INTERGROUP PHYLOGENIES IN DROSOPHILA AS DETERMINED BY COMPARISONS OF SALIVARY BANDING PATTERNS*

HARRISON D. STALKER

Department of Biology, Washington University, St. Louis, Missouri 63130

Manuscript received June 3, 1971

ABSTRACT

A salivary gland chromosome phylogeny is presented which summarizes the evolutionary relationships of twenty-two species belonging to the sub-genus Drosophila, and members of the twelve species groups: *D. melanica, D. repleta, D. carbonaria, D. polychaeta, D. annulimana, D. robusta, D. carsoni, D. virilis, D. funebris* and the "picture-wing," *D. mimica* and *D. crassifemur* groups (of Hawaii).——Photographic salivary chromosome maps were prepared for all twenty-two species studied. While the chromosomes of different species belonging to the same group can usually be homologized almost completely, so that construction of intragroup phylogenies is easy, chromosomes of members of different groups are so modified structurally that in most cases only short sections can be fully homologized, and these in only one or two chromosome elements.——Broadly homologous chromosome elements were compared for three species at a time, and on the basis of overlapping homologous sections, or overlapping inversions included within homologous sections, the trio of chromosomes, and the species to which they belonged can often be arranged in a two-step phylogenetic series. Detection of many such ordered trios permits construction of a single phylogenetic scheme encompassing all species.—— *D. nigromelanica*, of the *D. melanica* group is found to be chromosomally intermediate between the rest of its group and the species belonging to other groups, suggesting that it is the most nearly ancestral member of its group. When trios of species including *D. nigromelanica* and members of two other species groups are compared, it is found that in twelve of fourteen such comparisons the chromosomes of *D. nigromelanica* are structurally intermediate between those of the members of the other two species groups, indicating the central position of *D. nigromelanica* in the phylogeny as a whole.——Available cytological evidence indicates that among the nine continental groups studied, it is the *D. robusta* group which is chromosomally closest to the Hawaiian "picture-wing" groups. Among the members of the Hawaiian groups it is *D. primaeva* and *D. attigua* which are found to be closest to the continental species. This finding tends to confirm the earlier conclusion of CARSON and STALKER, based on different evidence, that the above two species were in an ancestral position in the Hawaiian phylogeny.——The relationship of the *D. robusta* and *D. melanica* groups demonstrated in this paper, the phylogenies within each of these two groups earlier worked out by NARAYANAN and by STALKER, and the present geographical distributions of the species within them, require that at least three Asiatic–New World migrations must have occurred during the evolution of the two groups.

IN the course of a series of studies of the *Drosophila melanica* species group, STALKER (1966) developed a photographic method for comparing species

* The work reported in this paper was supported by a grant from The National Science Foundation, NSF GB-7754.

banding patterns for those forms which could not be studied by production of hybrids. The ultimate aim of the development of this method was to determine the phyletic relationships of different species groups by photographic chromosome comparisons. The present paper describes such intergroup phyletic studies.

All species studied were members of the subgenus Drosophila. The choice of species used in the analyses was based on availability, possession of workable salivary chromosomes, and the representation of as many species groups as possible, especially those groups which, on the basis of their morphology, were believed to be closely related to the previously investigated *D. melanica* and *D. robusta* species groups. In this paper, all of the endemic Hawaiian species are, for the convenience of the reader, labelled "H". Thus, the Hawaiian species studied were: *D. primaeva-H*, *D. punalua-H*, *D. attigua-H*, *D. grimshawi-H*, *D. crucigera-H*, *D. amphilobus-H* and *D. mimica-H*. All of these species, with the exception of *D. mimica-H* and *D. amphilobus-H* belong to the so-called "picture-wings", which have been divided into five subgroups by CARSON and STALKER (1968, 1969). *D. mimica-H* belongs to a separate species group closely related to the picture-wings. The continental species studied were: *D. micromelanica*, *D. nigromelanica*, *D. melanura* and *D. pengi*, all of the *D. melanica* group; *D. funebris*, *D. multispina*, *D. subfunebris* and *D. macrospina* of the *D. funebris* group; *D. carbonaria* of the *D. carbonaria* group; *D. repleta* of the *D. repleta* group; *D. carsoni* of the *D. carsoni* group; *D. polychaeta* of the *D. polychaeta* group, *D. colorata* of the *D. robusta* group; *D. virilis* of the *D. virilis* group; and *D. gibberosa* of the *D. annulimana* group.

Much of the material was generously donated by other workers. Worthy of special mention in this respect are Dr. MARSHALL WHEELER, Dr. LYNN THROCKMORTON, Dr. HAMPTON CARSON and Miss KATHLEEN RESCH. The author is indebted to MARION L. STALKER for help in the preparation of plates, to Miss JEAN COUGHLIN for her skill and persistence in the preparation of chromosome smears, and to Miss ROMANY HUCK for much of the darkroom work involved in preparing maps and voucher prints. Finally, the author is indebted to Drs. HAMPTON CARSON and LYNN THROCKMORTON for their discussions and criticisms, although neither can be held responsible for the conclusions presented.

METHODS

Lactic-acetic-orcein salivary chromosome squashes were prepared for each species, and a large number of photomicrographs were made from these preparations. It was found that in most cases approximately 200 individual photographs were needed for the analysis of a species. From the original photographs maps were prepared for each chromosome element, an attempt being made to choose the most characteristic photographs for each map section. The photographic maps were mounted on opaque white plexiglass plates, each covered with a thin protective sheet of transparent plexiglass. The maps of homologous chromosomes from any three species being compared were temporarily assembled in a frame, the three being covered with a transparent cover sheet of plexiglass to be marked with a grease pencil as the analysis proceeded. As study of the three chromosomes revealed apparently homologous sections, these were marked on the cover sheet. Finally the original markings were corrected after numerous voucher photographs had been consulted, and the apparent homologies either confirmed or disproven. The confirmed homologies were then permanently recorded on prints of the original maps, and the

marked plexiglass cover sheet set aside in case further study of the three chromosomes was necessary at some later date. It should be emphasized that the determination of homologous regions was based on a study of the numerous voucher photographs, not on the maps, the latter being used simply as convenient and essential bookkeeping devices. With this method of analysis it is obvious that very short homologous regions, or those which were regularly indistinct in squash preparations could not be detected and studied.

RESULTS

The use of salivary chromosome partial homologies in the determination of phyletic relationships: When chromosome banding pattern comparisons are made between species of Drosophila belonging to the same species group it is frequently possible to identify homologous interspecific regions involving over 90% of the total chromosome material. In comparisons between species belonging to different species groups however, this is almost never possible, and if intergroup phyletic relationships are to be determined cytologically, these determinations must be based on a relatively small percentage of the total chromosome material.

In comparisons of the chromosomes of the *D. melanica* group species with those of species outside the group it soon became apparent that two chromosome elements were particularly useful, namely those labelled "XL" and 3 in the *D. melanica* group (STALKER 1966). "XL" is one arm of the two-element V-shaped *X* chromosome found in five of the members of the *D. melanica* group; in two other member species, and in many species outside the group, the *X* is a single element, and "XL" is in fact a separate autosome, or one arm of a two-element V-shaped autosome. Chromosome 3 in the *D. melanica* group is usually a single rod-shaped autosome.

The "XL" element in the *D. melanica* group shows banding pattern affinities with chromosome 2 of *D. colorata*; with chromosome 3 of *D. virilis*; with chromosome 4 of *D. repleta*; and with chromosome 5 of all of the Hawaiian endemics listed above.

Chromosome 3 of the *D. melanica* group shows affinities with chromosome 2 of the Hawaiian species; with chromosome 4 of *D. colorata*; and with chromosome 5 of *D. repleta* and of *D. virilis*.

