

XVII. Polytene Chromosome Relationships in Hawaiian  
Species of *Drosophila*. III. The *D. adiaastola* and  
*D. punalua* subgroups<sup>1</sup>

HAMPTON L. CARSON<sup>2,3</sup> AND HARRISON D. STALKER<sup>3</sup> (1968)

This paper is the third in a current series describing the sequential relationships in the giant chromosomes of the picture-winged members of the subgenus *Drosophila* in Hawaii. The species dealt with in this paper fall into two recognizable chromosome subgroups. Information is published here on nine species in the *D. adiaastola* subgroup and six in the *D. punalua* subgroup. Two of the species are as yet undescribed and have been tentatively designated as "A" and "B". Preliminary data have been published on four and three species respectively from these two subgroups (Carson, Clayton and Stalker, 1967).

MATERIALS AND METHODS

As in the first two papers in this series, the chromosome sequences in all species in all subgroups of picture-winged *Drosophila* are described in terms of an arbitrarily-chosen Standard. The chromosome arrangements found in the Auwahi, Maui strains of *D. grimshawi* have been so designated (Carson and Stalker, 1968a). Lower case letters have been used to denote inversions; those used in this paper, therefore, supplement those used in Carson and Stalker, 1968a and b. Tables 1 and 2 give the geographical origin and the number of chromosomes examined from each wild strain. Other details of the methods used will be found in the earlier papers of this series.

RESULTS

*Description of inversion break-points*

*The D. ADIAASTOLA subgroup*

The positions of the inversion breaks are given in Figures 1–3. The distribution of the inversions in the various species is given in Figure 6. In all photographs, the chromosomes are shown with their distal ends to the left. Figure 1 shows a photographic map of chromosome X (above) and chromosome 2 (below) of *D. adiaastola* of Maui. The arrangement shown in the X can be returned to the Standard, at the distal end, by making the inversions v, u and o in that order. The proximal end can be similarly returned by making y, w, x, k and i in that

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TABLE 1  
 Strains of *Drosophila* species of the *D. adiasiola* subgroup examined for giant chromosome sequences

Species	Locality and strains examined <sup>2</sup>	Number of wild chromosomes observed			
		Autosomes		X chromosomes	
		This paper <i>et al.</i> , 1967	Total	This paper <i>et al.</i> , 1967	Total
<i>adiasiola</i>	Waikamoi, Maui (4200') G59C3; J67C8; J75C7, 9; J76M11; J98A1; K72B5; L18P62, 63; L25P7 Kaulalewelewe, W. Maui (3000') J57B6 Paliku, Maui (6300') K22L7 Kipahulu Valley, Maui (3100') L5B21 Lanaihale, Lanai (3000') Katholena Gulch, Lanai (2100') K94G13 Total	34 4 4 4 .. 4 50	64 4 .. .. 30 98	26 3 3 3 .. 3 38	51 3 .. .. 23 .. 77
<i>ciliifera</i>	So. Hanalililo, Molokai (3000') C139.1A, B; G42B5	8	..	7	..
<i>clavisetae</i>	Waikamoi, Maui; J76M5; L18P12	4	8	4	8
<i>neogrimshawi</i>	Puu Lanipo, Oahu (2200') K53B2	4	..	3	..
<i>ochrobasis</i> <sup>1</sup>	Kipuka at B.M. 5108', Saddle Rd., Hawaii, K15N3; K33G1, 20; K46G1; L22G1, 5, P1 Hualalai, Hawaii (5300') G90A100 Alakahi Stream, Hawaii (3900') K77C6 Total	22 2 4 28	.. .. .. ..	17 1 3 21	.. .. .. ..
<i>peniculipedis</i> <sup>1</sup>	Waikamoi, Maui	..	4	..	3
<i>setosimentum</i> <sup>1</sup>	Awini Cabin, Kohala Mts., Hawaii (2000') J17H11 Puu Laalaau, Kohala Mts., Hawaii (4000') Waimea Res., Kohala Mts., Hawaii (3000') K78G3 E. of Puu Ohu, Kohala Mts., Hawaii (3500') K79G17, 20 Upper Olaa For. Res., Hawaii (4100') J48B8 Puu Huluhulu, Hawaii Volcano Nat. Park, Hawaii (3200') Kipuka at B.M. 5108', Saddle Rd., Hawaii L22G7 Pawaina, Hawaii (3000') K4B2 Mt. House, Hawaii (2800') L19B41 Total	4 4 4 8 2 8 4 4 4 42	.. .. .. .. .. .. .. .. .. ..	3 3 3 6 2 6 3 3 3 32	3 3 3 6 2 6 3 3 3 32
<i>spectabilis</i>	Puu Kolekole, Molokai (3500') Waikamoi, Maui; J76M20 Total	.. 2 2	2 8 10	.. 2 2	1 6 7
<i>new species "A"</i>	Pouli Stream, Hanalei Dist. Kauai (1500') L37G16, 17 Mt. Kahili, Kauai, L41G1 Total	8 4 12	.. .. ..	6 3 9	.. .. ..

