

## XV. Polytene Chromosome Relationships in Hawaiian Species of *Drosophila*. I. The *D. grimshawi* subgroup<sup>1</sup>

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

This paper is the first in a series which will describe the sequential relationships in the polytene chromosomes of certain Hawaiian Drosophilidae. The species concerned belong to the general picture-winged group of the subgenus *Drosophila* (Hardy and Kaneshiro, 1968).

In this publication, we present a chromosome atlas which will provide recognition characters for the polytene chromosomes of those species which are most closely related to *Drosophila grimshawi* (the *D. grimshawi* subgroup). Twenty-nine such species are included here. Subsequent papers will deal with the three other subgroups of picture-winged flies which may now be recognized. These are the *D. planitibia* subgroup, the *D. punalua* subgroup and the *D. adiantola* subgroup. A preliminary paper on the chromosomal relationships among Hawaiian *Drosophila* (Carson, Clayton and Stalker, 1967) dealt with members of all four subgroups; ten species of the *D. grimshawi* subgroup were included there. These species are shown at the base of the diagram (Figure 1) in the cited paper. Thus, data are being presented here for the first time for 19 species.

### MATERIALS AND METHODS

*Drosophila* specimens were collected on the six major Hawaiian Islands from 1963–68. Each wild female captured was placed as soon as possible on sugar food (Spieth, 1966) and returned to the laboratory in a refrigerated case.

Each female was then placed individually in a shell vial containing a special food mixture (Wheeler and Clayton, 1965). To each vial, a strip of tissue, moistened with a sterile mixture of Karo syrup, water and yeast extract, was added as an oviposition site. The laboratory temperature was held at approximately 63°F. From each such isofemale line, an attempt was made to obtain aceto-orcein smears of the salivary glands of seven F<sub>1</sub> larvae. This affords an approximately 63/64 probability that four wild autosomes and three wild X chromosomes of the parents have been observed, assuming there to have been only one wild male parent. When fewer than seven smears were examined, the first female larva was chosen and two wild autosomes and two wild X chromosomes were recorded from each such wild female.

Table 1 gives the geographical origin of the strains examined. Each locality is identified by its nearest position on U. S. Geological Survey topographical maps. When a locality is first mentioned in the Table, the approximate altitude of the

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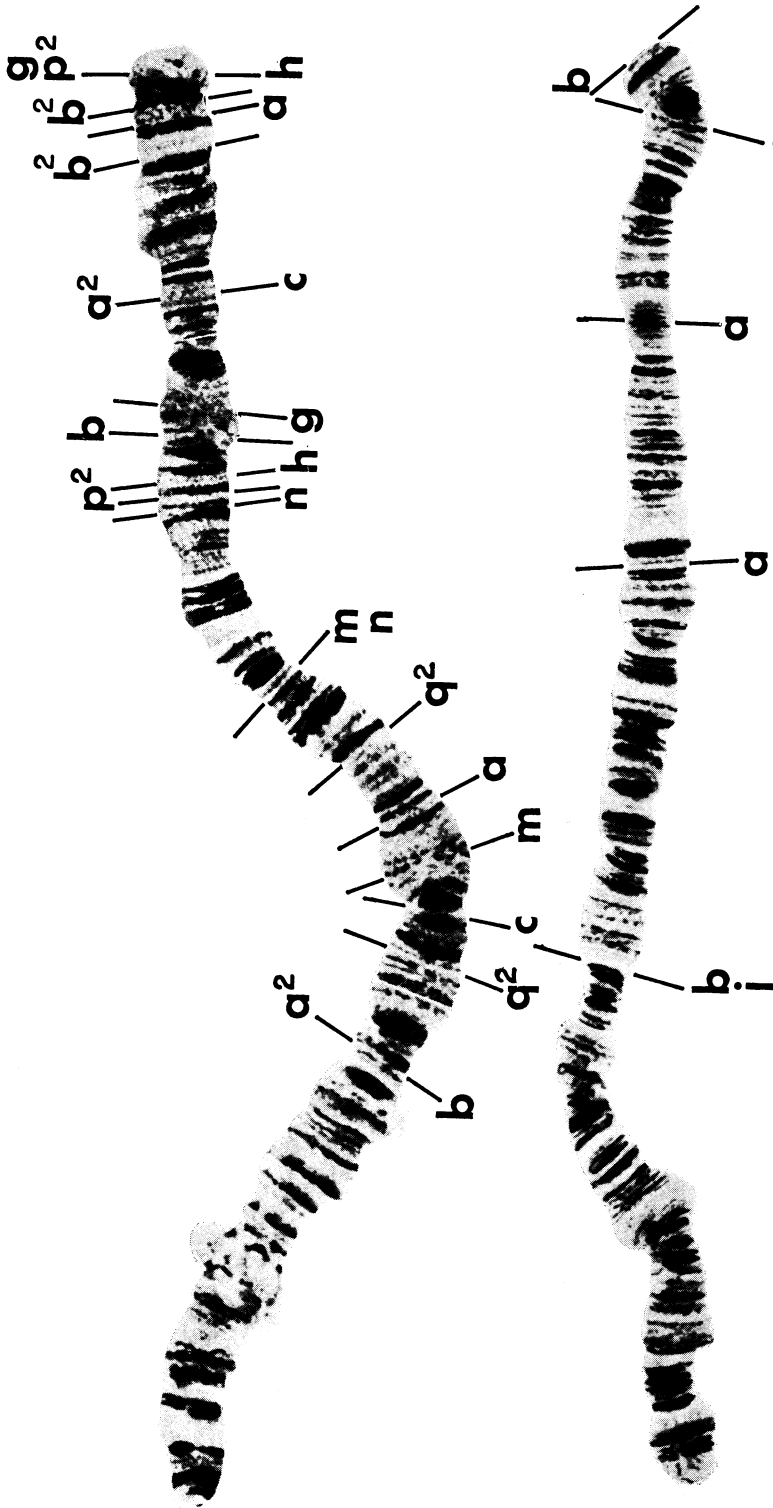


FIG. 1. Position of inversions in chromosome X (above) and chromosome 2. The photographs shown are of the Standard gene order of *Drosophila grimshawi* from Auwahi, Maui. The distal end of the chromosome is to the left in each case. Each inverted arrangement shown (see Figure 4) can be derived directly from this Standard by inverting the piece as indicated. The only exception to this is arrangement Xab of *Drosophila crucigera*. In this case, the Xa inversion must be followed by Xb, which overlaps it.

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collecting site is given. This is followed by strain numbers of the newly-examined strains. Those listed in Carson *et al.* 1967 are not repeated here. The right-hand columns show the numbers of wild chromosomes examined according to the method outlined in the previous paragraph.