Since the majority of the intergroup comparisons to be discussed involve one or more members of the *D. melanica* group, and since it has not yet been possible to apply a uniform intergroup chromosome labelling system, the chromosome designations as they are applied to the *D. melanica* group will be used throughout this paper for all species discussed.

If a given chromosome arm, e.g. "XL", is compared in three different species, it may be possible, by identifying certain homologous regions for the chromosomes of the three species, to determine which of the chromosomes is structurally, and thus presumably phylogenetically, intermediate to the other two. By an extension of this method to include trios of many different species, fairly complete chromosome phylogenies may be constructed showing both inter- and intragroup relationships.

For such an analysis only two patterns of partial homology are generally useful. The first of these, overlapping homologies, is illustrated in Figure 1, left

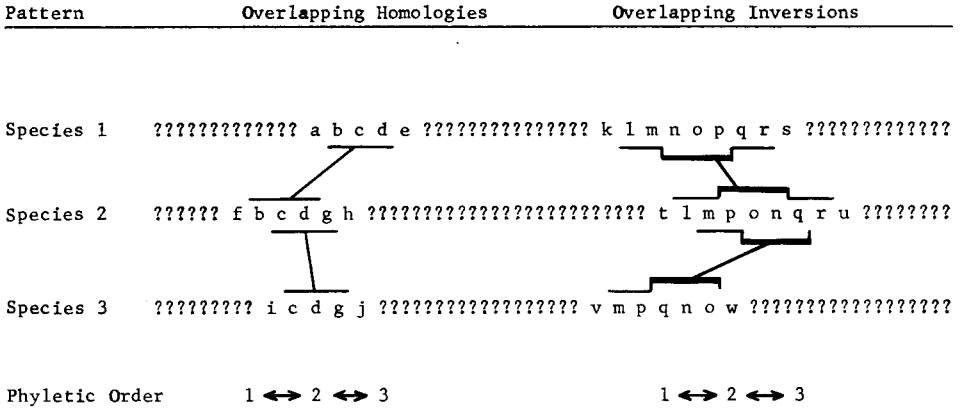

FIGURE 1.—Two diagnostically useful patterns of partial homology. The horizontal lines of symbols represent broadly homologous chromosomes of the three species 1, 2 and 3. Recognizably homologous regions are represented by underlined or overlined lower case letters. Thus, on the left side of the figure, the region b c d can be recognized only in species 1 and 2, while the shorter region c d can be recognized in all three species. Adjacent regions such as a, e, f, h, i and j, and those regions represented by ?????, can be recognized in one species only. The letters do not represent individual chromosome bands, but rather chromosome regions. The heavy bars on the right side of the figure indicate the limits of overlapping inversions within recognizably homologous regions. See text for further explanation.

side. In this figure the regions of chromosomes which cannot be homologized are represented by ??????, or by specific lower case letters appearing in only one of the three chromosomes. Underlined or overlined lower case letters appearing in two or more chromosomes in the figure represent those areas of the chromosome which can be homologized on the basis of the banding pattern. Thus, the region c d can be found in species 1, 2 and 3, while the more extensive region b c d occurs only in species 1 and 2. It will be noted that species 1 and 2 share the b c d region, while species 3 has the b c d region broken up by the removal of the b part, but resembles species 2 in having the c d g sequence, which is absent in species 1. Since the f b c d g h sequence in species 2 is clearly structurally intermediate between the a b c d e of species 1, and the i c d g j of species 2, the phyletic species order, based on this section is: 1 ↔ 2 ↔ 3. This conclusion of course rests on the assumption that inversions such as those that control the spatial relationship of b with c and of g with d will occur only once in the phylogeny under consideration. Figures 2, 3, 4 and 5 show examples of trios of species with partial overlapping homologies.

The other potentially useful (and essentially similar) partial homology is illustrated on the right side of Figure 1. Here two overlapping inversions (indicated by heavy bars) occur wholly within the recognizably homologous area. Such overlapping inversions can then be used in the usual way to establish the structural and phyletic chromosome order: 1 ↔ 2 ↔ 3 which, as in the case above, assumes the non-repeatability of the two inversions within the phylogeny under consideration.

The phyogenetic relationships within the D. melanica *group:* STALKER (1966)

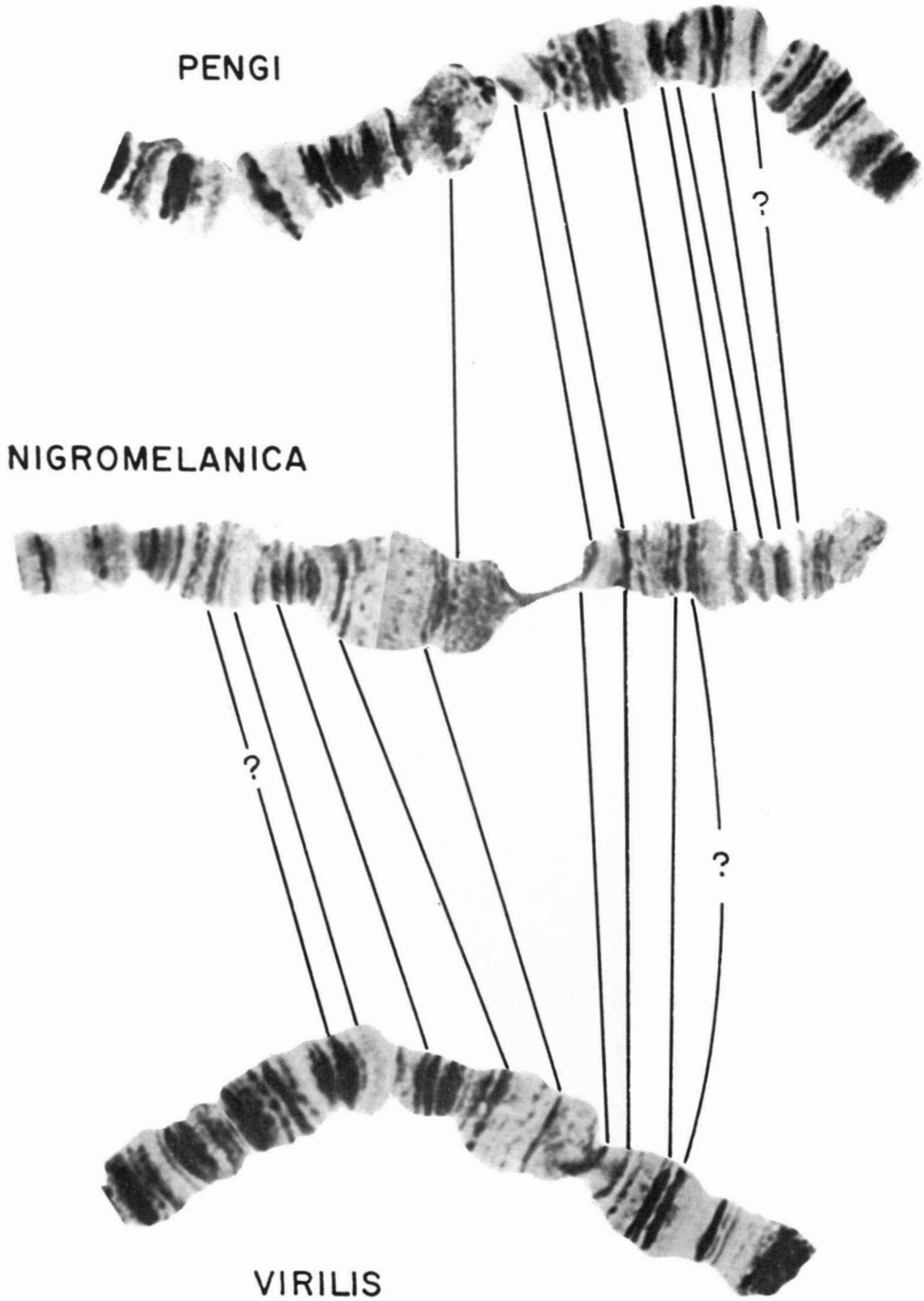

FIGURE 2.—Overlapping homologies in *D. pengi*, *D. nigromelanica* 3, and *D. virilis* elements. Homologous regions connected by heavy lines indicate a phylogenetic sequence with *D. nigromelanica* between the other two species. See text.

