaa, Kauai, Hawaii	18 iso-♀ lines	1967; C. Kanapi	....
3074.6	Manoa Valley, Oahu, Hawaii	22 iso-♀ lines	1967; S. Rockwood	....
3074.7	Wiliwilinui, Oahu, Hawaii	6 iso-♀ lines	1967; C. Kanapi	....
3045.5	Palmyra I., Line Is.	random	1962; W. S. Stone & M. R. Wheeler	....
3045.6	Palmyra I., Line Is.	random	1962; W. S. Stone & M. R. Wheeler	3045.6
3045.2	Pago Pago, Tutuila, Am. Samoa	random	1962; W. S. Stone & M. R. Wheeler	3045.2
3045.4	Taputimu, Tutuila, Am. Samoa	random	1965; W. S. Stone & C. P. Oliver	....
3045.7	Aopo, Savaii, Samoa	random	1965; W. S. Stone & C. P. Oliver	3045.7
3061.2	Aopo, Savaii, Samoa	random	1967; W. S. Stone & M. R. Wheeler	....
3045.8	Apia, Upolu, Samoa	random	1965; W. S. Stone & C. P. Oliver	3045.8
3045.9	Niue I.	random	1965; W. S. Stone & M. R. Wheeler	3045.9
3045.10	Tongatapu, Tonga Is.	random	1963; W. S. Stone & M. R. Wheeler	3045.10
3044.3	Coloisuva, Viti Levu, Fiji	random	1966; M. R. Wheeler	3044.3
3063.2	Nadarivatu, Viti Levu, Fiji	random	1967; W. S. Stone & M. R. Wheeler	....

TABLE 2—(Continued)

Strains of the *D. nasuta* subgroup species used in genetic tests and cytological investigations.

U. T. stock number	Locality collected	Type of stock; number of lines checked cytologically	Date collected and collector(s)	Line(s) used in crosses
3100.2	Korolevu, Viti Levu, Fiji	80 iso-♀ lines	1968; M. R. Wheeler	....
3071.6	Guam, Marianas Is.	60 iso-♀ lines	1968; H. L. Carson	3071.6
D. s. sulfurigaster				
3017.4	Kavieng, New Ireland	iso-♀ line	1961; M. Wasserman	3017.4
3019.8	Wau, New Guinea	iso-♀ line	1961; M. Wasserman	3019.8
3020.2	Brown River, Papua, New Guinea	iso-♀ line	1961; M. Wasserman	3020.2
3016.2	Madang, New Guinea	iso-♀ line	1961; M. Wasserman	3016.2
2372.16	Queensland, Australia	random	1955; W. B. Mather	2372.16
D. s. albostrigata				
3054.2	Cabuyao, Laguna, Luzon, Philippines	iso-♀ line	1966; M. Delfinado	3054.2
3138.2	Tagaytay, Luzon, Philippines	iso-♀ line	1966; L. H. Throckmorton & F. J. Lin	3138.2
3139.2	Luzon, Philippines	11 iso-♀ lines	1968; C. Kanapi	3139.2 ♀2, ♀3
3126.2	Baguio, Luzon, Philippines	2 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3126.2 ♀a
3056.2	Panitian, Palawan, Philippines	8 iso-♀ lines	1967; M. Delfinado	3056.2 ♀3, ♀6
3146.2	Los Banos, Luzon, Philippines	5 iso-♀ lines	1968; L. H. Throckmorton & F. J. Lin	3146.2 ♀43
3057.2	Ari Ksatr, Cambodia	27 iso-♀ lines	1967; M. Delfinado	3057.2 ♀9, ♀10, ♀12
3120.2	Siem Reap, Cambodia	3 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3120.2 ♀b, ♀c
3119.2	Semongok Forest Reserve, Sarawak	3 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3119.2 ♀a, ♀c
3121.2	Semongok Forest Reserve, Sarawak	49 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3121.2 ♀33, ♀52
3122.2	Brunei, Borneo	72 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3122.2 ♀44, ♀60
3124.2	Jesselton, Sabah, Sarawak	7 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3124.2 ♀2, ♀5
3033.17–.18–.49	Kuala Lumpur, Malaysia	3 iso-♀ lines	1962; M. Wasserman	3033.17

TABLE 2—(Continued)

Strains of the *D. nasuta* subgroup species used in genetic tests and cytological investigations.

U. T. stock number	Locality collected	Type of stock; number of lines checked cytologically	Date collected and collector(s)	Line(s) used in crosses
3116.2	Bon Chakkrarat, Thailand	43 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3116.2 ♀21, ♀29
3117.2	Bon Phra, Thailand	3 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3117.2 ♀a, ♀c
D. pallidifrons				
2535.4	Kolonia, Ponape, Caroline Is.	random	1959; W. S. Stone & M. R. Wheeler	2535.4
3131.2	Kolonia, Ponape, Caroline Is.	12-iso-♀ lines	1968; H. T. Spieth	....
D. albomicans				
3045.11	Okinawa, Ryukyu Is.	random	1966; T. Okada & O. Kitagawa	3045.11
3046.2	Wulei, Taiwan	14 iso-♀ lines	1966; L. H. Throckmorton	3046.2
3066.2	Taiwan	37 iso-♀ lines	1967; L. H. Throckmorton	....
3146.3	Taiwan	6 iso-♀ lines	1968; L. H. Throckmorton & F. J. Lin	....
3067.2	Pescadores Is. (Makung, Peng Hu)	iso-♀ line	1967; F. J. Lin	....
3116.3	Bon Chakkrarat, Thailand	26 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3116.3 ♀46
D. pulaua				
3121.5	Semongok Forest Reserve, Sarawak	iso-♀ line	1968; D. E. Hardy & M. Delfinado	3121.5
D. kepulauana				
3056.8	Panitian, Palawan, Philippines	iso-♀ lines	1967; M. Delfinado	3056.8
3122.3	Brunei, Borneo	4 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3122.3 ♀1, ♀5
D. kohkoa				
3129.2	Iwahig, Palawan, Philippines	iso-♀ line	1968; D. E. Hardy & M. Delfinado	3129.2
3121.3	Semongok Forest Reserve, Sarawak	17 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3121.3 ♀7, ♀13
3057.3	Ari Ksatr, Cambodia	12 iso-♀ lines	1967; M. Delfinado	3057.3 ♀2
3116.3	Bon Chakkrarat, Thailand	2 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3116.3 ♀55
3122.3	Brunei, Borneo	5 iso-♀ lines	1968; D. E. Hardy & M. Delfinado	3122.3 ♀6

Salivary gland preparations were made by dissecting third instar larvae in 45% acetic acid, fixing in 1 N HCl, staining in lacto-aceto-orcein stain and mounting in lacto-acetic acid (1 part lactic acid and 1 part 60% acetic acid). Squashing was as described for brain smears. Slides were examined and photographed on a Zeiss Phase Photomicroscope. Kodak Panatomic-X 35 mm film was used in photography.

Other procedures that helped separate the *D. nasuta* subgroup species were the sexual behavior pattern as observed by Speith (this Bulletin), the dissections of the genitalia by Kambysellis, and studies of protein variation by Johnson, *et al.* (1968) and Kanapi (1967) showing that there are different alleles and patterns of enzyme variability between some of the species.

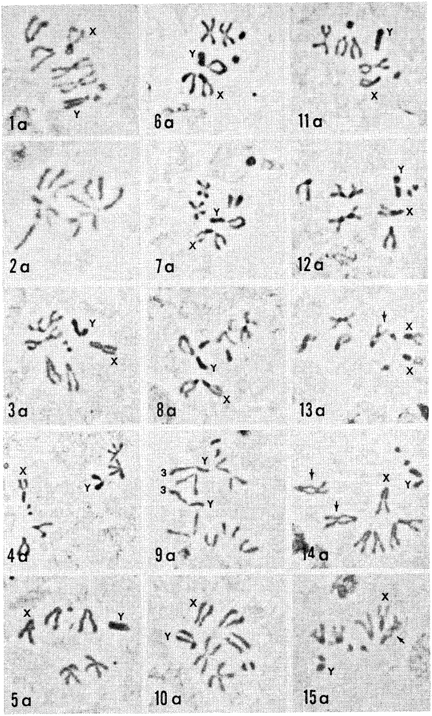
Metaphase Chromosome Configurations

The standard Hawaiian *D. s. bilimbata* strain, 3045.1, has the basic configuration of the *immigrans* group: a rod X and rod Y, a pair of dots (chromosome 4), a pair of V's (chromosome 2), and the double length rod (chromosome 3). This double length rod is composed of two of the five long rods of the primitive *Drosophila* karyotype which consists of five pairs of rods plus a pair of dots. It arose by a pericentric inversion of a two-element V or by a total translocation of a rod to the end of another.