TABLE 2

TABLE 2  
Strains of *Drosophila* species of the *D. punalua* subgroup examined for giant chromosome sequences

Species	Locality and strains examined <sup>2</sup>	Number of wild chromosomes observed								
		Autosomes		X chromosomes						
		This paper	Carson <i>et al.</i> 1967	Total	This paper	Carson <i>et al.</i> 1967	Total			
<i>basisetae</i>										
<i>paucipuncta</i>										
	Mountain House, Hawaii (2800') L19B33-36; L19G2	18	..	18	14	..	14	..	..	14
	Pawaina, Hawaii, K4B3	2	6	8	2	5	7	..	..	7
	Honaunau For. Res., Hawaii (2100')	..	40	40	..	30	30	..	..	30
	S. side of Hualalai, Hawaii (2500') K2B5	4	..	4	3	..	3	..	..	3
	Stainback Hwy., Hawaii (2100') K62G13, 14, 16	8	..	8	6	..	6	..	..	6
	Total	14	46	60	11	35	46	..	..	46
<i>prolaticilia</i> <sup>1</sup>										
	Pawaina, Hawaii	..	12	12	..	9	9	..	..	9
	Honaunau For. Res., Hawaii, J62B5, 11	4	..	4	4	..	4	..	..	4
	Kipuka (2200') Saddle Rd., Hawaii, J49B1, 4	4	..	4	3	..	3	..	..	3
	Hilo Watershed, Saddle Rd., Hawaii (2800') K78G5	4	..	4	3	..	3	..	..	3
	S. side of Hualalai, Hawaii, K2B6	2	..	2	2	..	2	..	..	2
	Mt. House, Hawaii, L19B1, 5, 12, 20	14	..	14	11	..	11	..	..	11
	Totals	28	12	40	23	9	32	..	..	32
<i>punalua</i>										
	Manoa Falls, Oahu (800') K24G7	4	4	8	3	3	6	..	..	6
	Palikey, Oahu (2700') K5C35-37; K20M1-3	18	16	34	14	13	27	..	..	27
	Wiliwilimui Ridge, Oahu (2100') K53B4-7, G2-4	26	4	30	20	3	23	..	..	23
	Mt. Kaala, Oahu (4000')	..	4	4	..	3	3	..	..	3
	Pupukea, Oahu (1900')	..	2	2	..	2	2	..	..	2
	Kului Gulch, Oahu (1000') K38G3, 27	8	..	8	6	..	6	..	..	6
	Kaau Crater, Oahu (1700') L24G2; L23G3	8	..	8	6	..	6	..	..	6
	Pukele Stream, Oahu (1000') K76G3	4	..	4	3	..	3	..	..	3
	Lulumahu Stream, Oahu (1000') K91G4	4	..	4	3	..	3	..	..	3
	Total	72	30	102	55	24	79	..	..	79
<i>uniseriata</i>										
	Kupaua Valley, Oahu (800') C144.5A	4	..	4	3	..	3	..	..	3
<i>new species "B"</i>										
	Mt. Kualapa, Kauai (1400') L45B2, 12, 13, 14; G7	20	..	20	15	..	15	..	..	15

<sup>1</sup> Shows intraspecific chromosomal polymorphism.  
<sup>2</sup> Strain numbers are entered only for newly-reported strains.