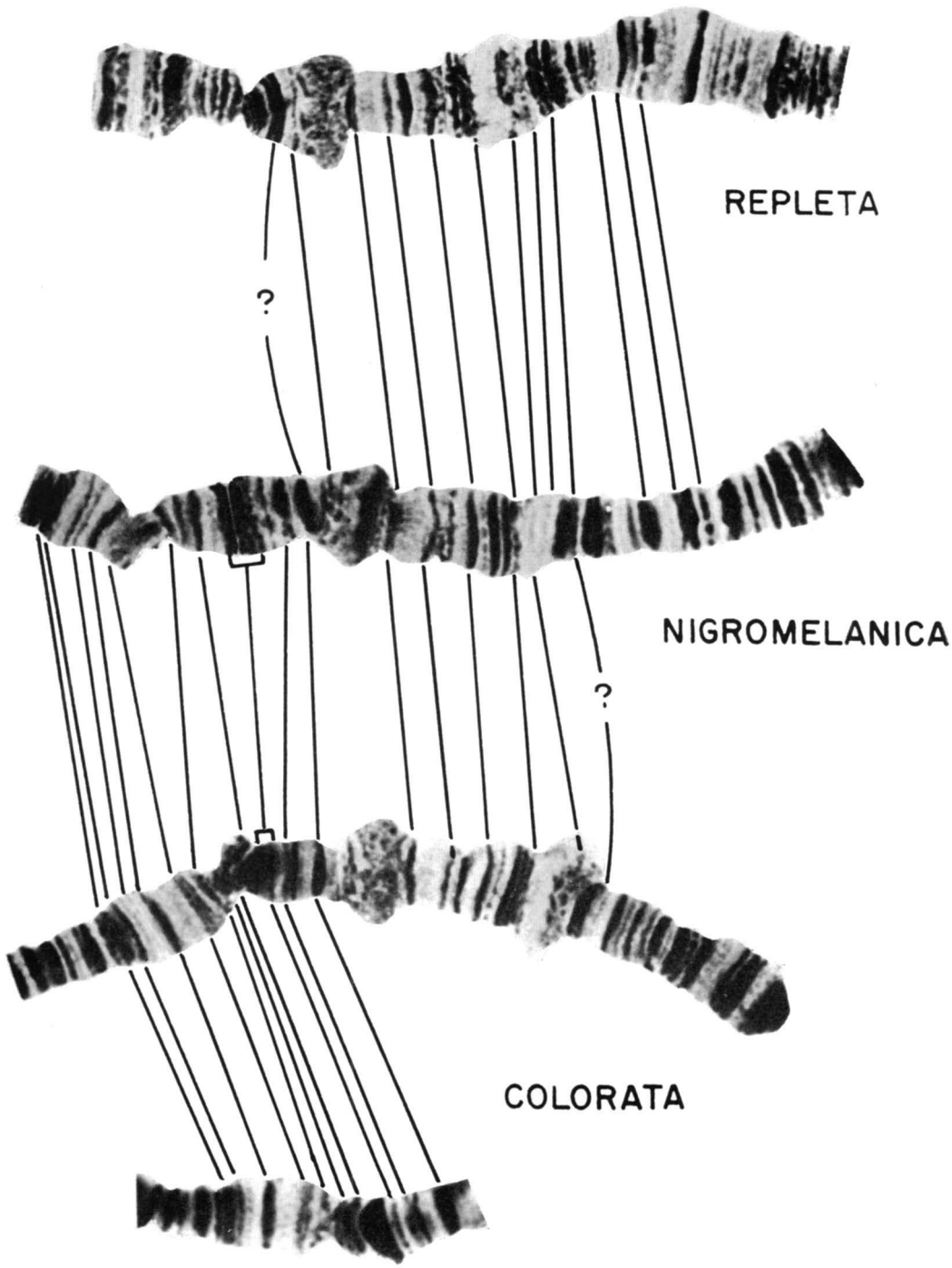

FIGURE 3.—Overlapping homologies in *D. repleta*, *D. nigromelanica* XL, and *D. colorata* elements. Homologous regions connected by heavy lines indicate a phylogenetic sequence with *D. nigromelanica* between the other two species. In this figure two examples of *D. colorata* are shown. See text.

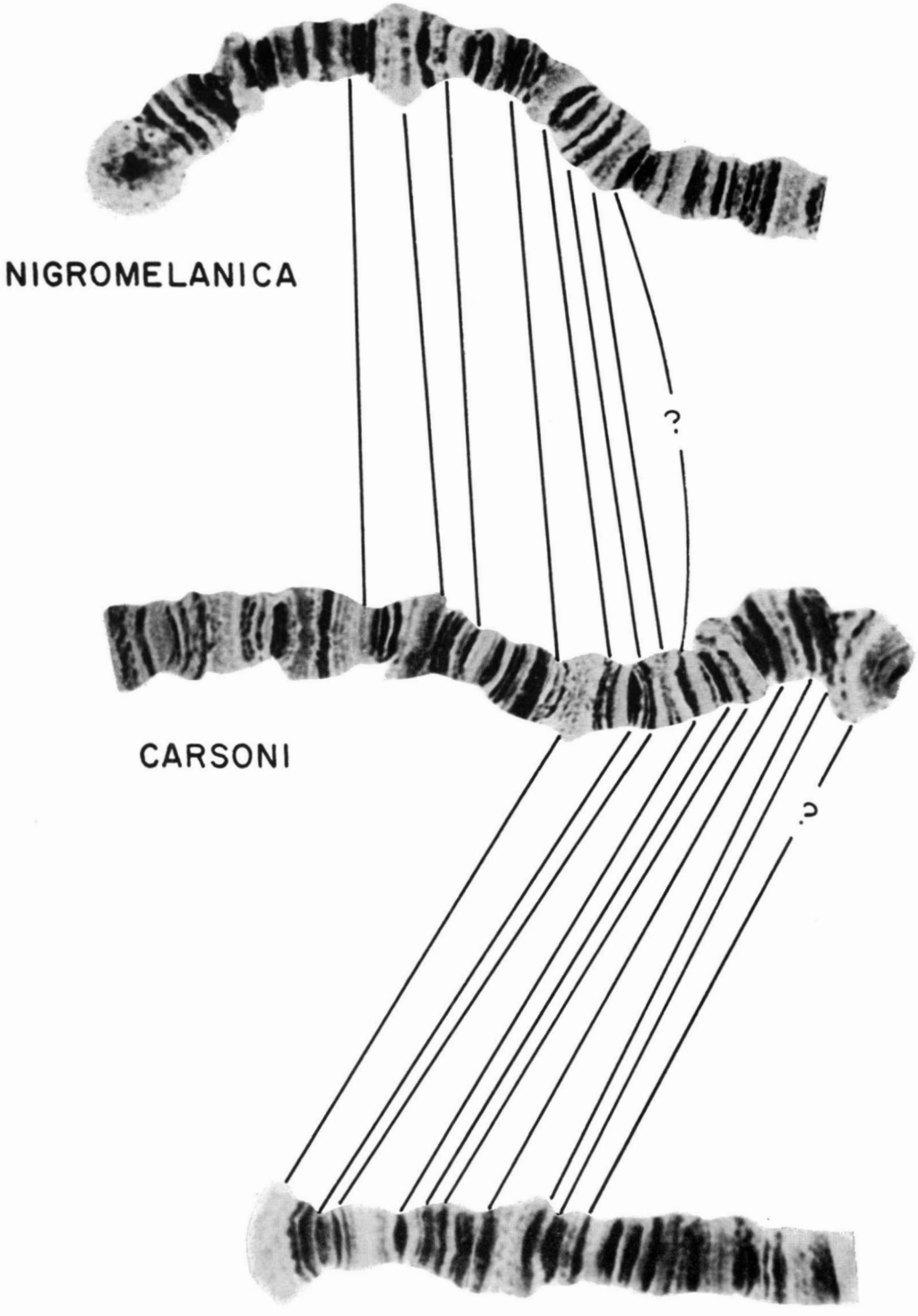

FIGURE 4. —Overlapping homologies in *D. nigromelanica* XL, *D. carsoni* and *D. polychaeta* chromosome elements. Homologous regions connected by heavy lines indicate a phylogenetic sequence with *D. carsoni* between the other two species. See text.

