

## VIII. Polytene Chromosome Relationships in Hawaiian Species of *Drosophila*. IV. The *D. primaeva* Subgroup.<sup>1</sup>

HAMPTON L. CARSON AND HARRISON D. STALKER<sup>2</sup> (1969)

This paper is the fourth in a series describing the giant chromosome relationships among members of the subgenus *Drosophila* endemic to Hawaii. The first three papers (Carson and Stalker 1968 a,b,c) have dealt with species characteristically having dark maculations on the wings (the "picture-winged" species). These fall into four rather close subgroups: I. The *D. grimshawi* subgroup (29 species), II. The *D. planitibia* subgroup (9 species), III. The *D. adiantola* subgroup (9 species) and IV. The *D. punalua* subgroup (6 species). Carson et al., (1970) have given preliminary data on fourteen more species of these subgroups bringing the totals to 36, 14, 11 and 6, respectively. This makes a total of 67 species which have been cytologically deciphered.

The present paper presents chromosome maps and cytological recognition features for two more species which can be linked with the picture-winged flies, despite the fact that they themselves lack wing maculations. These two species, *D. primaeva* and *D. attigua*, are both endemic to the wet forests of Kauai and are morphologically very close indeed. Despite the fact that these species are distinctive and superficially appear to be quite distant from the picture-wings, this paper documents the fact that the banding order of each of their chromosomes can indeed be completely interpreted according to the picture-wing Standard, *Drosophila grimshawi*. With the addition of these two species as subgroup V, the *D. primaeva* subgroup, the number of Hawaiian *Drosophila* related by completely-read polytene sequences totals 69 (see also Carson et al., 1970).

### MATERIAL AND METHODS

The methods followed have been described in detail in Carson and Stalker 1968a. Chromosomal data are given here for 26 wild strains of *D. primaeva* (Table 1) caught between December 1965 and August 1968. Each strain originated from a single wild female. Only a single wild specimen of *D. attigua* has been recognized. This strain (L41C12) was derived from a female captured by J. P. Murphy at Kahili, Kauai on March 19-20, 1968. A number of specimens of *D. primaeva* (e.g. L41C11,20,21, Table 1) were captured at the same time and in the same small baited area. *D. attigua* was described by Hardy and Kaneshiro 1969.

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This paper is dedicated to the memory of Wilson S. Stone, teacher, colleague and friend.

TABLE 1

Chromosome polymorphism in three populations of *Drosophila primaeva* on Kauai

locality and collection no.	no. of wild chromosomes tested		no. of gene arrangements observed in						
			chromosome X		chromosome 2		chromosome 4		
Waimea District	X	A	+	g <sup>2</sup>	g <sup>2</sup> 1 <sup>2</sup>	+	q	+	a <sup>2</sup>
Mohihi G20.3B	3	4	1	1	1	0	4	0	4
Kokee J8101	3	4	2	1	0	2	2	0	4
Total	6	8	3	2	1	2	6	0	4
	per cent		50.0	33.3	16.7	25.0	75.0	0	100
Koloa District									
Kahili L41011, 20,21; G8,11-14,16, 19,21,25,26; P4-6; M8J1,2.	48	68	0	4	44	53	15	66	2
	per cent		0.0	8.3	91.7	77.9	22.1	97.1	2.9
Hanalei District									
Fouli Stream L37B6; G2,3,7-9	18	24	0	15	3	18	6	24	0
	per cent		0.0	83.3	16.7	75.0	25.0	100.0	0.0

Photographic chromosome maps, prepared according to the method of Stalker (1965), have been made of *D. primaeva* and the sequences of this species and of *attigua* described in terms of the former (Figs. 1-3).

The sequences of these two species were compared both with the Standard *D. grimshawi* sequences and those of *D. picticornis*, *D. ornata* (new species "A", Carson and Stalker 1968 c) and other Kauai flies. This work was facilitated by the method of table-level matching of unknown sequences with photographic map cut-outs. This was done using a compound microscope fitted with a drawing tube (Wild-Heerbrugg Instruments Inc.) as described in Carson and Stalker 1968 a.