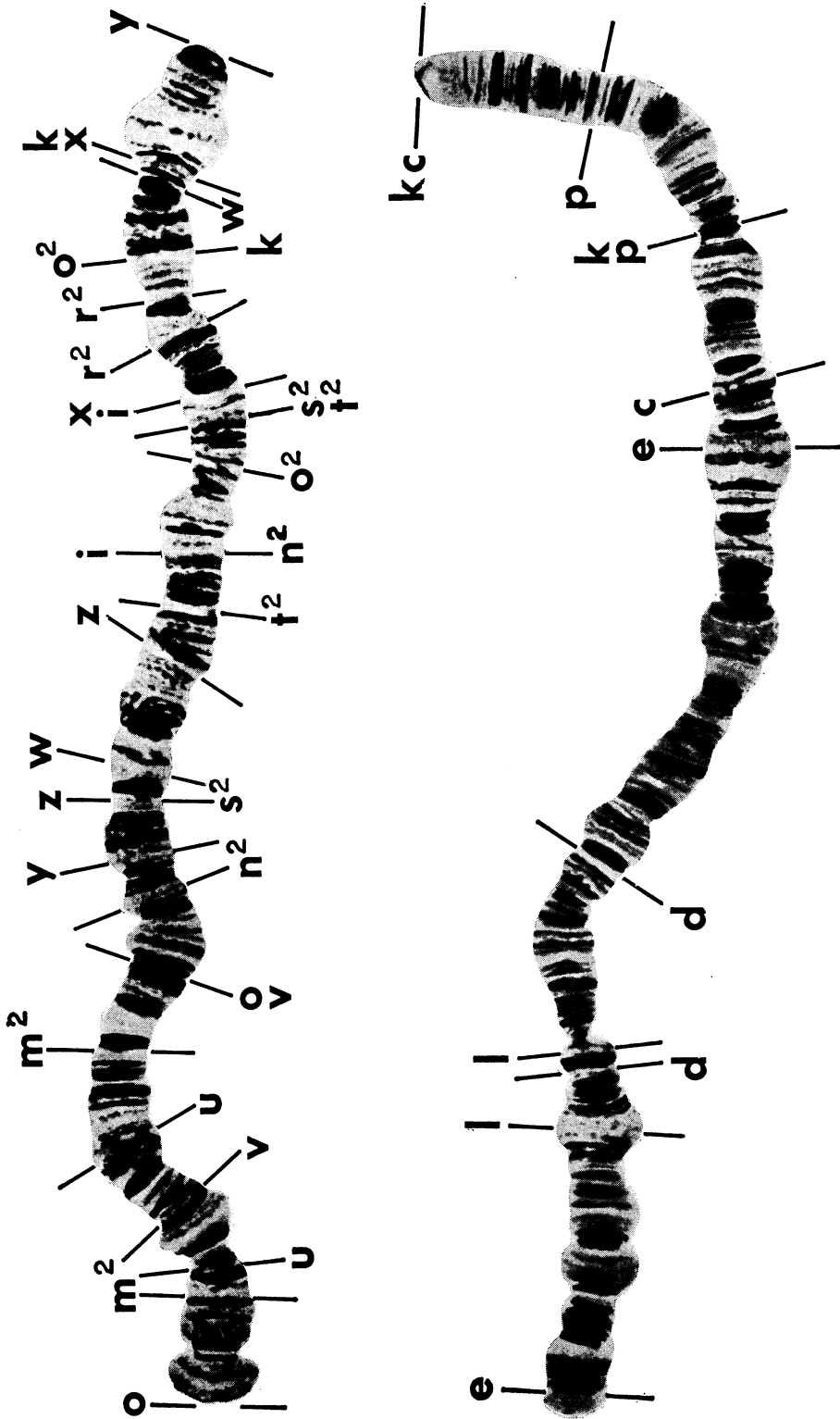


FIG. 1. Chromosome X (top) and chromosome 2 (below) of *D. adiasiola*. For details, see text.



FIG. 2. Chromosome 5 (top) and chromosome 3 (below) of *D. adiastrala*. For details, see text.

order. The break-points of inversions Xo and Xi and Xk are identical to those inversions as found in the *D. planitibia* subgroup; they are considered to be the same. Inversions Xz, Xm<sup>2</sup>, Xn<sup>2</sup> and Xo<sup>2</sup> are independent and are extensions of the basic *D. adiastrala* gene order. The order found in *new species* "A" from Kauai can be made by inverting Xx and Xw (Fig. 1) and then making the new inversions Xr<sup>2</sup>, Xs<sup>2</sup> and Xt<sup>2</sup>.

The lower photograph in Figure 1 shows chromosome 2 of *D. adiastrala*. Return to Standard can be achieved by making the independent inversions c and d. The inversions which are based on the *D. adiastrala* gene order and extend it are 2e, 2d, 2l, 2k and 2p. All represent single steps from the basic 2cd of *D. adiastrala*. 2p, however, is polymorphic within *D. ochrobasis*, which has fixed 2k. Accordingly, the former must have followed 2k in time (Fig. 6), unless the assumption is made that the whole phylogeny as presented starts with *D. ochrobasis*.

Figure 2 shows chromosome 5 (above) and 3 (below) of *D. adiastrala*. When 5f is inverted, the result will be the Standard 5. Chromosome 3 of *D. adiastrala* is shown in the lower photograph of Figure 2. Inversions 3k, 3j and 3f, made in any order, will produce the Standard arrangement. The inversion of 3f alone, however, will produce the arrangement 3jk which is precisely that which is found in *new species* "A" (Kauai). Further, 3f appears to be identical with 3f of *D. punalua* (see Figure 5, upper). The latter, however, lacks 3jk. 3l, 3u and 3t are each inversions extending the basic 3fjk gene order of *D. adiastrala*. Although 3t and 3l overlap, they have occurred independently in different species (see Fig. 6).

Figure 3 illustrates chromosome 4 of the *D. adiastrala* subgroup. The length of the photographs has necessitated an arbitrary division of each photograph into two parts. The situation in this chromosome is complex, but with an orderly application of the following information it will be possible to reconstruct the banding order of almost all fourth chromosomes in the subgroup. The gene order which has been invariably found in *D. adiastrala* is shown at the top of the figure. To return to the Standard *D. grimshawi* chromosome 4, inversion p should come first, followed by o and then b. 4q, which is independent, completes the restoration of Standard. Inversions 4r and 4s shown on the upper chromosome are independent extensions of the *D. adiastrala* gene order (see Fig. 6). To obtain the fourth chromosome gene order found in *new species* "A" from Kauai (4boy<sup>2</sup>), inversions 4p and 4q must be made and then 4v<sup>2</sup> added as an extension.

Both the middle and the lower chromosomes on Figure 3 represent that fourth chromosome gene order of *D. setosimentum* which is closest to *D. adiastrala*; it has the formula 4bopqb<sup>2</sup>c<sup>2</sup>d<sup>2</sup>e<sup>2</sup>f<sup>2</sup>n<sup>2</sup>o<sup>2</sup>. To return to the *D. adiastrala* arrangement, all of the inversions shown in the middle figure must be made. 4o<sup>2</sup> should come first, followed by 4b<sup>2</sup>. The rest are independent and can be made in any order. To permit further illustration of break-points, 4c<sup>2</sup> 4d<sup>2</sup> and 4e<sup>2</sup> are also shown in the upper figure as extensions of the *D. adiastrala* fourth chromosome. The lower chromosome (*D. setosimentum*) shows the same gene order as the middle one. In this case, however, the position of four inversions which have been found to be polymorphic within *D. setosimentum* are given. All of these have been observed in the homozygous state.