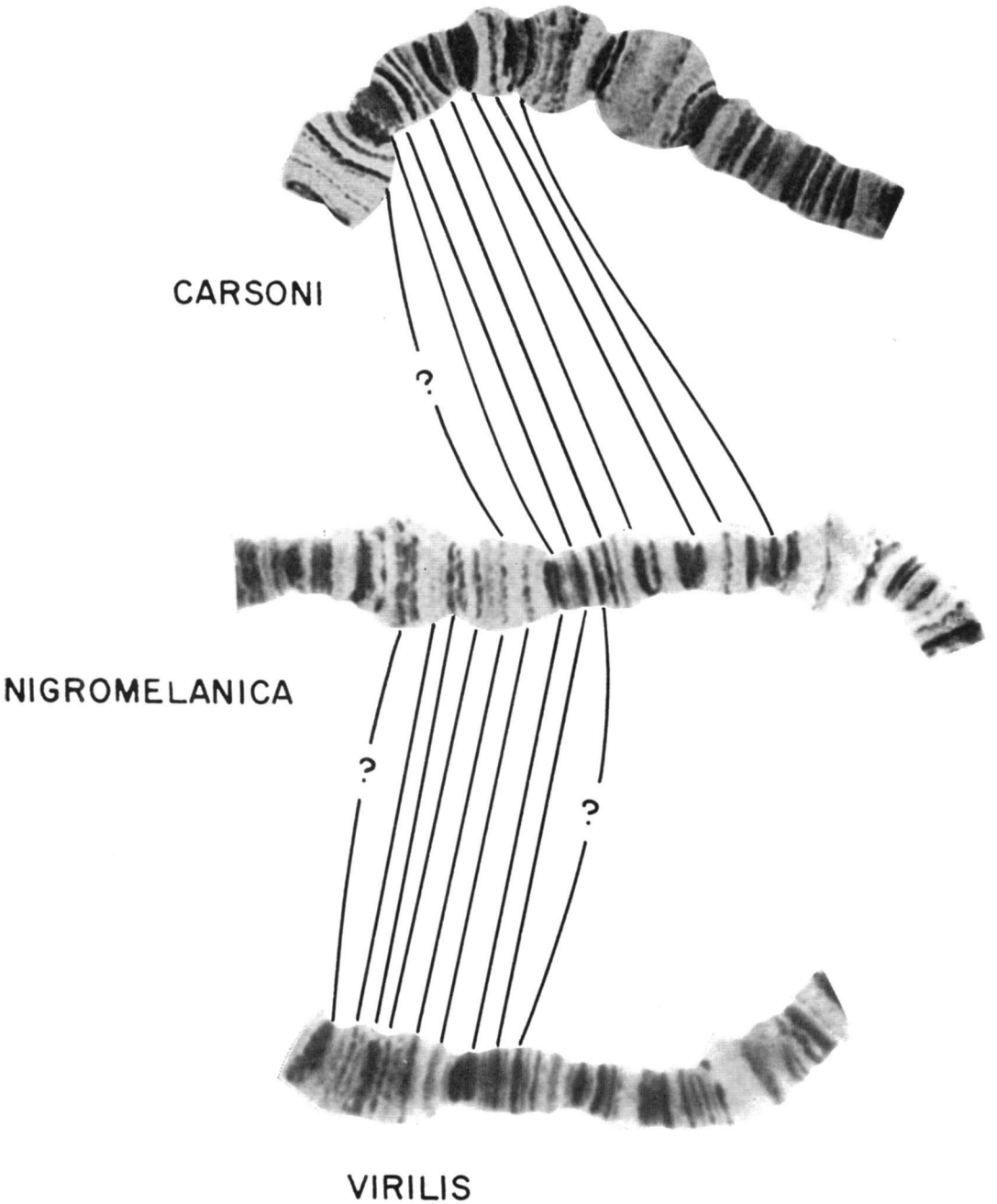

FIGURE 5.—Overlapping homologies in *D. carsoni*, *D. nigromelanica* 3, and *D. virilis* elements. Homologous regions connected by heavy lines indicate a phylogenetic sequence with *D. nigromelanica* between the other two species. See text.

analyzed the salivary chromosomes of the six New World members of the *D. melanica* group then available, and determined that these species could be represented phylogenetically in the form of a linear series: *D. nigromelanica–D. micromelanica–D. melanura–D. euronotus–D. paramelanica–D. melanica.* Of these six species, all but *D. micromelanica* have a two-element, V-shaped *X* chromosome. In *D. micromelanica* the *X* is a single-element rod, corresponding to XR in the other five species, while the arm which is XL in the other five appears as a rod-shaped autosome in *D. micromelanica.*

Since these results were published, a Japanese member of the group, *D. pengi,* has become available for study through the courtesy of Drs. T. OKADA and MARSHALL WHEELER. *D. pengi* differs from all other group members in having autosomal elements 2 and 3 fused to form a single V-shaped 2–3 compound autosome. However, this species resembles *D. micromelanica* in having a rod-shaped *X*, with the "XL" element appearing as a separate rod-shaped autosome.

WHARTON (1943) has described one strain of *D. micromelanica* in which fusion of two major autosomal arms existed. Unfortunately it is not known whether arms 2 and 3 were involved, as in *D. pengi,* and the strain in question is no longer available.

In comparisons of *D. pengi* with other *D. melanica* group species no very serious attempt has been made to work out homologies for the *X*-chromosome element (this element is traditionally refractory in interspecific comparisons), and only fragments of chromosome 2 have been homologized. However, complete interspecific homologies have been worked out for autosomes "XL", 4, and the 3 element of the 2–3 compound autosome.

When the trio: *D. pengi, D. micromelanica* and *D. nigromelanica* are compared for chromosome "XL" it is found that although *D. pengi* differs from both of the other species by a number of fixed inversions, these inversions do not overlap any of those by which *D. micromelanica* differs from *D. nigromelanica,* so a phyletic order cannot be determined. A similar, equally frustrating situation holds for chromosome 4. In the case of chromosome 3, the structural and phyletic order, based on an overlapping homology, is clearly: *D. pengi–D. micromelanica–D. nigromelanica,* (Table 1, comparison 11). The relationship of *D. pengi* to other members of the *D. melanica* group has been tested specifically in this study in the case of *D. melanura,* and may be inferred for the rest of the group on the basis of previously published data (STALKER 1966). When the trio: *D. pengi, D. micromelanica* and *D. melanura* are compared, the phyletic order is found to be: *D. pengi–D. micromelanica–D. melanura,* (Table 1, comparison 12). Thus, the seven species in the group may now be represented as shown in Figure 6.

