V. A Study of the Relationships of Hawaiian *Drosophila* Species Based on External Male Genitalia[1,2]

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Introduction

Sturtevant (1919) first mentioned the importance of the use of the external male genitalia of Drosophilidae as a taxonomic tool in distinguishing between closely related species. Since then, investigators such as Hsu (1949), Malogolowkin (1952, 1953), Nater (1953), Okada (1953, 1954, 1955), Spassky (1957), and Takada (1965, 1966, have made extensive studies of the external male genitalia of Drosophilidae.

Snodgrass (1957) states: "The great diversity in structural detail of the genitalia gives these organs a value for the identification of insect species almost equal to that of fingerprints for identification of human individuals. On the other hand, the very structural diversity of the organs makes it difficult to understand their fundamental nature and the homologies of their parts."

The lack of uniformity in the concept of homologies is certainly true in the case of the male genitalia of Drosophilidae. This has led to a confusing situation where a taxonomic specialist of one group of species adopts terminology which is unfamiliar to another specialist. Diagrammatic sketches (figs. 1.1 and 1.2) are presented herein to clarify any misinterpretation which may arise in reading this paper. The terminology used is based on Takada's (1966) study of the external male genitalia of Hawaiian Drosophilidae.

Carson *et al.* (1967) presented a diagram giving the relationships of 22 species of endemic *Drosophila* of Hawaii. This diagram is in the form of a phylogenetic tree and is based on the relationships of the polytene chromosome banding sequences of the 22 species with the sequences of *D. grimshawi* as the arbitrary standard. Carson and Stalker (1968a, b, c), have since expanded the phylogeny to include over 50 species of Hawaiian *Drosophila*. In most cases, chromosomally similar species are morphologically similar and vice versa. Carson's findings, however, also show that there is sometimes remarkable chromosomal similarity between species which show pronounced morphological differences; i.e., obvious differences in leg ornamentation, wing pattern, etc.

This study is an attempt to form species subgroups based on relationships of external male genitalia, especially the phallic organs. In addition, an attempt will be made to establish a correlation between the relationships of species based on

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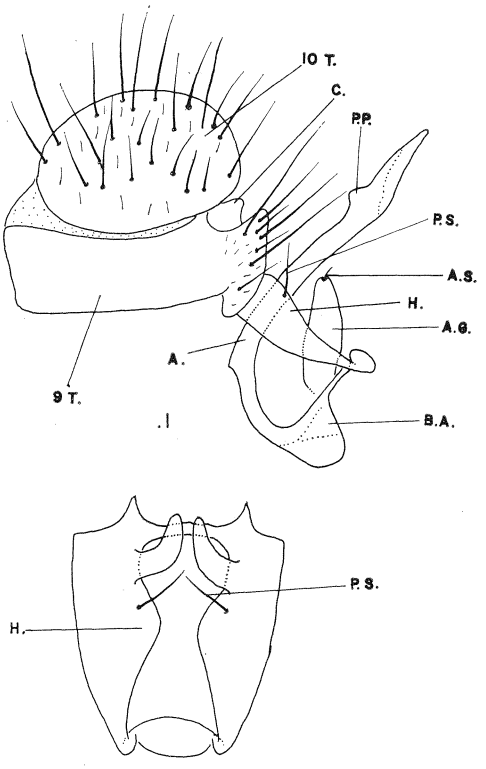

FIG. 1. Diagrammatic sketches of external male genitalia; .1 lateral view, .2 ventral view of hypandrium. A. aedeagus, A.G. anterior gonapophysis (paramere), A.S. apical sensillum, B.A. basal apodeme of aedeagus, C. clasper, H. hypandrium, P.P. preapical protuberance of aedeagus, P.S. paramedian spine, 9T. ninth tergum (genital arch), 10T. tenth tergum (anal plate).

male genitalia and relationships based on the banding sequences of the chromosomes. Therefore, species with large morphological differences, but with similar chromosomal makeup, will be shown to share similar genital apparatus. Also, species which may appear to belong to the same species subgroup based on external morphology, may in actuality belong to a separate subgroup based on genital characteristics. This is corroborated in most cases by the chromosomal relationships of the species.

All of the information on the chromosomal makeup of the species studied is taken from Carson's reports; however, not all of the species whose genitalia have been studied, have been studied by Carson. Based on genitalia relationships, it is possible to make some predictions as to the approximate placement of these species in Carson's phylogeny.

Most investigators have used genitalia characteristics to separate closely related species; *i.e.*, species which cannot be easily separated on the basis of external morphological characteristics. In this paper, however, emphasis will be placed on the usefulness of the similarities of the genital apparatus for assigning the different species to particular subgroups. All of the species studied here, are

considered to be distinct species based on consistent morphological characteristics; and in most cases, a study of the genital apparatus is not needed to distinguish between species. In fact, it was found that in many cases, there is a slight intraspecific variability in the shapes of the phallic organs and that the aedeagus of some specimens of a particular species show a strong tendency to resemble those of another species. This strongly supports the theories which will be presented later. It will be shown that despite the slight intraspecific variability which may be present in the shapes of the phallic organs, they are nevertheless consistent characteristics of the respective species subgroups.