## RESULTS

The X chromosome of *D. primaeva* differs in banding order from the Standard *D. grimshawi* (Carson and Stalker 1968 a) by a minimum of 10 fixed inversions (Figure 1, lower chromosome). Plotting of the break-points of these inversions reveals that three of them have identical break-points with three previously known inversions, namely, Xi, Xk and Xo. This is of very great interest in that it is precisely these three inversions which are common to the *D. adiaetola* and *D. planitibia* subgroups. The break-points of these inversions have been depicted in Figure 1 of Carson and Stalker 1968 b and Figure 1 of Carson and Stalker 1968 c. The eight remaining inversions, however, are new. The upper chromo-

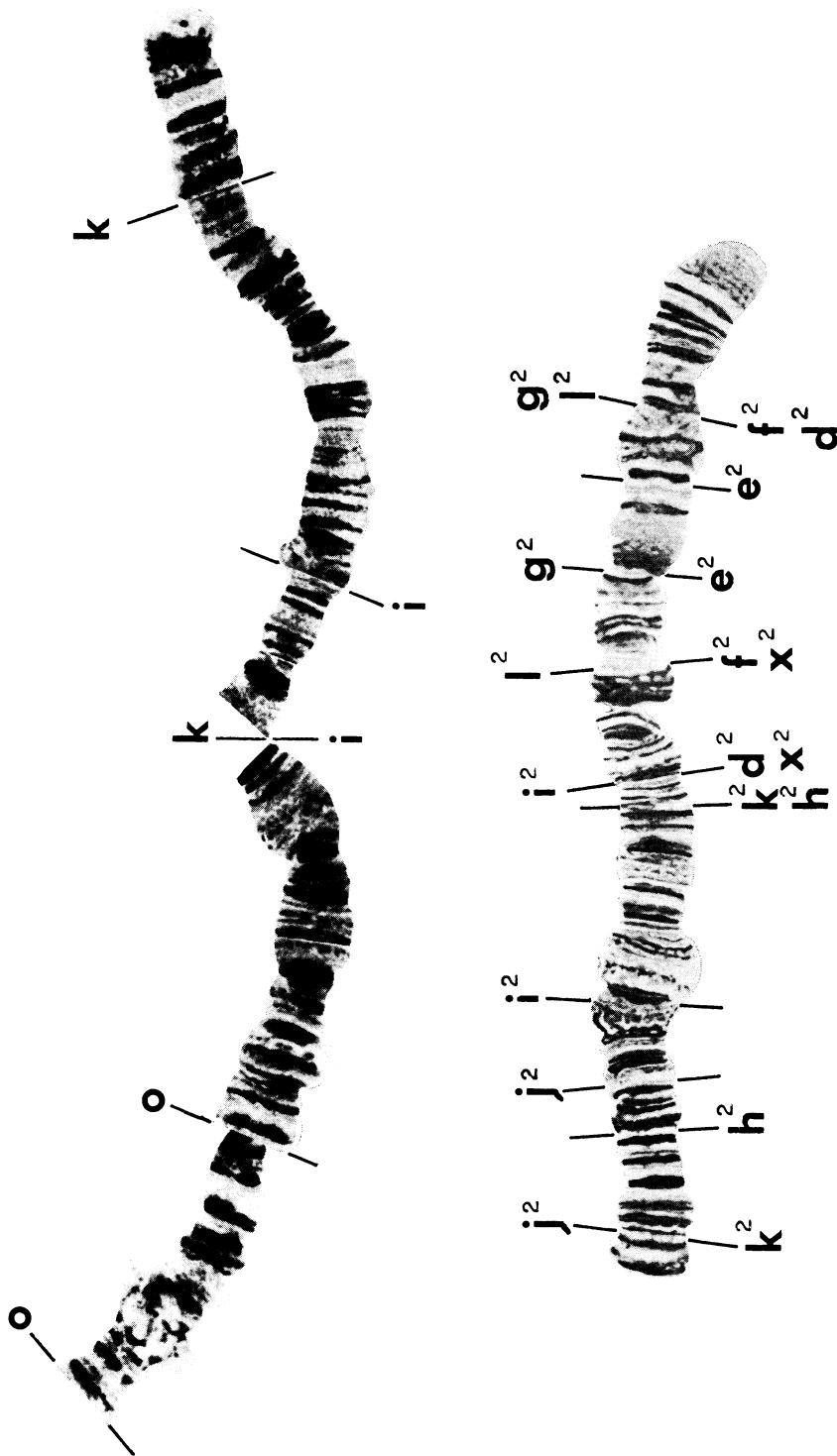


FIG. 1. Chromosome X (below) of *D. primaeva*. Above: X chromosome map of *D. grimshawi* with inversions Xiko. The distal ends in this and in Figs. 2 and 3 are to the left. For details, see text.