It is not possible at this stage to determine whether the *D. melanica* species group originated in the New World, and *D. pengi* represents a migrant to Asia via the Bering Bridge, or whether the early evolution of the group occurred in Asia, with *D. pengi* left behind. Despite the fact that the majority of the *D. melanica* group are presently found in the New World, the possibility of an Asiatic origin cannot be lightly dismissed, since, as will be shown below, the *D. melanica* group has strong cytological affinities to the *D. robusta* group, most of whose members are Asiatic.

TABLE 1

Phylogenetic order of trios of species as determined by analysis of recognizably homologous sections of salivary gland chromosomes. Endemic Hawaiian species are indicated by "H"

Comparison Number	Phylogenetic order of trio			Chromosome element	Homology patterns
1	*repleta*	*nigromelanica*	*micromelanica*	"XL"	1 overlapping
2	*gibberosa*	*nigromelanica*	*micromelanica*	"XL"	1 overlapping
3	*virilis*	*nigromelanica*	*micromelanica*	3	1 overlapping
4	*carsoni*	*nigromelanica*	*micromelanica*	"XL"	2 overlapping
5	*carbonaria*	*nigromelanica*	*micromelanica*	"XL"	3 overlapping
6	*polychaeta*	*nigromelanica*	*micromelanica*	"XL"	1 overlapping
7	*mimica-H*	*nigromelanica*	*micromelanica*	"XL"	1 overlapping
8	*primaeva-H*	*nigromelanica*	*micromelanica*	"XL"	1 overlapping
	primaeva-H	*nigromelanica*	*micromelanica*	4	1 overlapping
9	*colorata*	*nigromelanica*	*micromelanica*	"XL"	2 overlapping
	colorata	*nigromelanica*	*micromelanica*	3	1 overlapping
	colorata	*nigromelanica*	*micromelanica*	4	2 overlapping
10	*macrospina funebris multispina subfunebris*	*nigromelanica*	*micromelanica*	"XL"	1 overlapping
11	*pengi*	*micromelanica*	*nigromelanica*	3	1 overlapping
12	*pengi*	*micromelanica*	*melanura*	2	1 overlapping
	pengi	*micromelanica*	*melanura*	3	1 overlapping
13	*virilis*	*nigromelanica*	*pengi*	3	2 overlapping
14	*colorata*	*nigromelanica*	*pengi*	3	1 overlapping
15	*carsoni*	*nigromelanica*	*pengi*	3	1 overlapping
16	*carbonaria*	*nigromelanica*	*pengi*	"XL"	1 overlapping
17	*repleta*	*nigromelanica*	*colorata*	"XL"	1 overlapping
18	*repleta*	*nigromelanica*	*carbonaria*	"XL"	1 overlapping
19	*macrospina funebris multispina subfunebris*	*nigromelanica*	*repleta*	"XL"	1 overlapping
20	*virilis*	*nigromelanica*	*carsoni*	3	1 overlapping
21	*macrospina funebris multispina subfunebris*	*virilis*	*nigromelanica*	"XL"	1 overlapping
22	*carbonaria*	*nigromelanica*	*colorata*	"XL"	1 overlapping
23	*repleta*	*nigromelanica*	*carsoni*	"XL"	1 overlapping
24	*carbonaria*	*nigromelanica*	*carsoni*	"XL"	1 overlapping
25	*virilis*	*nigromelanica*	*polychaeta*	3	1 overlapping
26	*macrospina funebris multispina subfunebris*	*nigromelanica*	*polychaeta*	"XL"	1 overlapping
27	*carsoni*	*nigromelanica*	*primaeva-H*	"XL"	1 overlapping
28	*virilis*	*nigromelanica*	*repleta*	3	1 overlapping
29	*nigromelanica*	*carsoni*	*polychaeta*	"XL"	1 overlapping
30	*colorata*	*carsoni*	*polychaeta*	"XL"	1 overlapping
31	*colorata*	*mimica-H*	*amphilobus-H*	"XL"	1 inversion*
32	*colorata*	*primaeva-H*	*grimshawi-H*	"XL"	1 inversion*
33	*carsoni*	*colorata*	*primaeva-H*	"XL"	1 overlapping
34	all *melanica* group	*colorata*	*primaeva-H*	4	1 overlapping

TABLE 1—Continued

Comparison Number	Phylogenetic order of trio			Chromosome element	Homology patterns
35	*carbonaria* *repleta* *virilis* *colorata* *nigromelanica* *macrospina* *funebris* *multispina* *subfunebris* *polychaeta*	*primaeva-H* *attigua-H*	all other Hawaiian picture-wings	"XL"	1 inversion*
36	*colorata*	*nigromelanica*	*virilis*	"XL"	1 overlapping

* In comparisons 31, 32 and 35 the phylogenetic sequences are determined by presence or absence of inversion 5h found in most of the Hawaiian "picture-wing" group of species, but absent in *D. primaeva-H*, *D. attigua-H*, and a member of another Hawaiian group, *D. mimica-H*. Inversion 5h is also absent in all continental species listed in the above comparisons.

The relationships of the D. melanica *group to species of other groups:* As indicated above, five members of the *D. melanica* group show the *X*-autosomal fusion (*X*-A), while the other two, *D. pengi* and *D. micromelanica* have single-element, rod-shaped *X* chromosomes (*X*). In the earlier consideration of the group, before *D. pengi* had been studied, STALKER (1966) suggested that *D. micromelanica*, as the only member with the presumably primitive single-*X* condition, was the most nearly ancestral member of the group. This argument might now be extended to include *D. pengi* as a possibly ancestral species. If, in fact, either *D. pengi* or *D. micromelanica* occupied an ancestral position for the group as a whole, it would then be expected that in comparisons of this group with members of other closely related species groups, either *D. micromelanica* or *D. pengi* would be the *D. melanica* group members most closely related cytologically to the outside species, and that the chromosomes of either or both would be structurally intermediate between other members of the *D. melanica* group and the outside species.

When banding patterns in chromosomes "XL" and 3 are compared, it becomes

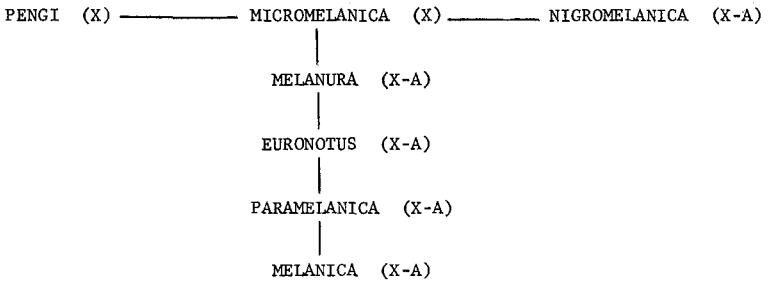

FIGURE 6.—The phyletic relationships of the members of the *D. melanica* species group, based on STALKER (1966) and data in this paper. (*X*) = single-element rod-shaped *X* chromosome, as in *D. pengi* and *D. micromelanica*. (*X–A*) = two-element *X* chromosome, corresponding to the *X* of *D. micromelanica* or *D. pengi* fused to an autosomal element "XL."

clear (Table 1, comparisons 1 through 10 and 13 through 16) that it is the chromosomes of *D. nigromelanica*, and not those of *D. pengi* or *D. micromelanica* which are closest to the outside species, and that the chromosomes of *D. nigromelanica* are structural intermediates between the chromosomes of outside species and those of *D. micromelanica*, *D. pengi*, and the other members of their group.

