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The Role of Heterochromatin in Karyotype Variation among Hawaiian Picture-Winged *Drosophila*

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ABSTRACT: A comparison is made of the amount and distribution of heterochromatin in the mitotic chromosomes of species of the Hawaiian picture-winged *Drosophila*, and metaphase configurations among these species are compared with those from other regions. Of 103 picture-winged species, only three species (*D. melanocephala*, *D. cyrtoloma*, and *D. prostopalpis*) have any metacentric or submetacentric chromosomes, and only six species have a haploid complement of six acrocentric chromosomes rather than the "primitive" karyotype of five rods and a microchromosome. All karyotype modifications among the picture-winged species can be explained on the basis of modifications in the amount and distribution of heterochromatin; there is no evidence of pericentric inversions or chromosomal fusion.

THE CYTOLOGICAL ANALYSIS of the chromosomes of the picture-winged species of Hawaiian *Drosophila* has spanned a period of over 20 years. During that time, the banding sequences in the polytene chromosomes (see Carson 1983 for a review) and the metaphase karyotypes (see Clayton and Guest 1986, Clayton and Wheeler 1975 for summaries) for 103 species have been determined. Carson (1983) was able to trace the sequential events in the speciation of these picture-winged species by the analysis of 213 paracentric inversions. He described 45 founder events that may account for the speciation on the islands of Oahu, the Maui complex, and Hawaii. The species on Kauai were considered to be ancestral species except for *D. crucigera* and *D. musaphilia*, which can be traced back to Kauai from the newer islands.

White (1978) discussed the presence of homosequential complexes in *Drosophila*, and indicated that most of these cases of apparently identical karyotypes will be shown to differ as techniques improve for determining the distribution of satellite DNAs in the heterochromatin or the amount and distribution of heterochromatin in mitotic chromosomes that cannot be analyzed from polytene chromosomes. An effort has been made here to consider the karyotypes in relation to the founder events described by Carson (1983) and to compare homosequential or closely related species on the basis of heterochromatin found in the mitotic chromosomes.

MATERIALS AND METHODS

The cytological material summarized here was prepared by the standard aceto-orcein staining technique or by one of the Giemsa methods described by Clayton (1985), resulting in G- or N-banding of the mitotic chromosomes. In many cases, the aceto-orcein neuroblast smears were prepared simultaneously with corresponding salivary gland preparations so that cytological data could be correlated. The photographic data were accumulated through the use of several different microscopes and camera accessories. Most of the photographs of Giemsa-stained chromosomes were taken through the Olympus Vanox microscope at an initial magnification of 750×. The descriptions of the karyotypes are the result of combining notes from data books, drawings, and photographs of the various picture-winged species.

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RESULTS AND DISCUSSION

The data on the karyotypes of the Hawaiian picture-winged species present a very conservative picture of karyotype modification, showing that chromosome rearrangements such as pericentric inversions and fusions are not required during speciation. A comparison of mitotic metaphases among 103 picture-winged species and known karyotypes of *Drosophila* (subgenus *Drosophila*) from other parts of the world are given in Table 1. Over 65% (159/244) of the nonendemic species have modified karyotypes; over 50% of these modifications can be accounted for by pericentric inversions and Robertsonian translocations (fusions). Among species of *Drosophila* from Hawaii, however, only seven of 148 species examined have karyotypes modified by fusions (Clayton 1976), and none of these is a picture-winged species. Only 8.7% (9/103) of the picture-winged species have karyotypes other than the "primitive," or basic, configuration of five pairs of rods and one pair of dots. The modifications can be accounted for by alterations in the amount and distribution of heterochromatin. There are six species with a haploid configuration of six rods; heterochromatin added to the microchromosome has resulted in the sixth rod. Three species have heterochromatic arms added, resulting in metacentric or submetacentric chromosomes. *Drosophila melanocephala* has a metaphase consisting of five pairs of rods and one pair of V-shaped chromosomes, while *D. prostopalpis* has a haploid configuration of four rods, one V-shaped chromosome, and one dot. The third species with metacentric chromosomes is *D. cyrtoloma*, which has a chromosome complement unique among all known *Drosophila* karyotypes. Heterochromatin has been added to every chromosome in the complement, resulting in a karyotype consisting of five pairs of V-shaped chromosomes and one pair of J-shaped chromosomes. Two species were described by Patterson and Stone (1952) as having five pairs of metacentric or submetacentric chromosomes; however, in both cases, pericentric inversions and fusions were involved in the formation of the V- or J-shaped chromosomes. *Drosophila annulimana*, with a haploid karyotype of one large V, three small V-shaped chromosomes, and one J-shaped chromosome, has a polytene complement of eight long arms and one short arm. The explanation for this configuration presented by Patterson and Stone involved the occurrence of three pericentric inversions, the fusion of an autosome to the dot, and the addition of heterochromatin. In the *D. nannoptera* karyotype, there are three pairs of large Vs and two smaller J-shaped chromosomes; the salivary gland chromosomes consist of six long arms and one short arm. This was interpreted as resulting from

TABLE 1

KARYOTYPES AMONG HAWAIIAN PICTURE-WINGED SPECIES AS COMPARED WITH NON-ENDEMIC SPECIES OF THE SUBGENUS *Drosophila*

KARYOTYPE	HAWAIIAN PICTURE-WINGED	*NON-ENDEMIC SPECIES
Basic (5R, 1D)	94 (91.3%)	85 (34.8%)
Added Heterochromatin:		
6R	6	10
5R, 1V	1	4
5V, 1J	1	0
4R, 1V, 1D or 4R, 1J, 1D	1	14
Subtotal	9 (8.7%)	28 (11.4%)
Fusions	0	53 (21.8%)
Other (Pericentric Inversions, etc.)	0	78 (32.0%)
Total Modifications	9/103 (8.7%)	159/244 (65.2%)

* Data from Clayton (1976)

one fusion, one pericentric inversion, and the addition of heterochromatin. In contrast, the chromosomes of *D. cyrtoloma*, based on evidence from the polytene data and Giemsa-stained mitotic chromosomes, resulted from the addition of a heterochromatic arm to each of the six chromosomes in the haploid complement. There is no evidence of pericentric inversions or fusions.

The basic, or "primitive," *Drosophila* haploid figure is considered to be comprised of four autosomal rods of approximately equal length, a rod-shaped X or Y chromosome, and the microchromosome. In an effort to establish differences among karyotypes of picture-winged species, all with the basic configuration, modifications have been summarized as follows: large dots, double-length rods, both large dots and double-length rods, and rods shorter than normal. All these are considered to be modifications resulting from changes in the amount and distribution of heterochromatin.

In Table 2, the distribution of different karyotypes is given by island or island complex. In addition to the categories given in the first table, the basic karyotype has been subdivided to note the variation in chromosome size or length. The presence of double-length rods accounts for a recognizable difference in the 5R, 1D karyotypes of 16 species of the picture-winged group, whereas noticeably large dots are present in only eight species. Four species possess both double-length rods and large dots. Slides prepared by the Giemsa techniques verify that these modifications are the result of added heterochromatin. The double-length rods are usually about 50% heterochromatin, and frequently possess a secondary constriction that results in a chromosome that may appear to be metacentric. It is important to verify the centromere location in these longer chromosomes by anaphase figures; all metacentric or submetacentric chromosomes described here have been verified by anaphases. Among the 103 species of picture-winged *Drosophila*, nine have modified karyotypes in which the primitive condition of 5R, 1D has been altered either by the presence of a sixth rod or by the presence of metacentric or submetacentric chromosomes. In addition, over 30 species have variations in chromosome length or size, although they retain the 5R, 1D configuration.

It may be noted that the three species with metacentric or submetacentric chromosomes are found on the Maui complex; no V- or J-shaped chromosomes have been described among picture-winged species on any of the other islands. The island of Kauai has the fewest modifications of the basic karyotype; of 12 species, only two can be distinguished from the basic configuration by the presence

TABLE 2

DISTRIBUTION OF KARYOTYPES BY ISLANDS OF HAWAII

KARYOTYPE	KAUAI	OAHU	MAUI COMPLEX*	HAWAII	TOTAL
Basic (5R, 1D)					
No modification	10	20	22	15	67
Large dots	1	2	1	4	8
Double-length rods	1	2	8	5	16
Large dots and double-length rods	0	1	2	1	4
Rods shorter than normal	0	1	2	0	3
Six-rod karyotype (6R)	0	3	2	1	6
Metacentric or submetacentric					
4R, 1V, 1D	0	0	1	0	1
5R, 1V	0	0	1	0	1
5V, 1J	0	0	1	0	1
Total species	12	29	40	26	107†

*Includes Molokai, Lanai, and Maui.