The gene sequences observed so far in all the chromosomes of all strains of *D. grimshawi* collected at Auwahi, Maui, have been arbitrarily chosen as Standard; it is exemplified by the laboratory strain known as G1, collected on June 17, 1965, by K. Y. Kaneshiro (see Table 1, Carson *et al.*, 1967). Each new sequence found in the same or some other species is then described in terms of what segments of the *D. grimshawi* Standard sequence would need to be inverted in order to produce the observed gene order. In both this paper and those on the other subgroups, no species has been included unless the gene orders in all its chromosomes can be completely and accurately described in terms of the *D. grimshawi* Standard sequences.

A set of photographic chromosome maps, made according to the method of Stalker (1965) was prepared for the *D. grimshawi* Standard. To facilitate comparison between chromosomes of unknown sequence and the Standard, a compound microscope fitted with a drawing tube (Wild-Heerbrugg Instruments, Inc.) was used. With this device, the image of the unknown sequence under the microscope can be observed as if projected onto a black table surface. A cut-out photograph of the homologous chromosomal Standard is matched, band for band, to this image at table level, after being adjusted to approximately the same magnification by a zoom lens. The positioning of the breaks for inversions fixed in the homozygous state can be accurately determined in this manner.

When a species or strain was found to be homozygous for an inversion relative to the Standard, a photographic print of the Standard map of the chromosome concerned was marked with the break points. The photograph was then cut by scissors at these points, the piece inverted and realigned with the rest of the chromosome with cellophane tape. Although occasional interspecific differences in puffing pattern were noted, these do not interfere with the usefulness of the maps made in this manner. These rearranged prints were then mounted and serve as base maps for the species concerned. They are on file at the Department of Biology, Washington University, and will be made available on request. Such maps, however, could be easily prepared from replications of the photographs in Figures 1-3.

## RESULTS

### *Description of inversion break-points*

The positions of the inversion breaks are given in Figures 1-3. On the figures, each chromosome is shown with its distal end to the left. Each inversion is represented by two break-points. Inversions are given letter designations, more or less in the order of discovery. The lower-case designations a-z have been used first. As more are necessary, the alphabet has been used a second time ( $a^2$ - $z^2$ ). Thus, inversions a and  $a^2$ , for example, are separate occurrences and bear no relationship to one another. In examining the figures, it will be noted that certain letters are not found; these have been retained for use for inversions in the other subgroups.

TABLE 1  
 Strains of *Drosophila* species of the *D. grimshawi* 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	Carson <i>et al.</i> 1967	Total
<i>D. balioptera</i>	Kaulalewele, W. Maui (3000') J57C4 Keanae Valley, Maui (1500') J51B8 Kipahulu Valley, Maui (2100') J88B16, 17 Halawa Valley, Molokai (1600') K86B1, 2 Kaiholena Gulch, Lanai (2400') K94G6 TOTAL	4 4 8 8 4 28	4 4 8 8 4 28	3 3 6 6 3 21	.	3 3 6 6 3 21
<i>D. bostrychia</i> <sup>1</sup>	Halawa Valley, Molokai, J97N2, 3 So. Hanahilohilo, Molokai (3000') J58M12 TOTAL	8 4 12	8 4 12	6 3 9	.	6 3 9
<i>D. ciliaticrus</i> <sup>1</sup> <i>D. conspiciua</i> <sup>1</sup>	Puu Laalaau, Kohala Mts., Hawaii (3300') J29B11 Pawaina, Hawaii (3000') Mt. House, Hawaii (2700') L19G4, 6; L19B22, 28 TOTAL	2 . 16 16	2 . 16 16	1 . 12 12	.	1 . 12 12
<i>D. crucigera</i> <sup>1</sup> <i>D. discreta</i> <sup>1</sup>	Oahu and Kauai, Totals Kaulalewele, W. Maui, J57C3 Waikamoi, Maui, C104.1; FA-1; PM-4, 13, 21-23, 29; K29L2; K73G1, 12 Kipahulu Valley, Maui (3000-4000') L1G64, 82, 83, 88, 90, 91, 94, 96, 97, 102; L4G3; L6N1; L11P4 TOTAL	4 38 46 88	4 38 46 88	3 30 35 68	.	3 30 35 68
<i>D. disjuncta</i> <sup>1</sup>	Kaulalewele, W. Maui Uluini Stream, Maui (1600') Keanae Valley, Maui, J51B12-15, 17 Kipahulu Valley, Maui, L1G41, 42, 44-47, 49, 51, 52, 54-56, 58, 61, 62, 65-68, 75, 76, 114; L4G5-8, 10, 12, 13, 15, 17-19; L5B9, 11; L6N51; L11P23 Waikamoi, Maui, J76M4; K25A1; K73B1 TOTAL	18 150 8 176	4 150 8 201	14 113 7 134	3 11 3 17	17 113 7 151

	176	25	201	134	17	151
<i>D. fasciculisetiae</i> <sup>1</sup>		28	28		21	21
<i>D. glabriapez</i>	4	40	44	3	30	33
<i>D. engyochracea</i>	2		2	2		2
	4		4	3		3
TOTAL	6		6	5		5
<i>D. gradata</i>	4		4	3		3
	6		6	5		5
	4		4	3		3
	6		6	5		5
	4		4	3		3
TOTAL	14		14	11		11
<i>D. grimshawi</i> <sup>1</sup>		48	48		24	24
	4	12	16	3	12	15
	2		2	1		1
		8	8		7	7
	24	4	28	15	3	18
		16	16		12	12
	4		4	3		3
	4		4	3		3
	16		16	12		12
	22	52	74	17	38	55
	4		4	3		3
	4		4	3		3
	4		4	3		3
	8		8	6		6
TOTAL	96	140	236	69	96	165
<i>D. hawaiiensis</i> <sup>1</sup>		6	6		5	5
	36		36	27		27
	4		4	3		3
	4		4	3		3
	8		8	6		6
TOTAL	52		52	39		39