Materials and Methods

Most of the genitalia studied were dissected from freshly killed specimens which were either collected in the field or taken from cultures reared at the Evolution of Hawaiian Drosophila Laboratory at the University of Hawaii. Some specimens were obtained from the general collection of Drosophilidae at the University of Hawaii. The species studied belong to the so-called "picture-winged" species (Hardy and Kaneshiro, 1968) of Drosophilidae. They are generally large species with markings at least on the base of the wing and the m-crossvein. They usually have ciliation on the front legs, and usually also have fleshy labella (*i.e.*, not modified with spines or setae) except for *D. neogrimshawi* in the *D. adiastola* subgroup. The species are listed in Table 1 according to species subgroups. The numbers correspond to the drawings of the respective genitalia in figures 2 through 9.

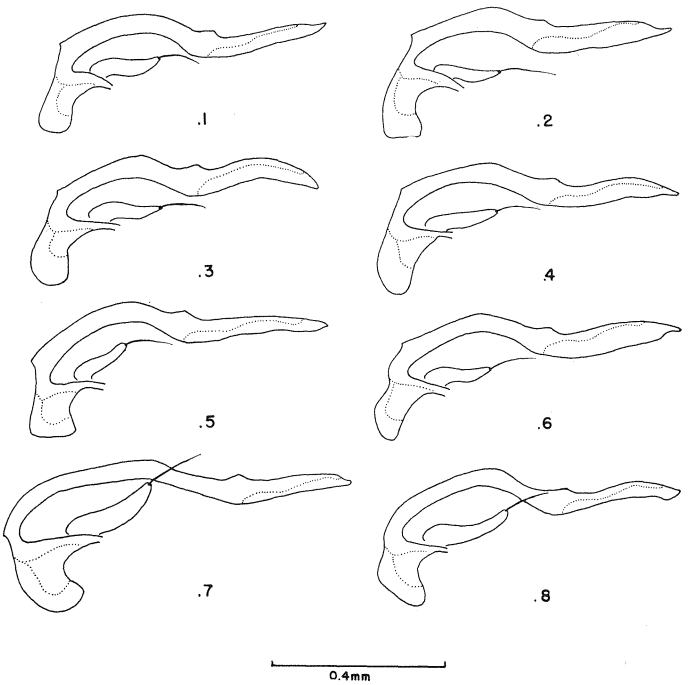

Fig. 2. Phallic organs of *D. adiastola* subgroup; .1 *D. adiastola*, .2 *D. cilifera*, .3 *D. peniculipedis*, .4 *D. spectabilis*, .5 *D. setosimentum*, .6 *D. ochrobasis*, .7 *D. clavisetae*, 8. *D. neogrimshawi*.

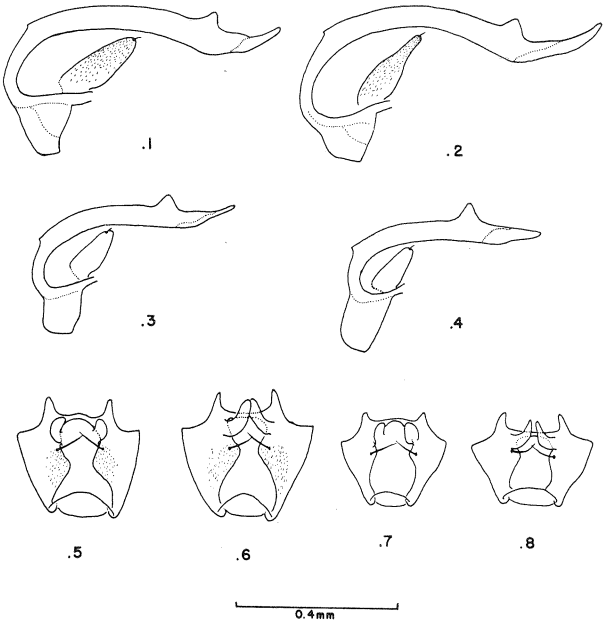

FIG. 3. Phallic organs of *D. paucipuncta* subgroup; .1 *D. paucipuncta*, .2 *D. uniseriata*, .3 *D. prolaticilia*, .4 *D. basisetae*. Ventral view of hypandrium of *D. paucipuncta* subgroup; .5 *D. paucipuncta*, .6 *D. uniseriata*, .7 *D. prolaticilia*, .8 *D. basisetae*.

It was necessary to mount the genitalia of each specimen on a slide in order to study them in detail.

The following is the procedure used in preparing the genitalic materials. It is a modification of the procedure used by Kambysellis and Wheeler (1966).

(1) The tip of the abdomen of the fly was clipped with a pair of microscissors, then placed in a test tube with 10% KOH, and boiled for 10- to 15-minutes.
(2) The KOH was then removed with a pipette and the material washed with water.
(3) The water was drained and replaced with a small amount of stain [four (4) parts of Gage's Stain (acid fushin, 0.5g; 10% HCl, 25.0cc; distilled water, 300.0cc) with one (1) part glacial acetic acid].
(4) The material immersed in the stain was then heated until boiling then the stain was replaced with 95% ethyl alcohol.
(5) The material was transferred to a small watch glass containing a drop or two of creosote.
(6) At this point, extraneous material was removed from the genitalia using a pair of fine probing needles.
(7) The cleaned material was then mounted on a slide using euparol as the medium.