Photographs and accompanying diagrams of the metaphase configurations of the *D. nasuta* subgroup species are shown in Figures 10 and 11. Great variation exists in the Y chromosome not only between the different species, but also within several of the species; therefore, the male set of chromosomes is represented in each instance. Where more than one type of karyotype was found in a species, an example of each is given. The variation and distribution of these metaphase karyotypes are summarized in Table 3.

Throughout the range of *D. s. bilimbata* the karyotype is like that of the Hawaiian strain, with the Y chromosome being a rod with a constriction that has been determined to be near the centromere. In the other two sub-species of *D. sulfurigaster*, the Y chromosome is variable. In *D. s. sulfurigaster*, it is modified to a V in the Australian strain and into a J in the New Guinea and New Ireland strains. In *D. s. albostrigata* there are three types of Y chromosomes: rod, J, and V. In the limited population samples from the Philippine Islands, all three types are represented from Luzon, and J- and V-shaped Y's are known from Palawan. In Sarawak, Malaysia, 13 lines showed configurations with a rod, and 38 had a J-shaped Y; in Brunei, Borneo, there were 8 lines with rod-shaped Y's found, and 23 with J's. In neither of these two areas were V-shaped Y's found. In Thailand and Cambodia, no rod-shaped Y's were found; Thailand had 16 lines with J's and 23 with V's while Cambodia had 13 lines with J's and 14 with V-shaped Y chromosomes.

Both *D. pallidifrons* and *D. pulaua* have the basic karyotype configuration, although the Y lacks the constriction of *D. s. bilimbata*. However, the samples are small; *D. pallidifrons* has been collected only in Ponape, and *D. pulaua* is represented by a single iso-female strain from Sarawak, Malaysia. The sample of *D. kepulauana* is greater but limited. The iso-female line from Palawan has the


1a
X
Y
6a
Y
X
11a
Y
X
2a
7a
Y
X
12a
Y
X
3a
Y
X
8a
Y
X
13a
X
X
4a
X
Y
9a
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Y
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Y
10a
X
Y
15a
X
Y

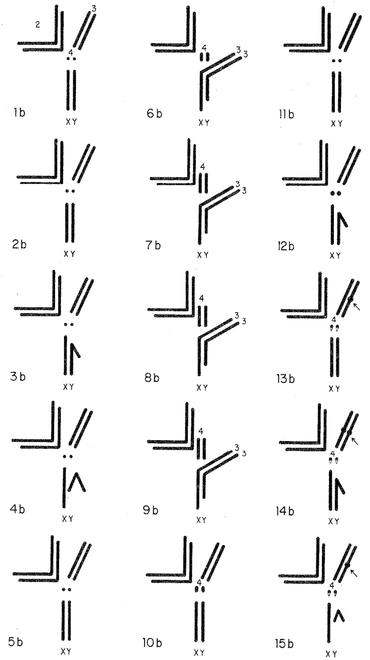

FIG. 10–11. Photographs (*a* series) and diagrammatic representations (*b* series) of metaphase configurations of the *nasuta* subgroup. Males are shown except in 13a. Some figures were photographed at anaphase. KEY: 1–4 *sulfurigaster*; 5 *pallidifrons* and *pulaua*; 6–9 *albomicans* (6, Thailand; 7–9, Taiwan); 10 *kepulauana;* 11–15 *kohkoa* (arrow indicates the pinched 3 chromosome).

TABLE 3

Variation and distribution of the Y Chromosome in the *D. nasuta* subgroup.

Location	Species	Rod	J	V	Y3	V-J
Hawaii	*D. s. bilimbata*	+*				
Palmyra	*D. s. bilimbata*	+				
Tutuila	*D. s. bilimbata*	+				
Savaii	*D. s. bilimbata*	+				
Upolu	*D. s. bilimbata*	+				
Niue	*D. s. bilimbata*	+				
Tonga	*D. s. bilimbata*	+				
Fiji	*D. s. bilimbata*	+				
Guam	*D. s. bilimbata*	+				
Luzon	*D. s. albostrigata*	1	5	12		
Palawan	*D. s. albostrigata*		1	8		
	D. kepulauana	1				
	D. kohkoa		1			
Malaya	*D. s. albostrigata*		+			
New Ireland	*D. s. sulfurigaster*		+			
Wau	*D. s. sulfurigaster*		+			
Papua	*D. s. sulfurigaster*		+			
Madang	*D. s. sulfurigaster*		+			
Australia	D. s. sulfurigaster			+		
Ponape	*D. pallidifrons*	+				
Okinawa	*D. albomicans*				+	
Pescadores	*D. albomicans*				+	
Taiwan	*D. albomicans*				57	
Cambodia	*D. s. albostrigata*		13	14		
	D. kohkoa	11		1		
Sarawak	*D. s. albostrigata*	13	38			
	D. kohkoa	4	3			
	D. pulaua	1				
Thailand	*D. s. albostrigata*		16	23		4
	D. albomicans				27	
	D. kohkoa	2				
Borneo	*D. s. albostrigata*	8	23			
	D. kepulauana	1	2			
	D. kohkoa	4	1			

* Random stock (+) used when iso-♀ lines were not available; numerals show number of iso-♀ lines checked.

primitive karyotype with a small amount of heterochromatin having been added to the dot chromosome (4) making it thicker and longer than the basic dot. In at least two iso-female lines from Brunei, Borneo, the Y chromosome is modified to a slight J shape. To be certain of this it has been necessary to check the anaphase instead of the metaphase configurations. An addition should be made to Figure 10–11 where only a rod is shown to represent the *D. kepulauana* Y.

Although in *D. kohkoa* many iso-female lines have a standard metaphase configuration, there are numerous deviations from it. Strains from Cambodia, Thailand, Sarawak, and Borneo often have either one or both of the double-length rod chromosomes (3) showing a pinched constriction which is always accompanied by the dot having a small amount of added heterochromatin which gives it a comma-shaped appearance. This "comma" dot is sometimes found without the pinched 3, but never *vice versa*. One line from Cambodia (♀ no. 24) has a small metacentric Y, and several lines from Sarawak and the only *D. kohkoa* from Palawan, P.I., have J-shaped Y chromosomes.

D. albomicans from Okinawa, Taiwan, the Pescadores Islands and Thailand has a remarkable karyotype. There has been heterochromatin added to the dot chromosome making it a heavy heterochromatic rod, and the X (and the Y) are fused with the double-length rod chromosome to place three of the primitive *Drosophila* rod elements in one very long J-shaped chromosome. This reduces the karyotype to three pairs of chromosomes. It is also observed that the terminal ends of the double-length third chromosome, which is fused to the Y, are always pinched together. The only variation in metaphase configuration for *D. albomicans* is seen in the strains from Thailand where not so much heterochromatin has been added to the dot chromosome.

Polytene Chromosome Analysis

The salivary gland chromosomes of the *D. nasuta* subgroup show four long arms and one small arm (Figure 12). Each has been numbered as the *D. immigrans* chromosomes were by Freire-Maia *et al.* (1953). The X chromosome is 1, 2L and 2R designate the left and right arms of the V of the metaphase configuration, 3 is the double-length rod chromosome and 4 is the small chromosome seen as a dot in the metaphase. Only the long arms have been investigated in this study. No centric heterochromatin is observed on the X or the 3; however, between the 2L and 2R, which are usually joined together, a small amount of heterochromatin has been noticed. Adjacent to it, a loop, which is not an inversion, is seen at the base of the 2R (Figure 13). We consider this "loop" area to be part of the 2R chromosome. When a salivary slide is squashed hard enough

FIG. 12. Salivary gland chromosomes of the *D. s. bilimbata* standard. The four long euchromatic arms are: the X, 2L, 2R with a characteristic "loop" at the base, and the double-length 3. The short arm is chromosome 4, the dot of the metaphase karyotype.

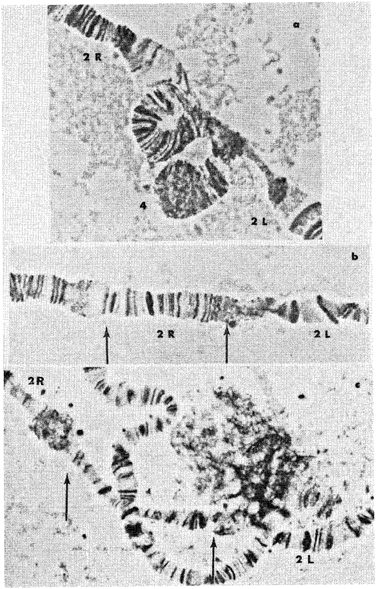

FIG. 13. a) An enlarged photograph of the characteristic "loop" observed in salivary gland preparations between chromosomes 2L and 2R. The small arm is chromosome 4.

b) The "loop" region, when stretched, is seen as the basal end of 2R. The small amount of centric heterochromatin observed is at the base of 2L. Arrows indicate limits of the "loop" region.

c) An unusually large amount of centric heterochromatin is seen at the base of 2L in the single iso-female *D. s. albostrigata* strain, 3116.2, ♀ no. 3. Arrows indicate limits of the stretched "loop" region which precisely coincides with the excessive heterochromatin sites. None is seen at the base of 2R except in this strain.

to stretch the chromosomes, the "loop" no longer appears as such in many cells, but as the proximal end of the 2R.

