XVI. Polytene Chromosome Relationships in Hawaiian Species of *Drosophila*. II. The *D. planitibia* subgroup[1]

HAMPTON L. CARSON[2,3] AND HARRISON D. STALKER[3] (1968)

This paper is the second in a series devoted to a description of the sequential relationships in the giant chromosomes of the picture-winged members of the subgenus *Drosophila* in Hawaii (Hardy and Kaneshiro, 1968). The species dealt with here are of particular interest in that included in the subgroup are some of the largest and most spectacular species in the entire family Drosophilidae from anywhere in the world. Most of them show an extra crossvein present in cell R_5 of the wing. Prior to the papers of Carson, Clayton and Stalker (1967) and Hardy and Kaneshiro (1968), which concur in ascribing these flies to the genus *Drosophila*, subgenus *Drosophila*, they had been considered under a separate generic name. This designation ("idiomyia") continues to be useful as a common name for these species.

This paper presents a chromosome atlas showing the relationships for nine species and gives a tentative interpretation of their origin, evolution and migration in the Hawaiian Islands. Carson, Clayton and Stalker (1967) made a preliminary report on five of these species (upper left-hand part of Figure 1 in that paper).

Materials and Methods

The methods followed have been described in the first paper of this series (Carson and Stalker, 1968). Table 1 gives the geographical origin and number of chromosomes observed in each of the wild strains examined. Its format follows the model established in the above paper.

In order to facilitate comparison of the chromosome arrangements of the different subgroups, the Standard *D. grimshawi* gene orders (Auwahi, Maui) are being used as Standards for the members of all four subgroups. Accordingly, all inverted sequences found in members of the *D. planitibia* subgroup are given letter designations supplementing those found in Carson and Stalker (1968).

Results

Description of inversion break-points

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 4. Figure 1 shows (from top to bottom) chromosome X of *D. picticornis* (Kokee, Kauai), *D. obscuripes* (Paliku, Maui) and *D. planitibia* (Waikamoi, Maui). The arrangement shown

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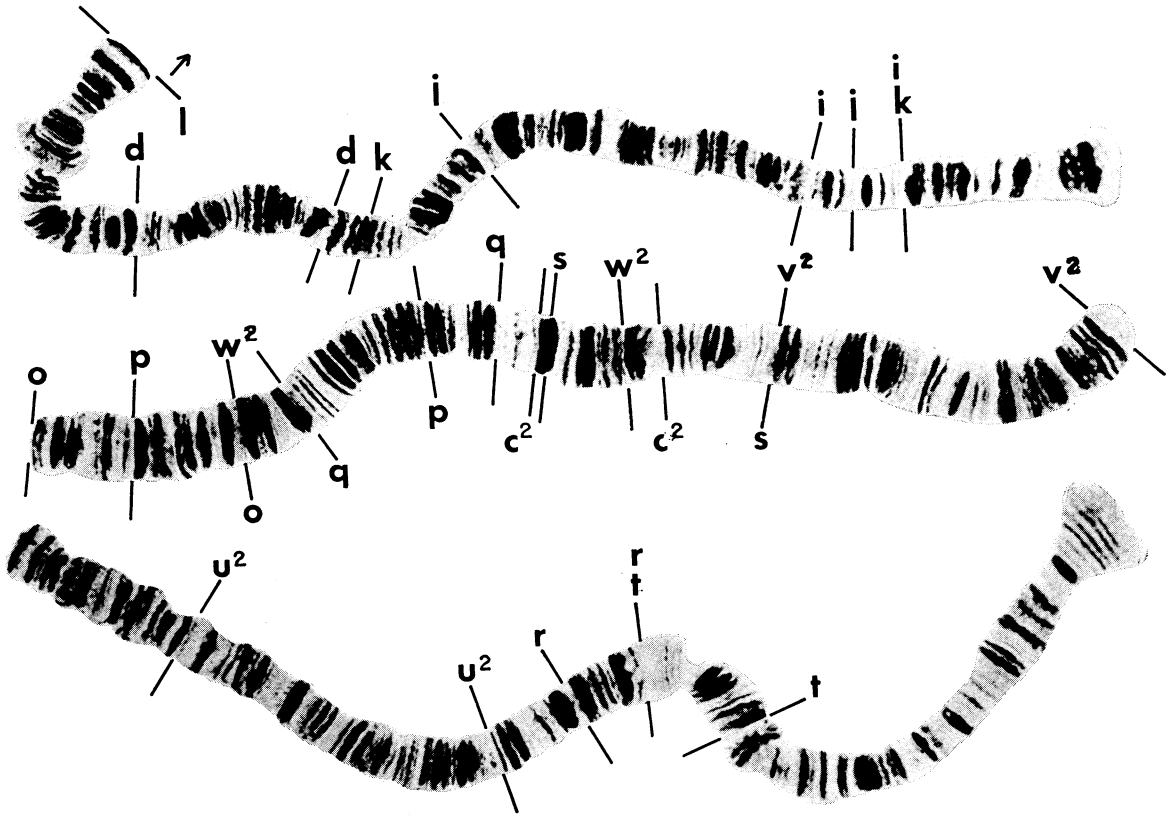