For most of the species studied, several specimens each were examined to study the variability which may exist in the structure of the genitalia within a species. For some of the species, only a very few or even only one specimen was available. Although the phallic organs of some specimens of a particular species had a strong

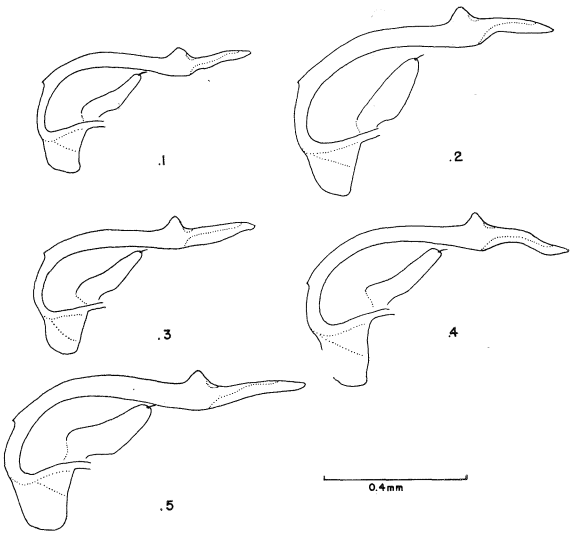

FIG. 4. Phallic organs of *D. pilimana* subgroup; .1 *D. pilimana*, .2 *D. glabriapex*, .3 *D. discreta*, .4 *D. fasciculisetae*, .5 *D. lineosetae*.

tendency to resemble those of another species, it was found that for this study, the intraspecific variability of the shape of the phallic organs was of little importance.

OBSERVATIONS

After making a detailed study of the external male genitalia of several "picture-winged" species of Hawaiian *Drosophila*, it became evident that there are characteristic similarities in the phallic organs among the species which indicated group relationships. The general shape of the aedeagus (especially the shape of the preapical protuberance), and the shape of the hypandrium and the ninth tergum (at least in the *D. paucipuncta* subgroup) are of special interest. Based on these characteristics, it was possible to set up at least eight species subgroups.

Except for the *D. paucipuncta* subgroup, the overall shapes of the hypandrium, the anal plate, and ninth tergum do not show striking characters which are of importance here. The shape of the anterior gonapophyses (parameres) seem to be a very useful character in distinguishing between two species which otherwise have very similar genital characters. The anterior gonapophyses are not important, however, for indication of any significant tendency in the formation of the species subgroups. In several species, there are minute setae in addition to the apical sensilla present on the surface of the anterior gonapophyses but these species do not appear to be very closely related (except *D. paucipuncta* and *D. uniseriata* in the *D. paucipuncta* subgroup; and *D. digressa* and *D. virgulata* in *the D. vesciseta* subgroup) as will be pointed out later.

In most of the species studied, there is a definite protuberance close to the apex of the aedeagus when viewed from the lateral aspect. Figures of the aedeagus of species such as *D. pilimana* (fig. 4.1), *D. grimshawi* (fig. 6.1), and *D. orphnopeza* (fig. 9.1) illustrate this characteristic. The shape and size of this protuberance are

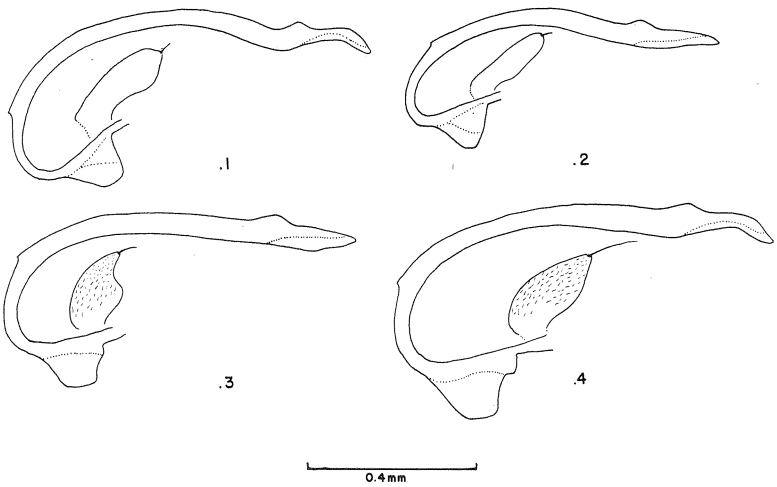

FIG. 5. Phallic organs of *D. vesciseta* subgroup; .1 *D. vesciseta*, .2 *D. hexachaetae*, .3 *D. virgulata*, .4 *D. digressa*.

very useful characteristics in grouping species which later were shown to have similar chromosomal makeup.

The eight species subgroups which include a total of 41 species will be identified by the name of the species most representative of each group as for example, the "*D. adiastola*" subgroup, and the "*D. hawaiiensis*" subgroup.

The *D. adiastola* Subgroup

This subgroup is comprised of eight species (Table 1). The characteristic features of the *D. adiastola* subgroup occur in the phallic organs. All species belonging in this subgroup have a characteristic bend at about the middle of the aedeagus (fig. 2); also, the preapical protuberance of the aedeagus is small and insignificant as compared to species such as *D. pilimana* (fig. 4.1), and *D. grimshawi* (fig. 6.1). The basal apodeme of the aedeagus is relatively narrow and elongate. The parameres of this subgroup are typically narrow and elongate and with an elongate apical sensillum. The other structures of the external genitalia are quite variable between species and may resemble those species from another subgroup.