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

Species	Locality and strains examined <sup>1</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>D. hexachaetae</i>	Pia Valley, Oahu (800') K37B3, 4	6	6	4	4
<i>D. hirtipalpus</i>	Waikamoi, Maui (4200') J66C4; K29J1	8	8	6	6
<i>D. limitata</i>	Keanae Valley, Maui C134.4A, E, F, G	16	16	12	12
	Kaulalewelewe, W. Maui, C109.3C; G80B6	4	4	3	3
	Kipahulu Valley, Maui, J88B11, 12	8	8	6	6
	TOTAL	28	28	21	21
<i>D. tiophallus</i>	Auwahi, Maui, G7.3C, E	8	8	6	6
<i>D. ochracea</i>	Pawaina, Hawaii	6	6	5	5
	Keaunou, Hawaii (2500') K2B1	2	4	2	4
	Mountain House, Hawaii, L19B29	4	4	3	4
	Honaunou For. Res., Hawaii	4	4	4	4
	Upper Oloa For. Res., Hawaii (4100') J48B5	4	10	3	7
	Puu Laalaaui, Kohala Mts., Hawaii (4000')	8	8	6	6
	Stainback Hwy., Hawaii (2100') K62G2, 7	8	8	6	6
	Saddle Rd., Hawaii (2200') J49B8-10; K32G1	16	16	12	12
	Mud Lane, Hawaii, (1900') K81G1	4	4	3	3
	Waimea Reservoir, Hawaii (3000') K78G10	4	4	3	3
	TOTAL	42	68	32	53
<i>D. odontophallus</i>	Auwahi, Maui, J10D2	4	4	3	3
<i>D. orphnopeza</i> <sup>1</sup>	Keanae Valley, Maui, C134.4D; J51B2	6	6	5	5
	Waikamoi, Maui, K75G9	2	2	1	1
	TOTAL	8	8	6	6
<i>D. orthofascia</i>	Auwahi, Maui, G7.2D; J10B5; J10G23-26	22	22	17	17
<i>D. pilimana</i>	Puu Kapu, Oahu (1000')	4	4	3	3
	Mt. Tantalus, Oahu (1400')	4	4	3	3
	Pupukea, Oahu (1900')	4	4	3	3
	Kului Gulch, Oahu (1000') K38G8, 10	8	8	6	6

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Kului Gulch, Oahu (1000') K38G8, 10

	Palikea, Oahu, G58E2; K5C32	6	12	6	5	5
	TOTAL	14	12	26	28	37
<i>D. recticilia</i> <sup>1</sup>	Waikamoi, Maui, G8.1H; J66C6; K72B4	12		12	9	9
	Kipahulu Valley, Maui, L3G2	4		4	3	3
	TOTAL	16		16	12	12
<i>D. silbarentis</i> <sup>1</sup>	Humuula Saddle, Hawaii (6500') K18A1, 2; K18M1, 3; K18N4; K47G2	24		24	18	18
<i>D. sproati</i>	Puu Laalaau, Kohala Mts. Hawaii, J20B7, 8, 11, 18; J20G19, 22-24; J20H11; J29B1, 9; J29H2, 4, 9	42		42	32	32
	Upper Olaa For. Res., Hawaii, K59G1	4		4	3	3
	Mountain House, Hawaii, L19B37, 38	8		8	6	6
	TOTAL	54		54	44	44
<i>D. pesiceta</i>	Kolea Stream, Maui (1600') K40G2	4		4	3	3
<i>D. villitibia</i>	Waikamoi, Maui, K39B10; K25G1; K75G13; K75P8, 9	18		18	12	12
<i>D. villosipedis</i>	Halemanu Valley, Kauai		20	20		15
	Kokee, Kauai		2	2		2
	TOTAL		22	22		17
<i>D. virgulata</i>	Waikamoi, Maui, J31C1	2		2	1	1

<sup>1</sup> Shows intraspecific chromosomal polymorphism.<sup>2</sup> Strain numbers are entered only for newly-reported strains.<sup>3</sup> Includes data from Carson, 1966.

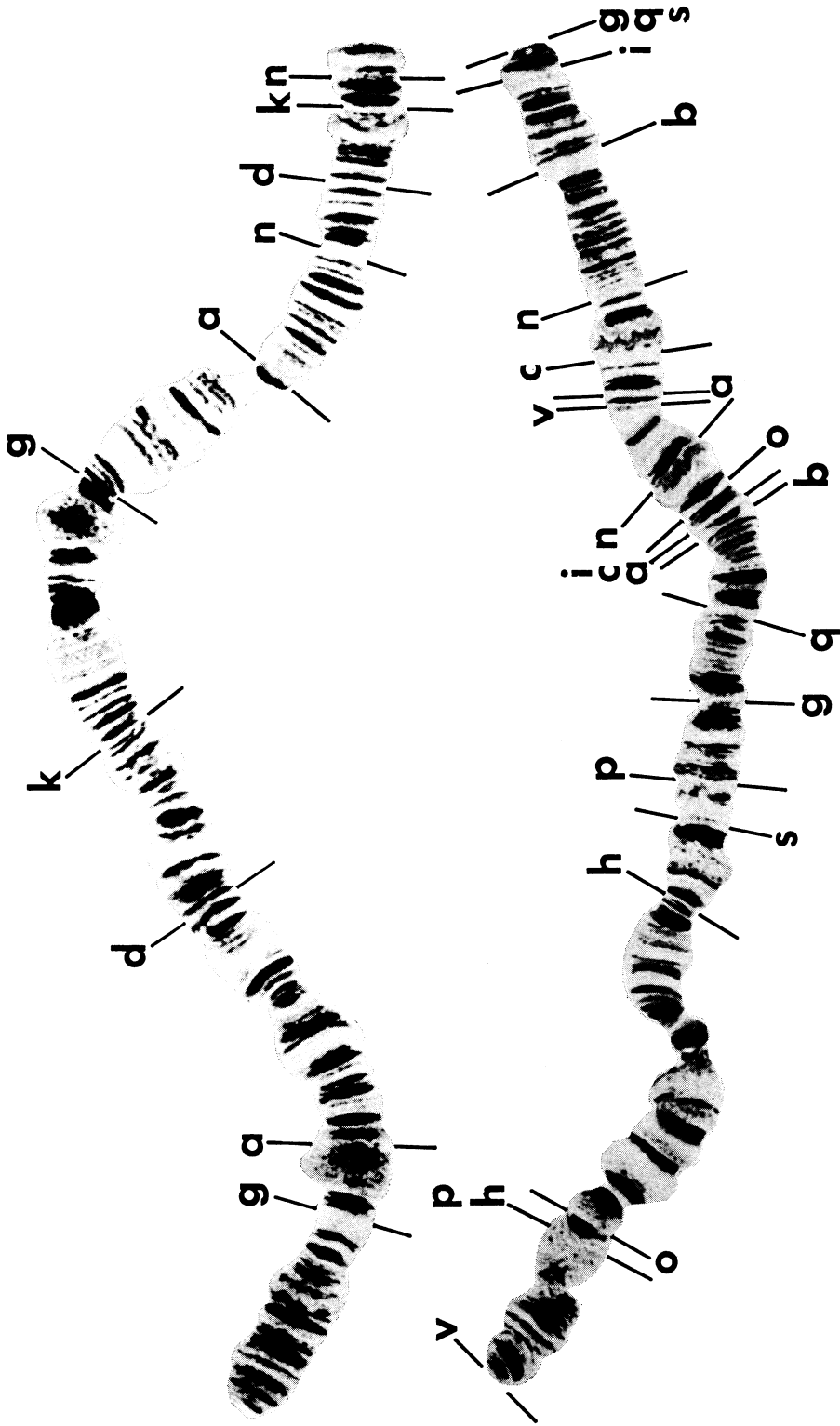


Fig. 2. Position of inversions in chromosome 5 (above) and chromosome 3. The photographs shown are of the Standard gene order of *Drosophila grimshawi* from Auwahi, Maui. The distal end of the chromosome is to the left in each case. Except as noted below, each inverted arrangement can be derived directly from this Standard by inverting the piece as indicated. 3g, however, must be inverted before 3s, which overlaps it; 3gs, furthermore, must precede 3v which overlaps them.



