

DROSOPHILA IMAII, A NEW SIBLING SPECIES RELATED TO
*DROSOPHILA BIFASCIATA*¹

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Drosophila bifasciata Pomini, which belongs to the *obscura* species group of the subgenus *Sophophora*, seems to be "trans-palaearctic," since it inhabits vast areas in Europe and Asia (Pomini, 1941; Burla, 1951). This species lives also in Japan, most commonly in cool and mountainous districts (Moriwaki et al., 1952, 1953, 1954). In some localities in the northern parts of Japan, e.g. Hokkaido, this species can be collected at low elevations in early summer. At six localities, so far confined to Hokkaido, some flies with a yellowish thorax color have occasionally been collected, designated provisionally as "yellowish" (*yh*), and regarded as mutant forms of the same species.

Interestingly, *yh* flies were always found together with what was believed to be wild type flies. Stimulated by this fact, the possibility of incipient reproductive isolation between *yh* and wild strains has been examined (Moriwaki and Saito, 1956; Ohnishi and Moriwaki, 1958). In addition to the thorax color, slight morphological differences and incomplete synapsis or specific configurations in salivary chromosomes of hybrid larvae have also been observed. Experiments and observations are reported below which show that the "yellowish" flies belong to a new sibling species related to *D. bifasciata*. This species is described and named below as *Drosophila imaii*.

GEOGRAPHIC DISTRIBUTION

The four main islands of Japan—Hokkaido, Honshu, Shikoku, and Kyushu, range from northeast to southwest, for a distance

of about 2000 km. As mentioned above, *D. bifasciata* is found chiefly in cool places. However, it has been collected also in the middle of Honshu in mountains in summer, and sometimes on the plains there in winter.

The "yellowish" sibling species has been obtained at six localities, Akkeshi, Wakoto, Abashiri, Nukabira, Nishitappu, and Nopporo, all in Hokkaido (Fig. 1). Remarkably, the yellowish species has never been captured in Honshu and southwards. Although it may still be premature to say how widespread their distribution is or what the relationship among these local strains of the yellowish species is, flies of this species were nearly always found together with typical *bifasciata* flies in the same locality, that is, sympatrically, as shown in Table 1, and on the same bait whenever captured.

MORPHOLOGY

In addition to the thorax color, the yellowish new species has one—not two—black stripes on the scutum, which expands posterolaterally forming a trident-like pattern (Fig. 2). The expression is not necessarily constant, depending on age, temperature, nutrition, and so on. Morphological differences between the new species and *D. bifasciata* flies were also examined with respect to some metric characters such as wing length (= body size), wing width, number of teeth in male sex combs, number of egg-guide bristles in females, and abdominal bristle numbers.

As compared with *D. bifasciata*, the new species is likely to be smaller in size, with slightly fewer teeth in the sex-combs, with more bristles on female egg-guides. Moreover, there are some differences involving abdominal bristles and wing size, as shown

¹This work was presented in part before the 11th International Congress of Genetics at Hague in Holland by Moriwaki and Kitagawa (1963).