The *D. paucipuncta* Subgroup

This subgroup is comprised of four species (Table 1). In this subgroup, the shapes of all the structures of the external male genitalia are very consistent. The aedeagus is relatively short, with a pronounced preapical proturberance and a broad but shortened basal apodeme, (fig. 3). The shape of the hypandrium is also characteristic in that the width at the widest points is about equal to or, as in *D. basisetae* (fig. 3.8), slightly greater than the length. In other picture-winged species, the length of the hypandrium is generally, at least one-half times greater than the width (fig. 1.1). In two species, *D. paucipuncta* (fig. 3.1), and *D. uniseriata* (fig. 3.2), the median surface of the hypandrium just ventrad to the paramedian spine is covered with minute setae. Also, in these two species, the apical

TABLE 1

Species Studied and Their Assignment to Different Subgroups

Fig. No.	*D. adiastola Subgroup*
2.1	*D. adiastola* Hardy
2.2	*D. cilifera* Hardy-Kaneshiro
2.3	*D. peniculipedis* Hardy
2.4	*D. spectabilis* Hardy
2.5	*D. setosimentum* Hardy-Kaneshiro
2.6	*D. ochrobasis* Hardy-Kaneshiro
2.7	*D. clavisetae* Hardy
2.8	*D. neogrimshawi* Hardy-Kaneshiro
	D. paucipunta Subgroup
3.1	*D. paucipuncta* Grimshaw
3.2	*D. uniseriata* Hardy-Kaneshiro
3.3	*D. prolaticilia* Hardy
3.4	*D. basisetae* Hardy-Kaneshiro
	D. pilimana Subgroup
4.1	*D. pilimana* Grimshaw
4.2	*D. glabriapex* Hardy-Kaneshiro
4.3	*D. discreta* Hardy-Kaneshiro
4.4	*D. fasciculisetae* Hardy
4.5	*D. lineosetae* Hardy-Kaneshiro
	D. vesciseta Subgroup
5.1	*D. vesciseta* Hardy-Kaneshiro
5.2	*D. hexachaetae* Hardy
5.3	*D. virgulata* Hardy-Kaneshiro
5.4	*D. digressa* Hardy-Kaneshiro
	D. grimshawi Subgroup
6.1	*D. grimshawi* Oldenberg
6.2	*D. disjuncta* Hardy
6.3	*D. bostrycha* Hardy
6.4	*D. crucigera* Grimshaw
6.5	*D. balioptera* Hardy
	D. hawaiiensis Subgroup
7.1	*D. hawaiiensis* Grimshaw
7.2	*D. recticilia* Hardy-Kaneshiro
7.3	*D. silvarentis* Hardy-Kaneshiro
7.4	*D. gradata* Hardy-Kaneshiro
7.5	*D. villitibia* Hardy
7.6	*D. hirtipalpus* Hardy-Kaneshiro
	D. ochracea Subgroup
8.1	*D. ochracea* Grimshaw
8.2	*D. limitata* Hardy-Kaneshiro
	D. orphnopeza Subgroup
9.1	*D. orphnopeza* Hardy-Kaneshiro
9.2	*D. sodomae* Hardy-Kaneshiro
9.3	*D. orthofascia* Hardy-Kaneshiro
9.4	*D. prostopalpis* Hardy-Kaneshiro
9.5	*D. engyochracea* Hardy
9.6	*D. sproati* Hardy-Kaneshiro
9.7	*D. villiosipedis* Hardy

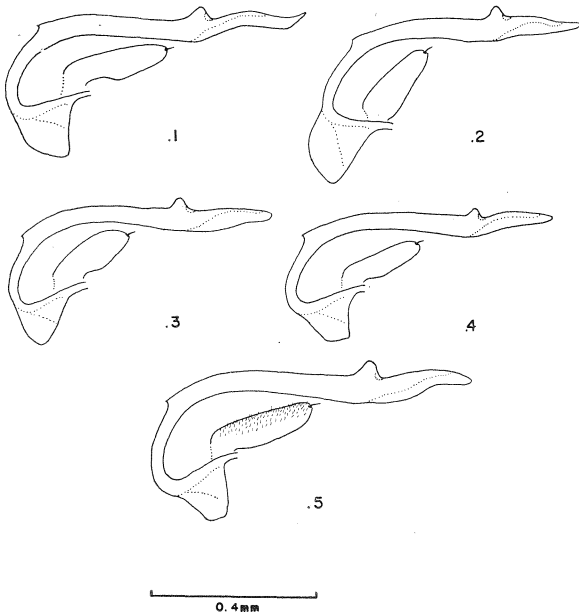

FIG. 6. Phallic organs of *D. grimshawi* subgroup; .1 *D. grimshawi*, .2 *D. disjuncta*, .3 *D. bostrycha*, .4 *D. crucigera*, .5 *D. balioptera*.

two-thirds of the parameres are covered with minute setae in addition to the minute apical sensilla (fig. 3.5 and 3.6). The other two species in this subgroup, *D. prolaticilia* (fig. 3.3), and *D. basisetae* (fig. 3.4) do not have setae on the median surface of the hypandrium (figs. 3.7 and 3.8) and the parameres, but the shape of the aedeagus and the hypandrium would definitely place them in this subgroup. The shape of the ninth tergum (or genital arch) of this subgroup also appears to be quite characteristic. It is narrow on the dorsal portion, gradually widening at the ventral margins.