†There are 103 different species of the picture-winged group. *Drosophila grimshawi*, *crucigera*, and *orthofascia* occur on more than one island.

of double-length rods or large dots. Three of the 29 species on Oahu have the chromosome complement of six rods; the heterochromatic nature of the sixth rod is easily observed in Giemsa preparations in which heterochromatin stains heavily. Several other species with the basic karyotype are differentiated by the presence of large heterochromatic dots or by variations in rod length. The islands of the Maui complex (Maui, Molokai, and Lanai) have the greatest variation resulting from added heterochromatin. Of the 40 species on these islands, 18 (45%) have karyotypes in which there are heterochromatin modifications. On Hawaii, the newest of the island chain, 11 of the 26 species have heterochromatin modifications; however, the basic karyotype has been changed from 5R, 1D in only one species. The other alterations involve variations in the size of dots and/or rods in the basic 5R, 1D karyotype.

Not included in Table 2 are the karyotypes in which at least one rod chromosome is somewhat longer than the other rods but is not double in length. These karyotypes have been tabulated in Table 3. One pair of longer rods is characteristic of many species; however, in some species, there are two or three pairs of longer rods. In the accumulated data, notations appear concerning the presence of one or more longer chromosomes in 50 of the species recorded from the different islands. As may be seen from the table, the longer rod has been identified as the X chromosome in 23 of these species. This was accomplished where both male and female larvae were identified by differences in the lengths of the X and Y chromosomes; usually, the heterochromatic nature of the Y chromosome made possible its identification, particularly where Giemsa preparations were examined. In aceto-orcein smears, it was possible, for some species, to correlate neuroblast preparations with the corresponding polytene chromosomes and determine sex by noting the single or double nature of the X chromosome. In addition, among the 16 species listed in Table 2 as having at least one pair of double-length rods, one of these long chromosomes was identified as the X chromosome in seven species. The length of these rods is associated with the presence of large blocks of heterochromatin adjacent to the centromere.

Carson (1983) placed the picture-winged species into five subgroups. Subgroup I, the *grimshawi* subgroup, consists of 62 species distributed on all the major islands. Three species of this subgroup are found on more than one island; *Drosophila crucigera* is found on both Kauai and Oahu, while *D. grimshawi* is present on Kauai, Oahu, and the Maui complex. *Drosophila orthofascia* may be found on both the Maui complex and Hawaii. Subgroup II, the *planitibia* subgroup, includes 17 species. This subgroup is represented on the island of Kauai by a single species, *D. picticornis*. The species of this subgroup on the other islands represent the largest *Drosophila* species found in Hawaii and are characterized by the presence of an extra cross-vein in cell R_5. Members of subgroup III, the *adiastola* subgroup, are represented on Kauai by one species, *D. ornata*. There are 14 species in this subgroup, with nine of the 14 found on the islands of the Maui complex.

TABLE 3

SPECIES WITH RODS LONGER THAN NORMAL BUT NOT DOUBLE-LENGTH

ISLAND	TOTAL NUMBER OF SPECIES	RODS LONGER THAN NORMAL			LONGER ROD IDENTIFIED AS X CHROMOSOME
		1 PAIR	2 PARIS	3 PAIRS	
Kauai	12	4	1	0	1
Oahu	29	11	2	1	6
Maui Complex	40	19	3	0	11
Hawaii	26	8	2*	0	5
Total	107†	42	8	1	23

*One longer chromosome identified as the X in both of these species.

†This includes *grimshawi*, *crucigera*, and *orthofascia* more than once as they occur on more than one island.

In contrast, subgroup IV, the *punalua* subgroup, consists of eight species, only one of which occurs in the Maui complex. This subgroup is represented on Kauai by *D. ocellata*. The two species, *D. primaeva* and *D. attigua*, both present on Kauai, are the only members of subgroup V, the *primaeva* subgroup.

Based on comparisons of banding sequences in polytene chromosomes using *grimshawi* chromosomes as the standard, Carson (1983) traced inversion sequences and was able to develop the concept of sequential speciation through the major islands based on 45 founder events. In the Appendix, species are listed by island or island complex and by subgroup. The comments concerning the karyotypes have been accumulated from the cytological data books (1963–1985), drawings, and photographs. Included in one column of the table is the identification of the founder as designated by Carson (1983) in his description of sequential speciation. Previously published metaphase configurations are tabulated in Clayton and Guest (1986) and Clayton and Wheeler (1975).

The 12 species of picture-winged *Drosophila* on Kauai all have the basic haploid configuration of 5R, 1D. There are no 6R forms and no species with metacentric chromosomes. Each subgroup is represented by only one member on Kauai except for groups I and V. All but two of the Kauai species are considered by Carson (1983) to be ancient, and their origins cannot be traced to a founder on the basis of polytene data. There are seven species belonging to the *grimshawi* subgroup, two of which are considered to have been derived from founders from other islands. *Drosophila crucigera* on Kauai is considered to be derived from founder #45 from Oahu. Although sharing similar polytene sequences with several other species, *crucigera* from Kauai was considered to be derived from a separate founder on the basis of behavioral patterns and chromosomal polymorphisms analyzed by Giddings and Carson (1982). No cytological differences were observed in the metaphases of this species from the two islands. *Drosophila musaphilia* on Kauai is considered a derivative from founder #12 from Maui since it shares inversions 2b, 3g, Xa^2, and 4u with other Maui species. *Drosophila grimshawi* was found on Oahu and the Maui complex as well as Kauai, and was cytologically similar from all these localities. The other members of the *grimshawi* subgroup on Kauai are single-island endemics, and they are among those species considered ancient. *Drosophila ornata*, the single representative of the *adiastola* subgroup, is characterized by the presence of large dots. A pair of double-length rods was described for *D. ocellata*, the single member of the *punalua* subgroup on Kauai (Clayton, 1969). In subgroup V, the *primaeva* metaphase can be distinguished from that of *attigua* by the presence of a pair of longer rods in the latter species. In *D. picticornis*, the *planitibia* subgroup species on Kauai, a longer rod with a secondary constriction has been identified as the X chromosome; the Y chromosome is largely heterochromatic and about equal in length to the X. In addition to *picticornis* and *attigua*, one pair of longer rods was noted in *ornata*, *grimshawi*, *musaphilia*, and *villosipedis*. This longer chromosome has been identified as the X in *D. grimshawi*.

The analysis of heterochromatin distribution using the Giemsa procedures has been limited almost entirely to available species in the *planitibia* subgroup and to closely related members of the *hawaiiensis* cluster of species in the *grimshawi* subgroup. Limited observations have been made on other species in the *grimshawi* subgroup and on some species in the *punalua* and *adiastola* subgroups.