FIG. 2. Position of the chromosome is to the left in each case. Except as noted below, each inverted arrangement can be derived directly from this Standard by inverting the piece as indicated. 3g, however, must be inverted before 3s, which overlaps it; 3gs, furthermore, must precede 3v which overlaps them.

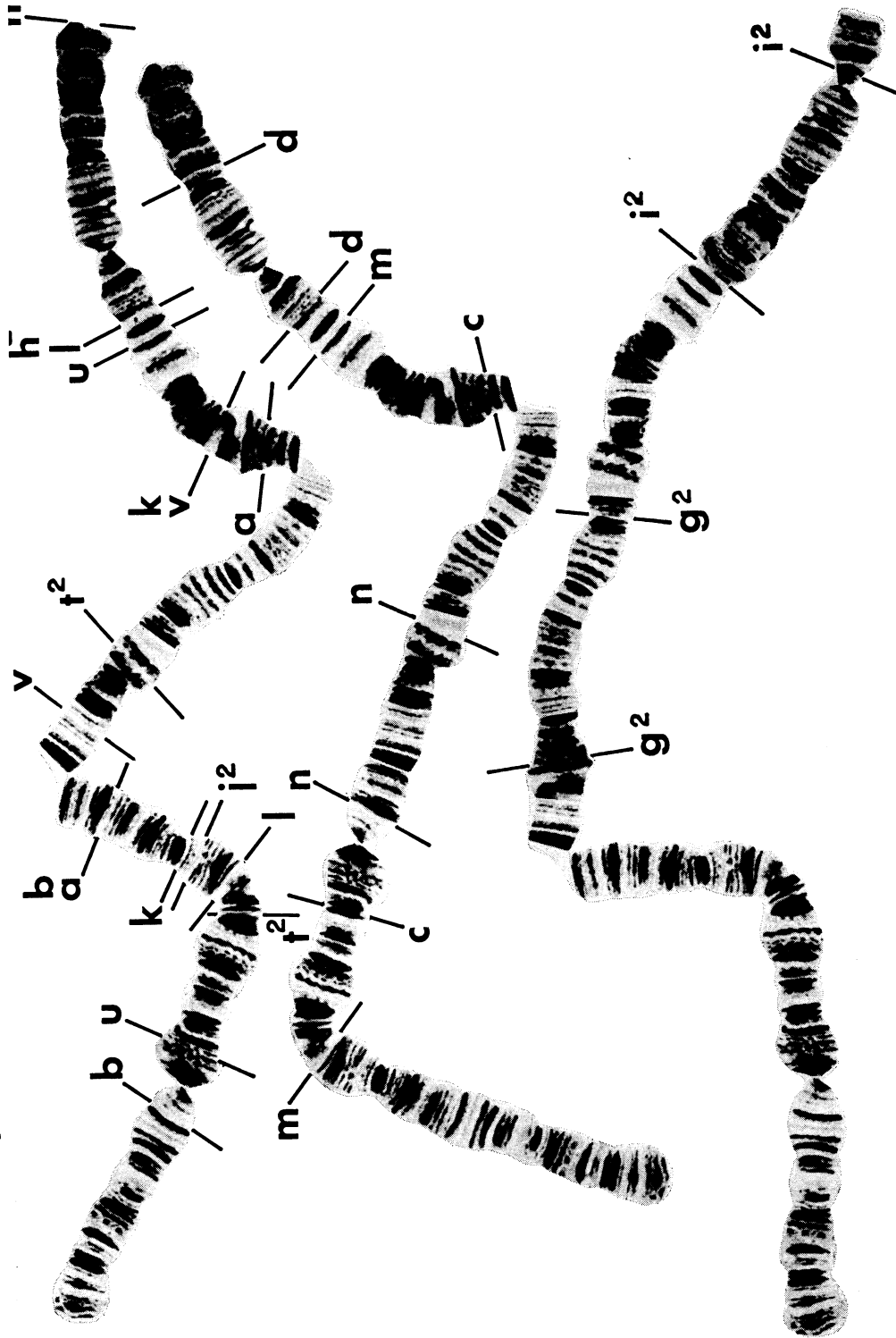
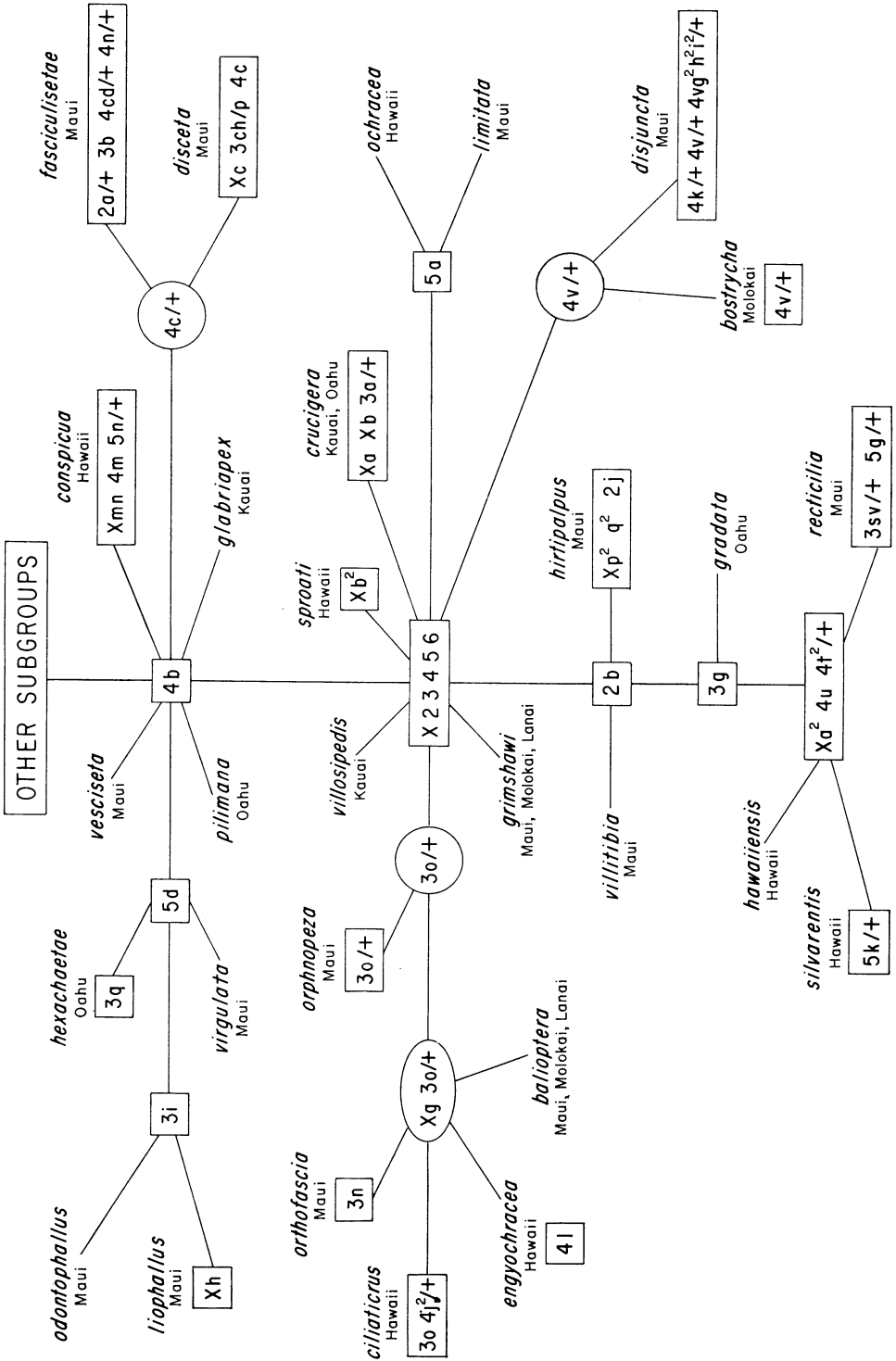