The number of inversion differences observed in the six species of the *D. nasuta* subgroup studied here varies greatly. Two of the species have the same sequence in all chromosomes as the *D. s. bilimbata* Hawaiian line used as the cytological standard. The four other species have sequences which differ from the standard by at least 39 fixed and heterozygous inversions. The majority of these sequences are found in the three species with silvery frons while the remainder occur within the subspecies of *D. sulfurigaster*. With completion of an analysis of the complexes on chromosome 3 and the X, there will be more than twice as many known rearrangements (fixed and heterozygous) in *D. kepulauana, D. albomicans* and *D. kohkoa* as in the two species with silvery orbits, *D. pulaua* and *D. sulfurigaster*, and the only species of the subgroup lacking the silvery markings, *D. pallidifrons*.

Figure 14 shows the approximate positions and sizes of the inversions which have been analyzed to date.

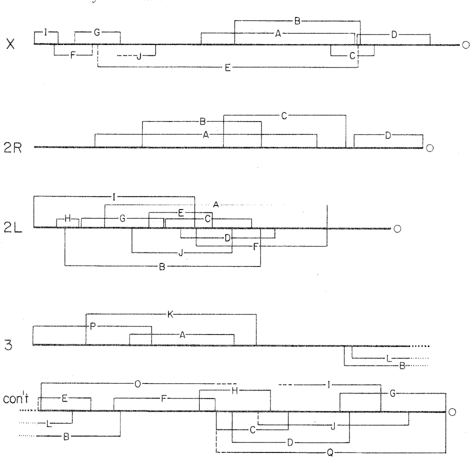

FIG. 14. Distribution of inversion breakpoints relative to the standard sequence. The open circles at the right ends represent the basal ends of the chromosomes.

TABLE 4

Chromosomal (inversion) variability in the *D. nasuta* subgroup

Species & Strains	Chromosomes X	2R	2L	3
D. sulfurigaster				
D. s. bilimbata	+	+	+	+
D. s. sulfurigaster				
New Ireland	+	+	D/+	+
Erown River, Papua, N. G.	+	+	+	+
Wau, Papua, N. G.	+	+	+	+
Madang, Papua, N. G.	D/+	+	+	+
Queensland, Australia	+	+	+	+
D. s. albostrigata				
Palawan, P. I.	C/+, F/+	B/+	A/+, C/+	A/+, C/+
Luzon, P. I.	C/+	+	A/+, C/+	A/+
Sarawak, Malaysia	B/+, C/+, Xs	B/+	A/+, C/+	A/+, C/+
Sabah, Malaysia	B/+, C/+, Xs	+	A/+, C/+	A/+, C/+
Brunei, Borneo	B/+, C/+, Xs	B/+	A/+, C/+	C/+
Continental Malaysia	C	B/+	A/+, C/+	A, C/+
Cambodia (Y = V)	+	A/+	A/+, B/+, G/+	A/+, B/+, C/+
Cambodia (Y = J)	C/+, F/+	A	A/+, G/+	+
Thailand (Y = V)	+	A/+	A/+, B/+, G/+	+
Thailand (Y = J)	+	A	A/+, B/+	+

TABLE 4—Continued

Species & Strains	Chromosomes X	2R	2L	3
D. pulaua	+	+	+	+
D. pallidifrons	+	+	+	+
D. kepulauana				
Palawan, P. I.	A, B, G, I, J	B, C	F	B, G, O, Q
Brunei, Borneo	B, G, I, J	B, C	A/+, F	A, B, C/+, G, O, Q
D. albomicans				
Okinawa	B, G, I, J	C	A/+, F, H/+	B, F, G, R
Taiwan	B, G, I, J	C	A/+, F, H/+	B, F/+, G, R
Pescadores Islands	B, G, I, J	C	F, H/+	B, F, G, R
Thailand	A/+, B, G, I, J	C/+	A/+, F, H/+, I/+, J/+	A/+, B/+, F/+, G, K/+
D. kohkoa				
Palawan, P. I.	B, C, I, J	C, D	A/+, E/+, F	A, D, H/+, R
Brunei, Borneo	B, C, I, J	C, D	A/+, E/+, F	A, D/+, E/+, H/+, I/+, J/+, K/+, R
Sarawak, Malaysia	B, C, I, J	C, D	A/+, E/+, F	A, D, E/+, J/+, K/+, L/+, O/+, R
Cambodia	B, C, E, I, J.	C, D	E/+, F	A, D, H/+, I/+, R
Thailand	B, C, E, I, J.	C, D	A/+, E/+, F	A, D, R

+ indicates the standard chromosomal sequence.
Capital letters alone indicate fixed inversion differences from the standard.
Capital letters/+ indicate inversion heterozygosity.

D. pallidifrons and *D. pulaua* are homosequential with the standard. No inversions were found in the 1959 laboratory stock of *D. pallidifrons* nor in the 12 iso-female lines collected in 1968, and none is present in the iso-female line of *D. pulaua*.

Of the 39 recorded inversions, 30 are found to be heterozygous in one or more of the four other species; 18 of these are found only as heterozygotes with no populations being fixed for the inverted sequence. Nineteen inversions are fixed in one or more localities and/or species, with 9 of these always fixed.

The three subspecies of *D. sulfurigaster* share none of the fourteen inversions found in this widespread species .All of these inversions exist heterozygous, but three of them are fixed in all strains from two different populations. *D. s. bilimbata* strains from twenty-one localities in the South Pacific islands have been extensively checked and each has been found to possess only the same sequence as the Hawaiian standard. Population samples of *D. s. sulfurigaster* are limited to one line each from five localities in the Australia-New Guinea area, but from larger samples, Mather (1962) and Clark (1957) reported no inversions. We have found two heterozygous inversions in this subspecies: there is one on the X chromosome of the Madang, New Guinea strain, XD, and one on the 2L of the New Ireland strain, 2LD; neither has been seen again in any of the other species of the subgroup.

The third subspecies, *D. s. albostrigata,* is quite polymorphic in all localities where it has been collected. A total of twelve heterozygous inversions distributed among all four long euchromatic arms have been analyzed, with three of them being fixed in certain continental populations. The two inversions on the 2R have not been found to exist in the same population. 2RA is seen only in this subspecies and only in the Thailand and Cambodia lines where it is heterozygous when the Y chromosome is a V but is homozygous in those lines where the Y chromosome is a J. 2RB is heterozygous in most of the island populations of

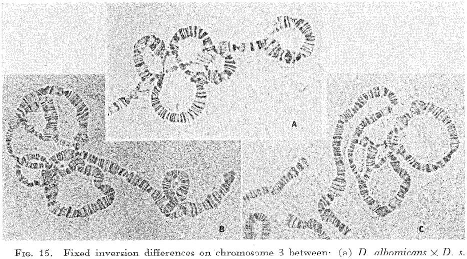

FIG. 15. Fixed inversion differences on chromosome 3 between: (a) *D. albomicans* × *D. s. albostrigata*; (b) *D. kohkoa* × *D. albomicans*; (c) *D. kepulauana* × *D. albomicans*.

D. s. albostrigata with Malaysia being the only continental area having it. Of the four heterozygous inversions on 2L, only 2LA is observed from all localities and is the only 2L inversion shared with other species. Moreover, it is found in all three other species which possess inversions. 2LC is common to the island members and continental Malaysia, 2G is found in strains from Cambodia and Thailand, and 2LB belongs only to the Thailand strains. Two of the three heterozygous inversions of the third chromosome, 3A and 3C, are widespread in this subspecies. They are seen in other species of the subgroup as is 3B, but Cambodia is the only locality where 3B has been observed in *D. s. albostrigata.* 3A of continental Malaysia is the only fixed inversion difference on the 3 from the standard. XC is found throughout the island populations and in a few continental areas; it is fixed in continental Malaysia. XB is limited to the island strains of *D. s. albostrigata* and both XB and XC are found as fixed inversions in one or more of the other species. XF, seen only in this subspecies, is present in lines from the Philippine Islands and Cambodia.