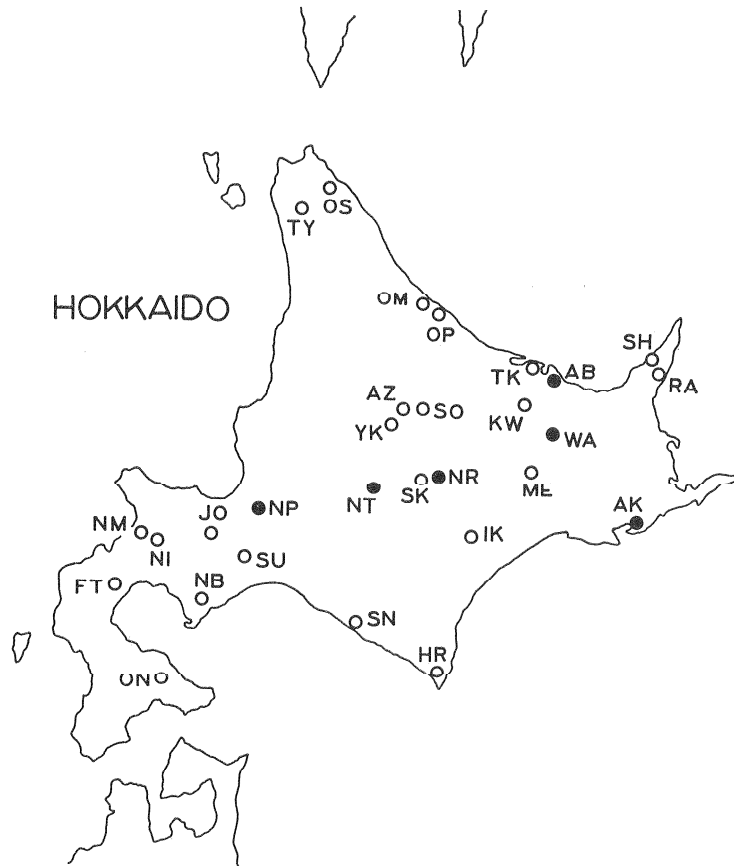


FIG. 1. Distribution of main collection localities of *D. bifasciata* in Hokkaido, Japan. ● Localities where the new species was collected.

in Table 2. Regarding the average number of abdominal bristles on 4th and 5th sternites in either females or males, no significant differences have been found between *D. bifasciata* and the new species, but the ratio of males to females manifests a significant difference. The wings in *D. bifasciata* are longer than in the new species, probably reflecting the body size difference.

Recently one of the authors (T. Okada) found a minor but important variation being characteristic of the male genitalia of the new species, the description of which will be given later.

REPRODUCTIVE ISOLATION

Sexual isolation.—First, the “male choice” method was used to test if sexual

isolation exists between *D. bifasciata* and the new yellowish species. Groups of 10 virgin females from strains of each species, aged for five to seven days at 20C, were placed in vials containing usual food, with an equal number of males of one or the other species. The exposure was 24 hours. Then the females were examined for the insemination. The isolation indices (I.I.) were computed according to the Stalker's formulæ (1942).

The crossability of *D. bifasciata* and the new species has also been examined in pair mating. Virgin yellowish females and *D. bifasciata* males were selected and aged for 10 days in isolation; over 3000 pair matings were then made and placed in vials with food, one pair per vial. Each vial was inspected after 10 days or more, to see if

TABLE 1. Numbers of individuals of the "yellowish" species and of *Drosophila bifasciata* collected in different localities.

Locality	Year	<i>bifasciata</i>	yellowish
Akkeshi	1951	++	++
Nishitappu	1953	++	+
Akkeshi	1954	+	+
Akkeshi	1961	47	2
Nishitappu	"	7	6
Nopporo	"	0	3
Nishitappu	1962	125	16
Nopporo	"	27	41
Nishitappu	1964	61	2
Nopporo	"	24	6
Abashiri	"	14	1
Nukabira	"	28	1
Wakoto	"	34	1

larvae had been produced in it. The numbers of emergent flies per mating have also been estimated.

Individual data are reported in Table 3a, b, and summarized in Table 4a with pooled data. Thus, the isolation index between the new species and *D. bifasciata* is on the average 0.83–0.84, showing a fairly strong sexual isolation. The percentage of the successful matings, that is, the crossability or fertility of the two species, is 2.86–5.04. However, the average number of progeny per mating of yellowish females with *D. bifasciata* males turns out to be about 77 flies per mating, thus showing a comparatively high fecundity. Here it is noteworthy that the fecundity is rather high regardless of the pronounced isolation and the low percentage of crossability.

Hybrid sterility.—Crosses of females of the new species with *D. bifasciata* males, as well as the reciprocal crosses, produce viable hybrids of both sexes. If hybrids are produced, the F₁ females are fertile

but the males are completely sterile. Motile spermatozoa are never found in the smears of the testes of these males.

The F₁ hybrid females were backcrossed to males of the new species and of *D. bifasciata*, respectively. On backcrossing, attention was paid to sexual isolation, crossability (fertility or hybrid sterility), and fecundity, similar to the parental crossing. These results are presented in Table 4b, comparing with the data in Table 4a.

As seen in Table 4b, considerable numbers of the F₁ hybrids resulted from the parental intercrosses between females of the new species and *D. bifasciata* males. The F₁ females, backcrossed to *D. bifasciata* or to the yellowish males, produce small progenies showing low fecundity, contrary to the parental crosses (Table 4a), while the sexual isolation between them seems to have decreased and the crossability restored.