The *D. pilimana* Subgroup

This subgroup is comprised of five species (Table 1). The phallic organs of this subgroup are characterized by the pronounced preapical protuberance (fig. 4), which is quite different from those of the *D. paucipuncta* subgroup. Also, the overall length of the aedeagus is longer than those of the *D. paucipuncta* subgroup relative to the basal apodeme. The parameres of this subgroup are typically narrow and elongate but in contrast to the *D. adiastola* subgroup, the apex is blunt and rounded rather than pointed. Also, the apical sensilla are minute rather than elongate.

The *D. vesciseta* Subgroup

This subgroup is comprised of four species (Table 1). The characteristic feature of this subgroup is the very narrow and elongate aedeagus with a small and insignificant preapical protuberance (fig. 5). Also, the parameres are broad and

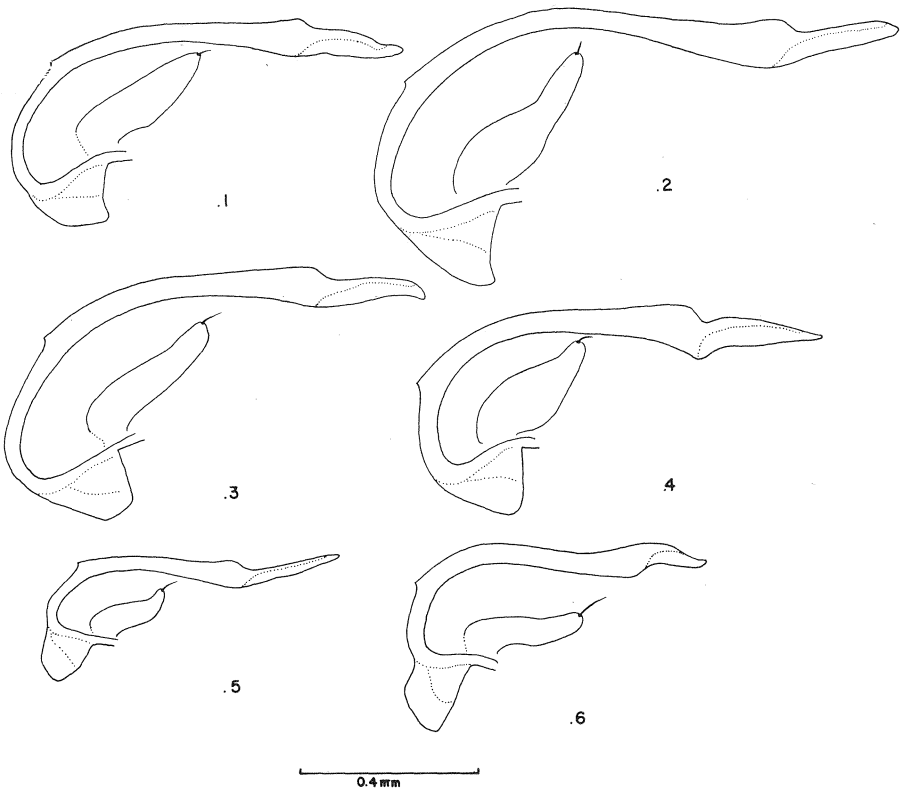

FIG. 7. Phallic organs of *D. hawaiiensis* subgroup; .1 *D. hawaiiensis*, .2 *D. recticilia*, .3 *D. silvarentis*, .4 *D. gradata*, .5 *D. villitibia*, .6 *D. hirtipalpis*.

have minute apical sensilla except those of *D. digressa* (fig. 5.4), which have elongate apical sensilla. The parameres of *D. virgulata* (fig. 5.3), and *D. digressa* (fig. 5.4) are covered with minute setae in addition to the apical sensilla while those of *D. vesciseta* (fig. 5.1) and *D. hexachaetae* (fig. 5.2) are bare except for the apical sensilla. All four species belong to the same subgroup on account of the shape of the aedeagus.

The *D. grimshawi* Subgroup

This subgroup is comprised of five species (Table 1). The phallic organs of these five species are characterized by the aedeagus having a significant preapical protuberance (fig. 6) which is quite similar yet readily distinguishable from those of the *D. pilimana* subgroup. The preapical protuberance is narrower at the base and more rounded at the apex rather than wide at the base and angular at the apex as in the *D. pilimana* subgroup. The basal apodeme is short and quite similar in shape to the *D. pilimana* subgroup in that it is truncate at the apex. The parameres are also quite similar to those of the *D. pilimana* subgroup although they appear to be somewhat more rounded at the apex. The dorsal surfaces of the parameres of *D. balioptera* (fig. 6.5) are covered with minute setae in addition to the

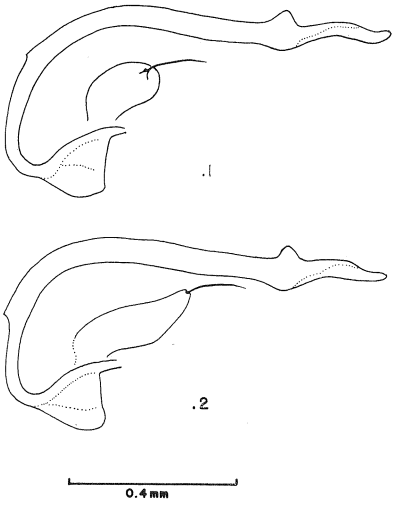

FIG. 8. Phallic organs of *D. ochracea* subgroup; .1 *ochracea*, .2 *D. limitata*.

apical sensilla while those of the remaining four species are bare except for the minute apical sensilla.