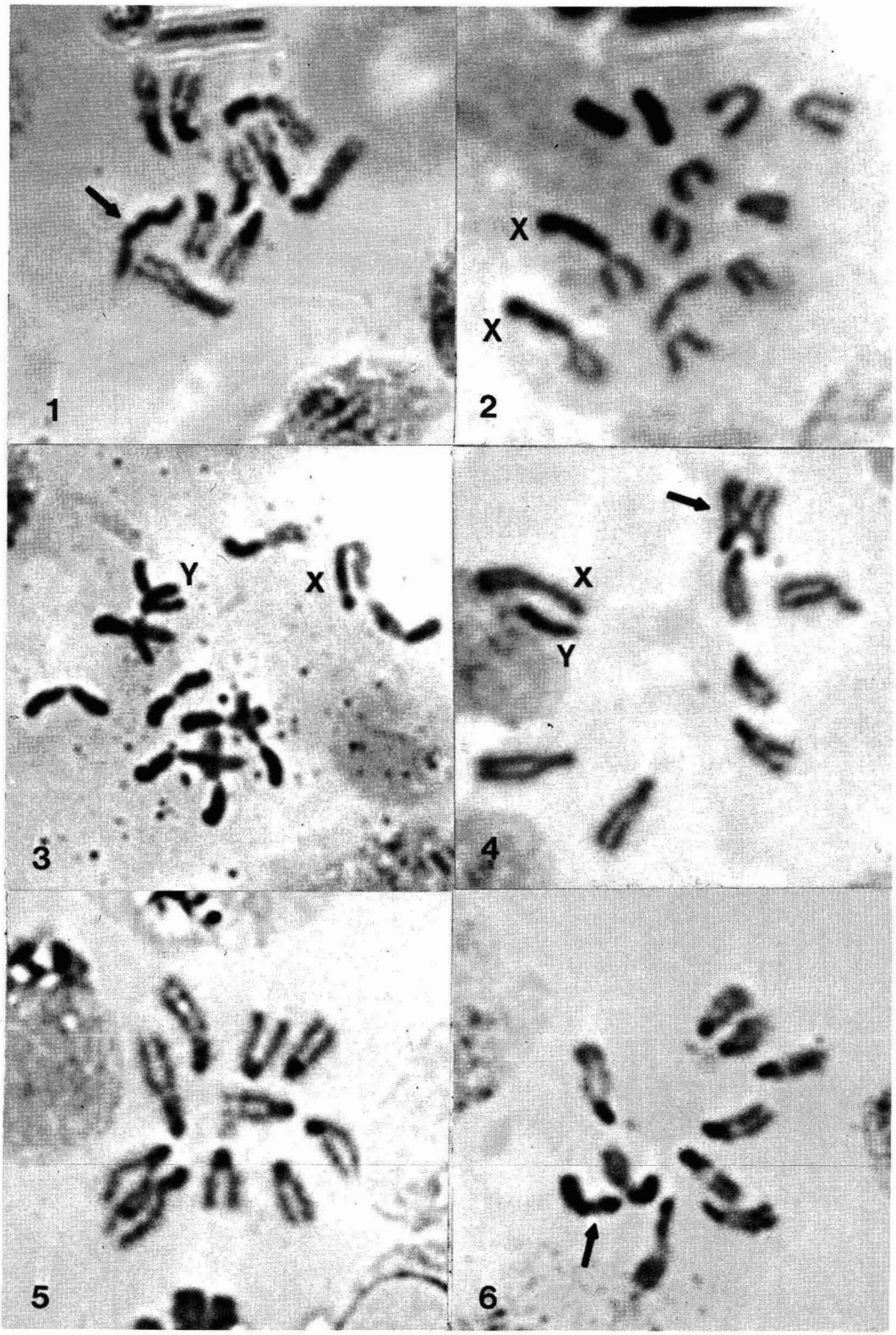
The *planitibia* subgroup consists of 17 species located on all the major islands. As mentioned previously, the species from Kauai, *D. picticornis*, is considered ancient, and its origin cannot be traced by polytene chromosome comparisons with other picture-winged species. However, the amount and distribution of heterochromatin in this species is typical of that observed in the acrocentric chromosomes of many of the picture-winged species. Each rod has constitutive heterochromatin adjacent to the centromere, and the Y chromosome is acrocentric and largely heterochromatic (Figure 1). On Oahu, there are four species of this subgroup: *substen-*

optera, derived from founder #2 from Kauai; *hemipeza* and *oahuensis* from founder #16 from Maui; and *nigribasis* from founder #26 from Maui. Material from these species was not available for heterochromatin analysis; however, on the basis of earlier observations from aceto-orcein material, no significant difference in chromosome length was noted. In *nigribasis*, one longer rod was paired with a rod of normal length, but for the other Oahu species, all rods were approximately equal in length.

The Maui complex has nine of the 17 species in the *planitibia* subgroup, all considered to have differentiated from a common founder, #25 from Oahu. Four of the species were studied with Giemsa procedures, *cyrtoloma*, *melanocephala*, *planitibia*, and *differens*. The other five species, *neoperkinsi*, *ingens*, *hanaulae*, *neopicta*, and *obscuripes*, have the basic 5R, 1D metaphase configuration; the only distinction noted from aceto-orcein preparations was the presence of slightly longer rods in three of the species. *Drosophila melanocephala* has a karyotype of four very short pairs of euchromatic rods, one pair of short heterochromatic rods, and one pair of sex chromosomes that are metacentric. In the female, both sex chromosomes have a euchromatic arm and a heterochromatic arm; in the male, the Y chromosome is V-shaped and almost entirely heterochromatic. The karyotype of the female is shown in Figure 2, where the absence of heterochromatin in the autosomes is evident. A detailed examination of the chromosomes of *D. cyrtoloma* has been described previously (Clayton 1985). The metaphase of this species consists of five pairs of V-shaped autosomes and a J-shaped X chromosome with a conspicuous satellite; in males, the Y chromosome is a small V-shaped heterochromatic chromosome. With the exception of the Y chromosome and the V-shaped sixth chromosome, each metacentric or submetacentric chromosome consists of one euchromatic arm and a heterochromatic arm (Figure 3). *Drosophila planitibia* from Maui and *D. differens* from Molokai are homosequential; no significant differences were noted in the amounts and distribution of heterochromatin in the mitotic chromosomes of these two species. In Figure 4, the chromosomes of *D. planitibia* are illustrated; the association of one pair of homologous autosomes indicated in this figure was consistent through all cells from this individual.

The remaining three species of the *planitibia* subgroup are found on Hawaii. *Drosophila silvestris* and *heteroneura* are homosequential and have a common founder, #17 from Molokai; whereas *D. setosifrons* is considered to be derived from founder #3 from Kauai. Only *D. silvestris* was analyzed by heterochromatin staining methods. The X chromosome is longer than the autosomes and possesses a secondary constriction; this constriction appears as an unstained region in Figure 5; the Y chromosome is a long heterochromatic rod, also with a secondary constriction, as may be seen in Figure 6. Carson and Bryant (1979) described differences between populations of *silvestris* from different localities on the island of Hawaii on the basis of ciliation on the front tibia. No cytological distinctions could be found between specimens from these different localities.

The *punalua* subgroup consists of eight species; although only one species, *D. prostopalpis*, was analyzed for heterochromatin, several of the other species could be distinguished on chromosomal features observed from aceto-orcein smears. Founder #7 from Kauai gave rise to three species on Oahu, *paucicilia*, *punalua*, and *uniseriata*. *Drosophila uniseriata* is easily distinguished from the other two homosequential species on Oahu by the presence of six pairs of rods; one pair of rods is double in length and may be confused with metacentric chromosomes. In *paucicilia* (5R, 1D), one pair of rods is almost double in length, a condition not observed in *punalua*. The X and Y chromosomes were identified in *punalua* as slightly longer rods; the X has a secondary constriction and the Y is largely heterochromatic, slightly longer than the autosomal rods. Therefore, we have distinguishing features for each of these three homosequential species. Three species of this subgroup, *basisetae*, *pauci-puncta*, and *prolaticilia*, are found on Hawaii.


1
2
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Y
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4
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Y
5
6

On the basis of aceto-orcein preparation only, differences in chromosome lengths could be noted; all three species have a haploid karyotype of five rods and one dot. In *paucipuncta*, all rods are approximately equal in length, while *prolaticilia* has one pair of double-length rods in the female and one double-length rod paired with a normal rod in the males. *Drosophila basisetae* has two pairs of very long rods with satellites; one of these long rods has been identified as the X chromosome since it is paired with a normal rod in males. The only species of the *punalua* subgroup found on the Maui complex is *D. prostopalpis*, the only other picture-winged species to have a karyotype including a metacentric chromosome. The karyotype of this species consists of four pairs of autosomal rods with very little heterochromatin, one pair of dots, and a pair of V-shaped sex chromosomes. As may be seen in Figure 7, the X consists of a metacentric chromosome with one heavily stained heterochromatic arm, while both arms of the Y chromosome are heterochromatic.

None of the 14 species of the *adiastola* subgroup was analyzed extensively, although a limited amount of material was available for the examination of chromosomes of *Drosophila spectabilis*. All species within this subgroup have the 5R, 1D configuration, although, on the basis of aceto-orcein smears, some differences in rod lengths were noted. The karyotype of *D. truncipenna* is quite distinct from other species in this subgroup; one pair of chromosomes is extremely large, while the remaining rods are very short. The dots in this species are quite small. Rod length varied with populations in *D. spectabilis*, *adiastola*, and *cilifera*. The two species of the *adiastola* subgroup from Hawaii, *setosimentum* and *ochrobasis*, were derived from founder #15 from Maui. Although chromosomal variations have been described among individuals and populations of both these species, no consistent species differences have been determined.

Cytological studies of the large *grimshawi* subgroup have been limited in most cases to the analysis of mitotic chromosomes of homosequential or closely related clusters of species that share common inversions. Ohta (1978) analyzed *grimshawi* populations from different localities on Kauai, Oahu, and the Maui complex. The populations on Maui and Oahu were considered to be derived, with the form on Kauai as ancestral. In the present study and Clayton (1985), no differences were observed in metaphases from the different localities. Baimai and Ahearn (1978) studied the relationships among three closely related *grimshawi* subgroup species, *disjuncta*, *affinidisjuncta*, and *bostrycha*. They described species differences in the amount and distribution of heterochromatin among these species.