In B₁ generation, produced by backcrossing F₁ females (yellowish × *D. bifasciata*) to *D. bifasciata* or to the yellowish species males, females were fertile but males were sterile. Only some males had motile sperms in their testes, and no males produced progeny. The situation changes in the second backcross, B₂. At least some of the B₂ males seem to be fertile, and brother × sister matings in the generation do produce offspring. This must be due to decreasing the proportion of foreign chromosomes in the individual owing to the repeated backcrosses.

CHROMOSOMES

The karyotype of *D. bifasciata* has been described by Buzzati-Traverso (1941). There are five pairs of chromosomes in

TABLE 2. Abdominal bristle numbers and wing sizes in *D. bifasciata* and *D. imaii*.

	Abdominal bristles			Wing	
	♀♀	♂♂	♂/♀	Length	Length/Width
<i>bifasciata</i>	34.66 ± 2.82	34.41 ± 2.24	.9928 ± .03	319.66	1.81
<i>imaii</i>	35.05 ± 0.64	31.90 ± 1.04	.9101 ± .03	271.05	1.80
Significance	None	None	***	***	None

TABLE 3. Isolation indices (a) and crossability (b) between *D. bifasciata* and *D. imaii*. The numbers of pair matings are shown in parentheses.

(a) Isolation indices:									
<i>D. bifasciata</i> ♀♀ and ♂♂									
<i>D. imaii</i> ♀♀	Akkeshi '61	Nishitappu '61	Roseppu '61	Nishitappu '61	Kunotori '61	Hakkoda '61	Akkeshi '51	Nishitappu '53	Weighted mean
Akkeshi '61	.86	1	1	.92	.67	1	.84	1	.87
Nishitappu '61	.72	.53	.91	.92	1	.78	1	.82	.80
Nopporo '61	.89	1	1	.73	.56	.80	.89	1	.83
Weighted mean	.79	.59	.94	.86	.74	.80	.86	.91	.83

(b) Crossability (%):								
<i>D. bifasciata</i> ♂								
<i>D. imaii</i> ♀	Akkeshi '51	Akkeshi '54	Tsukubasan '61	Akkeshi '62	Nishitappu '62	Nopporo '62	Sounkyo '62	Weighted mean
Akkeshi '61	3.08 (65)	12.00 (25)	0 (75)	0 (80)	0 (100)	1.43 (70)	—	1.45 (415)
Nishitappu '61	3.23 (155)	6.25 (48)	2.50 (40)	1.35 (74)	2.00 (50)	3.33 (30)	—	3.02 (397)
Nopporo '61	3.18 (157)	1.60 (125)	4.00 (75)	5.33 (75)	4.00 (50)	0 (100)	—	2.75 (582)
Nishitappu '62	8.00 (169)	5.55 (150)	4.62 (130)	9.66 (145)	11.98 (192)	10.63 (160)	9.00 (100)	8.80 (1046)
Nopporo '62	5.71 (175)	2.93 (171)	0 (50)	2.00 (100)	8.59 (163)	4.46 (112)	5.00 (100)	4.71 (871)
Weighted mean	5.13 (721)	4.05 (519)	2.70 (370)	4.43 (474)	7.21 (555)	5.08 (472)	7.00 (200)	5.04 (3311)

the diploid cells; the autosomes consist of three pairs of V- and one pair of dot-shaped chromosomes. The sex chromosomes are a V-shaped X and a rod-shaped Y. The correspondence between this karyotype and the nine elements in the salivary gland chromosomes (Fig. 3) has been established by Moriwaki and Kitagawa (1955). A composite map of the standard gene arrangement of the salivary chromosomes has been prepared, with a description of eleven different inversions detected in various geographic strains.

On the basis of characteristic features in salivary gland cells—that homologous discs of the giant chromosomes become associated during the somatic synapsis—a precise comparison of gene arrangements in the chromosomes of hybrids is possible. Accordingly, hybrid larvae between the yellowish species and *D. bifasciata* have been ex-

amined for configurations in their salivary chromosomes. The chromosome pairing often fails even when the gene arrangements of the homologous chromosomes appear to be almost similar (Fig. 4). A pattern of paired and unpaired sections can regularly be seen in the right arm of the fourth chromosome. A large inversion, characteristic of the new species has been found in the left arm of the second chromosome (Fig. 5).

DISCUSSION

The data presented above show that the new species can be distinguished from *D. bifasciata* by some morphological differences, though often with difficulty.