The remaining 25 analyzed inversions of the *D. nasuta* subgroup (Table 4) have been observed only in one or more of the three species characterized by more extensive silvery markings on the frons: *D. kepulauana*, *D. albomicans*, and *D. kohkoa*. Eighteen of the fixed 19 inversion differences found throughout the subgroup exist in these species, and they share one or more of the fixed inversion differences on three of the chromosome arms: 2RC, 2LF, and XB, XI, and XJ. Each of the three species has a set of fixed simple inversions or inversion

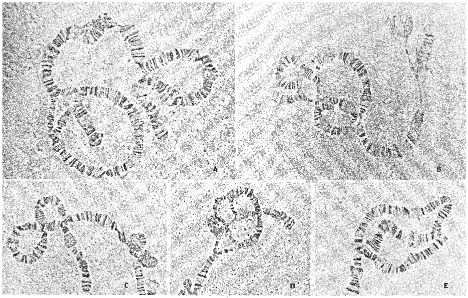

FIG. 16. Some inversion differences from the standard on chromosome 2L. (a) 2LA and 2RA of *D. s. albostrigata.* 2LA is polymorphic in *D. sulfurigaster, D. kepulauana, D. kohkoa* and *D. albomicans.* (b) Three polymorphisms of *D. albostrigata.* This complex is made up of 2LA, 2LB and 2LC. (c) 2LA and 2LC form this included configuration. (d) Inversion differences between *D. albomicans* and *D. albostrigata* include 2LA, 2LC and 2LF. (e) Two of the polymorphisms found in *D. albomicans* (Thailand). 2LI and 2LA form an overlapping configuration.

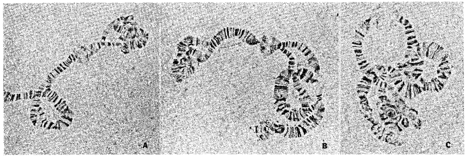

FIG. 17. Fixed inversion differences on the X chromosome between: (a) *D. albomicans* and *D. s. bilimbata*; (b) *D. kohkoa* and *D. kepulauana*; (c) *D. albomicans* and *D. kohkoa*.

complex differences, a few of which are shared by one of the two other species, but not by both of them. Six inversions which are polymorphic in *D. s. albostrigata* are also found in at least one of these species, where all but 2LA tend to be fixed. Thus, 31 of the 39 sequences which differ from the standard have been observed in at least one strain of one of these three species.

The population samples of *D. kepulauana* have at least ten fixed inversion differences from the standard. One of these inversions, XA, is found in the isofemale line from Palawan, Philippines (3056.8), and it has not been observed in any of the lines from Brunei, Borneo. Throughout this species the characteristic "loop" between chromosomes 2L and 2R was absent.

Strains of *D. albomicans* from Taiwan, Okinawa, and the Pescadores Islands are each fixed for the same four simple paracentric inversions, 2RC, 2LF, 3G, and XB, and for inversion complexes on the 3 and the X. In the strains from Thailand some of these inversions are found to be fixed while the others are heterozygous. Additional heterozygous inversions are observed in the Thailand population that are not found from any of the other localities where *D. albomicans* has been collected.

In *D. kohkoa* eight fixed simple paracentric inversions and two fixed inversion complexes are found in all lines of the species with the exception of the large XE which is fixed only in the continental population samples. Two inversions on the 2L, 2LA, and 2LF, are the only heterozygous inversions widely distributed throughout the range of *D. kohkoa*. However, in strains from Brunei, Borneo, and Malaysia seven heterozygous inversions have been observed on chromosome 3 which so far are limited to these lines.

Because of the chromosomal variability within *D. sulfurigaster*, *D. kepulauana*, *D. kohkoa*, and *D. albomicans*, Table 4 breaks down each species into areas of collection to show the intraspecific as well as the interspecific inversions of the *D. nasuta* subgroup, which have been analyzed at this time. Some of the inversions, inversion complexes, and crosses of the *D. nasuta* subgroup are shown in Figures 15–20.

HYBRIDIZATION EXPERIMENTS

A series of reciprocal crosses was made among laboratory stocks available in 1966. These stocks were primarily *D. sulfurigaster* including eight *D. s. bilim-*

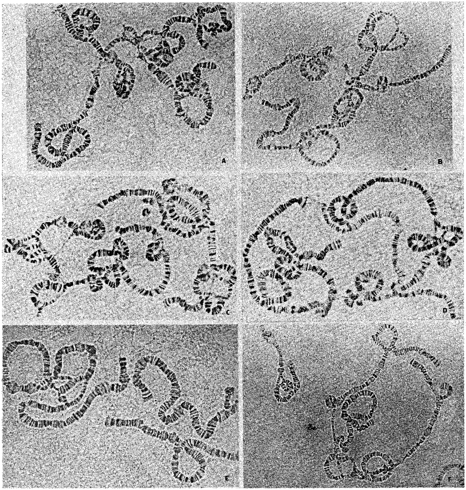

FIG. 18. Inversion differences in crosses between (a) *D. s. bilimbata* standard and *D. kepulauana*; (b) *D. kohkoa* and *D. albomicans*; (c) *D. albomicans* and *D. s. albostrigata*; (d) *D. s. bilimbata* standard and *D. albomicans*; (e) *D. kepulauana* and *D. albomicans*; (f) *D. kohkoa* and *D. kepulauna*.

bata, five *D. s. sulfurigaster*, and one *D. s. albostrigata.* The other random stocks used were one *D. pallidifrons* and two *D. albomicans* (Table 5). The complete series of crosses of *D. sulfurigaster* is presented to show the gradation of F_1 sterility between different sub-species of this species. Where *D. s. bilimbata* is the female of crosses to *D. s. albostrigata* or *D. s. sulfurigaster,* all crosses are fertile through F_1. Where *D. s. bilimbata* is the male of the crosses, the results agree with Clark (1957); when *D. s. sulfurigaster* lines from Australia (2372.16) or Madang, N. G., are the males of these crosses, $F_1 \times F_1$ is sterile. However, when *D. s. sulfurigaster* (3019.8) from Wau, N. G., and No. 3020.2 from Papua, N. G., are the males of these crosses, $F_1 \times F_1$ are consistently slightly fertile, and when the New Ireland (3017.4) line is used, all $F_1 \times F_1$ are very fertile. New Ireland is not reproductively isolated from either *D. s. bilimbata* nor *D. s. sulfurigaster*. $F_1 \times F_1$ from crosses with the *D. s. albostrigata* line from Malaysia (3033.18) behaved the same in crosses as when the *D. s. sulfurigaster* lines from Australia were used.

New material received from collections made in 1967 and early 1968 provided strains of *D. s. bilimbata* from Guam (3017.6) and population samples of *D. s. albostrigata* from Luzon, P. I., Palawan, P. I., and from Cambodia. The lines from Palawan and Cambodia were found to have both V- and J-shaped Y chromosomes; strains with each metaphase configuration type were selected to cross to some of the stocks used in crosses of Table 5. The latter strains consist of our standard, *D. s. bilimbata* Hawaii (3045.1), *D. s. sulfurigaster* Australia

♀ \ ♂	*D. sulfurigaster bilimbata*: Hawaii 3045.1	Tutuila 3045.4	Palmyra 3045.6	Savaii 3045.7	Upolu 3045.8	Niue 3045.9	Tonga 3045.10	Fiji 3044.3	*D. s. sulfurigaster*: New Ireland 3017.4	Wau, N. G. 3019.8	Brown River, N.G. 3020.1	Madang, N. G. 3016.2	Australia 2372.16	*D. s. albostrigata*: Cont. Malaysia 3033.18	*D. pallidifrons*: Ponape 2535.4	*D. albomicans*: Okinawa 3045.11	Taiwan 3046.2
D. sulfurigaster bilimbata																	
Hawaii 3045.1	\	F	F	F	F	F	F	F	F	F	F	F	F	F	f	F	F
Tutuila 3045.4	F	\	F	F	F	F	F	F	F	F	F	F	F	F	S	F	F
Palmyra 3045.6	F	F	\	F	F	F	F	F	F	F	F	F	F	F	S	F	F
Savaii 3045.7	F	F	F	\	F	F	F	F	F	F	F	F	F	F	f	F	F
Upolu 3045.8	F	F	F	F	\	F	F	F	F	F	F	F	F	F	S	F	F
Niue 3045.9	F	F	F	F	F	\	F	F	F	F	F	F	F	F	S	F	F
Tonga 3045.10	F	F	F	F	F	F	\	F	F	F	F	F	F	F	S	F	F
Fiji 3044.3	F	F	F	F	F	F	F	\	F	F	F	F	F	F	S	F	F
D. s. sulfurigaster																	
New Ireland 3017.4	F	F	F	F	f	f	F	F	\	F	F	F	F	F	S	F	F
Wau, N. G. 3019.8	f	f	f	f	f	f	f	f	F	\	F	F	F	F	S	F	F
Brown River, N.G. 3020.1	f	f	f	f	f	f	f	f	F	F	\	F	F	F	f	F	F
Madang, N. G. 3016.2	F	F	F	F	F	F	F	F	F	F	F	\	F	F	f	F	F
Australia 2372.16	F	F	F	F	F	F	F	F	F	F	F	F	\	F	S	F	F
D. s. albostrigata																	
Cont. Malaysia 3033.18	F	F	F	F	F	F	F	F	F	F	F	F	F	\	S	F	F
D. pallidifrons																	
Ponape 2535.4	f	f	S	f	f	S	f	S	f	S	S	S	S	S	\	S	S
D. albomicans																	
Okinawa 3045.11	f	f	f	f	f	S	f	f	f	f	f	f	f	f	S	\	F
Taiwan 3046.2	f	S	S	f	f	f	S	S	S	S	f	S	S	f	S	F	\