In the present study, available species that differ from *grimshawi* by the 2b inversion (referred to here as the *hawaiiensis* complex) have been examined using the Giemsa techniques. The three species, *villitibia* from Molokai, *lasiopoda* from Maui, and *flexipes*

FIGURE 1. *Drosophila picticornis* male (M11J3) from Kokee, Kauai; heterochromatic Y indicated by arrow. (G-Banding).

FIGURE 2. *Drosophila melanocephala* female (U16L5) from Waikamoi, Maui; X chromosomes indicated by "X". (G-Banding).

FIGURE 3. *Drosophila cyrtoloma* male (U16L5) from Waikamoi, Maui; sex chromosomes indicated by "X" and "Y". (G-Banding).

FIGURE 4. *Drosophila planitibia* male (U54B2) from Waikamoi, Maui; sex chromosomes indicated by "X" and "Y". Note pairing of homologous autosomes noted by arrow. (N-Banding).

FIGURE 5. *Drosophila silvestris* female (U28T2) from Kilauea Forest Reserve, Hawaii. (G-Banding).

FIGURE 6. *Drosophila silvestris* male (U28T2) from Kilauea Forest Reserve; heterochromatic Y is indicated by arrow. (N-banding).

from Oahu, are homosequential. No distinguishing features of mitotic chromosomes could be found to separate *villitibia* and *flexipes*, but *lasiopoda* has the 6R configuration. *Drosophila hirtipalpus*, *villitibia*, and *lasiopoda* have a common founder (#34) from Oahu. Cytological features make possible the distinction of these species from one another. *Drosophila hirtipalpus* (5R, 1D) has rods of approximately equal length and a pair of large dots; *villitibia* (5R, 1D) does not have large dots. *Drosophila lasiopoda*, a 6R species, has two pairs of rods that are shorter than normal, one pair of which is almost entirely heterochromatic (Figure 8). The X chromosome was identified as a longer rod, paired in males with a longer heterochromatic rod-shaped Y chromosome. One pair of autosomal rods is longer than normal; Giemsa staining indicated that about one-third of this chromosome is heterochromatic. *Drosophila formella* has the 5R, 1D configuration with large dots and large blocks of heterochromatin adjacent to the centromere of each rod. The Y is a long heterochromatic rod with a secondary constriction (Figure 9).

The remaining species of this *hawaiiensis* complex that have been examined by the Giemsa procedures share (in addition to the 2b inversion) inversions 3g, Xa^2, and 4u. Three species on Hawaii, *hawaiiensis*, *silvarentis*, and *heedi*, are homosequential but can be distinguished on the basis of their mitotic chromosomes. The basic 5R, 1D configuration is characteristic of *hawaiiensis* (Figure 10), with significant blocks of heterochromatin present on all chromosomes. *Drosophila silvarentis* (Figures 11, 12) also shows large blocks of heterochromatin in all acrocentric chromosomes; it is also characterized by the presence of large dots (not present in *hawaiiensis*) and by a metacentric or submetacentric Y chromosome. *Drosophila heedi* is the only picture-winged species on Hawaii with a karyotype other than five pairs of rods and one pair of dots; this species has six pairs of rods. As may be seen in Figures 13 and 14, the X chromosome is a double-length rod with a satellite, and the Y chromosome is a long heterochromatic rod with a large satellite. The heterochromatic sixth rod is slightly shorter than the other autosomes. Both *silvarentis* and *heedi* are collected from fluxes associated with *Myoporum* trees, but it has been shown (Kaneshiro et al. 1973) that niche separation between these two species is present. Both species are considered to have been derived from founder #19 from Maui.

On the Maui complex, *Drosophila recticilia* and *gymnobasis* differentiated from founder #11 from Oahu. The metaphases of these two species can be differentiated on the basis of the large dots in *gymnobasis* (Figure 15) and the presence of the double-length rods. The X was identified as a double-length rod with 40–50% heterochromatin; the Y is a shorter heterochromatic rod. The dots also appear to be largely heterochromatic. On the basis of aceto-orcein preparations only, the *recticilia* metaphase was described as five pairs of rods and one pair of dots, including

FIGURE 7. *Drosophila prostopalpis* male (S15B33) from Kaulalewelewe, West Maui; sex chromosomes indicated by "X" and "Y". (G-Banding).

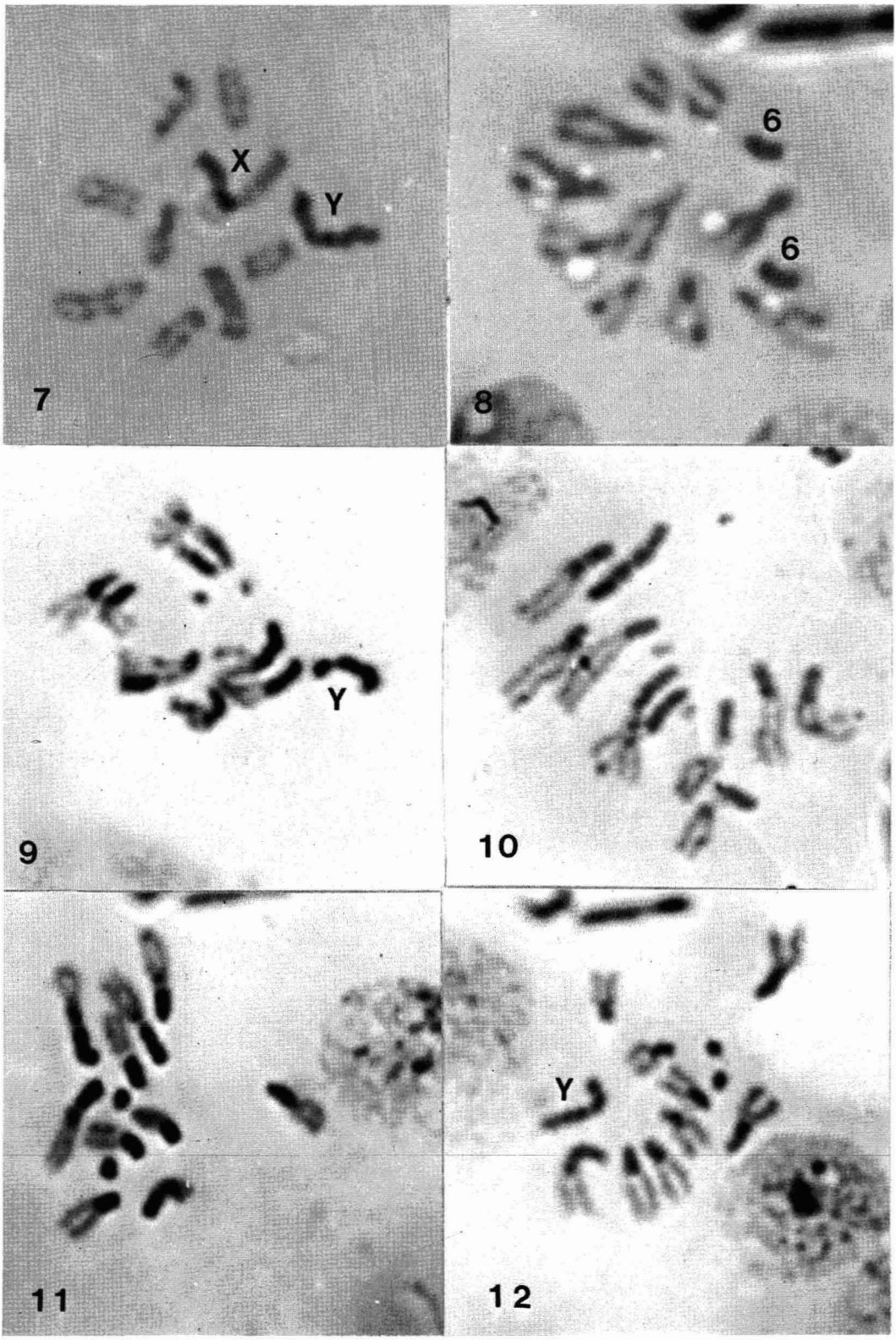
FIGURE 8. *Drosophila lasiopoda* female (Q52J11) from Waikamoi, Maui; note heterochromatic nature of sixth pair of rods indicated by "6". (G-Banding).

FIGURE 9. *Drosophila formella* male (M87G1) from 3 miles SW, Puuwaawaa, note heterochromatic Y chromosome with satellite, indicated by "Y". (N-Banding).

FIGURE 10. *Drosophila hawaiiensis* male (J14B8) from Kipuka Puaula, Hawaii; note large blocks of heterochromatin present on all acrocentric autosomes and the X chromosome. (N-Banding).