This finding raises an interesting problem. If indeed the single unfused *X* represents a primitive condition, then how can *D. nigromelanica* (*X*-A) occupy an intermediate position between *D. micromelanica* (*X*) or *D. pengi* (*X*) on the one hand, and nine outside species, representing eight species groups, on the other hand, when all of the outside species also have the single-*X* primitive condition? Stated differently, how can a "derived" (*X*-A) species form a link between the two "primitive" (*X*) species of the *D. melanica* group on the one hand, and nine "primitive" (*X*) outside species on the other?

It appears that this seeming inconsistency may be most readily explained (Figure 7) by accepting *D. nigromelanica* (*X*-A) as most nearly ancestral for its species group, and by assuming that the early ancestor of the species group as a whole (hypothetical I) was in fact (*X*), and that in some ancestral population the *X*-A fusion occurred and was carried, along with the original unfused *X*, in the same species, for a long period of time. This structurally heterozygous species (hypothetical II), now carrying both *X* and *X*-A, then gave rise to the modern *D. nigromelanica* (*X*-A) and to another species or sub-species (hypothetical III), still heterozygous (*X*/*X*-A). Hypothetical III produced modern *D. micromelanica* (*X*) and independently the chain of four (*X*-A) species: *D. melanura*, *D. euronotus*, *D. paramelanica* and *D. melanica*. For reasons given above, *D. pengi* (*X*) might be considered as having arisen from an early ancestral form of *D. micromelanica*. This scheme is illustrated in Figure 7.

The reasonableness of such an explanation of course depends on the likelihood

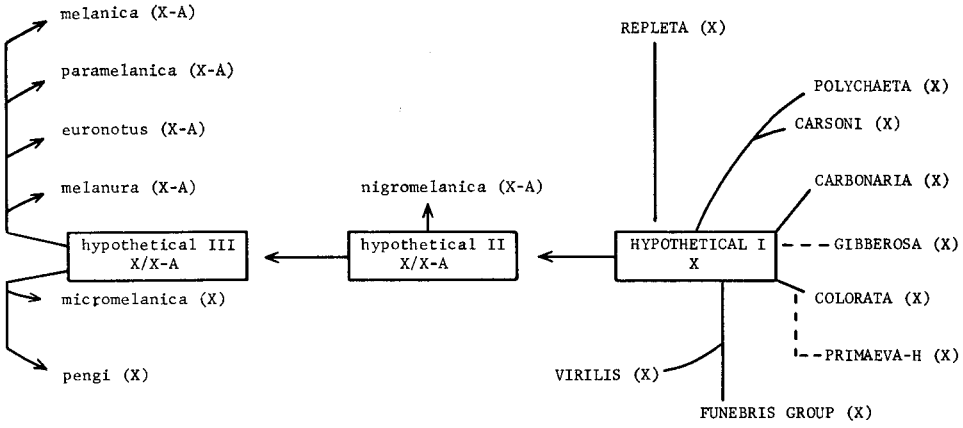

FIGURE 7.—Phylogenetic relationships of members of the *D. melanica* species group with species belonging to other groups. Species indicated by lower case letters are all members of the *D. melanica* group. The remaining nine species indicated by upper case letters belong to nine different species groups. See text.

of the establishment of a highly successful species carrying both *X* and *X*-A for a long period of time. This situation would be most likely to occur if hypothetical II and III were subdivided into a number of incompletely isolated sub-populations with some sub-populations primarily *X* and others primarily *X-A*. The survival of both karyotypes might then be assured by selection for one type or the other within sub-populations and/or the superiority of the structural heterozygotes in the zone of overlap.

In fact, in the New World species, *D. americana americana* and *D. americana texana*, exactly this situation exists today. The subspecies *D. americana* (*X*-A) is distributed over a broad range in northeastern and central United States, while *D. texana* (*X*) is found through the southeastern and south-central sector. The two ranges overlap broadly from south-central to mid-eastern United States. That the two subspecies interbreed at the zone of overlap has been proven by the repeated discovery of wild females with the heterozygous *X*/*X*-A karyotype. Such females have been found in or near Morrilton, Arkansas (STONE and PATTERSON 1947); St. Louis, Mo. (CARSON and BLIGHT 1952); and 550 miles to the east near Wooster, Ohio (STALKER 1939, unpublished). In the St. Louis area CARSON and BLIGHT found that of 25 strains, each of which had been derived from a single wild female, at least 14 were heterozygous (*X*/*X-A*). It is clear then that species such as hypothetical II and III above exist today, and presumably they could have existed in the past as well.

Phylogenetic relationships of species groups: When trios of species consisting of *D. nigromelanica* and representatives of two other species groups are compared, it is the general rule that in those cases in which a structural and phyletic order can be established, the chromosomes of *D. nigromelanica* are structural and phyletic intermediates *between* those of the species belonging to the other two groups. The pairs of outside species fitting this pattern of relationship through *D. nigromelanica* are: *D. repleta–D. colorata*; *D. repleta–D. carbonaria*; *D. repleta–D. funebris*; *D. repleta–D. carsoni*; *D. funebris–D. polychaeta*; *D. carbonaria–D. colorata*; *D. carbonaria–D. carsoni*; *D. virilis–D. carsoni*; *D. virilis–D. polychaeta*; *D. virilis–D. repleta*; *D. carsoni–D. primaeva-H* and *D. virilis–D. colorata*. See Table 1, comparisons 17 through 20, 22 through 28 and 36.

Thus, these eight non-*D. melanica* group species, representing eight different species groups, appear to be related to each other through an ancestral form (such as Hypothetical I), which is close to the modern *D. nigromelanica*. These relationships are indicated in Figure 7. In this figure the position of *D. gibberosa* is admittedly insecure, since the detectably homologous regions were so limited that it could not be placed in any phyletically ordered trio other than: *D. gibberosa–D. nigromelanica–D. micromelanica* (Table 1, comparison 2).

Certain trios listed in Table 1 do not fit the general rule concerning the intermediate position of *D. nigromelanica*. For example in the trio: *D. nigromelanica–D. carsoni–D. polychaeta* (comparison 29), the phyletic order is as listed, with *D. polychaeta* related to *D. nigromelanica* through *D. carsoni*. These three species are so represented in Figure 7. The phyletic trio: *D. colorata–D. carsoni–D. polychaeta* (comparison 30) fits the above arrangement.

In comparison 21 it is shown that the four members of the *D. funebris* group (*D. funebris*, *D. multispina*, *D. subfunebris*, *D. macrospina*) are all related to *D. nigromelanica* through *D. virilis*, and the *D. funebris* group is correspondingly placed beyond *D. virilis* in Figure 7.

It will be noted that in this figure *D. virilis* (and the more distal *D. funebris* group) are shown as related to *D. carbonaria* through Hypothetical I, although no trio of *D. virilis–D. nigromelanica–D. carbonaria* is presented in Table 1. The relative positions of *D. virilis* and *D. carbonaria* in the Figure are based on the following admittedly incomplete evidence. In the basal third of chromosome 3, *D. nigromelanica* and *D. virilis* share an homologous section (abcdef) of about 50 bands. In *D. carbonaria* inversions have broken up this homologous section so that only about a third of it (ab) can still be identified. Thus on the basis of chromosome 3, the phyletic *chromosome* order cannot be *D. nigromelanica– D. carbonaria–D. virilis*, since this would require an exact restoration of the missing (cdef) section in the transition from the *D. carbonaria* chromosome to that of one of the other two species. Similarly in chromosome "XL", *D. carbonaria* and *D. nigromelanica* are homologous over 90% of their length, while *D. virilis* shows extensive rearrangement, and is so broken up that only a number of short segments (making up about 50% of the total length) can be homologized with the other two species. This comparison indicates that the phyletic *chromosome* order cannot be *D. nigromelanica–D. virilis–D. carbonaria.* Thus the results of these two comparisons taken together are consistent with (but not complete proof of) the order on which Figure 7 is based, i.e., *D. carbonaria–D. nigromelanica–D. virilis.*

In addition to those intergroup comparisons listed in Table 1 and discussed above, many additional ones were made, but since they led to no definite phyletic orders, they are not included in the table. In summary, with the exception of the *D. virilis–D. funebris* branch, and the *D. carsoni–D. polychaeta* branch, all comparisons made between *D. nigromelanica* and members of other mainland species groups either indicated the central position of some ancestral form of *D. nigromelanica*, or due to insufficient detectable homologies, gave inconclusive results. No comparisons, with the two exceptions noted above, indicated that *D. nigromelanica* was *not* intermediate in a trio involving it and members of two other continental species groups.