TABLE 5. Results of 1966 crosses between the subspecies of *Drosophila sulfurigaster*, *D. pallidifrons* and *D. albomicans*. Symbols are as follows:
Capital F within black square: $P_1 \times P_1$ & $F_1 \times F_1$ fertile; **Lower-case f** in black square: $P_1 \times P_1$ fertile, $F_1 \times F_1$ slightly fertile; **Capital F** alone: $P_1 \times P_1$ fertile, $F_1 \times F_1$ sterile; **Lower case f** alone: $P_1 \times P_1$ slightly fertile; $F_1 \times F_1$ not tested; **S:** $P_1 \times P_1$ sterile.

(2372.16), and New Ireland (3017.4); these *D. s. sulfurigaster* lines show the two extremes in F_1 fertility and sterility between *D. s. bilimbata* and *D. s. sulfurigaster*. The *D. s. sulfurigaster*, Madang line, (3016.2), was also chosen to check further on a male:female sex ratio difference which was observed in crosses where the Madang stock furnished the male of the cross to strains from Malaysia (3033.17). The Malaysia strain was used to represent *D. s. albostrigata* (Table 6).

Results of tests with the strain from Guam placed it with the Polynesian *D. s. bilimbata*; genetically, it is like the Hawaiian standard, and in the two crosses to *D. s. albostrigata* males from Cambodia, the F_1 male:female sex ratio distortion was present.

The *D. s. albostrigata* lines from Palawan, Philippine Islands, and Cambodia and the single iso-female line from Luzon, Philippine Islands, showed the same

♀ \ ♂	*D. sulfurigaster bilimbata*: Hawaii 3045.1	Guam 3071.6	*D. s. sulfurigaster*: New Ireland 3017.4	Australia 2372.16	Madang, N. G. 3016.12	*D. s. albostrigata*: Cont. Malaysia 3033.18	Luzon 3054.2	Palawan 3056.2 #3	Palawan 3056.2 #6	Cambodia 3057.2 #9	Cambodia 3057.2 #10	Cambodia 3057.2 #12	*D. pallidifrons*: Ponape 2535.4	*D. kepulauana*: Palawan 3056.8	*D. albomicans*: Okinawa 3045.11	*D. kohkoa*: Cambodia 3057.3 #2
D. sulfurigaster bilimbata																
Hawaii 3045.1	\	F	F	F	F	F	F	F	F	F	F	F	f	S	F	S
Guam 3071.6	F	\	F	F	F	F	F	F	F	F	F	F	S	S	F	S
D. s. sulfurigaster																
New Ireland 3017.4	F	F	\	F	F	F	F	F	F	F	F	F	S	S	F	S
Australia 2372.16	F	F	F	\	F	F	F	F	F	F	F	F	S	f	F	S
Madang, N. G. 3016.12	F	F	F	F	\	F	F	F	F	F	F	F	S	S	F	S
D. s. albostrigata																
Cont. Malaysia 3033.18	F	F	F	F	F	\	F	F	F	F	F	F	S	f	F	f
Luzon 3054.2	F	F	F	F	F	F	\	F	F	F	F	F	S	S	F	S
Palawan 3056.2 #3	F	F	F	F	F	F	F	\	F	F	F	F	S	S	F	f
Palawan 3056.2 #6	F	F	F	F	F	F	F	F	\	F	F	F	S	S	F	f
Cambodia 3057.2 #9	F	F	F	F	F	F	F	F	F	\	F	F	S	S	F	S
Cambodia 3057.2 #10	F	F	F	F	F	F	F	F	F	F	\	F	S	S	F	S
Cambodia 3057.2 #12	F	F	F	F	F	F	F	F	F	F	F	\	S	S	F	S
D. pallidifrons																
Ponape 2535.4	f	S	S	S	S	S	f	S	S	S	S	S	\	S	S	S
D. kepulauana																
Palawan 3056.8	S	S	S	f	S	S	S	S	S	S	f	S	S	\	F	S
D. albomicans																
Okinawa 3045.11	f	f	f	f	f	f	f	f	f	f	f	f	f	F	\	f
D. kohkoa																
Cambodia 3057.3 #2	S	S	S	f	S	S	S	f	S	S	S	S	S	S	F	\

TABLE 6. Results of crosses (1967–May, 1968) between additional strains of *D. s. albostrigata* to the "standard" *D. s. bilimbata*, and to *D. s. sulfurigaster*, *D. pallidifrons*, *D. kepulauana*, and *D. albomicans*. Fertility-sterility symbols are as in Table 5.

F_1 sterility that the limited material from continental Malaysia had shown. Seven crosses to *D. s. sulfurigaster* males had the disturbance of male:female sex ratio. Since these crosses were mass matings there was no test of the percent of fertile pairs, but the crosses of *D. s. bilimbata* and *D. s. sulfurigaster* to *D. s. albostrigata* strains were slow in showing fertility and in several instances had to be repeated. Dissections of females to check the spermathecae and seminal receptacles for sperm showed that failure to mate was high (Table 12).

Drosophila collections in 1968 enabled us to extend the boundaries of *D. s. albostrigata* to include Sarawak, Malaysia; Sabah, Malaysia; Brunei, Borneo; and Thailand. In studying individual iso-female lines it was discovered that twelve lines from Sarawak, Malaysia had a sex ratio imbalance which was predominantly female. All collection sites and karyotypes are represented in the reciprocal crosses of these strains to the standard Hawaiian strain (Table 7). Additional crosses among *D. s. albostrigata* lines were made totaling 100 in all;

Metaphase Y Karyotype	♀ \ ♂	*D. s. bilimbata* Hawaiian Standard 3045.1	*D. s. sulfurigaster* New Ireland 3017.4	*D. s. sulfurigaster* Australia 2372.6	*D. s. albostrigata* Cabuyao Laguna, Luzon 3054.2	Tagaytay, Luzon 3138.2	Luzon 3139.2 #2	Baguio Benquet 3126.2a	Baguio Benquet 3126.2b	Los Baños 3146.2 #43	Palawan 3056.2 #3	Palawan 3056.2 #6
	D. sulfurigaster bilimbata											
I	Hawaiian Standard 3045.1	\	F	F	F	F	F	F	F	F	F	F
	D. s. sulfurigaster											
↾	New Ireland 3017.4	F ✓	\	F	F	F	F	F	F	F	F	F
Λ	Australia 2372.6	F	F	\	F	F	F	F	F	F	F	F
	D. s. albostrigata											
I	Cabuyao Laguna, Luzon 3054.2	F ☆	F	F	\	F ✓	F ✓	F ✓	F ✓	F ✓	F ✓	F ✓
Λ	Tagaytay, Luzon 3138.2	F ☆	F	F ☆	F ✓	\	F ✓	F ✓	F ✓	F ✓	F ✓	F ✓
Λ	Luzon 3139.2 #2	F ☆	F ☆	F ☆	F ✓	F ✓	\	F ✓	F ✓	F ✓	F ✓	F ✓
↾	Baguio Benquet 3126.2a	F ☆	F	F	F ✓	F ✓	F ✓	\	F ✓	F ✓	F ✓	F ✓
Λ	Baguio Benquet 3126.2b	F ☆	F ☆	F	F ✓	F ✓	F ✓	F ✓	\	F ✓	F ✓	F ✓
↾	Los Baños 3146.2 #43	F	F	F	F ✓	F ✓	F ✓	F ✓	F ✓	\	F ✓	F ✓
Λ	Palawan 3056.2 #3	F ☆	F	F	F ✓	F ✓	F ✓	F ✓	F ✓	F ✓	\	F ✓
↾	Palawan 3056.2 #6	F ☆	F	F	F ✓	F ✓	F ✓	F ✓	F ✓	F ✓	F ✓	\

TABLE 7. Results of crosses of strains of *D. s. albostrigata* from the Philippines, to the "standard" *D. s. bilimbata* and to *D. s. sulfurigaster*. Fertility-sterility symbols are as in Table 5. Starred squares = sex ratio distortion was observed in hybrids; checked squares = normal sex ratio in hybrids.

a complete series was not felt necessary, because no deviations were found from previous crosses made within this subspecies. The earlier analysis of the single line from Luzon, P. I., was supported by genetically similar lines from four other locations in Luzon (Table 7). Among crosses with these lines to *D. s. bilimbata* males, some of the more extreme examples of the unusual male: female F_1 sex ratio are seen; for example, 3138.2♀ × Hawaiian standard male produced 198 F_1♂ : 2 F_1♀, and in 3126.2a♀ × Hawaiian ♂, there were 280 F_1♂ : 52 F_1♀.