Sexual isolation seems to function effectively to separate the new species genetically from *D. bifasciata* in nature. The crossability between the two is low owing to the sexual isolation, while the fecundity

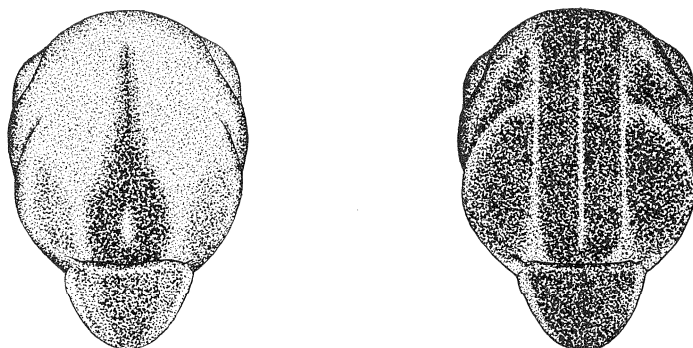


FIG. 2. Thorax pattern, morphological characteristics, of *D. imaii* (left), compared with *D. bifasciata* (right).

estimated from the numbers of progeny per mating in the intercrosses is rather high. Concerning the lack of correlation between the sexual isolation, the crossability and the fecundity, Baker's report (1947) may be referred to. Studying the isolating mechanisms between *D. arizonensis* and *D. mojavensis*, Baker showed that there is no correlation between the degree of insemination (sexual isolation) and the number of progeny (fecundity) in the interspecies crosses, and that in some cases both are negatively correlated, as contrasted to a positive correlation seen in the intraspecies crosses.

Hybrid sterility, another important reproductive isolating mechanism, is also clearly observed, hybrid males being sterile, although the hybrid females are fertile and produce backcross progenies with males of

the parental species. Carmody et al. (1962) have found that "the two isolating mechanisms, sexual isolation and hybrid sterility are quite different in genetic basis." In the present case, the hybrid males between *D. bifasciata* and the new species are completely sterile. This should be very effective in limiting the gene exchange between them, reinforcing the sexual isolation.

Since these two isolating mechanisms function to isolate genetically the new species from *D. bifasciata*, the fact that both coexist sympatrically in several localities is understandable. Moreover, the findings of incomplete pairing of the homologous chromosomes in the salivary gland cells of their hybrid larvae supplies further evidence for the interpretation. Equally decisive evidence of the absence of gene exchange between *D. bifasciata* and the yel-

TABLE 4. Estimates of reproductive isolation between *D. bifasciata* and *D. imaii*.

Crosses	Sexual isolation (I.I.)	Crossability (or fertility) (%)	Hybrid sterility	Fecundity No. of flies Mating
(a)				
Control { <i>bif.</i> ♀ × <i>bif.</i> ♂	.14	83.56	—	97.47
Intracrosses { <i>imaii</i> ♀ × <i>imaii</i> ♂	-.10	88.67	—	77.00
Intercrosses { <i>imaii</i> ♀ × <i>bif.</i> ♂	.83	5.04	—	76.93
{ <i>bif.</i> ♀ × <i>imaii</i> ♂	.84	2.86	—	—
(b)				
Backcrosses { (<i>imaii</i> ♀ × <i>bif.</i> ♂) F ₁ ♂ × <i>bif.</i> ♀	—	0.00	All sterile	—
" F ₁ ♀ × <i>bif.</i> ♂	-.07	36.01	Fertile	8.52
" F ₁ ♂ × <i>imaii</i> ♀	—	0.00	All sterile	—
" F ₁ ♀ × <i>imaii</i> ♂	.67	19.71	Fertile	17.87