The *D. hawaiiensis* Subgroup

This subgroup is comprised of six species (Table 1). The species in this subgroup are characterized by the absence or very small preapical protuberance of the aedeagus (fig. 7). This character alone will readily separate this subgroup from the others. In *D. gradata* there appears to be a depression at the position where the protuberance normally occurs (fig. 7.4). The parameres of this subgroup are typically broad at the base and narrowing at the apex and with minute apical sensilla. The basal apodeme is typically triangular in shape and is very short in relation to the length of the aedeagus.

The *D. ochracea* Subgroup

This subgroup is comprised of only two species (Table 1). These two species appear to be closely related due to the overall shape of the aedeagus (fig. 8). Specifically, there is a bend at about the middle of the aedeagus plus another bend at the junction of the basal apodeme. Also, the swelling ventrad to the preapical protuberance of the aedeagus is characteristic of the two species. The parameres of these two species, however, are different from each other. In *D. limitata* (fig. 8.2), they are elongate and narrow at the apex; in *D. ochracea* (fig. 8.1), short and quite rounded at the apex. The parameres of both species have elongate apical sensilla but those of *D. ochracea* are on short projections which are somewhat subapical.

The *D. orphnopeza* Subgroup

This subgroup is comprised of seven species (Table 1). In the previous subgroups, there were several characteristics which were used to group the species

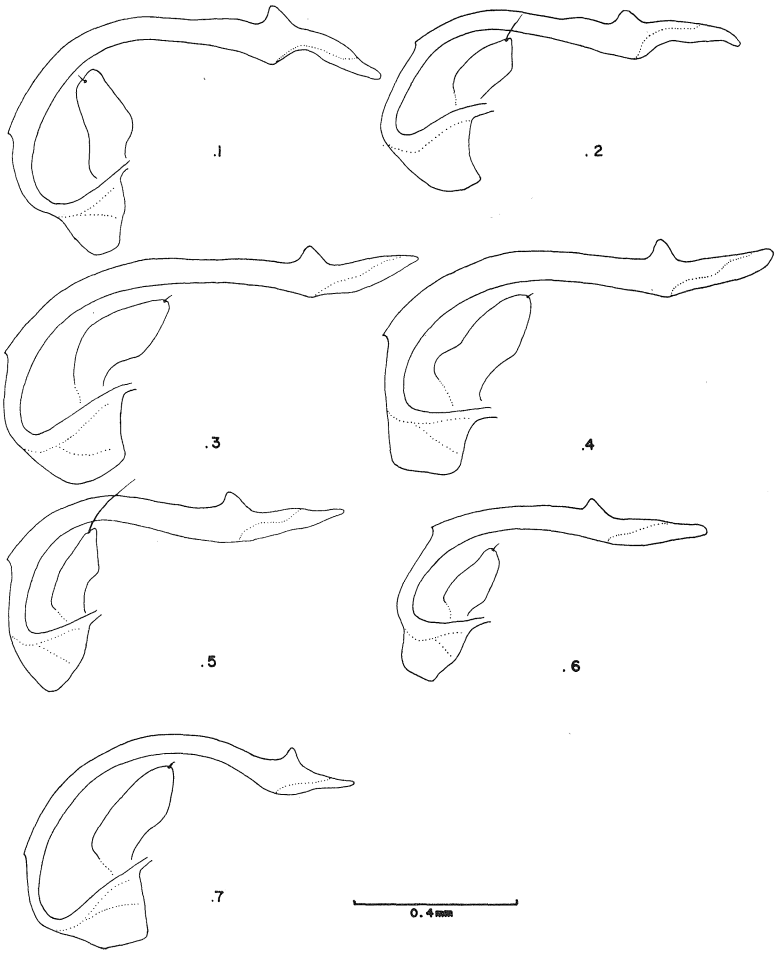

FIG. 9. Phallic organs of *D. orphnopeza* subgroup; .1 *D. orphnopeza*, .2 *D. sodomae*, .3 *D. engyochracea*, .4 *D. sproati*, .5 *D. orthofascia*, .6 *D. prostopalpis*, .7 *D. villosipedis*.

into their respective subgroups. This was not the case for the *D. orphnopeza* subgroup. There is one character which is shared by all of them; i.e., the preapical protuberance is slanted toward the base, each species to a different degree (fig. 9). A distinct similarity in the shape of the aedeagus can be seen in *D. engyochracea* (fig. 9.3), and *D. sporati* (fig. 9.4); *D. orphnopeza* (fig. 9.1) and *D. sodomae* (fig. 9.2); and *D. orthofascia* (fig. 9.5), and *D. prostopalpis* (fig. 9.6). It would appear that there are three separate subgroups involved; however, due to the intraspecific variability of the shape of the aedeagus of *D. orthofascia* (of which several specimens were available), a definite relationship between the species of this subgroup can be seen. Although the genitalia of *D. prostopalpis* and *D. sodomae* were studied from single specimens, the very fact that they resemble *D. orthofascia* and *D. orphnopeza* respectively, would be enough evidence to include them in this subgroup. Undoubtedly, if more specimens were available, a more concrete relationship between the species of this subgroup could be made.