FIGURE 11. *Drosophila silvarentis* female (U87Cα) from Ahumoa, Hawaii; note presence of large microchromosomes. (N-Banding).

FIGURE 12. *Drosophila silvarentis* male (U87Cα) from Ahumoa, Hawaii; note J-shaped Y chromosome indicated by "Y". (N-Banding).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12

two longer or double-length rods with secondary constrictions. Baimai (1977), using a Giemsa technique, determined the distribution of heterochromatin in the mitotic chromosomes of this species and correlated metaphase configurations with the presence of certain inversions in chromosome 3. In addition to the other inversions mentioned for the *hawaiiensis* complex, *recticilia* and *turbata* share inversion 5g. The latter species also has the 5R, 1D metaphase with one pair of rods greater than double in length.

Variation in the size or length of chromosomes has been noted within species, either varying with different populations or with different individuals with a single population. Some of these cases have been correlated with cytological features found in the polytene chromosomes. Clayton (1971) described the presence of two or three double-length rods in samples of *disjuncta* from Kipahulu Valley, Maui, and these longer chromosomes were correlated with the presence of particular inversions (Baimai 1975*a*). The present study included the examination of *Drosophila disjuncta* from Waikamoi, Maui; from this collection there also appear to be differences in the mitotic chromosomes that may be associated with inversions (Figure 16). The double-length X and Y chromosomes can be identified, as well as one chromosome with a block of heterochromatin not seen in the remaining autosomes. Similarly, the metaphases of *D. formella* varied in appearance; Baimai (1975*b*) found that the appearance of certain mitotic chromosomes can be related to the presence of certain inversions in this species also. Other variations in the number of double-length rods have been recorded for several species. In *D. spectabilis*, metaphases from collections at Hanaula, West Maui, showed one pair of double-length rods; while strains collected from Koolau Gap, Waikamoi, and Ainahou Valley had three pairs of double-length rods. The chromosomes of a strain of this species from Waikamoi are shown in Figure 17, where three pairs of long rods are visible. *Drosophila adiastola* from Kaulalewelewe and Hanaula, West Maui, had one pair of double-length rods; while no double-length rods were recorded from collections at Waikamoi and Waihoi Valley. In *D. cilifera*, all rods were described as equal in length in collections from Hanaula, West Maui; while a double-length rod with a secondary constriction was identified as the X chromosome in a collection from Mapuleha Gulch, Molokai.

In *Drosophila spaniothrix* from Oahu, variation was found among individuals within a single locality. Either one pair of double-length rods or two pairs of double-length rods plus a single double-length rod were found among material examined from Kaunala Gulch, Oahu. Similarly, in *D. gymnobasis*, most slides examined showed metaphases with two pairs of double-length rods, but in some slides, only one pair of double-length rods could be seen; both types were collected

FIGURE 13. *Drosophila heedi* female (W5L-Flux 4) from Ahumoa, Hawaii; X chromosome identified by presence of large blocks of heterochromatin and satellites. Heterochromatic rod-shaped chromosome 6 indicated by arrows. (G-Banding).

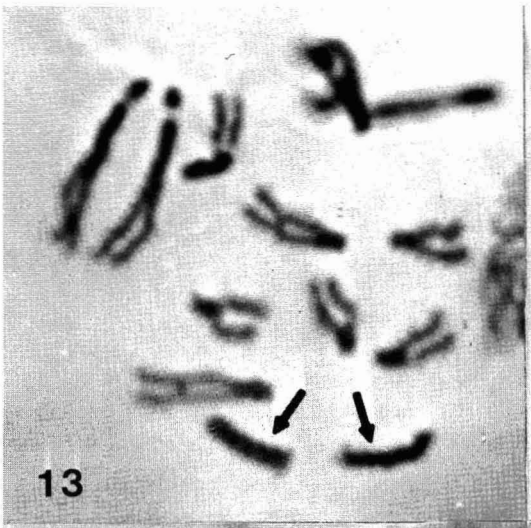
FIGURE 14. *Drosophila heedi* male (W5L-Flux 4) from Ahumoa, Hawaii; sex chromosomes identified by "X" and "Y". (G-Banding).

FIGURE 15. *Drosophila gymnobasis* female (M64G2) from Auwahi, Maui; sex chromosomes indicated by "X". (G-Banding).

FIGURE 16. *Drosophila disjuncta* male (W6L14–17) from Waikamoi, Maui; chromosome indicated by arrow differs from other autosomes. (G-Banding).

FIGURE 17. *Drosophila spectabilis* female (W6L10–12) from Waikamoi, Maui; note three pairs of double-length rods. (G-Banding).

FIGURE 18. *Drosophila silvestris* female (U51Y32) from Hualalai, Hawaii; eleven rods present. Note the heterochromatic nature of the extra rod indicated by arrow. (N-Banding).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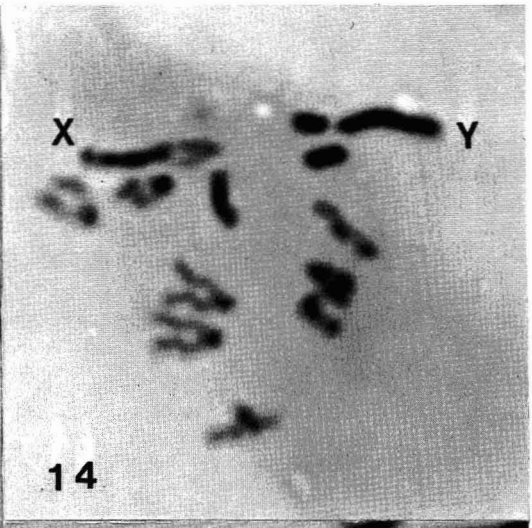
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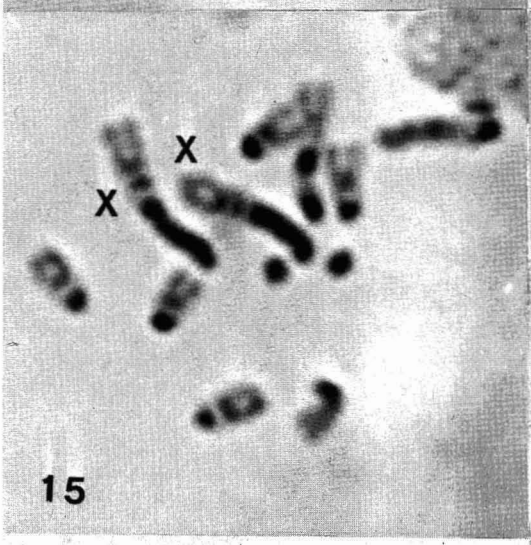
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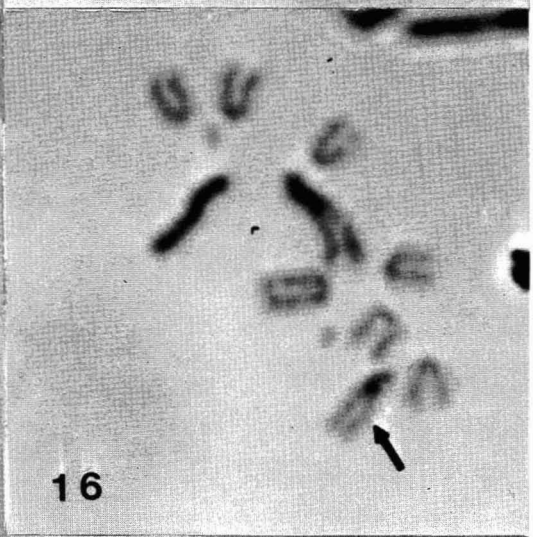
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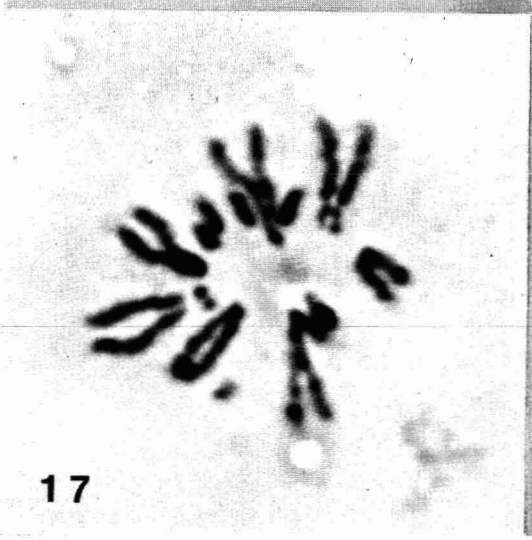
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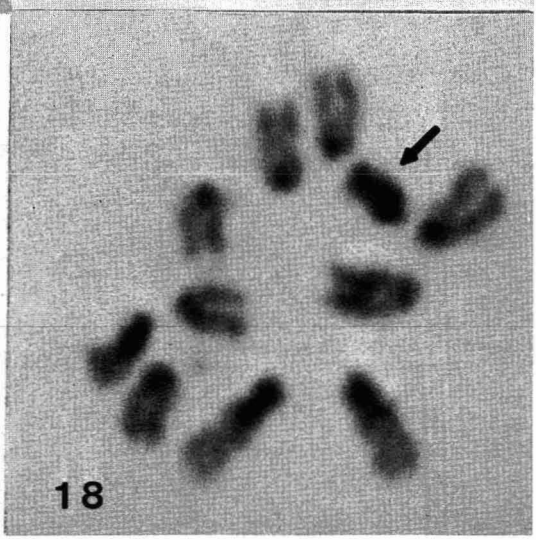
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from Auwahi, Maui. In *D. liophallus* from Kawela Gulch, Molokai, and *D. setosimentum* from several localities on Hawaii, the rod length was variable.