Only three iso-female *D. s. albostrigata* lines were established from the 1968 collection at Siem Reap, Cambodia. Table 8 shows that they are like the earlier lines from Ari Ksatr, Cambodia, and again the abnormal F_1 sex ratio is seen.

Examples of the F_1 sex ratio differences which have been observed in the crosses between members of the subspecies of *D. sulfurigaster* are listed in Table 9. In each of these crosses the number of F_1 males is significantly higher than the number of F_1 females. There were both abnormal males and females as

Metaphase Y Karyotype	♀ \ ♂	*D. s. bilimbata* Hawaiian Standard 3045.1	*D. s. sulfurigaster* New Ireland 3017.4	*D. s. sulfurigaster* Australia 2372.6	*D. s. albostrigata* Ari Ksatr, Cambodia 3057.2 #7	Ari Ksatr, Cambodia 3057.2 #9	Ari Ksatr, Cambodia 3057.2 #10	Ari Ksatr, Cambodia 3057.2 #12	Siem Reap, Cambodia 3120.2a	Siem Reap, Cambodia 3120.2b
	D. sulfurigaster bilimbata									
I	Hawaiian Standard 3045.1	╲	F	F	F	F	F	F	F	F
	D. s. sulfurigaster									
↾	New Ireland 3017.4	F	╲	F	F	F	F	F	F	F
Λ	Australia 2372.6	F	F	╲	F	F	F	F	F	F
	D. s. albostrigata									
Λ	Ari Ksatr, Cambodia 3057.2 #7	F ☆	F	F	╲	F	F	F	F	F
Λ	Ari Ksatr, Cambodia 3057.2 #9	F ☆	F	F	F	╲	F	F	F	F
↾	Ari Ksatr, Cambodia 3057.2 #10	F ☆	F	F	F	F	╲	F	F	F
↾	Ari Ksatr, Cambodia 3057.2 #12	F ☆	F	F	F	F	F	╲	F	F
Λ	Siem Reap, Cambodia 3120.2a	F ☆	F	F	F	F	F	F	╲	F
↾	Siem Reap, Cambodia 3120.2b	F ☆	F	F	F	F	F	F	F	╲

TABLE 8. Results of crosses of 1967–1968 strains of *D. s. albostrigata* originating from Cambodia. Fertility-sterility symbols are as in Table 5; starred squares = sex ratio distortion present.

well as some intersex flies in several of the crosses. Further data on different aspects of the sex ratio factor will be published at a later date.

The species from Ponape, *D. pallidifrons,* was the only Pacific Island population sample from the Stone-Wheeler collections of 1955–1966 which did not fit into the *D. s. bilimbata* subspecies of *D. sulfurigaster*, and from none of the ensuing collections was this type seen from any locality but Ponape. The original strain, 2535.4, was crossed to 26 lines of the different species of the subgroup (Tables 5 and 6) with at least fifteen mass matings per cross. P_1 fertility was very low, and dissections of females for the presence of sperm showed almost complete sexual isolation between *D. pallidifrons* and each of the other species. From the 500 or more pairs per cross no more than two F_1 flies were recovered from any one cross.

Stock No. 3056.8 was the only iso-female strain from Palawan, P. I., with silvery markings all over the frons from the 1967 collection. Reciprocal crosses to *D. albomicans* from Okinawa (3045.11) went readily and were fertile through the F_2 showing the two types to be closely related. However, they did not behave the same in crosses to the other species (Tables 6 and 10). The 1969 lines from Brunei, Borneo, were placed in the same group with the Palawan 3056.8 strain after cytological and genetic tests. The Borneo lines used in the crosses were 3122.3♀ No. 1 and ♀ No. 5. When crossed to *D. albomicans* they differed from 3056.3 in that the $F_1 \times F_1$ were sterile. This type has now been named *D. kepulauana.*

In 1967 the only strains collected of *D. kohkoa* were Cambodia, 3057.3 ♀ No. 2 was the line chosen to use in crosses to the material available at the time (Table 6). P_1 sexual isolation was seen between this *D. kohkoa* strain and *D. sulfurigaster*, *D. pallidifrons,* and *D. kepulauana.* In 1968 *D. kohkoa* from Sarawak, Malaysia, Brunei, Borneo, Palawan, Philippine Islands, and Thailand

Table 9

Sex ratio distortion among F_1 hybrids from crosses within *D. sulfurigaster*.

P_1 Cross	No. of F_1♀♀	No. of F_1♂♂	% ♂♂ of F_1 progeny	P_1 Cross	No. of F_1♀♀	No. of F_1♂♂	% ♂♂ of F_1 progeny
D. s. albostrigata ♀♀				*D. s. albostrigata* ♀♀			
× *D. s. bilimbata* ♂♂				× *D. s. bilimbata* ♂♂			
3616.2 # 29 × 3045.1	51	172	92.0	3126.2a × 3045.1	52	280	84.3
3116.2 # 21 × 3045.1	17	171	91.0	3033.18 × 3045.1	91	199	68.6
3117.2a × 3045.1	159	387	70.9	3054.2 × 3045.1	4	15	78.9
3117.2c × 3045.1	49	165	77.1	3120.2a × 3045.1	5	85	94.4
3119.2c × 3045.1	29	130	81.8	3120.2b. × 3045.1	18	69	79.3
3121.2 # 52 × 3045.1	90	226	71.5	3057.2 # 10 × 3045.1	47	92	66.2
3122.2 # 60 × 3045.1	27	64	70.3	3057.2 # 7 × 3045.1	158	342	68.4
3122.2 # 44 × 3045.1	59	134	69.4	*D. s. albostrigata* ♀♀			
3124.2b × 3045.1	64	174	73.1	× *D. s. sulfurigaster* ♂♂			
3124.2 # 2 × 3045.1	42	245	85.4	3192.2 # 2 × 3017.4	112	227	67.0
3124.2 # 5 × 3045.1	56	183	76.6	3139.2 # 2 × 2372.16	207	541	72.3
3139.2 # 2 × 3045.1	60	187	75.7	3126.2b × 3017.4	82	148	64.3
3138.2 × 3045.1	2	198	99.0	3033.18 × 3016.2	5	242	98.0
				3138.2 × 2372.16	5	72	93.5

was collected and representative strains from each locality were crossed to the other species of the subgroup (Table 10). Sexual isolation was not so great at the P_1 level as with the Cambodia line, but there was F_1 sterility in all interspecific crosses.

D. albomicans from Okinawa (3045.11) and Taiwan (3046.2) were crossed extensively to *D. sulfurigaster* (Table 5). P_1 *D. albomicans* females were slightly fertile in these crosses if enough mass matings were attempted. The few F_1 recovered were backcrossed to both parent strains. F_1 females backcrossed were fertile; F_1 males backcrossed were sterile. The reciprocal matings, using *D. albomicans* males to *D. sulfurigaster* females were fertile in all cases but with F_1 sterility.* Backcrosses were fertile using F_1 females; with F_1 males of *D. sulfurigaster* they were sterile, and with F_1 males of *D. albomicans* they were slightly fertile (Table 12). *D. albomicans* was also found in the Pescadores Islands and all tests showed it to be like the lines from Okinawa and Taiwan. In crosses to

♀ \ ♂	*D. sulfurigaster bilimbata*	*D. kepulauana*			*D. kohkoa*						*D. albomicans*	
	Hawaii 3045.1	Palawan 3056.8	Borneo 3122.3 #1	Borneo 3122.3 #5	Palawan 3129.3	Sarawak 3121.3 #7	Sarawak 3121.3 #13	Cambodia 3057.3 #2	Thailand 3116.3 #55	Sarawak 3122.3 #6	Thailand 3116.3 #46	Okinawa 3045.11
D. sulfurigaster bilimbata												
Hawaii 3045.1	\	S	S	f	F	F	F	S	f	F	F	F
D. kepulauana												
Palawan 3056.8	S	\	F	F	F	f	f	f	f	f	F	F
Borneo 3122.3 #1	f	F	\	F	f	f	f	S	f	f	F	F
Borneo 3122.3 #5	S	F	F	\	f	f	f	S	f	f	F	F
D. kohkoa												
Palawan 3129.3	S	f	f	S	\	F	F	F	F	F	F	F
Sarawak 3121.3 #7	f	f	S	S	F	\	F	F	F	F	F	S
Sarawak 3121.3 #13	S	f	S	S	F	F	\	F	F	F	S	f
Cambodia 3057.3 #2	S	f	F	f	F	F	F	\	F	F	F	f
Thailand 3116.3 #55	f	f	f	f	F	F	F	F	\	F	F	S
Sarawak 3122.3 #6	S	f	f	S	F	F	F	F	F	\	F	S
D. albomicans												
Thailand 3116.3 #46	f	F	F	F	F	f	f	f	f	f	\	F
Okinawa 3045.11	S	F	F	F	f	f	f	f	f	S	F	\

TABLE 10. Results of crosses between *D. kepulauana*, *D. kohkoa*, and *D. albomicans* and the "standard" *D. s. bilimbata*. Fertility-sterility symbols are as in Table 5.