FIG. 1. Top: X chromosome of *D. picticornis*. When the inversions marked are made in a specific order, the arrangement of bands will be converted into *D. grimshawi* Standard (for details, see Text). Middle: X chromosome of *D. obscuripes*. When inversions q, p, o (in that order) and s, j, k and i (in that order) are made, the Standard *D. grimshawi* sequence will result. The other inversions marked are additional inversions based on the *D. obscuripes* gene order (for details, see Text). Bottom: X chromosome of *D. planitibia*. When inversions r and t are made, the result will be the *D. obscuripes* gene order. Xu^2 is an additional inversion based on the sequence found in *D. neoperkinsi* (or *D. obscuripes*).

TABLE 1

Strains of Drosophila species of the *D. planitibia* subgroup examined for giant chromosome sequences

Species	Locality and strains examined[2]	Number of wild chromosomes observed					
		Autosomes			X chromosomes		
		This paper	Carson *et al.* 1967	Total	This paper	Carson *et al.* 1967	Total
D. hemipeza	Palikea, Oahu (2800′)	..	10	10	..	7	7
D. heteroneura[1]	Hualalai, Hawaii (5300′) G90A104	4	..	4	3	..	3
D. nigrifacies[1]	Hualalai, Hawaii, K3B1	4	..	4	3	..	3
	Upper Olaa For. Res., Hawaii (4100′)	..	6	6	..	5	5
	Honaunau For. Res., Hawaii (2100′) J62B2	4	..	4	3	..	3
	Kulani, Hawaii (5100′) K64G3	4	..	4	3	..	3
	Kipuka at B.M. 5108′, Saddle Rd., Hawaii, K15N1, 5; K33G3; K46G6; K64G6, 8; K66P2, 5–7, B.M. 5108′	40	..	40	30	..	30
	Total	52	6	58	39	5	44
D. neopicta[1]	Kipahulu Valley, Maui (6000′) L13G12–21 (mass)	4	..	4	3	..	3
	Paliku, Maui (6300′) K22L20–24 (mass)	4	..	4	3	..	3
	Total	8	..	8	6	..	6
D. oahuensis	Wiliwilinui Ridge, Oahu (2100′) J25H3	2	..	2	2	..	2
D. obscuripes[1]	Paliku, Maui: K22L1–7 (mass); K22L34–38 (mass)	8	..	8	6	..	6
D. neoperkinsi	Kipahulu Valley, Maui, L13G9–11 (mass)	4	..	4	3	..	3
	Waikamoi, Maui (4200′)	..	2	2	..	2	2
	Total	4	2	6	3	2	5
D. picticornis	Halemanu Valley, Kauai (3400′)	..	68	68	..	51	51
	Kumuwela Ridge, Kauai (3600′)	..	22	22	..	18	18
	Kokee, Kauai (3600′) J95C8; K51C2	6	8	14	5	6	11
	Mohihi, Kauai (3500′) G83C2	4	..	4	3	..	3
	Total	10	98	108	8	75	83
D. planitibia	Waikamoi, Maui J66C16; J67C5; J91A1	12	12	24	9	9	18
	Kaulalewelewe, W. Maui (3000′)	..	2	2	..	2	2
	Total	12	14	26	9	11	20

[1] Shows intraspecific chromosomal polymorphism.
[2] Strain numbers are entered only for newly-reported strains.