Fig. 3. Position of inversions in chromosome 4. The top photograph depicts the Standard gene order of *Drosophila grimshawi* from Auwahi, Maui. The middle photograph shows arrangement 4b and the inversions based on it. The bottom photograph depicts the arrangement 4vh<sup>2</sup> of *D. disjuncta* and the inversions based on it.



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In a number of instances, one of the breaks of each of two different inversions appears to have occurred at precisely the same point on the chromosome (e.g. the distal breaks of Xb and Xa<sup>2</sup>, Fig. 1). This is true, of course, only within the limits of the observational technique. Even though two different breaks can be located as occurring between the same two visible bands, this may not mean that they are at the same position at the molecular level. It will also be noted that one break of an inversion sometimes occurs at the proximal end, presumably in the heterochromatin, whereas the other is within the euchromatin. Thus, inversions Xg, Xh and Xp<sup>2</sup> (Fig. 1) all have one break-point which visually coincide at the base of the chromosomes. It is probable, however, that each of these breaks lies in a different position within the proximal heterochromatin. These points, however, cannot be mapped on the polytene chromosomes.

#### *Relationships of the species based on banding sequence*

The phenomenon of inversion-sharing makes it possible to arrange the species studied according to their sequential similarities. Such an arrangement will be found in Figure 4. The inversion method does not permit a direction of evolution to be specified without outside information so that although the phylogenetic relationships are clearly specified, it is not possible to designate any one species or region of the chart as primitive. The Standard sequence X23456 has been arranged for convenience, in a box in the center of Figure 4. The microchromosome (6) has not been systematically studied.

As was pointed out by Carson, Clayton and Stalker (1967) it will be seen that a number of species are basically homosequential, that is, having identical gene orders in all chromosomes. Thus, *D. pilimana*, *D. vesciseta* and *D. glabriapex* all have the formula X234b56 (Fig. 4). Similarly, if intraspecific chromosomal polymorphism is disregarded, there are five species which have the Standard karyotype, X23456. These are *D. grimshawi*, *D. disjuncta*, *D. bostrycha*, *D. orphnopeza* and *D. villosipedis*. *D. hawaiiensis*, *D. recticilia* and *D. silvarentis* likewise share a common basic sequence, Xa<sup>2</sup>2b3g4u56. *D. ochracea* and *D. limitata* are also homosequential but the latter, like *D. villitibia*, has six rods at metaphase rather than the five rods and a dot characteristic of all other species of the subgroup. In *D. silvarentis*, however, the microchromosomes are larger than usual and the condition approaches that of six rods (Clayton, 1968).

In four instances on Figure 4, hypothetical populations have been proposed (encircled). The assumption that such populations exist (or existed) is made necessary by the fact that a certain gene order may be fixed in one species but not in the other. Thus, *D. fasciculisetae*, for example, is heterozygous for inversion 4c but the latter is fixed in *D. discreta*. *D. bostrycha* and *D. disjuncta* are unusual

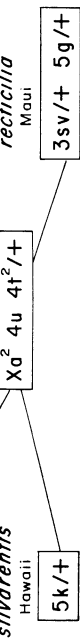


FIG. 4. Chromosomal relationships among 29 species of Hawaiian *Drosophila* belonging to the *D. grimshawi* subgroup. Letters appearing singly represent fixed inversions; when otherwise (e.g. "4a/+"), this denotes an intraspecific polymorphism. Read the formula to reach species cumulatively by following the line from the *D. grimshawi* Standard (box at center); e.g. *D. conspiciua* has the formula Xmn 2 3 4bm 5 6. Boxes denote present species; hypothetical populations are encircled. The island origin of each species is entered below its name. *D. balioptera* lacks inversion 3o.

TABLE 2  
Chromosome inversions in 29 species of the *D. grimshawi* subgroup

Chromosome	X	2	3	4	5	
Number of fixed inversions	11	2	5	4	2	24
Number of inversions polymorphic within a species	0	1	5	11	3	20
Totals	11	3	10	15	5	44

in that they stem from an ancestral population which was also heterozygous for 4v.

### Intraspecific polymorphism

Of the 29 species, 17 are without chromosomal polymorphism, although for most of the species in this category the data are not extensive. Species having intraspecific chromosomal polymorphism may be identified on Figure 4 because each such inversion is given a letter as usual and then is followed by “/+”, the “plus” symbol indicating the basic arrangement for the species, including, of course, any inversions which may have become fixed different from Standard in that same species. Thus, for example, “4a/+” in *D. grimshawi* serves to indicate that the 4th chromosome of this species is polymorphic within the species, the two alternate arrangements being + (Standard chromosome 4) and gene arrangement

TABLE 3  
Chromosomal polymorphism within 4 species of *Drosophila* of the *D. grimshawi* subgroup

Species and locality	Total chromosomes observed (N)	Inverted chromosomes		
<i>D. discreta</i>	N	3p		
Kipahulu Valley, Maui	46	0		
Waikamoi, Maui	38	0		
Kaulalewelewe, W. Maui	4	1		
Total	88	1		
Per cent		1.1		
<i>D. fasciculisetae</i>	N	2a	4cd	4cdn
Waikamoi, Maui	44	28	23	6
Per cent		63.6	52.3	13.6
<i>D. hawaiiensis</i>	N	4t <sup>2</sup>		
Kipuka Puaulu, Hawaii	36	1		
Honaunau For. Res., Hawaii	6	0		
Kipuka Ki, Hawaii	4	0		
Kipuka Kekake, Hawaii	4	0		
Puu Oo—Volcano Trail, Hawaii	8	0		
Total	58	1		
Per cent		1.7		
<i>D. silvarentis</i>	N	5k		
Humuula Saddle, Hawaii	24	1		
Per cent		4.2		

4a. The distribution of fixed and polymorphic inversions among the 29 species is given in Table 2.