* In these crosses, where *D. albomicans* was the male, a sex ratio difference was also seen, but it was the opposite of that seen between *D. sulfurigaster* subspecies crosses. Here the females were in excess of the males, but there is insufficient data to be presented at this time.

collections from 1967 and 1968, the Okinawa stock (3045.11) was used to represent the species (Tables 6 and 10), and the 1968 *D. albomicans* strain from Thailand (3116.3 ♀ No. 46) was also tested in the latter series of crosses.

Our population sample of *D. pulaua* consisted of one iso-female line from the Semongok Forest Reserve in Sarawak, Malaysia (3121.5). It was crossed to members of each of the other species (Table 11). All crosses showed P_1 reproductive isolation with the exception of those in which *D. pulaua* was the male in crosses to the subspecies of *D. sulfurigaster*, and F_1 hybrids of these crosses were fertile. In crosses to *D. s. sulfurigaster* males, the sex ratio was normal, but *D. pulaua* females × *D. s. albostrigata* males showed an imbalance of males over females. In the four crosses made, the percent of F_1 hybrid males ranged from 80.3% to 97.7% of the total progeny of each cross.

In initial crosses if no offspring (larvae, etc.) were obtained, a sample of the females was dissected from some of the crosses and their spermathecae and seminal receptacles checked for sperm. In most of the cases P_1 crosses that failed to produce offspring were the result of sexual isolation, *i.e.*, the females had apparently not mated since there were no stored sperm. This was true for several hundred females dissected from various cross combinations. These tests showed that the *D. pallidifrons* did not mate often with any of the other species.

TABLE 11

Crosses of *D. pulaua* to other members of the subgroup, illustrating the high degree of reproductive isolation.

P_1 Cross	Fertility P_1	Fertility F_1	P_1 Cross	Fertility P_1	Fertility F_1
D. s. bilimbata ♀ × *D. pulaua* ♂	fertile	fertile	*D. albomicans* ♀ × *D. pulaua* ♂	slightly fertile	sterile—all F_1 flies abnormal
D. pulaua ♀ × *D. s. bilimbata* ♂	sterile	none	*D. pulaua* ♀ × *D. albomicans* ♂	slightly fertile	sterile
D. s. sulfurigaster ♀ × *D. pulaua* ♂	fertile*	fertile	*D. kepulauana* ♀ × *D. pulaua* ♂	sterile	none
D. pulaua ♀ × *D. s. sulfurigaster* ♂	sterile	none	*D. pulaua* ♀ × *D. kepulauana* ♂	sterile	none
D. s. albostrigata ♀ × *D. pulaua* ♂	fertile*	fertile	*D. kohkoa* ♀ × *D.pulaua* ♂	sterile to slightly fertile	too few to test
D. pulaua ♀ × *D.s. albostrigata* ♂	sterile	none	*D. paulau* ♀ × *D. kohkoa* ♂	sterile to slightly fertile	too few to test
D. pallidifrons ♀ × *D. pulaua* ♂	slightly fertile	too few to test			
D. pulaua ♀ × *D. pallidifrons* ♂	slightly fertile	too few to test			

* Crosses where sex ratio distortion was observed:

3054.2♀ × D. pulaua 3121.5♂	13 F_1♀, 98 F_1♂
3017.4♀ × D. pulaua 3121.5♂	157 F_1♀, 191 F_1♂
3119.2a♀ × D. pulaua 3121.5♂	52 F_1♀, 212F_1♂
3117.2a♀ × D. pulaua 3121.5♂	6 F_1♀, 35 F_1♂
3122.2 # 60♀ × D. pulaua 3121.5♂	1 F_1♀, 42 F_1♂

Most females without offspring in crosses of *D. albomicans* (Okinawa) females to *D. sulfurigaster* males were unmated; one female in 32 was inseminated. Also 2 in 17 females were fertilized in a cross between *D. albomicans* (Okinawa) and *D. pallidifrons*. The *D. albomicans* species was not only isolated by sexual isolation, but by F_1 sterility as well. This conclusion was also reached by Wakahama *et al.* (1968). *D. kepulauana* is almost completely sexually isolated from all other species except *D. albomicans*; when 156 females of crosses to *D. sulfurigaster* were dissected, there were no sperm, and 46 females of crosses to *D. kohkoa* showed no sperm. In crosses between the subspecies of *D. sulfurigaster* and the two Cambodian lines of *D. kohkoa* (3057.3 ♀ No. 2 and No. 24) 226 females were dissected, and there were no sperm. An occasional offspring was obtained in some of these crosses, but sterile crosses usually resulted from sexual isolation.

When F_1 hybrids were obtained from crosses and found to be sterile when inbred, some of these flies were dissected. In crosses of *D. kohkoa* using strain 3057.3 ♀ No. 2 and No. 24 to *D. albomicans* (Okinawa), 33 F_1 females had no sperm although 31 F_1 males with them had non-motile sperm. In crosses between *D. albomicans* and *D. s. bilimbata*, 41 F_1 females were unfertilized although 7 of 13 F_1 males had motile sperm. This is one of the series of crosses where some of the backcrosses were fertile when $F_1 \times F_1$ did not produce offspring (Table 12).

TABLE 12

Results of F_1 backcrosses from reciprocal crosses between *D. sulfurigaster* and *D. albomicans*.

P_1 cross: *sulfurigaster* ♀♀ × *albomicans* ♂♂

P_1 stocks crossed	F_1 backcrosses to maternal type		F_1 backcrosses to paternal type	
	♀♀	♂♂	♀♀	♂♂
Hawaii × Okinawa	S[1]	F[2]	sF[3]	F
Savaii × Okinawa	S	F	sF	F
Luzon × Okinawa	S	F	sF	F
Madang × Okinawa	S	F	sF	F
Malaysia × Okinawa	S	F	sF	F
Australia × Okinawa	S	F	sF	F

P_1 cross: *albomicans* ♀♀ × *sulfurigaster* ♂♂[4]

P_1 stocks crossed	F_1 backcrosses to maternal type		F_1 backcrosses to paternal type	
Okinawa × Savaii	S	F	S	F
Okinawa × New Ireland	S	F	S	—
Okinawa × Madang	S	F	S	F

1 Sterile.
2 Fertile.
3 Slightly fertile.
4 These crosses went very poorly; the ones listed are the only ones in which there were enough F_1 to make a backcross test. *D. albomicans* was represented in all cases by stock No. 3045.11.

Discussion

Population samples of the *D. nasuta* subgroup of the *D. immigrans* group of species collected from twenty-seven localities in the South Pacific and Southeast Asia have proved of considerable variability and interest. This report primarily presents the results of taxonomical comparisons, genetic tests and cytological analysis that led to our classifications within this subgroup. Together with the findings on sexual behavior by Spieth (this Bulletin), each line of investigation supports the other in dividing this divergent material into six separate species.

After compiling information on all related *D. nasuta* forms described since the description of *D. nasuta* by Lamb, 1914, and studying the above mentioned data, we have classified the six species here reported on as *D. sulfurigaster, D. albomicans, D. pulaua, D. pallidifrons, D. kepulauana, and D. kohkoa*, the latter four being new species.

The division of *D. sulfurigaster* into three subspecies, *D. s. bilimbata, D. s. sulfurigaster* and *D. s. albostrigata* was made primarily on cytogenetic analysis; the patterns of F_1 reproductive isolation, F_1 sex ratio distortion and chromosomal rearrangements compliment one another in supporting this decision. It was also compatible with the geographic isolation and distribution of members of this species. Spieths' observations on sexual behavior within the species fall into three types which overlap the subspecies as they are seen from cytogenetic tests. However, differences between subspecies are not distinct in all respects as they evolve toward becoming separate species. Characteristically subspecies remain open systems during the process of speciation, but it is doubtful that there is an opportunity for the isolated island populations of *D. sulfurigaster* to have more than rare, if any, interbreeding. Of the many strains tested of *D. sulfurigaster* from twenty-four localities, only the one from the island of New Ireland shows no distinct sexual isolation from any strain of the whole species. Although it has not become reproductively isolated, this strain does show the sex ratio distortion characteristically found among F_1 hybrids from crosses between the different subspecies of *D. sulfurigaster*.