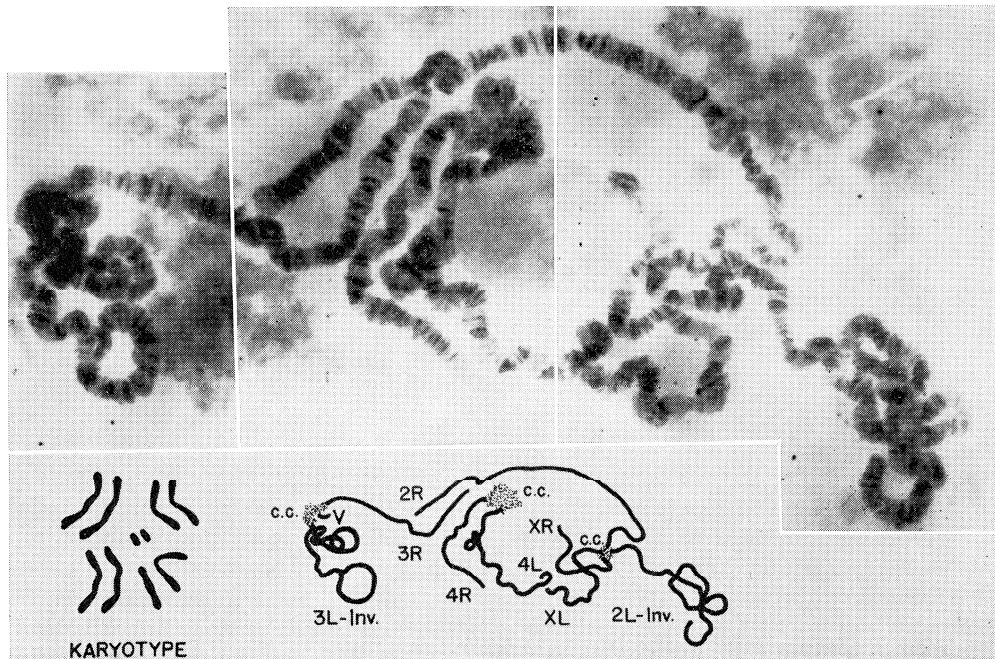


FIG. 3. Salivary chromosomes of a typical *D. bifasciata*.

lowish species in nature is seen. By examining the preparations of salivary gland cells of each species, it is clear that each contains characteristic inversions which are usable as cytological markers. So far as examined, there exists in nature no individual suggestive of introgression accompanied with any recombination of the cytological markers, and neither one of natural hybridization.

In view of this evidence, it appears certain that the extent of reproductive isolation between the new species and *D. bifasciata* is sufficient to maintain them sympatrically. Thus it seems safe to conclude that the new species is in fact a species distinct from, although related to *D. bifasciata*.

A formal description of the new species, *D. imaii*, is accordingly given below.

Drosophila imaii Moriwaki and Okada, sp. nov.

Male and female.—Body about 2–2.5 mm in length, dark yellowish brown to brownish black. Antenna with second joint mostly black, narrowly pale apically. Arista with about three upper and two lower branches besides a moderate or large

fork. Palpus with only one prominent terminal seta. Periorbit pollinose black. Front dull black, anteriorly as broad as median length, posteriorly half as broad as head width. Carina high, long. Anterior reclinate orbital two-fifths as long as posterior reclinate, which is slightly longer than proclinate. Vibrissa long, other orals fine.

Mesonotum dark brown, medially with a broad anteriorly narrowing black longitudinal stripe, laterally with diffuse dark patches before and behind suture. Scutellum dark brownish black, unicolorous. Thoracic pleura darker than mesonotum. Humeral two, long. Acrostichal hairs in eight rows. Length distance of dorsocentrals half cross distance. Anterior scutellars convergent, as long as posteriors, which are nearer to each other than to anteriors. Sterno-index about 0.5. Legs dark greyish brown, femora darker. Wings hyaline, venation as in *D. bifasciata*. C3-fringe on basal one-third. Halteres yellowish white. Abdominal tergites black, anterolateral margin of the fourth to sixth tergites of female narrowly yellow.

Periphallallic and phallic organs closely similar to those of *bifasciata*, decasternum or the bridge connecting claspers (Fig. 7) parallel-sided, broader than in *bifasciata*. Egg-guide as in *bifasciata*.

Holotype (male) and Allotype (female).—In a stock originated from a single fertilized female collected at Nishitappu, Hokkaido, August 22, 1953 (Kazuo Moriwaki). Deposited at the Biological Department, Tokyo Metropolitan University.