Discussion

In the *D. adiastola* subgroup, there are two (2) species *D. clavisetae* and *D. neogrimshawi* which were until recently considered to belong to a different genus, *Idiomyia*. Hardy (1965) stated that the only reliable character which will separate *Idiomyia* from *Drosophila* is the presence of an extra crossvein in cell R5 of the wing. Interestingly, however, Carson et al. (1967) presented chromosomal evidence which indicate that *Idiomyia* is cogeneric with *Drosophila*. Consequently, *Idiomyia* has been sunk as a synonym of *Drosophila* by Hardy and Kaneshiro (1968). During this study, it was found that these two species definitely belong in the *D. adiastola* subgroup on the basis of the male genitalia. This is corroborated by Carson's chromosomal relationship. It should be restated here that the possibility that morphologically (external) dissimilar species actually being closely related, i.e., if based on male genitalic characters is evidenced strongly by the *D. adiastola* subgroup and is corroborated by Carson's chromosomal relationships.

In the *D. paucipuncta* subgroup, the three species *D. paucipuncta*, *D. uniseriata*, and *D. basisetae* could very easily be considered to belong to the same subgroup on the basis of external morphology. The male genitalia shows that they are closely related species; and Carson's chromosomal study shows that *D. paucipuncta* and *D. uniseriata* are chromosomally homosequential. However, *D. prolaticilia* would probably have been placed in a separate subgroup from these three on the basis of the wing markings and general body shape. The male genitalia show that *D. prolaticilia* is nonetheless closely related to the *D. paucipuncta* subgroup. Chromosomally, it has only one fixed inversion difference from *D. paucipuncta* and *D. uniseriata*. Here again is good evidence that species which can be shown to be closely related species on the basis of male external genitalia, especially the phallic organs, despite other morphological differences, can also be shown to be chromosomally closely related.

Carson et al. (1967) showed that *D. punalua* is chromosomally homosequential with *D. paucipuncta* and *D. uniseriata* of the *D. paucipuncta* subgroup. The shape of the aedeagus of *D. punalua* appear to fit the characteristics of the *D. paucipuncta* subgroup; however, the shape of the hypandrium and ninth tergum, which are consistent characteristics of the *D. paucipuncta* subgroup, are very different in *D. punalua*. The hypandrium is at least one-half times longer than wide in *D. punalua*, and the ninth tergum is of uniform length; i.e., not narrowing at the dorsal portion. Therefore, although chromosomally *D. punalua* is homosequential with *D. paucipuncta* and *D. uniseriata* and although some genitalic characteristics indicate a possible relationship of *D. punalua* to others of the *D. paucipuncta* subgroup, because of the difference in the hypandrium and the ninth tergum, *D. punalua* will not be included in this subgroup. This does not necessarily contradict Carson's chromosomal evidence because it has been shown that speciation and evolution of the picture-winged species of Hawaiian *Drosophila* may in a number of cases be based on mutational changes occurring at the submicroscopic level rather than on changes in the sequences of the chromosomal bandings (Carson et al. 1967). Therefore, based on genitalic characteristics, two species which are chromosomally homosequential may be shown to belong to two

separate subgroups; i.e., each is more closely related to another species with which it may not be chromosomally homosequential but to which it is similar in genitalic characteristics. Similar cases will be presented to emphasize this situation.

In the *D. pilimana* subgroup, *D. lineosetae* has not yet been studied by Carson; but on the basis of the shape of the aedeagus, it is predicted that chromosomally it will certainly belong to this subgroup. Chromosomally, *D. pilimana* and *D. glabriapex* are homosequential (Carson and Stalker, 1968). The preapical protuberance of the aedeagus of *D. pilimana* is somewhat variable. In some specimens, the aedeagus tends to resemble and in some cases is indistinguishable from that of *D. glabriapex*. Morphologically, these two species are also very similar but are distinct species, based on consistent morphological (external) characteristics.

In the *D. vesciseta* subgroup, there are two species, *D. vesciseta* and *D. hexachaetae*, which are small; and the other two, *D. virgulata* and *D. digressa*, which are at least twice as large. It is difficult to visualize that these four species belong to the same subgroup. Not only are there extreme size differences between the small and large species, but there are also distinct differences in the leg ornamentation and wing markings. The phallic organs, nevertheless, show that these four species definitely belong to a single subgroup. Carson's chromosomal study shows that *D. hexachaetae* is indeed closely related to *D. virgulata*. Also, *D. vesciseta* is shown to differ from *D. virgulata* by only one chromosomal inversion and from *D. hexachaetae* by only two chromosomal inversions. However, he has also found that *D. vesciseta* is chromosomally homosequential with *D. pilimana* and *D. glabriapex* of the *D. pilimana* subgroup. This does not contradict the genitalic evidence which places *D. vesciseta* in a separate subgroup from that of *D. pilimana* and *D. glabriapex*, because it has been shown that speciation of picture-winged species of Hawaiian *Drosophila* is not always accompanied by changes in the banding arrangements of the polytene chromosomes. In a number of cases, speciation has occurred due to mutational changes on the submicroscopic level of the chromosomes without alteration of the banding sequences. Therefore, it can be shown that chromosomally homosequential species may belong to separate subgroups on the basis of genitalic evidence. The shape of the aedeagus of *D. vesciseta* shows that it is more closely related to species of the *D. vesciseta* subgroup rather than those of *D. pilimana* subgroup. Therefore, in this particular situation, the genitalia evidence provide supplementary information on the relationships of these species.