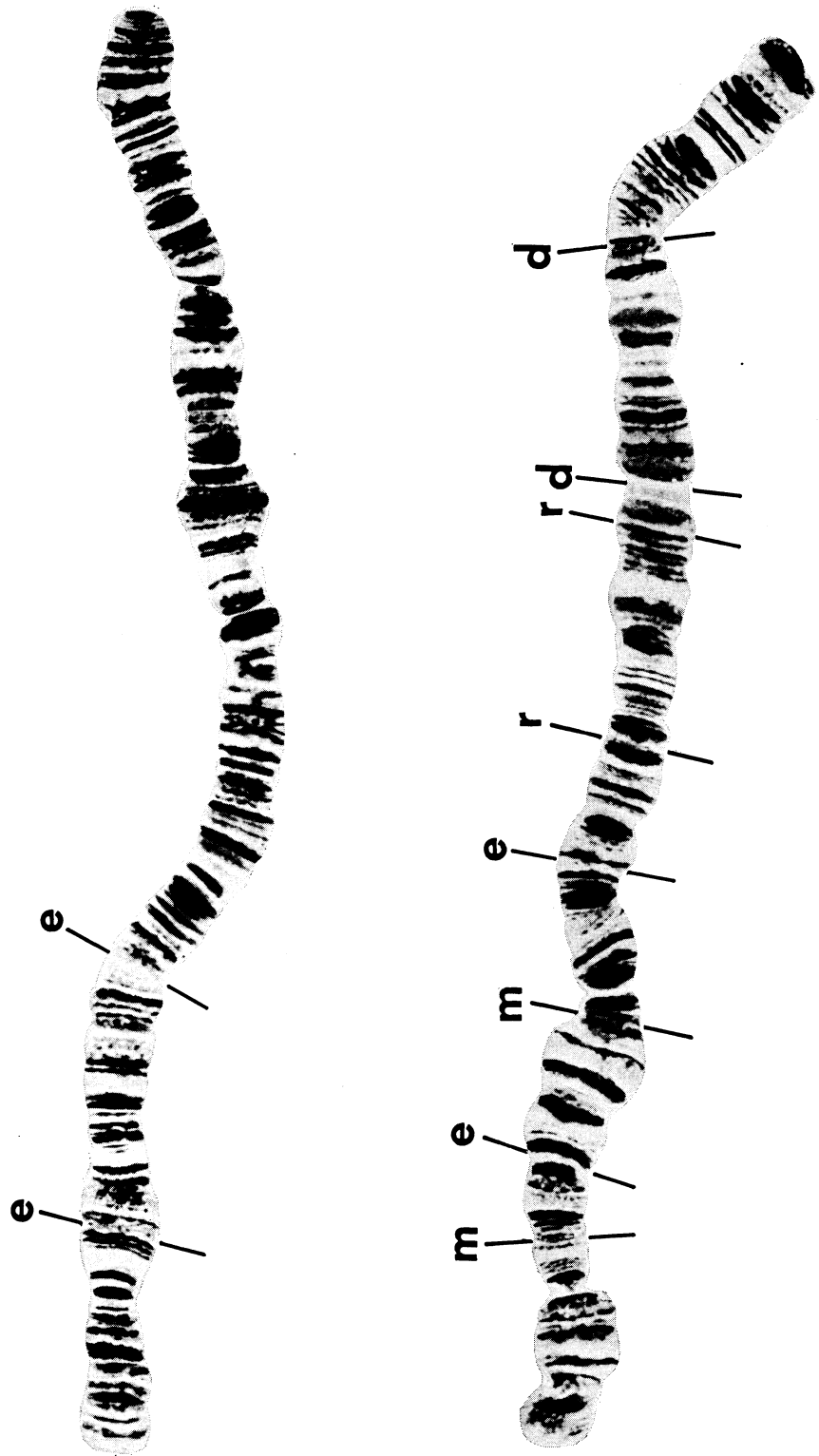

FIG. 2. Chromosome 5 (top) and chromosome 3 of *D. obscuripes*. Chromosome 5 has the Standard gene order; e is based on it. Chromosome 3 has the 3d arrangement; it can be converted to Standard by inverting this piece. For details, see Text.