The relationships of continental and Hawaiian species: In regard to the relationships of Hawaiian and continental species groups two questions are of special interest. First, which of the Hawaiian species are most closely related to continental species, and thus presumably most nearly ancestral among the Hawaiian group? Second, which of the continental species groups are closest to the Hawaiian species, and thus possibly ancestral to the Hawaiians?

Attempts were made to find continental species which showed chromosomal affinities to the various members of the Hawaiian species for which chromosomal maps were available, and when such promising species were found trios consisting of two continental and one Hawaiian species were analysed in an attempt to establish a phylogenetic relationship, and specifically to determine which of the two continental species was phylogenetically intermediate, and thus closest to

the Hawaiian species. Only three such direct phylogenetic relationships could be established. One of these (Table 1, comparison 34) indicated that *D. colorata* of the *D. robusta* group was an intermediate between the Hawaiian species, *D. primaeva-H* and all of the members of the *D. melanica* group. Another, (comparison 27) indicated that *D. nigromelanica* of the *D. melanica* group was closer to *D. primaeva-H* than was *D. carsoni*. Since it had already been established that *D. polychaeta* was related to *D. nigromelanica* through *D. carsoni* (Figure 7; Table 1, comparison 29) the results of comparisons 27 and 34 further indicated that *D. colorata* is closer to the Hawaiian species than are the members of the *D. melanica*, the *D. carsoni* and the *D. polychaeta* groups. Comparison 33, in which it was shown that *D. colorata* was closer to *D. primaeva-H* than was *D. carsoni*, fitted the above conclusions.

Since (comparison 22) it has been shown that *D. carbonaria* is related to *D. colorata* through *D. nigromelanica*, it may also be concluded that *D. colorata* is closer to the Hawaiian species than is *D. carbonaria*. Since *D. virilis* is related to *D. colorata* through *D. nigromelanica*, it may be assumed that *D. colorata* is closer to the Hawaiian species than *D. virilis* (and *D. funebris*).

Finally, since *D. repleta* is related to *D. colorata* through *D. nigromelanica* (comparison 17), it may be assumed that *D. colorata* is closer to *D. primaeva-H* than is *D. repleta*. In summary: it appears that of the continental species groups considered, the *D. robusta* group (represented by *D. colorata*) is a more reasonable choice as an Hawaiian ancestral group than are the *D. melanica*, *D. carbonaria*, *D. carsoni*, *D. polychaeta*, *D. virilis*, *D. funebris* or *D. repleta* groups.

The problem of determining the ancestral species among the endemic Hawaiian picture wings was approached in two quite different ways. CARSON and STALKER (1968, 1969) developed a single phylogenetic scheme encompassing all of the 68 species then available. In the course of this study CARSON, while working in Hawaii, had provisionally concluded that on the basis of its position in the phylogenetic scheme, and because of its geographic position in the northwestern island of Kauai, *D. primaeva-H* was most nearly ancestral for the picture-wing groups taken together. STALKER, working in St. Louis at the time, had independently come to the same conclusion on the basis of comparisons between Hawaiian and continental banding patterns. Further study appears to confirm this joint conclusion, and more recently a second primitive Hawaiian species, *D. attigua-H*, has been found by CARSON, also from Kauai. *D. attigua-H* which is adjacent to *D. primaeva-H* in the Hawaiian phylogeny presently appears to have equally close continental affinities.

The basis for the conclusion that *D. primaeva-H* (and *D. attigua-H*) are intermediates lying between other Hawaiian picture-wing species and the continental species (and are therefore primitive for the Hawaiian picture-wing phylogeny) is the distribution of inversion 5h in chromosome element "XL" (corresponding to chromosome 5 in the Hawaiian species) Inversion 5h is present in all of the Hawaiian picture-wings except *D. primaeva-H* and *D. attigua-H*. It is absent in all continental species groups in which the pertinent homologous region could be identified: the *D. carbonaria*, *D. repleta*, *D. virilis*, *D. colorata*, *D. melanica*, *D. polychaeta* and *D. funebris* groups (Table 1, comparisons 32 and 35). This

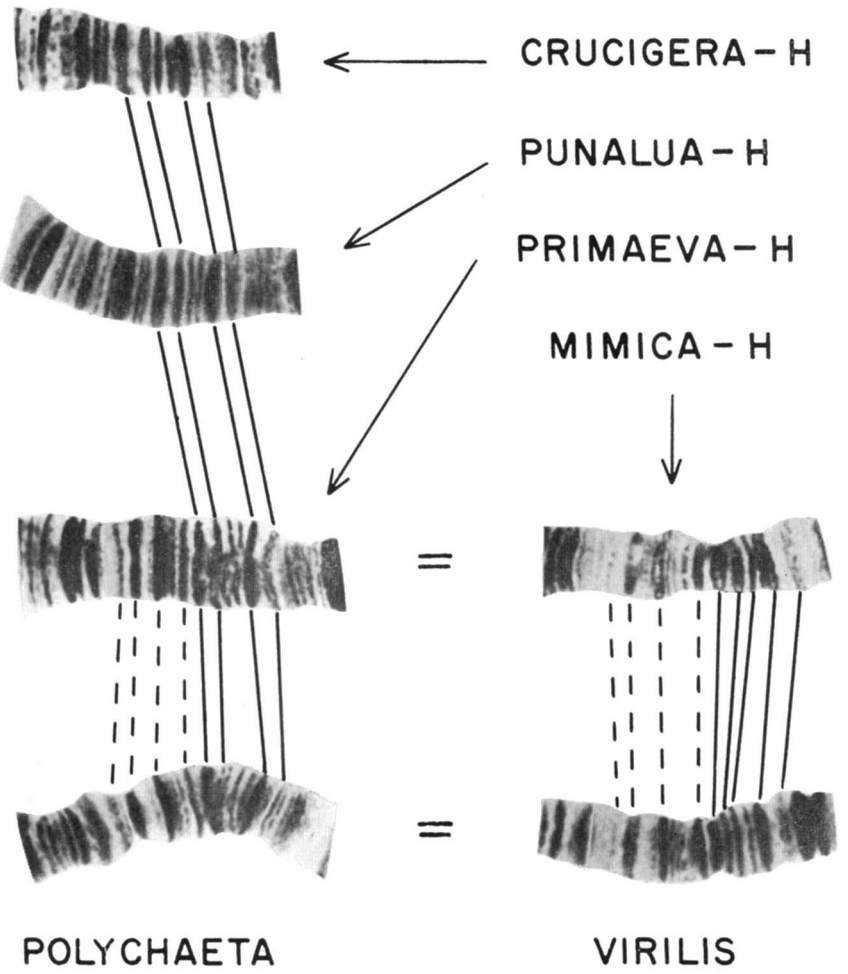

FIGURE 8.—Association of "XL" inversion 5h with certain continental and Hawaiian species. The short homologous region for the various species is indicated by the connecting solid and dashed lines. Between the continental species *D. polychaeta* and *D. virilis*, and the Hawaiian species *D. primaeva-H*, the homologous segment is longer than that between *D. primaeva-H* and the *D. punalua-H*, *D. crucigera-H* pair. This reduction in the extent of the homologous segment within the Hawaiian species is the result of the occurrence of inversion 5h in the Hawaiian phylogeny, resulting in the displacement of the left end of the homologous segment (that indicated by dashed lines). Inversion 5h is present in all of the species of the Hawaiian "picture-wing" group with the exception of *D. primaeva-H* and *D. attigua-H* (not shown in this Figure). The absence of 5h in *D. primaeva-H* and *D. attigua-H* and many (apparently all) of the continental species. indicates the relatively close affinity of *D. primaeva-H* and *D. attigua-H* to the continental species, as well as the ancestral position of those two species within the Hawaiian phylogeny. *D. mimica-H* is a member of an entirely different Hawaiian species group, and also shows the primitive absence of the 5h inversion. See text and Table 1, comparisons 31, 32 and 35.