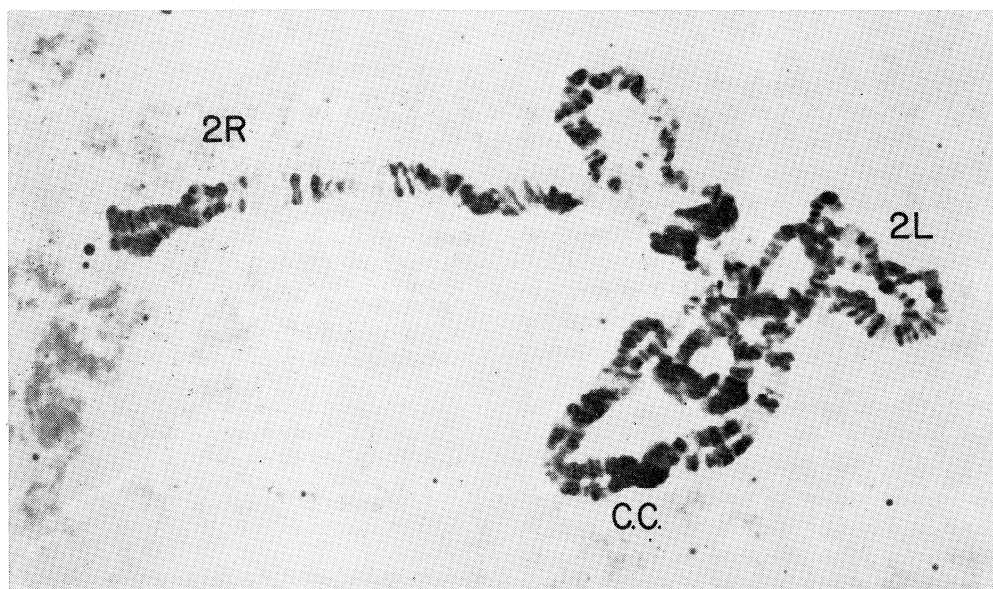


FIG. 4. Configurations in salivary chromosomes of hybrid between *D. imaii* and *D. bifasciata*; Incomplete pairing along the full length.

Distribution.—Hokkaido (see details in the text).

Relationships.—Closely allied to *D. bifasciata*, similar in general structures as well as male and female genitalia. Distinguished from *bifasciata*, however, in the mesonotal dark stripe: one median, anteriorly narrowing, in the present species; two, anteriorly not narrowing, in *bifasciata*. Decasternum or the bridge connecting claspers in male (Fig. 6) is parallel-sided and broader than in *bi-*

fasciata. Further descriptions of morphological and genetic differences between the two species appear in the text.

SUMMARY

Drosophila bifasciata is widespread in Japan, found chiefly in cooler and mountainous regions. In several localities in Hokkaido, flies with a yellowish coloration of the thorax were found, always together with the typical *D. bifasciata*. The “yellowish” was originally considered to be a mutant form of *D. bifasciata*, although certain minor morphological differences in addition to the coloration have been found. A more careful investigation has demonstrated that two reproductively isolated sibling species are involved.

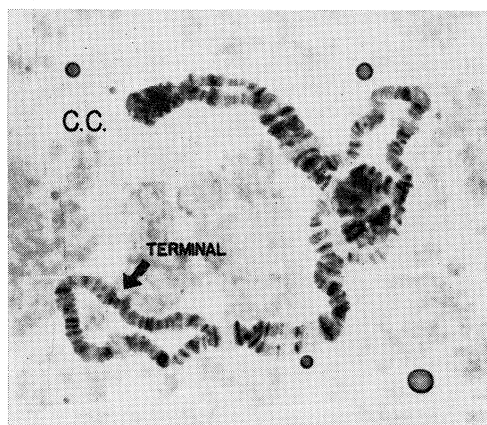


FIG. 5. Configurations in salivary chromosomes of hybrid between *D. imaii* and *D. bifasciata*; A newly found inversion, probably double-inverted, specific to “yh.”

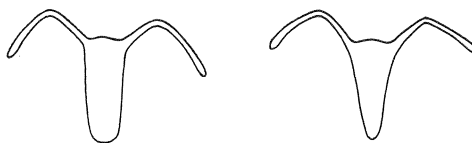


FIG. 6. Decasternum or the bridge connecting claspers in *D. imaii* (left) and *D. bifasciata* (right).

The females of the yellowish species cross to *D. bifasciata* males; the reciprocal cross succeeds less easily. Indices of sexual isolation of 0.8 and higher are obtained in the crosses. The hybrid progenies consist of completely sterile males and partially fertile females. Widespread failure of pairing is observed between the homologous chromosomes in the cells of the larval salivary glands in the hybrids. The two species are each polymorphic for several inversions. No individual has been found in nature with a cytological configuration suggesting a natural hybridization of the two species.

In view of the evidence summarized above, the yellowish form must be recognized as a sibling species, distinct from *D. bifasciata*. The new species is given the name of *Drosophila imaii*.

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