Because most Hawaiian species of *Drosophila* are difficult to obtain in large numbers, quantitative data on intraspecific chromosomal polymorphism are lacking for most species. Nevertheless, considerable information exists for *Drosophila crucigera* (Carson, 1966 and unpublished data). Table 3 presents quantitative data for those polymorphic species from which 20 or more wild chromosomes have been observed. This table, however, does not include *D. crucigera* and the three very close species *D. grimshawi*, *D. bostrycha* and *D. disjuncta*. Polymorphism in these species is extensive. Quantitative data are being actively gathered and will be presented in subsequent papers.

#### DISCUSSION

Attempts to estimate the number of species of Drosophilidae in the Hawaiian Islands must still be made very cautiously. Suffice it to say that large flies of the picture-winged type, belonging to the subgenus *Drosophila*, will eventually form a fairly small segment, perhaps twenty per cent, of the entire fauna. Perusal of the papers of Hardy (1965, 1966) and Hardy and Kaneshiro (1968) as well as examination of undescribed material in collections suggests that between 105 and 110 species of this major group have actually been collected. The number of species which have been assayed cytologically at the time of writing stands at 51; 29 in the *D. grimshawi* subgroup, 9 in the *D. planitibia* subgroup, 5 in the *D. punalua* subgroup and 8 in the *D. adistola* subgroup. Some of the species are extremely rare in collections and the cytological assay has sometimes been made from single strains only (see Table 1). Quite a number of species are difficult to breed in the laboratory; these appear, however, to be distributed in all subgroups and the numbers given above surely reflect real differences in the size of the subgroups.

Most of the 29 species have a fixed difference from the Standard *D. grimshawi* of less than three inversions; *D. discreta* has the greatest amount of fixed difference from Standard (5); four species differ by four inversions. The maximum difference between any two species within the subgroup is 10 inversions. This is a remarkably small amount of interspecific sequential variation, far less than that found between species in the *repleta* group (Wasserman, 1960), the *virilis* group (Patterson, and Stone, 1952) or the *melanica* group (Stalker, 1966). The Hawaiian species, furthermore, even within the homosequential species clusters, are for the most part highly distinctive entities morphologically. So far, not a single case has emerged which might be properly described as sibling (morphologically cryptic) species. This is in contrast to the situation regarding a number of the species from the continental groups mentioned above.

The phenomenon of homosequential species (Carson, Clayton and Stalker, 1967) is well exemplified in this subgroup. In a number of cases there has been no apparent change during speciation in either gross metaphase karyotype or in the gene order of any chromosome. As was pointed out in the paper above, this supports the idea that a considerable amount of speciation and evolution can be based entirely on mutational changes occurring at the submicroscopic level.

*Origin and evolution of the D. GRIMSHAWI subgroup*

As has been pointed out several times, this discussion is being confined to specimens for which full cytological data are presently available. Such data are of necessity rather restricted and many specimens for which no cytological data exist are, of course, present in large numbers in collections. At the present time, these records, both published and unpublished, do not appear to alter in any significant way the geographical facts and hypotheses now to be discussed.

Definitive interpretation of the geographical interrelationships of the Hawaiian flies must await fuller information. Such information, however, will not be available for a period of some years. In order to form a framework of thought about these complex data, the authors present the following tentative working hypothesis which can be strengthened, altered or abandoned as more compelling data are gathered.

Figure 5 depicts the six major islands. On each island is entered the number of species of the *D. grimshawi* subgroup which is found there and for which cytological data exist. The Island of Maui has the largest number of species (15), followed by Hawaii (7), Oahu (4), Molokai (3), Kauai (3) and Lanai (2). This distribution may reflect intensity of collecting to some extent but there is little question that these differences represent a real situation, with Maui having the richest fauna.

Thus, it may be seen that the entire subgroup is archipelago-wide, that is, at least some representatives are found on all six major islands. Despite this, however, no certain case is yet known of a single species which ranges over all islands. The most widespread species for which pertinent cytological and genetic data are available are *D. grimshawi* and *D. balioptera* which are on Maui, Molokai and Lanai (b and g, Figure 5) and *D. crucigera* on Oahu and Kauai (c, Figure 5). Carson (1966) presented data which indicate that *D. crucigera* is indeed one species on the two islands. Further unpublished data on interfertility bear out this conclusion. No systematic study of *D. balioptera* and *D. grimshawi* from the three islands where they occur has been made, but every indication points to the conspecificity of all strains.

The relatively depauperate nature of this subgroup on Molokai (3 species) and Lanai (2 species) is noteworthy. Both islands were at one time joined to Maui (Stearns, 1966). Other than the two species mentioned above, the only other species of this subgroup which has been cytologically checked from Molokai is *D. bostrycha*. This species is extremely closely related to the Maui species, *D. disjuncta*. In fact, these two share an identical chromosomal polymorphism (4v/+), a situation which is certainly a very rare one. Not only is this the only case of interspecific polymorphism-sharing in this subgroup, but very few cases exist among continental species (Carson, 1959, 1964).

The greatest amount of sequential divergence in the subgroup, 10 fixed inversions, may be exemplified by the two Maui species *D. recticilia* and *D. discreta*. In fact, these two species are sympatric in Kipahulu Valley and at Waikamoi. Despite this, however, the wide dispersion of at least some members of the subgroup is underscored by the fact that both Standard and "4b" species are found on Kauai (*D. villosipedis* and *D. glabriapex*, respectively).