*Drosophila ochrobasis*, like *D. setosimentum*, has 4b<sup>2</sup> in fixed condition. Certain

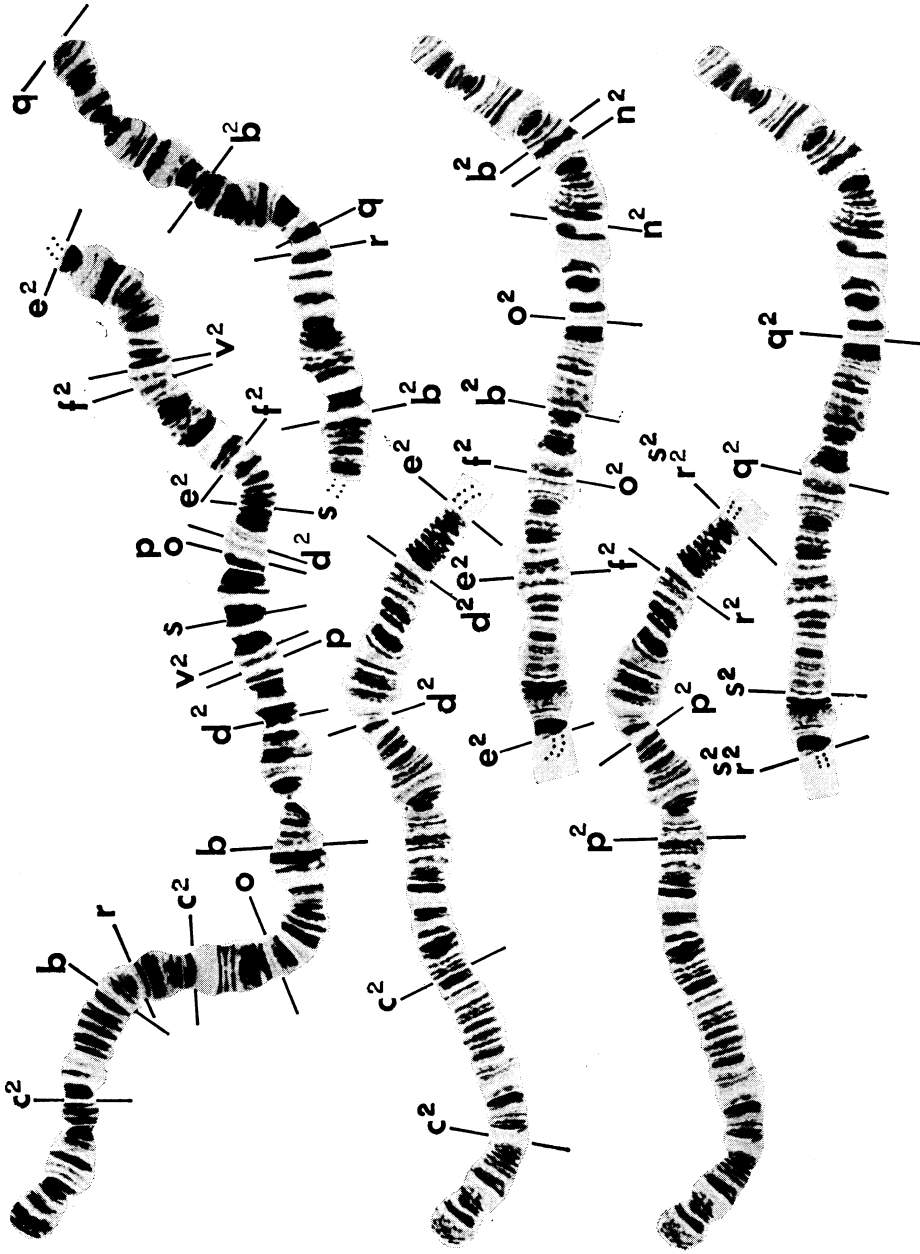


FIG. 3. Chromosome 4 (top) of *D. adiastrata*, chromosome 4 of *D. setosimentum* (middle and bottom). For details, see text.

fourth chromosomes of *D. ochrobasis* differ from *D. adiastrata*, furthermore, only by  $4b^2$ . This arrangement can be visualized in Figure 3 by making all the inversions in the *D. setosimentum* chromosome (middle) except  $4b^2$ . *D. ochrobasis*, however, has in its populations at least two more extensively rearranged fourth chromosomes. These form complex heterozygotes proximally with the above chromosome. Neither of these has been found in the homozygous state and their full band ordering has not been deciphered. These chromosomes, however, all

clearly show the  $4c^2$  gene order. The undeciphered portion, due to about four short additional inversions, is located in the proximal two-thirds of the chromosome, proximal to the proximal break of  $4c^2$ . Further study of the polymorphism in these two species is in progress.

#### The *D. PUNALUA* subgroup

The positions of the inversion breaks are given in Figures 4 and 5. The distribution of the inversions in the various species is shown in Figure 6. Figure 4 shows the X (above) and 4 (below) of *D. punalua* of Oahu. When the independent inversions Xe and Xf are made, the result will be the *D. grimshawi* Standard. The lower chromosome in this figure is the fourth. It can be changed back to the Standard order by making inversions 4e, 4f, 4g and 4b. The latter, which is shared by most members of all of the other three subgroups, is probably the oldest of the four. 4b and e must precede  $4u^2$ , a new extension found in *D. basisetae*.