In the *D. pilimana* subgroup, close examination of the intraspecific variability of the shape of the preapical protuberance of the aedeagus and the overall shape of the aedeagus show that there is a slight resemblance to those of the *D. grimshawi* subgroup. Interestingly, Carson's chromosomal study shows that there is only one chromosomal inversion difference between *D. pilimana* and *D. grimshawi*. There appears to be a definite relationship between the two subgroups; however, the general shape of the preapical protuberance of the aedeagus will readily differentiate between the two subgroups. Specifically, in the *D. pilimana* subgroup, the preapical protuberance is in general, wider at the base and typically more angular rather than rounded at the apex. The fact that there is a tendency for the shape of the aedeagus in some specimens of the *D. pilimana* subgroup to

resemble those of the *D. grimshawi* subgroup but not vice versa, gives reason to believe that these two subgroups are indeed closely related subgroups but are probably separate subgroups.

In the *D. grimshawi* subgroup, *D. grimshawi*, *D. bostrycha*, and *D. disjuncta* are chromosomally homosequential species. However, *D. orphnopeza* and *D. villosipedis* of the *D. orphnopeza* subgroup are also homosequential with these three species. The external male genitalia show that the latter two species definitely belong to a separate subgroup from the first three. Also, chromosomally, *D. balioptera* shares a common inversion with *D. orthofascia* and *D. engyochracea* which would make *D. balioptera* appear to belong to the *D. orphnopeza* subgroup. However, the male genitalia of *D. balioptera* would definitely place this species in the *D. grimshawi* subgroup; and chromosomally, *D. balioptera* differs from *D. grimshawi* by only one inversion. Both of these situations do not contradict the chromosomal evidence in the evolution of these species. Rather, the genitalia evidence certainly appears to supplement the chromosomal evidence and perhaps provides a better picture of the relationships between these species.

It was stated earlier that in some species, there are setae on the parameres. The species with minute setae on the parameres are *D. paucipuncta* and *D. uniseriata* of the *D. paucipuncta* subgroup; *D. virgulata* and *D. digressa* of the *D. vesciseta* subgroup; and *D. balioptera* of the *D. grimshawi* subgroup. These five species in their respective subgroups do not, however, share any other similarities in their phallic organs and therefore are not considered to be related to each other on the basis of the setae on the parameres.

White (1968) states that there are only two "sure" exceptions to the generalization, that as far as the higher animals are concerned, even the most closely related species are usually different in chromosomal karyotypes. These two exceptions occur in certain complexes of *Drosophila*. Wasserman (1962) presented evidence that *D. mulleri*, *D. aldrichi*, and *D. wheeleri* of the *D. mulleri* complex do not differ at all in the banding sequences of their polytene chromosomes. Carson et al. (1967) found that this same situation occurs in several species complexes of Hawaiian *Drosophila*. All of these species which have been found to be chromosomally homosequential, have been described as distinct species on the basis of consistent morphological characteristics, and in most cases, the species concept has been verified by behavioral studies and hybridization experiments. Carson has shown that speciation in the picture-winged species of Hawaiian *Drosophila* has resulted in pronounced morphological diversity but with remarkable stability of the chromosomal banding arrangements. The phallic organs of these species are also very "stable" characteristics of the species subgroups. Species which may be morphologically (external) very different may be shown to belong to the same subgroup based on their genitalic characteristics. In cases where chromosomally homosequential species occur, the genitalic characteristics supplement the chromosomal evidence in the relationships of the species involved. This situation is illustrated in the case where *D. vesciseta* is shown to be chromosomally homosequential with *D. pilimana* and *D. glabriapex* of the *D. pilimana* subgroup but is found to be more closely related to *D. virgulata* and *D. hexachaetae* on the basis of genitalic characteristics.

Conclusion

In this study it was found that grouping the picture-winged species of Hawaiian *Drosophila* on the basis of external male genitalia closely resemble the groupings based on chromosomal evidence. Thus, in cases where the chromosomal banding arrangements of a particular species cannot be studied due to difficulty in rearing in the laboratory, a careful study of the phallic organs of a field-collected adult male, would help to determine its relationship to other species. There have been several instances where formerly only a single male specimen represented the whole of the collection of a particular picture-winged species and its relationship to a particular group of species was determined on the basis of its genital apparatus. Later, when females of this same species were collected and when chromosomal evidence became available, its relationship to the same group of species (as had been determined on the basis of genitalic characteristics) was verified. Therefore, the study of the external male genitalia can play an important role in the study of the evolution of Hawaiian Drosophilidae.

In a few instances, the subgroupings may not appear to be on as sound a basis as the others. This was due to a lack of a sufficient number of specimens of several species. With more material, these subgroupings would no doubt be more firmly established.

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