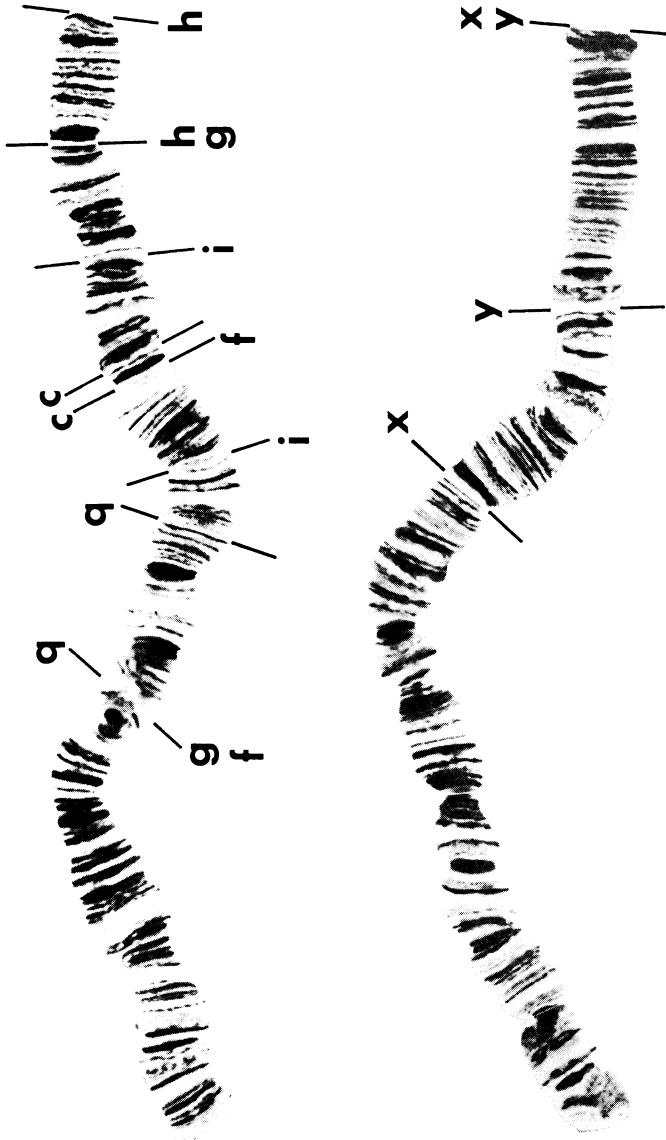


FIG. 2. Chromosome 2 (above) and 3 (below) of *D. primaeva*. To return chromosome 2 to Standard, see text. Chromosome 3 of *D. primaeva* is Standard. When inversions 3x and 3y are made, the order is converted to that of *D. altigua*.

some of Figure 1 shows a photographic map of the Standard *D. grimshawi* X chromosome which has been cut and refitted to give it the order represented as Xiko. Were the inversions marked to be made, the order would be identical with the *D. grimshawi* Standard (see Carson and Stalker 1968 a). The lower figure shows the *D. primaeva* X chromosome. It may be converted to Xiko fairly easily, because the inversions occur in two separate groups, those which are distal, i.e. in the region of Xo, and those which are proximal, i.e. in the region of Xi and k. Thus, to achieve the Xo order,  $k^2$  and  $j^2$  should be made first. As  $k^2$  is made, the proximal break of  $h^2$  should be carried along with the proximal break of  $k^2$ . The next inversion to be made is  $i^2$ . The  $d^2$  and  $x^2$  breaks should not be moved along