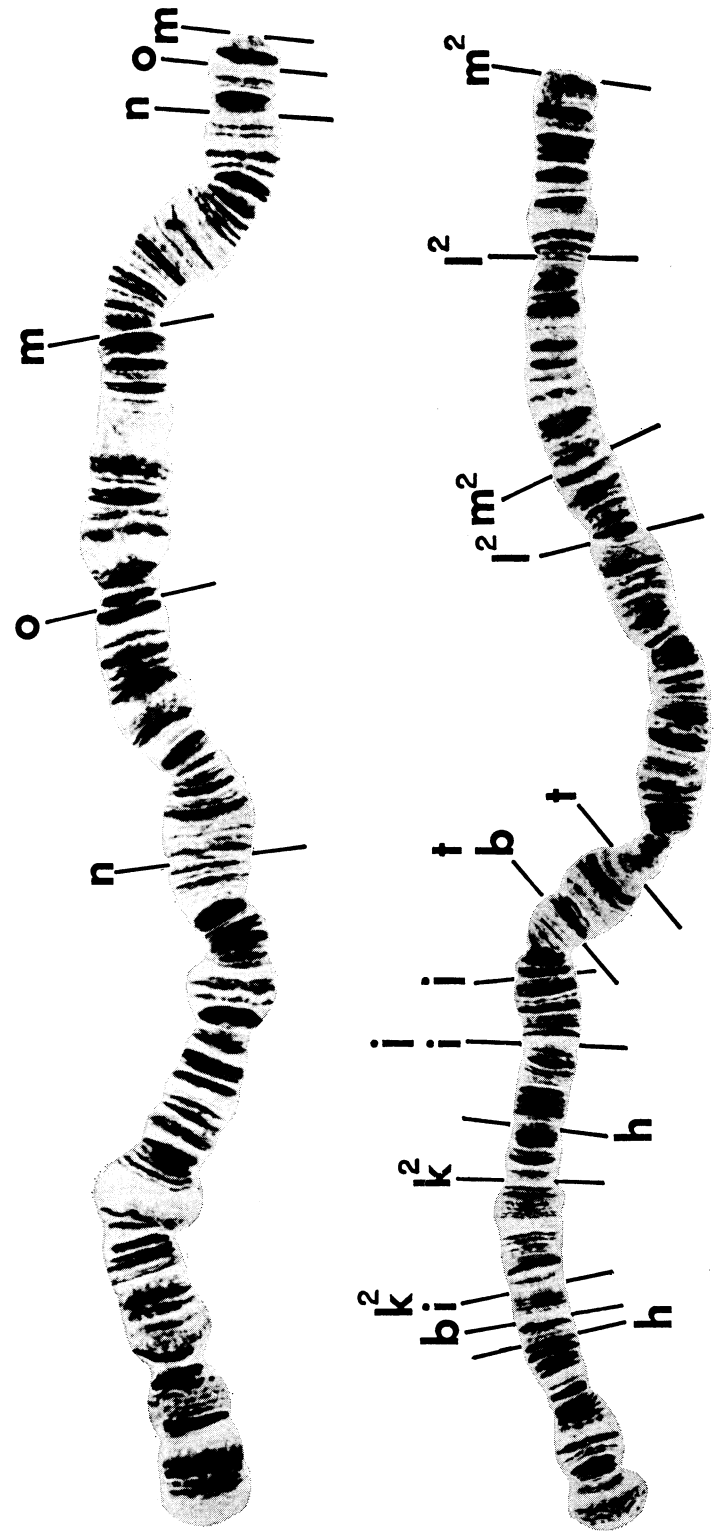

FIG. 3. Chromosomes 2 (top) and 4 of *D. obscuripes*. Chromosome 2 is Standard. For ordering of inversions based on it, see Text. The arrangement shown in chromosome 4 is 4b; by making the b inversion, the Standard *D. grimshawi* gene order will result. Because of overlapping inversions, the *D. picticornis* order should be obtained by starting with 4b, then inverting h, i and j in that order (for details, see Text).