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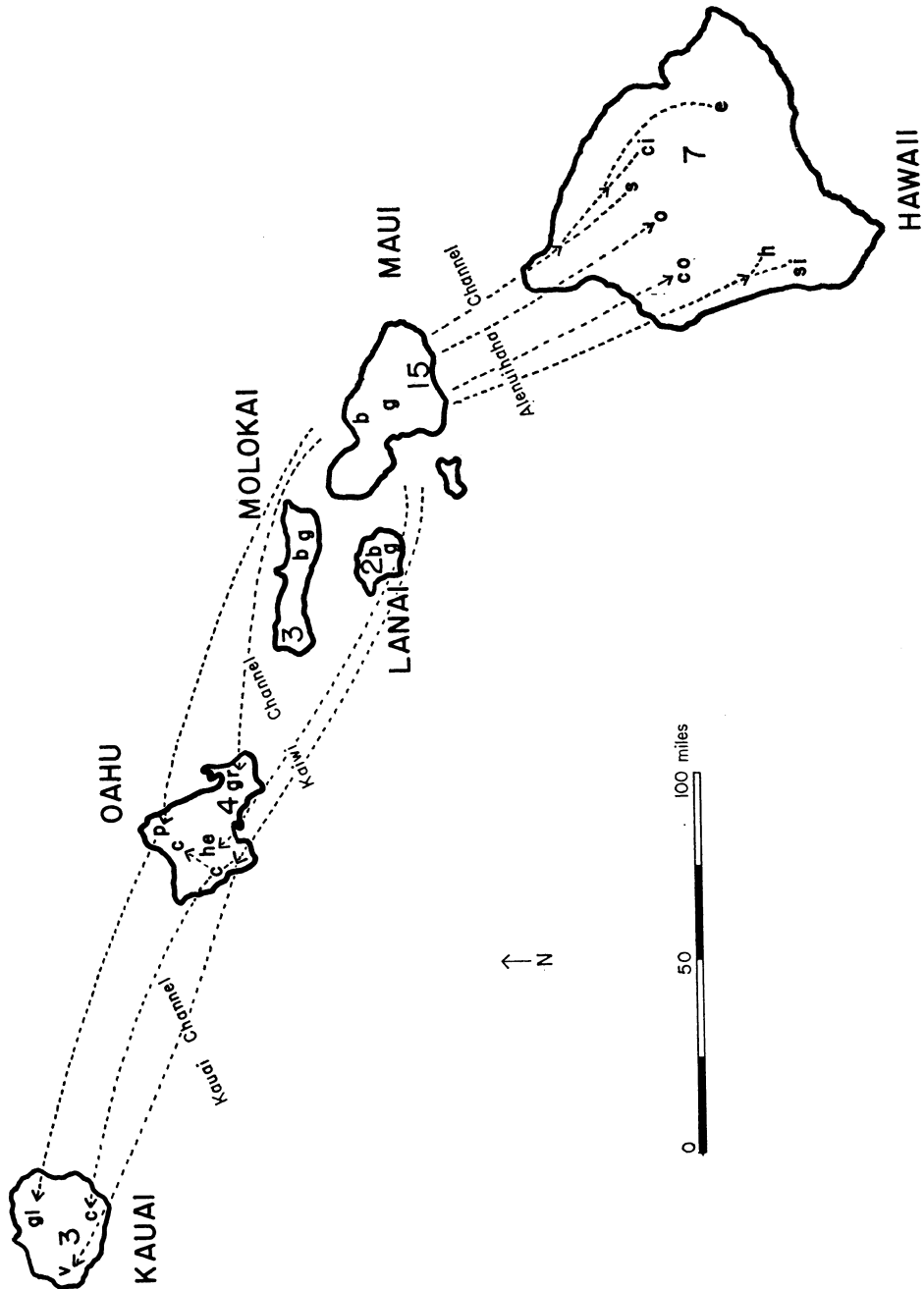


FIG. 5. Origin, migration and speciation of the *D. grimshawi* subgroup on the principal islands of Hawaii. Superimposed on each island is the number of species of the subgroup found there. b = *D. balioptera*, c = *D. crucigera*, ci = *D. ciliaticrus*, co = *D. conspicua*, e = *D. engyochracea*, g = *D. grimshawi*, gl = *D. glabriapez*, gr = *D. gradata*, h = *D. hawaiiensis*, he = *D. hexachaetae*, o = *D. ochracea*, p = *D. pilimana*, s = *D. sproati*, si = *D. silvarentis*, v = *D. villosipedis*. Arrows represent proposed separate colonizations. For details, see text.

TABLE 4

Fixed gene arrangements not found in any Maui species

Gene arrangement	Species in which arrangement is found	Island
4l	<i>D. engyochracea</i>	Hawaii
Xmn. 4m	<i>D. conspicua</i>	Hawaii
Xb <sup>2</sup>	<i>D. sproati</i>	Hawaii
3q	<i>D. hexachaetae</i>	Oahu
Xa	<i>D. crucigera</i>	Waianae Range, Oahu; Kauai
Xab	<i>D. crucigera</i>	Koolau Range, Oahu

The Island of Maui not only has the largest number of species but it also has somewhere among its species *all* of the fixed gene arrangements which are shared by two or more species. Such arrangements, of course, are basic to the phylogeny. The best way to visualize this is to consider those fixed gene arrangements *not* found on Maui. Only 8 such arrangements are known (Table 4), and they are of course without exception unique to the species in which they occur. Accordingly, these facts suggest that Maui is the center for the evolution of this subgroup and that the faunas of the other islands were established by colonizations from Maui.

In applying this hypothesis to the data, we may begin with the most interesting stepwise phylogeny shown at the bottom of Figure 4. This involves the complex of six species near to *D. hawaiiensis*. This complex could have originated on Maui from a *D. villitibia*-like ancestor, giving rise subsequently to *D. gradata* of Oahu and *D. recticilia* of Maui. This direction for the chromosomal evolution is suggested because the two species found on the most recent volcanic areas of Hawaii (the south and eastern areas, including the volcanoes Mauna Kea, Mauna Loa, Kilauea and Hualalai) are homosequential with each other and with *D. recticilia*. These species (*D. hawaiiensis* and *D. silvarentis*, h and si, Figure 5) would appear to have been directly derived by recent speciation from a migrant from a *D. recticilia*-like ancestral population on Maui (arrow, Figure 5). This part of Hawaii is of Pleistocene Age (Stearns, 1966) and species unique to it would of necessity be newly-derived in time, thus giving direction to the phylogeny.

Also striking is the fact that the chromosome formula of *D. hawaiiensis* and *D. silvarentis* (Xa<sup>2</sup>2b 3g 4u) includes four inversions unknown among any of the other five members of the fauna of Hawaii. Accordingly, there must have been other independent crossings of the Alenuihaha Channel during the colonization of Hawaii in the Pleistocene.

A second such crossing must have been accomplished by an ancestor of *D. conspicua* (co, Figure 5), a species which carries arrangement 4b, not possessed by any other member of the subgroup on Hawaii. The species on Maui closest to such an ancestor is *D. vesciseta*.

Neither of the above species has been found in the Kohala Mountains, a slightly older area but still of Pleistocene Age, at the North-West corner of Hawaii. The two colonizations just discussed, therefore, probably were made directly into the newer areas (Figure 5).



The data require at least two more colonizations of Hawaii from Maui. *D. ochracea*, which is widespread in both the Kohalas and the newer areas of Hawaii, carries the distinctive arrangement 5a, which it has in common with *D. limitata* of Maui. Thus, the ancestors of *D. ochracea* must have arisen from a third independent colonization of Hawaii.

Another species known chromosomally from Hawaii is *D. ciliaticrus*. This species has two Maui gene arrangements, Xg and 3o, so that its ancestor must have arisen from a fourth channel crossing.