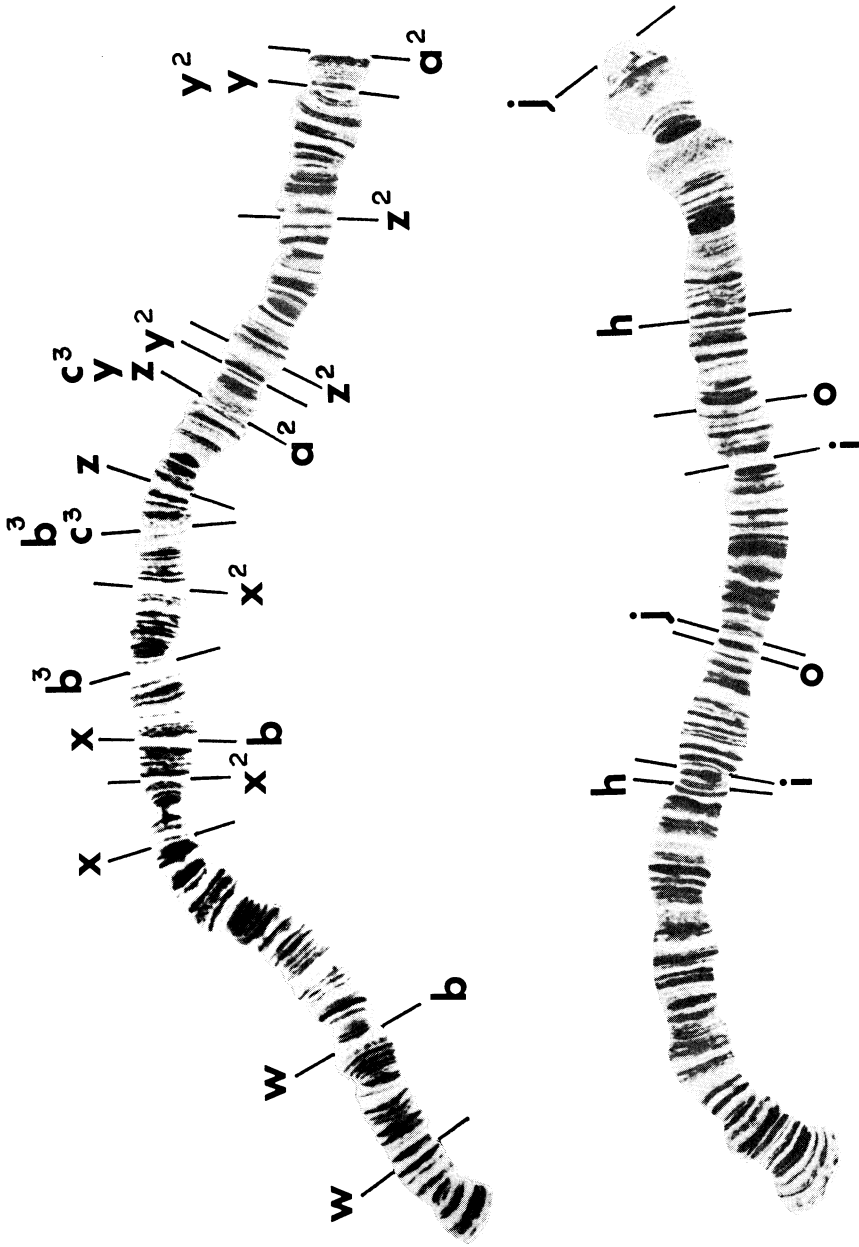


FIG. 3. Chromosome 4 (above) and 5 (below) of *D. primaeva*. For details, see text.

with  $i^2$ . Finally, when  $h^2$  is made, the  $X_o$  order is obtained. To obtain the  $X_{ik}$  order at the proximal end, starting with the *primaeva* X,  $f^2$ ,  $d^2$  and  $e^2$  should be made in any order.

Three alternative sequences occur in the X chromosome of *D. primaeva*, resulting in considerable chromosomal polymorphism in natural populations. The order which is closest to Standard *D. grimshawi* is  $X_{ikod^2e^2f^2h^2i^2j^2k^2}$ . The second order differs from the above by a single inversion,  $X_{g^2}$  and the third differs from the second by having another inversion,  $X_{l^2}$ , in addition to  $X_{g^2}$ .  $X_{l^2}$ , however, has not

been found apart from  $Xg^2$ . These three gene orders are referred to as +,  $Xg^2$  and  $Xg^2l^2$  respectively (see Table 1).