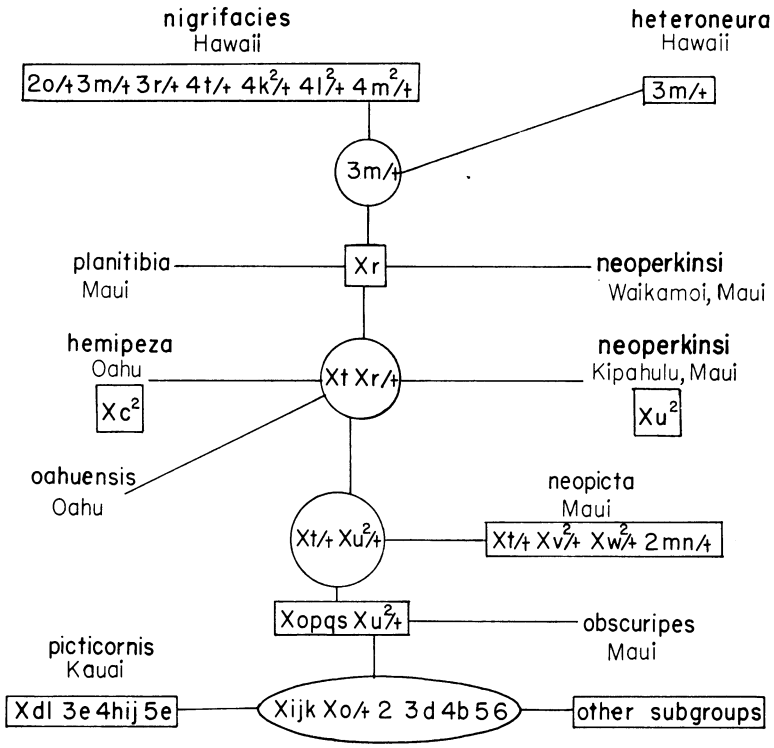

FIG. 4. Chromosomal relationships among nine species of the *D. planitibia* subgroup. Letters appearing singly represent fixed inversions whereby the arrangement differs from the *D. grimshawi* Standard. Read the formula for each species cumulatively by following the line from the basic formula at the bottom of the figure (Xijk Xo/+ 2 3d 4b 5 6). For example, *D. hemipeza* has the formula: Xijkopqstc² 2 3d 4b 5 6. Boxes denote present species; hypothetical populations are encircled. The chromosomally distinct populations of *D. neoperkinsi* are tentatively considered as subspecies.

in *D. picticornis*, relative to the *D. grimshawi* Standard (Figure 1, Carson and Stalker, 1968) is Xdijkl. In order to derive the Standard sequence, inversion Xj should be made first, followed by k and i (in either order). The independent inversion Xd must also be made. The aberration marked l is interpreted as a single-break change which has resulted in the deletion of approximately five bands from the distal end of the Standard chromosome.

Figure 1 (middle photograph) shows the X chromosome of *D. obscuripes* from Paliku, Maui (Xijkopqs). To convert this arrangement to the *D. grimshawi* Standard, inversion s should be made first; this should be followed by j, k and i as in *D. picticornis*. The distal end may be converted to Standard by inverting q, p and o in that order. The other inversions marked represent extensions of the *D. obscuripes* gene order. The lower photograph in Figure 1 shows the order found in *D. planitibia* (Xijkopqstr). If inversions r and t are made, the arrangement will be converted into the *D. obscuripes* gene order. Xu^2 is an extension of the *D. planitibia* (or *D. obscuripes*) gene order. In this and the other figures of the chromosomes, the distal end is to the left.

Figure 2 shows chromosomes 5 (above) and 3 (below) of *D. obscuripes*. The order shown in chromosome 5 is identical to that of the *D. grimshawi* Standard. The order shown in chromosome 3 is 3d. Accordingly, the arrangement may be converted into the *D. grimshawi* Standard by inverting the 3d section. The rest of the inversions marked represent extensions of the 3d gene order.