Figure 5 shows chromosome 3 (above) and 5 (below) of *D. punalua*. When inversion 3f is made, the result will be the Standard 3. It should be noted that this inversion appears to be identical with that found in the *D. adiastrala* subgroup (see Figure 2, lower). Chromosome 5 (below) shows the Standard gene order in *D. punalua*. 5b, 5c and 5m are independent extensions of the Standard order found within several species of the subgroup. Chromosome 2 throughout the subgroup is identical in gene order to the Standard and has not been illustrated.

#### Fixation and polymorphism for inversions

Except for 5m and 5c in *D. prolaticilia*, the *D. punalua* subgroup species lack intraspecific chromosomal polymorphism. This fact is shown in Table 3, which also summarizes the data on both fixed and polymorphic inversions in all four

TABLE 3

Chromosome inversions in 53 species of picture-winged *Drosophila* from Hawaii. Inversions common to two or more subgroups are not included more than once

Chromosome	No. of species	X	2	3	4	5	Total
Number of fixed inversions:							
<i>D. grimshawi</i> subgroup	29	11	2	5	4	2	24
<i>D. planitibia</i> subgroup	9	11	0	2	3	1	17
<i>D. adiastrala</i> subgroup	9	10	5	5	8	1	29
<i>D. punalua</i> subgroup	6	2	0	1	4	1	8
Total	53	34	7	13	19	5	78
Number of inversions polymorphic within a species:							
<i>D. grimshawi</i> subgroup	..	0	1	7	11	3	22
<i>D. planitibia</i> subgroup	..	4	3	2	4	0	13
<i>D. adiastrala</i> subgroup	..	2	1	1	9	1	14
<i>D. punalua</i> subgroup	..	0	0	0	0	2	2
Total	53	6	5	10	24	6	51
Grand Totals	..	40	12	23	43	11	129



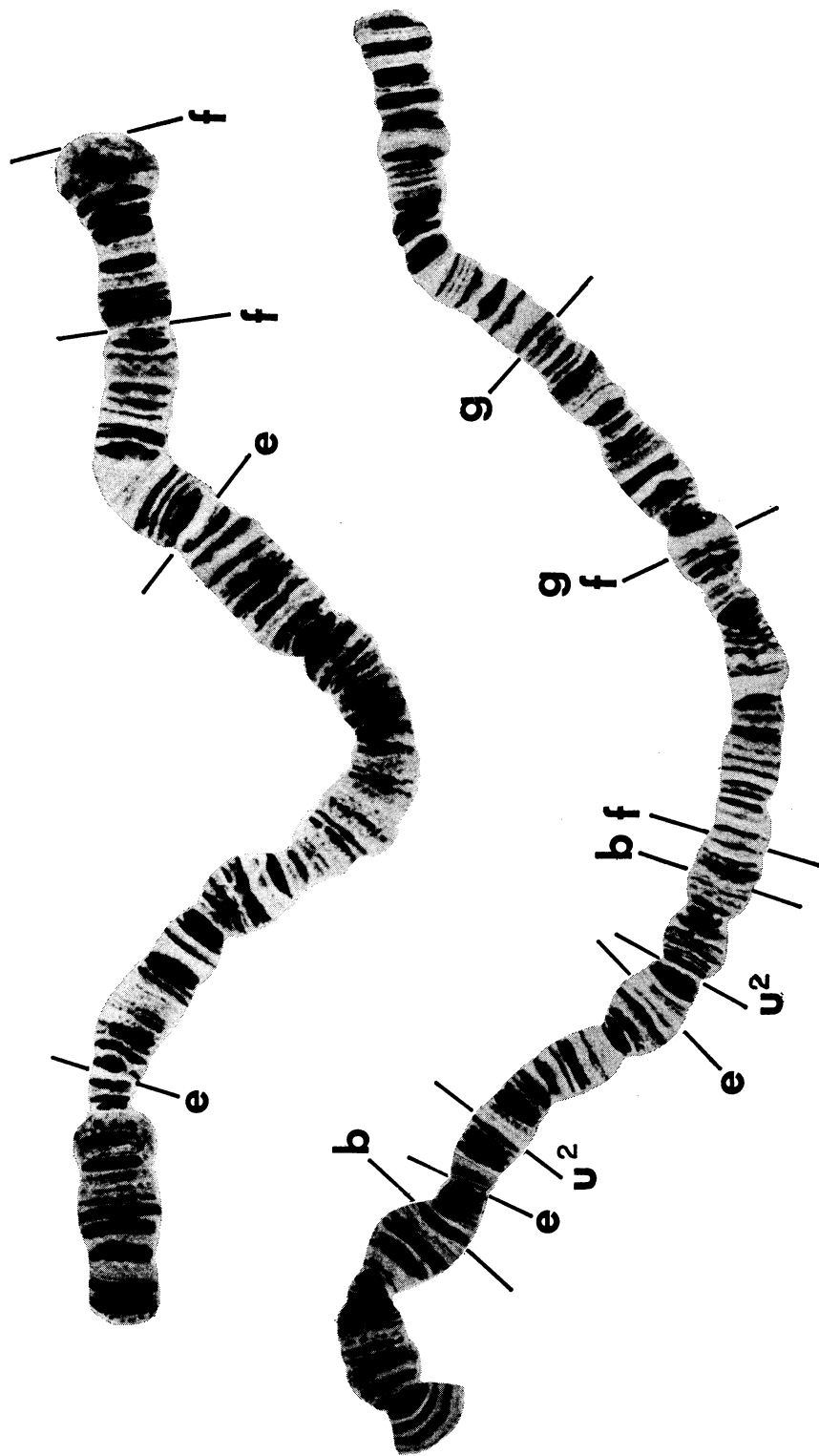


FIG. 4. Chromosome X (top) and chromosome  $\beta$  (below) of *D. punalua*. For details, see text.