*D. engyochracea*, found only in a single *kipuka* in the Kilauea area, also has Xg, so that it seems likely that it arose from the same *ciliaticrus*-like ancestor. *D. engyochracea*, however, lacks the 3o arrangement which we assumed came over the channel from Maui with the ancestor. This situation may be resolved by suggesting that the original migrant was heterozygous for 3o (3o/+), that 3o became fixed in *D. ciliaticrus* and that the progenitor of *D. engyochracea* ended up with the Standard chromosome 3 in the fixed state. This hypothesis is strengthened by the fact that despite the small number examined, *D. orphnopeza* of Maui is heterozygous for 3o at the present time (see Figure 4).

Although at first glance, *D. sproati* would appear to have arisen from a fifth colonization, one further and rather simple assumption could render it derivable from the common ancestor of *D. ciliaticrus* and *D. engyochracea*. Thus, if the ancestral migrant was heterozygous for Xg as well as 3o (Xg/+ 3o/+), the karyotype found in *D. sproati* could have resulted from the fixation of the Standard X and 3rd chromosomes in populations descended from the colonizer. This scheme has been tentatively adopted and is diagrammed in Figure 5.

With the exception of *D. engyochracea*, these latter species are currently found both in the Kohalas and in the newer areas of Hawaii. The data presently available provide no indication of whether the original colonizations were into the Kohalas, with subsequent spread elsewhere, or whether these species reached the Kohalas by spreading from colonizations which were originally into the newer areas.

In summary, then, we may say that the seven species found on Hawaii fall into four chromosomally distinct groups. These are: 1) *D. hawaiiensis*-*D. silvarentis*, 2) *D. conspicua*, 3) *D. ochracea* and 4) *D. sproati*-*D. ciliaticrus*-*D. engyochracea*. Since the closest common ancestors for these groups are found on Maui rather than on Hawaii itself, the derivation of the fauna of Hawaii requires a minimum of four separate colonizations across the Alenuihaha Channel from Maui. Two (numbers 1 and 2) of these were probably made directly into the newer areas of Hawaii. Except to stress this point, the positioning of the beginnings and the ends of the arrows joining the two islands in Figure 5 are not to be considered significant.

Because of the fact that Molokai and Lanai were apparently joined to Maui at one time, colonization from Maui across the channels now separating them is not a necessary assumption, although this possibility cannot be excluded.

With regard to the *D. grimshawi* subgroup on Oahu and Kauai, the formulation given in the preceding paragraphs would require that at least four interisland colonizations occurred between the Maui complex and Oahu (arrows, Figure 5). The precise points of origin are not known. These would involve a 4b 5d

ancestor for *D. hexachaetae* (he, Figure 5), a 4b ancestor for *D. pilimana* (p, Figure 5), a 2b 3g ancestor for *D. gradata* (gr, Figure 5) and a Standard ancestor for *D. crucigera*.

If, disregarding for the sake of the argument earlier cited evidence to the contrary, the assumption is made that the evolutionary order is from Oahu to Maui, a difficulty arises in explaining the origin of certain Maui flies. Thus, sequence 2b (apart from the associated 3g in *D. gradata*) would have to be hypothesized as existing on Oahu in order to appear in *D. villitibia* and *D. hirtipalpus* on Maui. *D. recticilia* of Maui would have to arise from a separate 2b 3g migrant from Oahu. If, on the other hand, it is assumed that the evolutionary order is from Maui to Oahu, this difficulty disappears. The 2b species which lack 3g would, according to this scheme, be more primitive and would be found only on Maui. Oahu, accordingly, could have been colonized by a single 2b 3g ancestor from Maui.

Three more interisland migrations would be necessary for the colonization of Kauai from Oahu. These would involve a 4b ancestor for *D. glabriapex* (gl, Figure 5) and a Standard ancestor for *D. villosipedis* (v, Figure 5). The scheme suggests that *D. crucigera* colonized Kauai without speciation, carrying Xa and 3a/+ from the Waianae Range (southwest Oahu). The Koolau Range of Oahu, where the overlapping inversion Xb is found fixed on an Xa arrangement (Xab) probably derived its *D. crucigera* population from the Waianaes (see Carson, 1966).

A further discussion of these topics, and a suggestion for the source of the original colonization of Maui will be presented in later papers of this series.

#### SUMMARY

The polytene chromosome sequences of 29 species of picture-winged Hawaiian *Drosophila* comprising the *D. grimshawi* subgroup are described. Polytene karyotypes within the subgroup are remarkably stable despite the morphological diversity of the species. Thus, only 24 inversions have been fixed among the 29 species. Stability is further emphasized by the fact that 12 species are homosequential; these occur in groups of 4, 3, 3 and 2. Twelve species show intraspecific chromosomal polymorphism.

The subgroup and its major chromosomal types are widespread over the six major islands but with the greatest concentration of species present on the Island of Maui. All the interspecific fixed inversion variability shared by two or more species is present in Maui species. Based on these facts, it is suggested that the subgroup may have had its origin on Maui and undergone extensive speciation there. Four of the seven species from the geologically recent island of Hawaii are more closely related chromosomally to Maui species than they are to each other. Accordingly, the population of Hawaii thus appears to have involved at least four colonizations from Maui; two of these were apparently directly into the geologically more recent areas. Although Molokai and Lanai may have been populated from Maui by terrestrial spread when these islands were joined, the hypothesis presented suggests that Oahu and secondarily Kauai were colonized by migrants of this subgroup from the Maui complex.

## ACKNOWLEDGMENTS

The work for this series of papers has been supported by the Evolution and Genetics of Hawaiian Drosophilidae Project, Grant No. GM10640-04 and -05 to the University of Hawaii from the National Institutes of Health; by GB-3147 to Washington University and GB-711 to the University of Texas from the National Science Foundation.

Many individuals have contributed time and effort to this work. In addition to those mentioned in Carson *et al.* 1967, we are further indebted for laboratory and/or field assistance to O. M. Barber, W. Batungbacal, J. Coughlin, A. Cowan, J. Ellison, G. Gerstenberg, M. P. Kambysellis, C. Kanapi, G. Kawanishi, H. Kircher, A. Kuniyuki, N. Lee, P. S. Nair, G. Ota, S. Rockwood, L. Stelzer, S. Shiinoki, J. A. Tenorio, and J. M. Tenorio. Our special thanks go to Joyce Sato, who has prepared, with great skillfulness, almost all the slides made since October, 1966. Special acknowledgment is due also to Rae Tanabe for her tenacious work in rearing the larvae from difficult species. We also wish to thank Romany Huck for her excellent photographic work. Figures 4 and 5 were prepared by Geraldine Oda. Finally, we wish to acknowledge with utmost appreciation the superior field work of Kenneth Y. Kaneshiro, without whose efforts many of these species would have remained unknown.

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