In most collections on Oahu, *Drosophila crucigera* had the basic configuration without modification. However, preparations from Mauna Kapu and Palikea Trail had metaphases with very large dots, quite distinct from those of other localities. Collections from Kaau Crater Trail yielded chromosome preparations that were variable; both normal dots and large dots were described from this population. The *D. crucigera* from Kauai did not have the large dots. *Drosophila setosimentum* metaphases from different collecting areas showed the same type of variation. Large dots were present in karyotypes from Moanuiahea, Pawaina, and Pauahi, while those from Kipuka 9 (BM 5108 ft., Saddle Road), Mountain House, Haleuanui, and Kipuka at 4140 ft. did not have the large dots.

During the 20-year period of karyotype analysis, abnormalities in chromosome numbers also have been observed. Several polyploid larvae were found in which all dividing cells had the increased chromosome number. A triploid has been described from larvae of *Drosophila murphyi* and *truncipenna*, while a tetraploid larva of *D. neoperkinsi* was recorded. The presence of an extra rod or the absence of one rod has been described in several species. Of particular interest is the occurrence of 11 rods and two dots in samples of *D. silvestris* from two different localities. Clayton (1971) described the presence of 11 rods in an individual larva in a collection from Puu Laalaau, South Kohala Mountains, Hawaii. During the recent examination of material prepared by Giemsa procedures, a similar condition was found in *D. silvestris* from Hualalai, Hawaii. This extra rod was found to be heterochromatic; a single heterochromatic rod was present in mitotic figures from the female (Figure 18), while two rods were heterochromatic in males. Among slides of *D. silvestris* from the Kilauea Forest Reserve, one individual was found in which all dividing cells had only nine rods and two dots. Larvae of *D. prolaticilia* and *D. neoperkinsi* also have been described with karyotypes of nine rods and two dots.

During the examination of larvae brought into the laboratory directly from *Myoporum* fluxes, a number of abnormalities were found among dividing cells of *silvarentis* larvae. Among abnormalities recorded were extremely large dots, three dots or four dots instead of the normal two, one additional rod, an extra V-shaped Y chromosome, and tetraploidy. Such abnormalities found among larvae brought into the laboratory from the field may have been from larvae that would not have survived beyond this immature stage.

Yoon and Richardson (1978*b*) described ectopic pairing in Hawaiian *Drosophila* in which heterochromatic sites paired although they were nonhomologous. White (1973) noted that ectopic pairing is a general feature of heterochromatin, found particularly in polytene chromosomes of *Drosophila*; he also described a condition in which heterochromatic regions of different chromosomes adhere temporarily during prophase of meiosis. Yoon and Richardson found that ectopic pairing in polytene chromosomes of four species in the *D. hystricosa* subgroup formed pseudochromocenters. They also observed ectopic pairing in heterochromatic regions of mitotic chromosomes in *D. biseriata*. This author has not observed such pairing among somatic mitoses of picture-winged species although blocks of heterochromatin are characteristic of the chromosomes of a number of these species. The only instance that may be considered a case of unusual pairing among heterochromatic regions was reported from meiotic cells in *D. cyrtoloma* adult males (Clayton 1985).

Six species of the picture-winged group have the haploid configuration of 6R, the microchromosome having been modified by the addition of heterochromatin to form acrocentric chromosomes. Each of these species is a member of a homosequential cluster of species in which polytene chromosomes are identical, and the only cytological distinction is in mitotic figures. Yoon and Richardson (1978*a*) considered each homo-

sequential set of species that included a 6R form to be located at the terminus of a branch of the chromosome phylogeny. Their "simplified" version of the Carson and Kaneshiro (1976) phylogeny does indeed show six distinctly separate groups of homosequential species as terminal clusters. However, in the chromosomal phylogenies of Carson (1983) and Carson and Kaneshiro (1976), two of these six sets of species occur on the same branch of the phylogeny (Figure 19). Differing from the standard polytene banding pattern of *Drosophila grimshawi* by the 2b inversion is a cluster of four species, *D. villitibia*, *D. formella*, *D. lasiopoda*, and *D. flexipes*. As may be seen in the figure, *flexipes*, *villitibia*, and *lasiopoda* are homosequential, and *formella* differs by only two additional inversions. *Drosophila lasiopoda* is the 6R species in this cluster. On the same 2b branch of the phylogeny, there is an additional group of five homosequential species; Yoon and Richardson (1978*a*) placed this group into a separate branch in their version of the chromosomal phylogeny. These five species, however, share the 2b inversion and have, in addition, inversions 3g, Xa^2, and 4u. The polytene banding patterns of *D. gymnobasis*, *musaphilia*, *hawaiiensis*, *silvarentis*, and *heedi* are homosequential; *D. heedi* is the 6R species in this cluster.

In addition, five homosequential species share inversions 4b, 5d, and 3i; the 6R species in this cluster is *Drosophila psilophallus* from Oahu. This group of species is not terminal on that branch of the phylogeny. *Drosophila gymnophallus* and *D. liophallus*, which are homosequential, share the inversions with *tarphytrichia*, *spaniothrix*, *odontophallus*, *psilophallus*, and *macrothrix*, and have, in addition, inversion Xh. It therefore appears that homosequential clusters that include 6R species do not always occur at the terminus of a branch, as proposed by Yoon and Richardson (1978*a*). These authors proposed that the 6R configurations in these homosequential clusters may represent "dead-ends" in speciation or, perhaps, early stages of divergence; after heterochromatin is distributed among the chromosomes, inversion differences may result in the disappearance of these homosequential clusters.

Based on Carson's chromosomal phylogeny and his analysis of founder events (Carson 1983), the modern picture-winged species are represented as terminal in branches of the phylogenetic tree. Carson's hypothesis is based on the differentiation of new species on each island or island complex following successful colonization by one or a few founders. In this context, the conclusion by Yoon and Richardson (1978*a*) that each 6R species is terminal on a branch of the chromosome phylogeny is not considered by this author as being a unique feature of 6R species. What does seem to be a relevant feature of homosequential clusters is the differentiation in metaphase karyotypes that has occurred in several cases when more than one homosequential species in a cluster inhabits a single island or island complex.

Four of the six homosequential clusters that include 6R species have two or more species from one island or island complex. In each of these four cases, the metaphase configurations differ from one another sufficiently to distinguish these species on the basis of metaphase karyotypes alone. The other two homosequential clusters include two or three species with no more than one species per island. As described earlier, the homosequential cluster of species that share the 2b inversion includes two species from the Maui complex, *Drosophila villitibia* from Molokai and *D. lasiopoda* from Maui. Although homosequential and derived from a common founder (#34), these two species may be differentiated on the basis of metaphase configuration. Similarly, among the species sharing inversions 3g, Xa^2, and 4u in addition to the 2b inversion, there are three homosequential species on Hawaii. These three species also may be distinguished on the basis of metaphase karyotypes. In this same set of homosequential species is *D. gymnobasis* from Maui; it differs from *D. recticilia*, also from Maui, only by the 5g inversion. These two species differ in the size of the dot chromosome in mitotic figures; *D. gymnobasis* has a large heterochromatic dot while *D. recticilia* has a small dot.