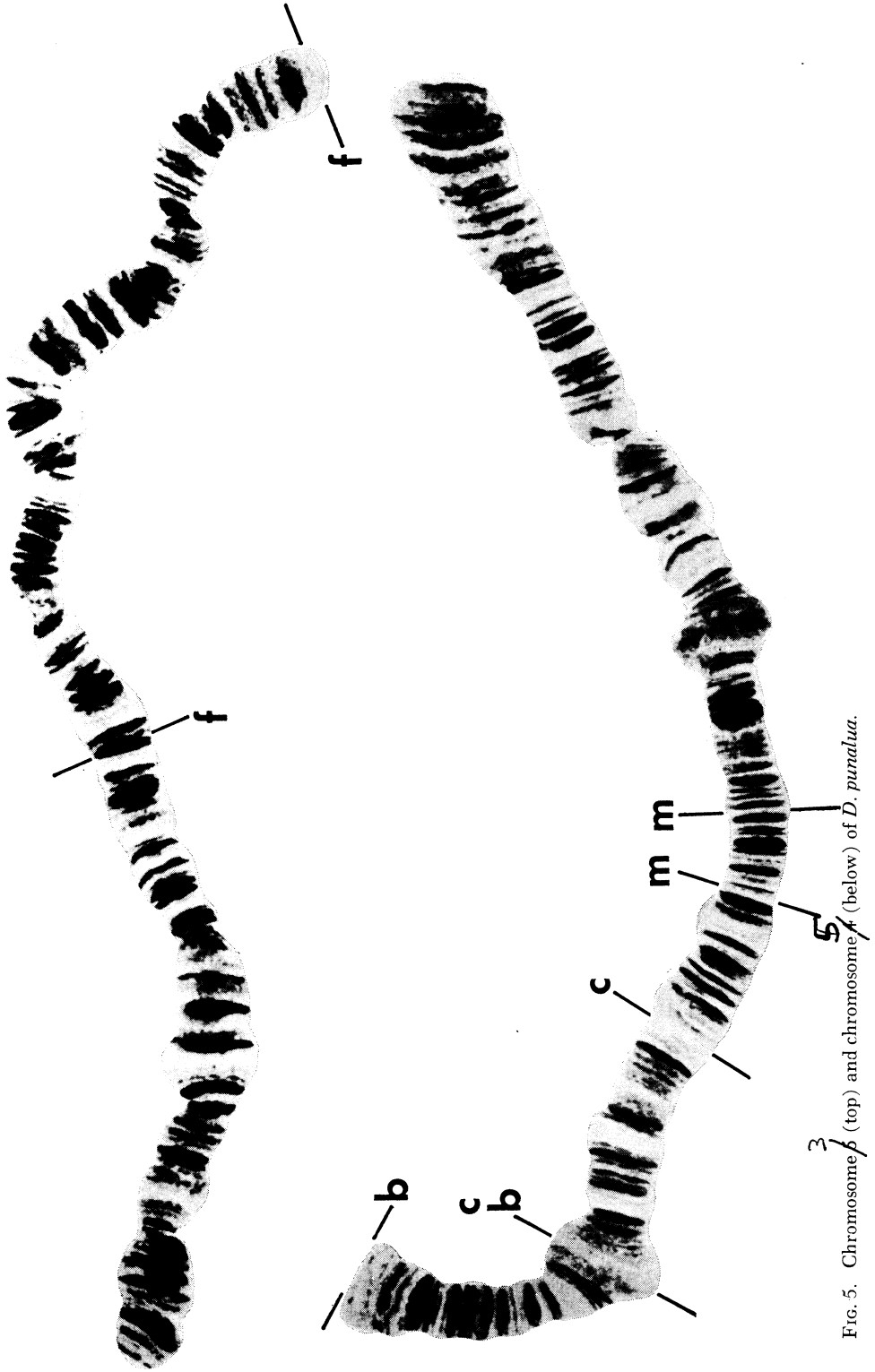


FIG. 5. Chromosome 3 (top) and chromosome 5 (below) of *D. punalua*.

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subgroups. It is noteworthy that chromosome 2 and 5 are involved in inversions less frequently than the others. Chromosome X is the leader in fixed inversions but does not show polymorphism as frequently as chromosome 4. The latter, with 24 polymorphisms in 53 species is by far the most frequent chromosome to be so involved.