Kanapi (1967) studied isozyme differences in three species of the subgroup, using starch gel electrophoresis. Although the species terminology was not known at that time, she was able to divide the 24 strains into three "groups"; these correspond to our species identifications as follows: Group I = *pallidifrons* (one strain used); Group II = *albomicans* (two strains); Group III = *sulfurigaster* (five strains of *s. sulfurigaster*, two strains of *s. albostrigata*, and 14 strains of *s. bilimbata*). She found *pallidifrons* to be the most distinct, with a unique Esterase F form, an absence of activity at the Leucine Aminopeptidase (LAP A) zone, and an Acid Phosphatase (ACPH B) band which, although classed as *Acph* B^1 consistently showed reduced activity in the faster bands of the *Acph* B^1 triplet. The strains of *albomicans* were alike electrophoretically. Their unique male-specific alcohol-inhibited esterase, *Est C′*, set them apart from all other forms. They also showed two *Esterase F* alleles (*Est* F^3 and *Est* F^5) which were not seen in any other strains. All strains of *sulfurigaster* were essentially indistinguishable, no evidences of geographic subspeciation being noted. The electrophoretic pattern for alcohol dehydrogenase in this species was found to differ from that of the other two.

In view of the interesting differences which were found by Kanapi, it seems especially desirable to try this or similar techniques on the other members of the *nasuta* subgroup.

The overlapping geographical distribution of the *D. nasuta* subgroup species is of interest. The populations of the small isolated islands of the South Pacific are each of one species per island. But in several other areas the unusual occurrence is seen of two and sometimes three species, so closely related that they could be called sibling species, co-existing together. *D. s. albostrigata* and *D. kohkoa* were found together in the majority of the localities where either was collected as in Brunei, Borneo, and Sarawak, Malaysia, and Palawan, Philippine Islands, and Cambodia and Thailand. Only in the very small sample from continental Malaysia and in the limited lines from Luzon, P. I., was *D. s. albostrigata* found without *D. kohkoa*. *D. kohkoa* was never collected without *D. s. albostrigata*. In three of the collections which included these two species, a third species was also found. In the Semongok Forest Reserve, Sarawak, Malaysia, *D. pulaua* was present; in Bon Chakkrarat, Thailand, *D. albomicans* was also found; and in Brunei, Borneo, they co-existed with *D. kepulauana*. In Palawan, P. I., *D. s. albostrigata*, *D. kohkoa* and *D. kepulauana* were all collected, but the latter was not present in the same population sample. Wherever collected, *D. kohkoa* and *D. kepulauana* were found to co-exist sympatrically with at least one other species of the subgroup, and in none of the instances of two or three species occupying the same niche was there any evidence that there was any gene flow between them.

Over 800 crosses were attempted during this investigation to determine intra- and interspecific reproductive isolation. Small mass matings of at least 100 pairs of flies were made in intraspecific crosses and from 300 to 1,000 pairs in interspecific crosses. The data is summarized in Tables 5 through 11 showing that the six species demonstrate a range from partial to almost complete reproductive isolation between one another. Among the F_1 hybrids obtained, two different sex ratio distortions were observed. In crosses between the subspecies of *D. sulfurigaster* the percentage of F_1 males was much greater than F_1 females; in interspecific crosses to *D. albomicans* males, more F_1 hybrid females were obtained. Both of these conditions are being investigated.

In different respects this scattered, isolated complex of species resembles several subgroups in the *D. repleta* group of species (Wasserman, 1963), the *D. virilis* group of species (Patterson and Stone, 1952; Stone, Guest and Wilson, 1960) or the *D. paulistorum* group (Dobzhansky and Pavlovsky, 1967; Kastritsis, 1966). Some species of *D. nasuta* are also comparable to those of the *D. ananassae* complex of species found in many of these areas (Stone, Wheeler, Wilson, et al., 1966; Spieth, 1966; and Futch, 1966). These isolated island populations receive few migrants and, even on large islands, are often decimated by the effects of typhoons. The latter could be the case in the islands of Yap and Palau where Carson collected in 1968 and expected to, but did not find any representative of the *D. nasuta complex*. Stone has pointed out that such factors contribute in a major way to the rapid divergent evolution of populations. For example, on Ponape the new species, *D. pallidifrons*, was collected and has been found only on this isolated island, and it is almost completely genetically isolated from the other subgroup species.

The *D. repleta* group of species (Wasserman, 1963) consists of many desert adapted populations that often exist in small numbers. Some of these forms occur in other areas also. The cytological differences between species are often small and many species have not been demonstrated to have heterozygous inversion systems. The Pacific island populations of *D. s. bilimbata*, *D. pallidifrons*, and *D. pulaua* of the *D. nasuta* subgroup are similar to these. Another similarity to the *D. repleta* group is the presence of a chromosomal polymorphism in several descendent species. The inversion 2LA is found as a heterozygote in *D. kohkoa*, *D. kepulauana*, *D. albomicans*, and *D. s. albostrigata*; 3A is heterozygous in *D. s. albostrigata* and in *D. albomicans* from Thailand. These are exceptions to the general rule as seen in studies by Carson (1959) where descendent species are not expected to retain an inversion polymorphism.

D. nasuta subgroup populations of the Philippine Island-Sarawak area and Southeast Asia, which have 28 known heterozygous inversions, can be compared to the *D. virilis* group and the *D. paulistorum* group (Kastritsis, 1967) which have considerable inversion polymorphism. All of these groups have species with varying degrees of isolation, and all utilize various types of isolation mechanisms. Between the species of the *D. nasuta* subgroup, isolation is both genetic and behavioral.

The *D. repleta* group and the *D. immigrans* group (Mather, 1962) have cases of added heterochromatin and chromosomal fusion. In the *D. nasuta* subgroup added heterochromatin to the dot chromosome (4) of the metaphase karyotype is a fixed characteristic of *D. kepulauana* and *D. albomicans*. A small amount has been added in *D. kepulauana* making the dot appear larger; in strains of *D. albomicans* from Thailand, it appears as a short rod; and in all strains of *D. albomicans* from Okinawa, Taiwan and the Pescadores Islands, it appears as a heavy longer rod. Lines of *D. kohkoa* often have a small amount of heterochromatin added to the dot giving it a comma-shaped appearance. *D. albomicans* is unusual in having a fusion which combines three of the five primitive rod elements of the genus *Drosophila* into one chromosome. In many cases (Patterson and Stone, 1952) it has been observed in *Drosophila* that the dot chromosome has fused with or has been transferred bodily to an autosome or the sex chromosomes. Were this to happen in one of the *D. albomicans* island populations and become established, it would produce a *Drosophila* with only two pairs of chromosomes.

The characteristics of at least one of the species of the *D. nasuta* subgroup support the view of Carson (1955, 1959) and DaCunha, Dobzhansky, Pavlovsky, and Spassky, (1959) regarding the extent of inversion polymorphism being greater toward the center of the population distribution while marginal populations tend to be chromosomally homozygous. In *D. sulfurigaster* all population samples of the subspecies *albostrigata* from Sarawak, which seems to be the central area of geographical distribution of this subspecies, show a high degree of polymorphism. There are ten heterozygous inversions plus a heterozygous complex on the X chromosome. In the marginal South Pacific islands, all *D. s. bilimbata* populations are monomorphic, and the Australia-New Guinea area has very slightly polymorphic populations; one inversion has been seen in the strain from New Ireland and one in the line from Madang, New Guinea. Mather (1962) has reported no polymorphisms in collections from this area. In the continental localities from which there are lines of *D. s. albostrigata*, there is a slight decline in polymorphism. Collections have been extensive toward the northern and eastern limits of the distribution of this subgroup, but on the continent they have been limited to the coastal area; therefore, the lines studied are not necessarily marginal to the west.

Further comment on the implications of chromosomal rearrangements in the *D. nasuta* subgroup will be reserved for a later date. Due to the wealth of material acquired over a short period of time, the cytological complexities encountered, and some unique facets of the investigation, considerable studies remain in progress or are yet to be carried out. It is hoped it will be possible to coordinate a chromosomal phylogeny compatible with the independent investigations on genitalia differences, and patterns of courtship and mating behavior, because all indications are that this species cluster ranks with that limited number of groups of species with a sufficient range of presently existing and available material necessary to determine the phylogenetic relationships.

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