The *Drosophila attigua* X chromosome is without polymorphism. It has been found to have the  $Xg^2$  in fixed condition but it lacks  $Xl^2$ . It also has in fixed condition the short inversion  $Xx^2$  (Figure 1, lower).

The arrangement in chromosome 2 of *D. primaeva* that is closest to Standard *grimshawi* is 2cfgh. This order is shown on the photographic map (top, Figure 2). To return to Standard, make inversion h (moving the break-point of g to the proximal end), then make g (moving the break-point of f to the proximal end). Following this, make f (moving the break-point of c to the proximal end). When c is made, the Standard *D. grimshawi* sequence results. Inversion 2c appears to be identical with the same inversion found in the *adiastola* subgroup (Carson and Stalker 1968 c, Fig. 1). Inversion 2q is polymorphic within *D. primaeva*.

The *D. attigua* second chromosome lacks 2h and 2g but has in addition a new inversion 2i. It thus has the formula 2cfi. This can be made from Figure 2 by first removing h and g as described in the preceding paragraph and then inverting 2i.

Chromosome 3 of *D. primaeva* is the same as Standard *D. grimshawi*. *D. attigua* is 3xy; if these two inversions, which are shown on Figure 2, lower, are made, the *attigua* arrangement will result.

Chromosome 4 of *D. primaeva* differs from Standard *D. grimshawi* by five inversions. Its formula is 4bwxyz (Figure 3, upper). To return to the common arrangement 4b (and ultimately to Standard *D. grimshawi*) 4w and 4x should be made without moving the b points. When 4b is then made, the result is Standard at the distal end. When the tandem inversions 4y and 4z are made, the proximal end will also be returned to Standard. Inversion 4a<sup>2</sup> is polymorphic within *D. primaeva*. The fourth chromosome of *D. attigua* has the formula 4bwxx<sup>2</sup>y<sup>2</sup>z<sup>2</sup>. It may be visualized by first making the 4y inversion, carrying along the 4y<sup>2</sup> break-point so that it comes to be the distal one of the two 4y<sup>2</sup> breaks. Then make 4y<sup>2</sup> followed by 4z<sup>2</sup>. 4c<sup>3</sup> and 4b<sup>3</sup> are polymorphic tandem inversions found within *D. attigua*.

Chromosome 5 of *D. primaeva* (Figure 3, lower) differs from Standard *D. grimshawi* by three inversions; its formula is 5hij. This order may be converted to Standard by first making 5j, then 5h and 5i. *D. attigua* lacks 5j but has 5o. It may be derived from the *D. primaeva* order shown in Figure 3, lower, by first making 5j and then inverting 5o using the 5hi gene order as a base.

Chromosomal polymorphism exists within *D. primaeva*. The species is difficult to obtain in most of the areas of easy access on Kauai and the data presented in Table 1 are based on small samples and are preliminary, at best. Nevertheless, certain features are of interest. The *primaeva* Standard X is found only at the Waimea District stations;  $Xg^3$  appears to be the principal arrangement at the Hanalei District site, whereas  $Xg^2l^2$  is predominant at Kahili. The only known strain of *D. attigua* proved to be polymorphic for two fourth chromosome inversions. Both species have a metaphase consisting of 5 rods and 1 dot (Clayton 1968, 1969).

#### DISCUSSION

Stalker (1968 and cited in Carson et al. 1970) has compared the banding sequences of giant chromosomes from Hawaiian and non-Hawaiian *Drosophila*.

His analysis shows that certain sequences of *D. primaeva* can be matched with homologous sequences in certain continental forms of the subgenus *Drosophila*, notably the predominantly Palearctic *robusta* group. Such a banding sequence, common to two such widely separated species groups, is judged as primitive. As at least one such sequence (5h) is broken up by an inversion within Hawaiian species other than those of the *D. primaeva* subgroup, these facts may be used to give direction to the chromosomal phylogeny within Hawaii. The flies of the *D. primaeva* subgroup indeed appear to be the closest, of the 69 which have been analyzed, to a hypothetical ancestor for the Hawaiian flies.