homologous chromosome region is illustrated in Figure 8. In this figure it will be noted that the full homologous section is found in *D. primaeva-H* and *D. attigua-H* and in the continental species, but only part of it can be seen in the

remaining Hawaiian picture-wings. The reduction of the homologous section is the result of occurrence of the 5h Hawaiian inversion.

It is of some interest that the absence of the important 5h inversion in *D. primaeva-H* and *D. attigua-H* exists also in the Hawaiian endemic *D. mimica-H*, a member of a different group (see comparison 31, in which 5h is absent in *D. colorata* and *D. mimica-H* but present in *D. amphilobus-H*). This finding indicates that the *D. mimica-H* group is related to the picture-wings through an ancestral form close to *D. primaeva-H* and *D. attigua-H*, and is additional indirect evidence for the ancestral position of these two species.

DISCUSSION

Although the phylogenetic scheme presented in this paper satisfies available cytological data, it should be emphasized that the study has dealt primarily with New World forms. The missing steps in the present scheme may well involve species and even species groups from other areas. The need for caution in interpreting a phylogeny within a single continent is pointed up by the following example.

NARAYANAN (1970) in an extensive analysis of six members of the *D. robusta* group has presented convincing evidence to support the group phylogeny indicated in a simplified form in Figure 9 below. As indicated earlier (see Figure 7), *D. nigromelanica* is a member of the *D. melanica* group which is cytologically closest to *D. colorata* of the *D. robusta* group (and as far as is now known, is equally close to *D. moriwakii* of that group). Thus in Figure 9 the two groups are shown connected through *D. nigromelanica*, with the nearest relative in the *D. robusta* group arbitrarily chosen as *D. colorata* (rather than *D. moriwakii*), because of its sympatry with *D. nigromelanica*.

It will be noted in Figure 9 that since one member of the *D. melanica* group, and four members of the *D. robusta* group are Asiatic, while all other species studied are from the New World, at least three separate migrations between the two continents (presumably via the Bering Bridge) are required. Had the study of these two species groups been restricted to New World forms, the intermediate stages in the *D. robusta* phylogeny and the required three crossings of the Bering Bridge would have been missed entirely. It is still not clear whether the immediate ancestor of the two groups was an Asiatic or a New World form.

Evidence of a different nature, which likewise supports the inference of intercontinental migration is of course available from a study of the geographic distributions of members of species groups as these groups are determined by morphological criteria (see for example, PATTERSON and STONE 1952). However in morphological studies, even when these are amplified by investigation of hybridization capabilities, it is rarely possible to determine the details of intragroup relationships or the detailed patterns of migration, as has been possible by cytological analysis of the *D. robusta* and *D. melanica* groups.

If at least three intercontinental migrations occurred during the evolution of only two species groups, it may be inferred that a good deal of migration has occurred during the establishment of the various species groups in the subgenus

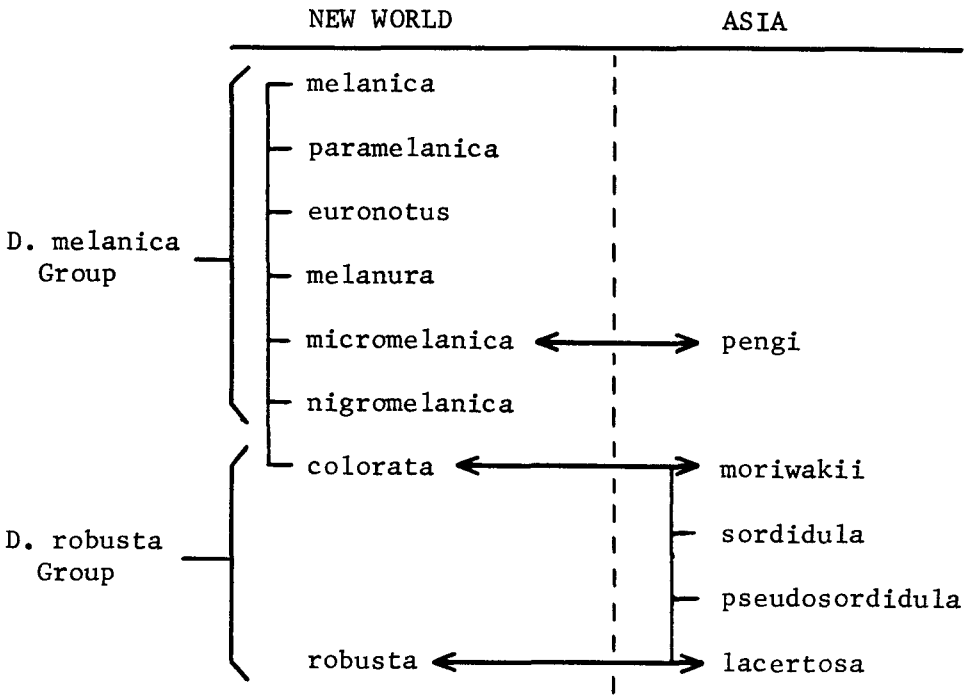

FIGURE 9.—Simplified phylogenies of the *D. melanica* and *D. robusta* species groups showing the intergroup relationships and the continental distributions. It will be noted that regardless of the ancestral origin of the two groups, at least three intercontinental migrations were involved in their evolution. Based on the data presented in this paper and on NARAYANAN (1970).

Drosophila, and that therefore the intergroup relationships may be more complex than those presented in this paper.

LITERATURE CITED

CARSON, H. L. and W. C. BLIGHT, 1952 Sex-chromosome polymorphism in a population of *Drosophila americana*. Rec. Gen. Soc. Amer. **21**: 16.

CARSON, H. L. and H. D. STALKER, 1968 Polytene chromosome relationships in Hawaiian species of Drosopshila. Univ. Tex. Publ. No. 6818. I. The *D. grimshawi* subgroup. 335–354. II. The *D. planitibia* subgroup. 355–365. III. The *D. adiastola* and *D. punalua* subgroups. 367–380. ——, 1969 Polytene chromosome relationship in Hawaiian species of Drosophila. IV. The *D. primaeva* subgroup. Univ. of Tex. Publ. No. 6918. 85–94.

NARAYANAN, Y., 1970 The phylogenetic relationships of the members of the *Drosophila robusta* group. Washington University Ph.D. dissertation.

PATTERSON, J. T. and W. S. STONE, 1952 *Evolution in the genus Drosophila*. Macmillan Co., New York.

STALKER, H. D., 1966 The phylogenetic relationships of the species in the *Drosophila melanica* group. Genetics **53**: 327–342.

STONE, W. D. and J. T. PATTERSON, 1947 The species relationships in the *virilis* group. Univ. Tex. Publ. No. 4729: 157–160.

WHARTON, L. T., 1943 An analysis of the metaphase and salivary chromosome morphology within the genus Drosophila. Univ. Tex. Publ. No. 4313: 282–319.