DISCUSSION

*Relationships of the species based on banding sequence*

These relationships are presented in Figure 6. As in previous diagrams of this sort (see Carson, *et al.*, 1967 and Carson and Stalker, 1968a and b), certain hypothetical populations are proposed as necessary common ancestors for existing species. In a number of instances, these populations are assumed to have been heterozygous for one or more inversions which have subsequently become homozygous as the species were formed. Inversion-sharing can, of course, also be explained by hybridization after the formation of the species. Intrinsically, the data do not speak against this hypothesis. Until such time, however, as evidence for such interspecific hybridization in nature is obtained, the hypothesis that inversion-sharing is due to common descent will be favored.

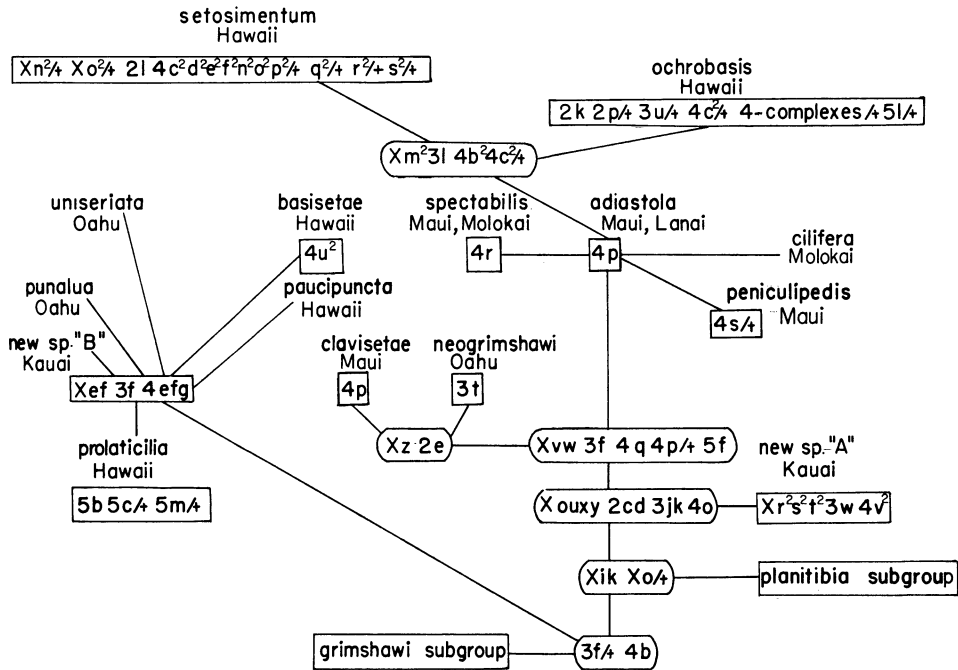


FIG. 6. Chromosomal relationships among nine species of the *D. adiastrala* subgroup (right) and six species of the *D. punalua* subgroup (left). Letters appearing singly represent fixed inversions whereby the arrangements found differ from the *D. grimshawi* Standard. For details, see text.

Fig. 5. Chromosome 4 (top) and chromosome 4 (below) of *D. punalua*.

*Geographical distribution, migration and evolution of the  
D. ADIASTOLA and D. PUNALUA subgroups*

At the bottom of Figure 6, two hypothetical populations are proposed. These populations could have served as points of origin of all four subgroups, as indicated in the figure. Evidence is strong (see Carson and Stalker, 1968b) that the *D. planitibia* subgroup had its main differentiation on Maui, following migration to that island from Kauai by a *D. picticornis*-like ancestor (Xijk 3d 4b). The inversion-sharing data, however, give no very good clues to the island origin of the *D. punalua* and *D. grimshawi* subgroups. On other grounds, however, it has been tentatively suggested that the latter group arose on Maui (Carson and Stalker, 1968a). The *D. punalua* subgroup is remarkable in its chromosomal uniformity in the face of wide distribution in the islands (with the curious exception of Maui) and the wide morphological divergence among its essentially homo-sequential members.

On the other hand, the discovery and analysis of a new species ("A") of the *D. adiaastola* subgroup on Kauai has shed considerable light on the origin of this subgroup. It is of particular interest that this Kauai species shares Xi, Xk, Xo and 4b with *D. picticornis*, of the *D. planitibia* subgroup, but at the same time it has much in common with *D. adiaastola* of Maui. It is hard to escape the conclusion