The formulae of the eleven cytologically deciphered species from Kauai are arranged in a relationship diagram on Figure 4. This material is assembled from

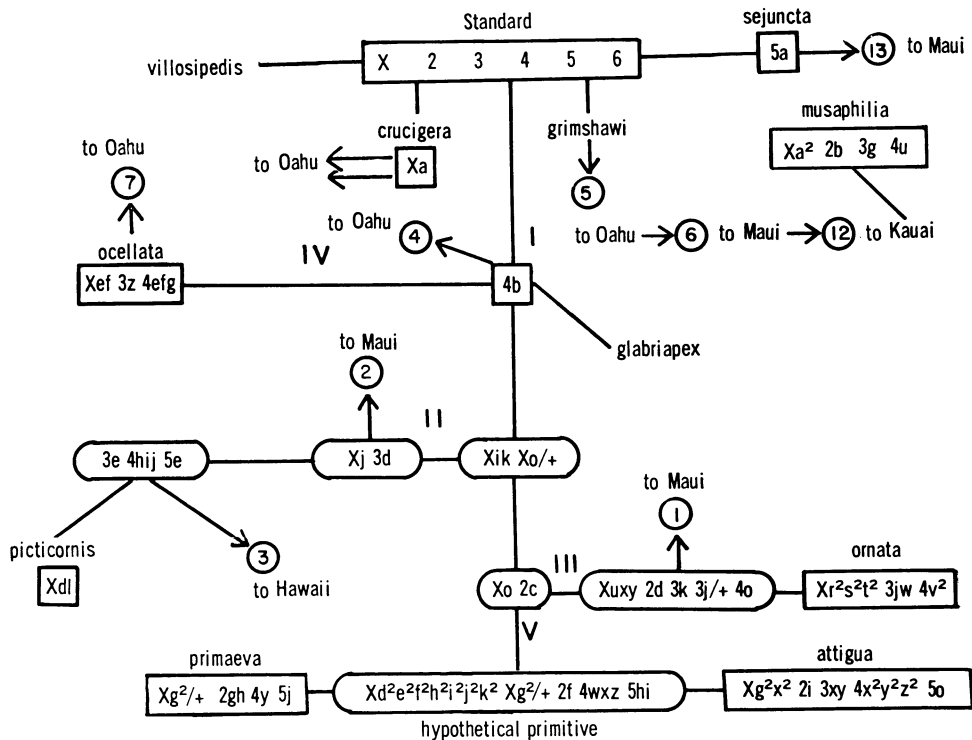


FIG. 4. Polytene chromosome relationships between the *D. primaeva* subgroup V (bottom) and nine other species of subgroups I, II, III and IV on the Island of Kauai. Read the inversion formula for each species cumulatively by following the line from the *D. grimshawi* Standard (box, upper center) e.g. *D. ocellata* has the formula Xef 2 3z 4befg 5 6. Arrows leading to encircled numbers indicate hypothetical founders which go to the other islands. For details, see text.

similar diagrams given in Carson and Stalker 1968 a,b and c and Carson et al. 1970. As in those diagrams, boxes with rectangular ends show the actual species; boxes with rounded ends denote hypothetical populations.

Inspection of the bottom line of formulae in Figure 4 shows that *D. attigua* and *primaeva* have a series of inversions in common. This permits the specification of a hypothetical primitive sequence not only for these two species but also

for all of the 67 other species which have been cytologically deciphered. The formula for this primitive is rather lengthy because its sequence is described in terms of inversion differences from the arbitrary Standard at the top of Figure 4. The Standard was chosen some years ago before the *D. primaeva* subgroup species were known.

All of the species and species groups of which the polytene chromosomes have been studied may be derived from this primitive sequence. Thus, the *adiastola* subgroup (III) is considered to have arisen from a hypothetical intermediate of the formula Xiko 2c 3 4b 5 (Figure 4). The *adiastola* species group includes 11 species of which only one, *D. ornata*, is found on Kauai. As has been described elsewhere (Carson et al 1970) the species of the eastern islands were apparently derived from an interisland founder (no. 1, Fig. 4) which went directly to Maui from Kauai.