Three homosequential species from Oahu

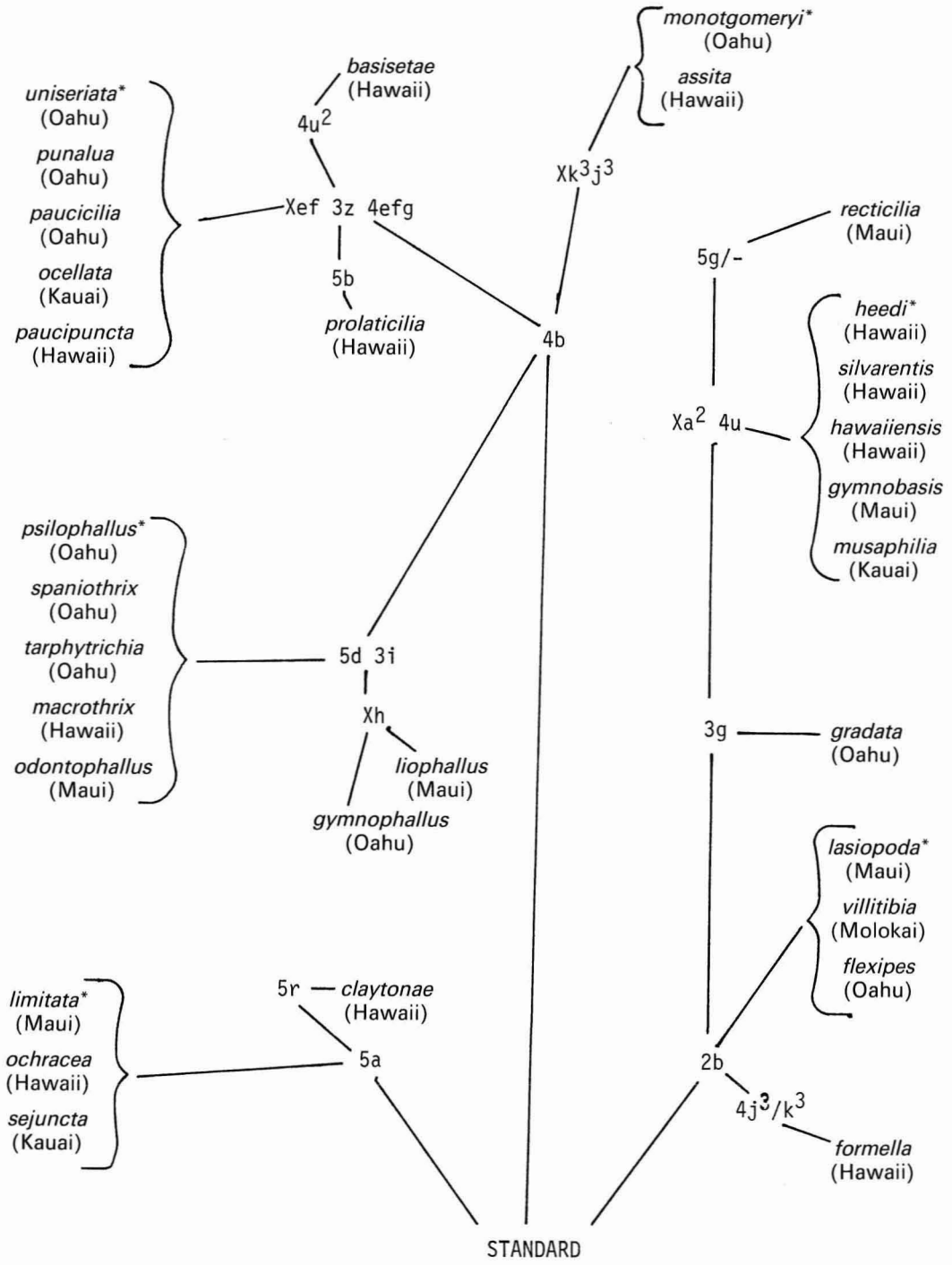

FIGURE 19. Chromosomal phylogeny modified from Carson (1983) showing homosequential clusters of species that include one species with the 6R karyotype. (The 6R species are indicated by *.)

that share inversions 4b, Xef, 3z, and 4efg differentiated from a common founder (#7). One of these, *Drosophila uniseriata*, has a 6R configuration; the other two, *D. punalua* and *D. paucicilia*, are 5R, 1D species and may be differentiated on the basis of chromosome length. A fourth homosequential species in this cluster is *D. paucipuncta* from Hawaii, a 5R, 1D species that differs from *D. basisetae* by the $4u^2$ inversion and from *D. prolaticilia* by the 5b inversion.

It appears that when several species on one island or island complex have differentiated from a single founder, diversity among these species is present in the form of paracentric inversions and/or heterochromatin modifications such as 6R, large dots, and variations in chromosome length.

It is of interest to note that the three species with metacentric chromosomes are all inhabitants of the Maui complex. *Drosophila cyrtoloma* and *D. melanocephala* are members of the *planitibia* subgroup, and *D. prostopalpis* is the only species of the *punalua* subgroup located on the islands of the Maui complex. In this group of islands, where the largest number of picture-winged species are found, the greatest number of heterochromatin modifications also are found. This allows cytological differentiation of homosequential or closely related species.

Although no conclusion can be reached at this time concerning the origin of the added heterochromatin, it may be noted that in both *melanocephala* and *prostopalpis* (Figures 2 and 7) large amounts of heterochromatin are associated with the modified chromosomes, while the remainder of the acrocentric chromosomes have relatively small amounts of heterochromatin. This is not the case in *Drosophila cyrtoloma*, however, in which all chromosomes have added heterochromatic arms.

In summary, metaphase karyotype variations resulting from differences in amount and distribution of heterochromatin make possible the cytological distinction among homosequential and closely related species. With more refined heterochromatin techniques it may be possible to demonstrate additional cytological differences among these closely related species of the Hawaiian picture-winged *Drosophila*.

APPENDIX

SUMMARY OF COMMENTS ON KARYOTYPES OF HAWAIIAN PICTURE-WINGED *Drosophila*

ISLAND/SUBGROUP/ SPECIES/KARYOTYPE	FOUNDER*	COMMENTS
Kauai (12 species)		
primaeva subgroup		
primaeva (5R, 1D)		All rods approximately equal in length
attigua (5R, 1D)		Two pairs of rods slightly longer, not double in length
planitibia subgroup		
picticornis (5R, 1D)		Secondary constrictions observed in 1–3 pairs of rods
adiastola subgroup		
ornata (5R, 1D)		Large dots; one pair of rods almost double in length
punalua subgroup		
ocellata (5R, 1D)		One pair of rods double in length
grimshawi subgroup		
crucigera (5R, 1D)	45	No modification noted; no large dots
glabriapex (5R, 1D)		No modification noted
grimshawi (5R, 1D)		One pair of rods slightly longer
micromyia (5R, 1D)		No modification noted
musaphilia (5R, 1D)	12	One pair of rods longer, not double in length; secondary constrictions
sejuncta (5R, 1D)		No modifications noted
villosipedis (5R, 1D)		One pair of rods almost double in length

APPENDIX (continued)

ISLAND/SUBGROUP/ SPECIES/KARYOTYPE	FOUNDER*	COMMENTS
Oahu (29 species)		
planitibia subgroup		
hemipeza (5R, 1D)	16	All rods approximately equal in length
oahuensis (5R, 1D)	16	All rods approximately equal in length
nigribasis (5R, 1D)	26	One longer rod paired with normal rod; dots very small
substenoptera (5R, 1D)	2	Secondary constrictions in two pairs of rods; all rods approximately equal in length
adiastola subgroup		
neogrimshawi (5R, 1D)	14	Two pairs of rods longer but not double in length
touchardiae (5R, 1D)	23	No modification noted
punalua subgroup		
paucicilia (5R, 1D)	7	One pair of rods almost double in length
punalua (5R, 1D)	7	One longer rod with secondary constriction paired with longer rod or one pair of longer rods with constrictions
uniseriata (6R)	7	One pair of rods double in length
grimshawi subgroup		
aglaia (5R, 1D)	4	One pair of rods slightly longer
distinguenda (5R, 1D)	4	No modifications noted
divaricata (5R, 1D)	4	No modifications noted
gymnophallus (5R, 1D)	4	No modifications noted
hexachaetae (5R, 1D)	4	No modifications noted
inedita (5R, 1D)	4	Very large dots; all rods approximately equal in length
montgomeryi (6R)	4	All rods approximately equal in length
pilimana (5R, 1D)	4	One pair of rods longer, not double in length; secondary constrictions
psilophallus (6R)	4	All rods approximately equal in length
spaniothrix (5R, 1D)	4	Variable; one pair of double-length rods; three pairs of double-length rods or two pairs and a single double-length rod; secondary constrictions in all double-length rods
tarphytrichia (5R, 1D)	4	Rods approximately equal in length; secondary constrictions in one pair of rods
atrimentum (5R, 1D)	5	One pair of rods longer but not double in length
crucigera (5R, 1D)	5	Large dots from Palikea and Mauna Kapu only; variable dot size from Kaau Crater Trail; X is slightly longer rod; Y is normal-length heterochromatic rod
flexipes (5R, 1D)	5	One pair of longer rods or one longer rod paired with normal rod
gradata (5R, 1D)	5	One pair of rods slightly longer but not double in length
obatai (5R, 1D)	5	One pair of rods longer with secondary constrictions (bend easily and may be confused with Vs); two other pairs of rods longer but not double in length
grimshawi (5R, 1D)	40	One pair of longer rods or one longer rod paired with normal rod; in early drawings (Molokai and Lanai) longer rod shown with secondary constriction
turbata (5R, 1D)	42	Large dots; one pair of rods greater than double in length and largely heterochromatic
reynoldsiae (5R, 1D)	43	One pair of rods approximately half-length (Peacock Flat); two pairs of rods almost double in length (Makaleha Valley)
sobrina (5R, 1D)	44	Two pairs of rods longer, one pair almost double in length; one pair with secondary constrictions; one pair of short rods
Maui Complex (40 species)		
planitibia subgroup		
cyrtoloma (5V, 1J)	25	One arm of each chromosome is heterochromatic; X is J-shaped with conspicuous heterochromatic satellite; Y is heterochromatic V
differens (5R, 1D)	25	All rods approximately equal in length
hanaulae (5R, 1D)	25	One double-length rod paired with a normal rod