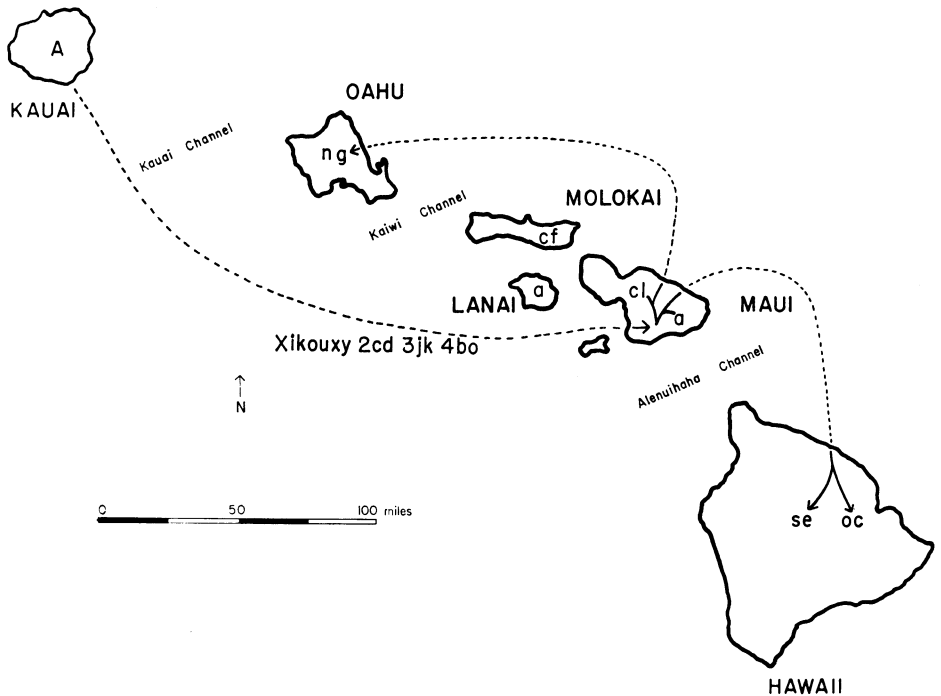


FIG. 7. Migration of the *D. adiaastola* subgroup on the principal islands of Hawaii. Following an early colonization of Maui from Kauai, *Drosophila neogrimshawi* (ng) apparently reached Oahu by northwestward migration of a colonizer. A similar colonization appears to have led to the population ancestral to *D. setosimentum* (se) and *D. ochrobasis* (oc) of Hawaii. A = new species "A", a = *D. adiaastola*, cf = *D. cilifera*, cl = *D. clavisetae*.

that both species "A" and *D. adiaestola* are descended from a common Kauai ancestor (Xikouxy 2cd 3jk 4bo, Figure 6). Furthermore, it seems likely that the *D. adiaestola* species cluster on Maui (Figure 6) arose from a colonizer from Kauai which of necessity must have been different from the *D. picticornis*-like ancestor referred to previously (Figure 7).

The curious but rather close relationship between *D. adiaestola* and *D. clavisetae* requires that several ancestral populations of the two were heterozygous for 4p. These species, nevertheless, are quite similar cytologically. This is interesting because both *D. clavisetae* and *D. neogrimshawi* have an extra crossvein in cell R<sub>5</sub> (see Hardy, 1965, 1966). This recalls the situation in the *D. planitibia* subgroup, a cytologically distinct array which includes extra-veined species. The conclusion that these extra crossveins have arisen independently in the two lineages is inescapable. This fact further speaks against the retention of a generic name for those species having extra crossveins (see Carson, *et al.*, 1967).

Carson and Stalker (1968b) showed that the *D. planitibia* subgroup species found on Oahu are most closely related to Maui species rather than the Kauai member of this subgroup. In a similar manner, the Oahu member of the *D. adiaestola* subgroup (*D. neogrimshawi*) is not intermediate between the Kauai and Maui faunas. This species has both the extra crossvein and two specific inversions (Xz and 2e) in common with *D. clavisetae* of Maui. Thus, it is related specifically to this peculiar Maui species. That it arose from a northwestward colonizer from Maui is strongly indicated (Figure 7).

Intraspecific chromosomal polymorphism is absent in *D. adiaestola* (Table 1) and, with the exception of *D. peniculipedis*, none of the Maui-complex species shows any such variation. Furthermore, despite pronounced morphological divergence, especially between *D. clavisetae* and *D. adiaestola*, the number of fixed inversion differences is small in all the Maui-complex species. Accordingly, the sharp cytological divergence of the two species on the Island of Hawaii, *D. ochrobasis* and *D. setosimentum*, is remarkable. Thus, *D. setosimentum*, which is almost indistinguishable from *D. adiaestola* in the female, has ten fixed inversion differences from the latter, including seven in chromosome 4. Both Hawaii species, furthermore, show extensive chromosomal polymorphism which will be the subject of further investigation.

As in the *D. grimshawi* and *D. planitibia* subgroups, the *D. adiaestola* subgroup members found on the geologically new island of Hawaii probably arose by simple colonization across the Alenuihaha Channel between these two islands (Figure 7). The two species could have originated from a single colonizer having the approximate formula, superimposed on the *D. adiaestola* basic arrangements, of X<sub>m</sub><sup>2</sup> 3l 4b<sup>2</sup> 4c<sup>2</sup>/+ (Figure 6). Both species are present in the Kohala Mountains as well as being quite widely distributed elsewhere. *D. ochrobasis*, however, appears to occupy higher altitudes and the two are apparently only rarely sympatric.

#### SUMMARY

The polytene chromosome sequences of 15 species of picture-winged Hawaiian *Drosophila* are described. These comprise the *D. adiaestola* subgroup (9 species)

and the *D. punalua* subgroup (6 species). Two of the former and four of the latter are homosequential. Although the island origin of the very uniform *D. punalua* subgroup is in doubt, the Kauai member of the *D. adiaastola* subgroup has chromosomal conditions which suggest an ancestral position. As in the *D. planitibia* subgroup, it appears that an early *D. adiaastola*-like colonizer reached Maui from Kauai. During or following a speciation episode on Maui, at least one further colonizer reached Oahu and one reached Hawaii.

Seventy-eight (78) fixed and fifty-one (51) polymorphic inversions have been found in the 53 species belonging to the four subgroups of the picture-winged species of the Hawaiian Islands.

#### ACKNOWLEDGMENTS

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