Figure 3 shows chromosomes 2 (above) and 4 (below) of *D. obscuripes*. Chromosome 2 is identical in order to that of the *D. grimshawi* Standard. The three inversions shown are polymorphic within several species (see Figure 4); all represent extensions of the Standard gene order. Inversion 2m must precede 2n, which overlaps it. Chromosome 4 depicts the 4b gene order; accordingly, in order to derive the Standard *D. grimshawi* gene order, this inversion must be made. The rest of the inversions are extensions based on the 4b gene order. The *D. picticornis* gene order can be obtained from the 4b gene order by first making inversion h and following with the tandem inversions i and j. The rest of the inversions are based directly on the 4b arrangement; although l^2 and m^2 overlap, each is derived separately from a 4b chromosome.

By the proper and orderly application of the information presented in Figures 1–3, all chromosome types in the whole subgroup may be derived. The sequential karyotype of each species is given in Figure 4.

A summary of the fixed and polymorphic inversions in the *D. planitibia* subgroup is given in Table 2. Inversion 4b is excluded from the count because it is shared with a number of species of the *D. grimshawi* subgroup and was included in the count given in Table 2, of Carson and Stalker (1968). Except for *D. picticornis* all of the fixed interspecific inversion variation in the *D. planitibia* subgroup is confined to the X chromosome.

Relationships of the species based on banding sequence

Based on the phenomenon of inversion-sharing, the nine species of the subgroup have been arranged according to their sequential relationships (Figure 4). As in the consideration of previous diagrams of this sort, no region or species can be designated as primitive without information extrinsic to the chromosomal data themselves. Arguments that *D. picticornis* and *D. obscuripes* are closest to the primitive ancestors will be presented in the discussion. Suffice it to point out here, however, that five hypothetical populations have been included as necessary links between existing species. Thus, *D. picticornis* shares Xijk and 3d with all the species from *D. obscuripes* towards the top of Figure 4. Arrangement 4b is found

TABLE 2

Chromosome inversions in nine species of the *D. planitibia* subgroup. Inversion 4b has been excluded from the count (see text)

Chromosome	X	2	3	4	5	Total
Number of fixed inversions	11	0	2	3	1	17
Number of inversions polymorphic within a species	4	3	2	4	0	13
Total	15	3	4	7	1	30

in all members of the *D. planitibia* subgroup as well as in at least some members of all three other subgroups. As will be shown in a forthcoming paper on the *D. adiastola* subgroup, members of that group not only have 4b but have fixed Xi,Xk and Xo as well. These relationships will be diagrammed more fully in a later paper.

Disregarding intraspecific polymorphism, there are three homosequential pairs of species diagrammed in Figure 4. These are: 1) *D. planitibia* and *D. neoperkinsi* (Waikamoi, Maui race), 2) *D. neopicta* and *D. obscuripes* and 3) *D. nigrifacies* and *D. heteroneura*. The latter share a common polymorphism (3m/+) recalling the condition found in *D. bostrycha* and *D. disjuncta* (Carson and Stalker, 1968).

Intraspecific Polymorphism

Of the nine species, four show intraspecific chromosomal polymorphism. *D. nigrifacies* of Hawaii is the most variable species yet found in any of the subgroups. There are seven intraspecific autosomal inversions (Figure 4); although most of them are short, they are distributed through three of the four autosomes. Despite the small sample, *D. neopicta* of Maui has been found to be polymorphic for three X chromosome and two autosomal inversions. One of these inversions (Xt) is fixed in six other species, including two from Maui, two from Oahu and two from Hawaii (Figure 4). Chromosomally, *D. obscuripes* of Maui is very close to *D. neopicta*. Nevertheless, it lacks Xt but is polymorphic for another X chromosome inversion, Xu^2. This latter, although it has not been found in *D. neopicta* is present in the fixed state in the specimens of *D. neoperkinsi* examined from Kipahulu Valley, Maui.

The situation with respect to *D. neoperkinsi* is most interesting and puzzling. As can be seen on Figure 4, at Waikamoi, Maui, gene arrangement Xr is fixed, whereas at Kipahulu Valley, on the same island, populations lack Xr but have fixed Xu^2. Furthermore, Clayton (1968) has found that the metaphases are different; whereas Waikamoi flies show 5R1D (five rods and one dot), Kipahulu specimens show 5V1J (five V and one J-shaped chromosome). No heterozygotes for either salivary or metaphase differences have been found. Despite these striking cytological differences, no consistent morphological difference, even in male genitalia, has been found (K. Y. Kaneshiro, personal communication). For the present, these populations will be considered as chromosomal races. All other species in this subgroup show 5R1D.