APPENDIX (continued)

ISLAND/SUBGROUP/ SPECIES/KARYOTYPE	FOUNDER*	COMMENTS
ingens (5R, 1D)	25	One pair of rods longer but not double in length
melanocephala (5R, 1V)	25	Metacentric has one heterochromatic arm; one pair of rods heterochromatic; remaining rods very short
neoperkinsi (5R, 1D)	25	All rods approximately equal in length
neopicta (5R, 1D)	25	All rods approximately equal in length
obscuripes (5R, 1D)	25	One pair of rods longer but not double in length
planitibia (5R, 1D)	25	All rods approximately equal in length
adiastola subgroup		
adiastola (5R, 1D)	1	One pair of double-length rods from Kaulalewelewe and Hanaula; one pair longer rods but no double-length rods from Puu Kukui, Waihoi Valley, Waikamoi
clavisetae (5R, 1D)	1	One pair of longer rods, not double in length, or one longer rod with secondary constriction paired with normal rod
cilifera (5R, 1D)	1	One pair of rods unequal in length; longer, almost double-length rod, with a secondary constriction
hamifera (5R, 1D)	1	One pair of rods slightly longer but not double in length
paenehamifera (5R, 1D)	1	All rods approximately equal in length
peniculipedis (5R, 1D)	1	One pair of rods longer but not double in length
spectabilis (5R, 1D)	1	One pair of double-length rods from Hanaula; three pairs of double-length rods from Ainahou Valley and Waikamoi
truncipenna (5R, 1D)	1	One pair of extremely large rods, largely heterochromatic; other rods shorter than normal
varipennis (5R, 1D)	1	One pair of rods longer, almost double in length
punalua subgroup		
prostopalpis	24	One arm of metacentric chromosome is heterochromatic; configuration verified by anaphase
grimshawi subgroup		
affinidisjuncta (5R, 1D)	6	No double-length rods (as *disjuncta* from Kaulalewelewe)
balioptera (5R, 1D)	6	One longer rod identified as X paired with normal-length rod Y
bostrycha (5R, 1D)	6	All rods approximately equal in length
disjuncta (5R, 1D)	6	One pair double-length rods; in some slides a third single double-length rod from Kipahulu Valley only; in other areas one pair rods slightly longer but not double in length
orphnopeza (5R, 1D)	6	One pair double-length rods; one pair longer but not double-length from Waikamoi only
orthofascia (5R, 1D)	6	All rods approximately equal in length
sodomae (5R, 1D)	6	All rods approximately equal in length
discreta (5R, 1D)	8	Two pairs of longer rods in females; in males, one pair of longer rods and one longer rod paired with normal rod
fasciculisetae (5R, 1D)	8	Same as described above for *discreta*
lineosetae (5R, 1D)	8	One pair longer rods or one longer rod paired with normal rod
vescisetae (5R, 1D)	8	One pair longer rods or one longer rod paired with normal rod
virgulata (5R, 1D)	9	One pair of rods longer but not double-length; two pairs of short rods
gymnobasis (5R, 1D)	11	Large dots; two pairs of double-length rods or large dots and one pair double-length rods (both collections from Auwahi)
recticilia (5R, 1D)	11	Two pairs of double-length rods or one pair of double-length rods and one double-length rod paired with longer rod
limitata (6R)	13	One pair slightly longer rods; one pair of short heterochromatic rods
odontophallus (5R, 1D)	28	One pair rods almost double in length; second pair longer with secondary constrictions, often appearing V-shaped
liophallus (5R, 1D)	29	Two pairs of rods shorter than normal; appears to be variable
hirtipalpus (5R, 1D)	34	All rods approximately equal in length; large dots from Hanaula, not Waikamoi
lasiopoda (6R)	34	One pair heterochromatic rods shorter than other autosomes; XY longer rods, Y largely heterochromatic

APPENDIX (continued)

ISLAND/SUBGROUP/ SPECIES/KARYOTYPE	FOUNDER*	COMMENTS
villitibia (5R, 1D)	34	One pair longer rods or one longer rod paired with normal rod
grimshawi (5R, 1D)	41	One pair longer rods; in early drawings (1965) from Molokai and Lanai, longer rods had secondary constrictions
Hawaii (26 species)		
planitibia subgroup		
setosifrons (5R, 1D)	3	One pair rods longer but not double in length; in males longer rod paired with normal-length heterochromatic Y
heteroneura (5R, 1D)	17	Very small dots; all rods approximately equal in length
silvestris (5R, 1D)	17	One pair longer rods or one longer rod with secondary constriction paired with shorter rod
adiastola subgroup		
setosimentum (5R, 1D)	15	Large dots present from Moanuiahea, Pawaina, Pauahi; no large dots from Kipuka 9, Mountain House, Laupahoehoe, Haleuanui, Kipuka at 4140′; variable in different localities and among individuals: double-length rods, longer rods but not double in length, all rods normal in length
ochrobasis (5R, 1D)	15	In female, one pair of double-length rods; in male, one double-length rod paired with rod of normal length; one pair of longer rods, not double in length from Kipukas 9 and 14
basisetae (5R, 1D)	10	Two very long pairs of rods with satellites (rod shape verified by anaphase); in males one double-length rod paired with rod of normal length
paucipuncta (5R, 1D)	10	All rods approximately equal in length
prolaticilia (5R, 1D)	10	One pair double-length rods or one double-length rod paired with rod of normal length
alsophila (5R, 1D)	18	One pair of rods with satellites; two pairs of longer rods with secondary constrictions
conspicua (5R, 1D)	18	One pair of rods longer but not double in length
silvarentis (5R, 1D)	19	Very large dots; Y is a J-shaped, heterochromatic chromosome
heedi (6R)	19	One pair of rods double in length with satellites; in male, one double-length rod with satellite paired with heterochromatic double-length rod
murphyi (5R, 1D)	20	One longer rod with secondary constriction paired with a heterochromatic longer rod in males; in females, one pair of longer rods with secondary constrictions. Larger dots from Lalakea Stream
pullipes (5R, 1D)	21	All rods approximately equal in length
claytonae (5R, 1D)	22	One pair of rods almost double in length
ochracea (5R, 1D)	22	One pair of rods almost double in length
assita (5R, 1D)	27	One pair of double-length rods or one double-length rod paired with rod of normal length; double-length rod has a satellite
digressa (5R, 1D)	30	Large dots; two pairs of rods almost double in length
macrothrix (5R, 1D)	31	One double-length rod paired with normal rod, long rod frequently bent, resembling V-shape; Y is rod shorter than X
hawaiiensis (5R, 1D)	32	All rods approximately equal in length
psilotarsalis (5R, 1D)	33	All rods approximately equal in length
formella (5R, 1D)	35	Large dots; one pair of longer rods or one longer rod paired with heterochromatic Y
engyochracea (5R, 1D)	36	Small dots; two pairs of longer rods
orthofascia (5R, 1D)	37	All rods approximately equal in length
ciliaticrus (5R, 1D)	38	All rods approximately equal in length
sproati (5R, 1D)	39	One pair rods longer but not double in length; in males, one longer rod paired with normal-length rod

* Founder number is based on Carson (1983).

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