Stemming from a very similar ancestor (Xik Xo/+ 2 3 4b 5) was a line of descent which gave rise to modern *D. picticornis* of Kauai, the only member of the *D. planitibia* subgroup (II) on that island. Like the *adiastola* subgroup, this subgroup is extensively developed on the other islands (13 species). These are among the most remarkable drosophilines in the world attaining a very large size and having an extra wing-vein in cell R-5. As in the *adiastola* case, these flies are considered to be descended from a single founder (no. 2, Fig. 4) reaching Maui directly and spreading to Oahu and Hawaii from there (see Carson et al. 1970).

The remainder of Fig. 4 is self-explanatory. The founder numbers conform to those used in Carson et al. (1970). The *punalua* subgroup apparently arose on Kauai and is represented by present-day *D. ocellata* (new species "B", Carson and Stalker 1968 c). The *D. grimshawi* subgroup consists of six species on Kauai. All but one can be derived from Kauai ancestors; the exception is *D. musaphilia*. This species has intermediate forms (having 2b, 3g or both) on Oahu and Maui. Accordingly, *D. musaphilia* is considered to be the only Kauai picture-winged species which arose from a re-invasion of Kauai from the east (see upper right, Fig. 4 and Carson et al. 1970). *D. crucigera* has apparently colonized Oahu twice from Kauai, without undergoing speciation in either instance.

The facts presented in this paper point to the arrival on Kauai of a primitive founder, probably during the Pliocene. There then ensued a small proliferation of species, at least of picture-wings. This speciation episode appears to be reflected today by the five subgroups of picture-wings found there. Of these five subgroups, only the *grimshawi* subgroup underwent a modest secondary episode of speciation, resulting in *D. grimshawi*, *crucigera*, *sejuncta* and *villosipedis*. Although Kauai has played a key role in the ancestry of Hawaiian *Drosophila*, the number of species resulting from the early speciation episode was small, possibly as small as six species but not more than ten. This is far fewer than the number of comparable species (59) on the three other major island masses (Oahu, Maui complex and Hawaii). As Kauai is the oldest island (see Carson et al. 1970), it is tempting to suggest that most of its species are also old in the geological sense. This is in strong contrast to the situation relative to the easternmost island (Hawaii). This island, which is of late Pleistocene age and still has two active volcanoes, has 17 cytologically deciphered species in the picture-winged group. That



these species are indeed new in the geological sense is unequivocal. Each is endemic to the island and the origin of most can be traced by chromosomal techniques directly to founders stemming from related but specifically different populations on Maui, 30 miles away.

#### SUMMARY

The polytene chromosomes of two species of *Drosophila* endemic to Kauai, *D. primaeva* and *attigua* are described. They are related to the picture-winged flies and constitute subgroup V, the *D. primaeva* subgroup. The chromosomes can be completely described in terms of the *D. grimshawi* Standard sequences from which *D. primaeva* differs by 23 fixed inversions. *D. primaeva* is polymorphic in chromosomes X, 2 and 4 and appears to show different frequencies in different populations on Kauai. *D. attigua* Hardy and Kaneshiro is a sibling species of *D. primaeva*. It differs from the latter by 13 fixed inversions and is known only from a single strain captured sympatrically with *primaeva*.

The *D. primaeva* subgroup is judged to include the most primitive of the species so far studied on the basis of the similarity of certain of its polytene sequences to continental members of the subgenus *Drosophila*. A phylogenetic scheme is proposed whereby all five subgroups of picture-winged flies evolved on Kauai from a *primaeva-like* ancestor. It is suggested that a modest burst of speciation at a relatively early time (Pliocene?) produced the species we now recognize as belonging to different subgroups. From this set of not more than ten species, at least seven migrants reached the islands to the east and then underwent exuberant further speciation, producing more than fifty species of picture-wings on the Maui complex, Oahu and Hawaii.

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## ERRATA

The caption for Figure 4, *Studies in Genetics* IV, 1968, p. 375, should read: "Chromosome X (top) and chromosome 4 (below) of *D. pinalua*. For details, see text."

The caption for Figure 5, p. 376, of the same article should read: "Chromosome 3 (top) and chromosome 5 (below) of *D. pinalua*."