DISCUSSION

Origin and evolution of the D. planitibia *subgroup in Hawaii*

As has been stressed by Carson and Stalker (1968) the present series of papers serve the primary purpose of placing on record the basic chromosomal data for those species which have so far been examined. In considering the following discussion, three points in particular must be borne in mind. First, of the species examined, in most cases only a small number of strains have been studied. Secondly, a number of species are known, in all subgroups, for which no cytological data have yet been obtained. In the third place, the probability of the

existence of undiscovered new species remains high, in view of the rapid discoveries of the last few years (see Hardy and Kaneshiro, 1968).

Accordingly, the following hypotheses are advanced by the authors with caution, and should be viewed by the reader as tentative. Certain of the facts already available, however, are of such key importance to the understanding of the origin and evolution of these flies that some preliminary discussion here is mandatory.

Each of the nine species in the *D. planitibia* subgroup are strikingly different from one another morphologically. This is true even of the two members of each of the three homosequential pairs. *D. heteroneura*, for example, is characterized by an extraordinary lateral production of the compound eyes of the males, a character wholly lacking in *D. nigrifacies* (see Hardy, 1965). Eight of the nine, furthermore, have an extra crossvein in cell R_5 of the wing. There is one exception to this; *D. picticornis* of Kauai lacks this crossvein. Chromosomally, however, this species shares five inversions with the rest of the subgroup, indicating its basic affinity with them. As *D. picticornis* lacks the extra crossvein, it must be more like the ancestral populations from which the extra-crossveined flies evolved; that is, it must be the most primitive of this series of nine species. The cytological position of this species (Figure 4) also points to the same conclusion, namely, that *D. picticornis* occupies an ancestral position. *D. picticornis*, furthermore, is the only member of this subgroup known from Kauai, the northernmost and geologically oldest of the main islands (Stearns, 1966). Despite fairly intensive collecting, no extra-vein fly has ever been caught on Kauai.

The two extra-vein species closest to *D. picticornis* are *D. obscuripes* and *D. neopicta* of Maui. These two species are necessary chromosomal intermediates between *D. picticornis* and the species found on Oahu and Hawaii. The clue to this is that these latter species all have fixed inversion Xt. This arrangement is lacking in *D. obscuripes* of Maui and, most importantly, it is present in heterozygous state in *D. neopicta* of Maui. Accordingly, the Oahu and Hawaii species must have arisen from a *D. neopicta*-like ancestral population presumably having its origin on Maui, since this is the only island from which *D. neopicta* is presently known.

These facts, therefore, suggest that this subgroup originated on Kauai at some remote time and that an ancestor bearing chromosome arrangements Xijk 3d and 4b, migrated directly to Maui where the major evolution of the extra-vein flies apparently occurred (Figure 5). This migration could have gone from Kauai to Oahu and thence to Maui with the Oahu populations having been lost at a later time or as yet undiscovered.

This ancestor, furthermore, could serve not only for the origin of the *D. planitibia* subgroup but also for the *D. grimshawi* subgroup. Carson and Stalker (1968) presented independent evidence that this latter subgroup originated on Maui and then spread to the other islands from there.

The general hypothesis dealing with the origin and migration of the members of this group is depicted on the map, Figure 5. The migrant from Kauai is shown colonizing Maui directly and then giving rise to *D. planitibia* on the one hand (pl) and *D. neopicta* (n) on the other. Descendants of *D. neopicta*-like popula-

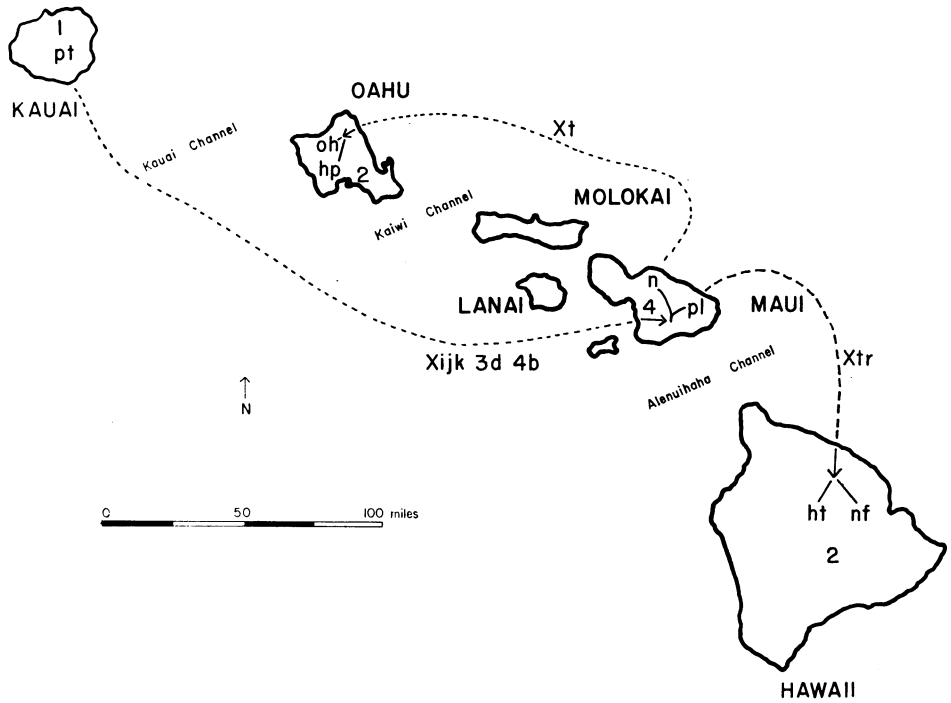

FIG. 5. Origin, migration and speciation of the *D. planitibia* subgroup on the principal islands of Hawaii. Superimposed on each island is the number of species of th esubgroup found there. hp = *D. hemipeza*, ht = *D. heteroneura*, n = *D. neopicta*, nf = *D. nigrifacies*, oh = *D. oahuensis*, pt = *D. picticornis*. The pertinent chromosomal composition of the proposed migrants is placed adjacent to the arrows. For details, see Text.

tions, in which Xt was fixed, are then supposed to have given rise, by northwestward colonization, to the present-day Oahu species of the subgroup, *D. oahuensis* and *D. hemipeza* (o and hp).

On Hawaii, the two homosequential species found there, *D. heteroneura* and *D. nigrifacies* have been found only in the newer areas of the island, that is, neither species has so far been taken in the Kohala Mountains, a slightly older Pleistocene range at the northwest corner of the island. Both species, furthermore, show, in addition to Xt, a distinctive inversion Xr, known elsewhere only from the two most advanced species on Maui, *D. planitibia* and *D. neoperkinsi* (Waikamoi race). Accordingly, a single colonization of a *D. planitibia*-like ancestor across the Alenuihaha Channel separating Maui and Hawaii is suggested, with subsequent very recent speciation during the Pleistocene.

Accordingly, the history of this subgroup, like the *D. grimshawi* subgroup (Carson and Stalker, 1968), appears to have had its major evolution on Maui. The minimum number of colonizations by members of the two subgroups combined is as follows: Kauai to Maui (1), Maui to Oahu (5), Maui to Hawaii (5). Further information on these topics will be presented in forthcoming papers.

Summary

Polytene chromosome banding sequences are described for nine species related to *D. planitibia* of Maui. Eight of the nine species are unusual among drosophilids in that they show an extra vein in cell R_5 of the wing. The only member of the group found on the geologically oldest island, Kauai, has the usual wing venation. Nevertheless, this species, *D. picticornis*, shares five fixed inversions with the extra-veined flies. Although intraspecific polymorphism is extensive in several species, fixed differences, except for *D. picticornis*, are confined to the X chromosome. The species closest to *D. picticornis* are *D. obscuripes* and *D. neopicta* of Maui. Both of these are chromosomal intermediates between *D. picticornis* on the one hand and the Oahu and Hawaii members of the subgroup on the other. Accordingly, it is postulated that an ancestral form, carrying gene arrangements Xijk 3d and 4b colonized Maui at an early time and that the main evolution of the subgroup occurred there. Colonization of Oahu and Hawaii is supposed to have been effected by migrants from Maui.

Acknowledgments

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