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Genitalia of F₁-Hybrids within the *Cardini* Species Group of *Drosophila*

With 5 Text-figures

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In recent years, knowledge of the structure of sclerotized organs of the genitalia of the family Drosophilidae has become increasingly important as an auxiliary element in genetics; it has offered a new approach to the analysis of problems in relation to evolution and phylogeny. The male genitalia of drosophilid flies were first described and employed by Sturtevant (1919) as useful tool in delimiting species, between *Drosophila melanogaster* and *D. simulans*, subgenus *Sophophora*, genus *Drosophila*. That use has also been recognized by many other workers; among them Dobzhansky and Bridges (1928), Frolova and Astarov (1929), Gleichauf (1936), Malogolowkin (1953), Nater (1953), Burla (1954), Okada (1956), Basden (1961), Wheeler, Takada and Brncic (1962) and Wheeler and Takada (1963) are notable, all of them having been made extensive studies of species of the Drosophilidae, based on the structure of both the external apparatus and the copulatory organs of males. Comparative studies of the male genitalia have been made by Hsu (1949) based on the external genital apparatus, and by Okada (1954, 1955) with special regard to the copulatory organs.

On the other hand, the ovipositor of female drosophilid flies has been fragmentarily investigated by several workers, since the first work published by Sturtevant (1921) on North American species of *Drosophila*. Recently the ovipositor of the Drosophilidae of Japan has been described by Okada (1956), and that of South American species of *flavopilosa* species group by Wheeler, Takada and Brncic (1962).

It has long been recognized that interspecific hybrids furnish valuable material for the study of comparative genetics and phylogeny. Heed *et al.* (1957, 1959, 1962) found the small island population of species of the *cardini* species group.

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subgenus *Drosophila* in the West Indies to be an excellent source of material for studying speciation and evolution in such geographically isolated populations of relatively few individuals. In the course of his work with the *dunni* complex. Heed has completed hybridization tests among all of the species and subspecies of *dunni* subgroup, thus far known. Further, Futch (1962) has hybridized eight currently known species of the *cardini* subgroup. These tests included intra-subgroup crosses in all possible combinations and also some intersubgroup crosses between members of the *cardini* subgroup and three members of the *dunni* subgroup.

In view of the need to inquire into sclerotized organs of the genitalia of drosophilid flies, the present author undertook a study of the structure of the genitalia of the F_1 -hybrid of *Drosophila*. The present paper describes the genitalia of F_1 -hybrids produced between four members of the *cardini* subgroup and two members of the *dunni* subgroup.

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MATERIALS AND METHOD

The parental species and adult F_1 -hybrids were derived directly from those maintained as stock at the University of Texas Laboratory. Stock numbers and collection localities are listed in Table 1.

Table 1
List of the stocks used in this study, with their geographic origin
and the University of Texas stock number

subgroup	species	locality	stock number
<i>cardini</i>	<i>acutilabella</i>	St. Vicent, Cuba	2380.2
"	<i>polymorpha</i>	El Recuerdo, Colombia	H 186.49
"	<i>neomorpha</i>	Cerro la Campana, Panama	H 183.4
"	<i>neocardini</i>	Sao Paulo, Brazil	H 340.7
"	<i>parthenogenetica</i>	Atlixco, Mexico	1802.17
<i>dunni</i>	<i>a. arawakana</i>	Gadalupe, F. W. I.	H 252.7
"	<i>belladunni</i>	Hardware Gap, Jamaica	H 356.3

The methods used for demonstrating genital structure are quite simple. The flies were dissected on a slide, and the terminal segments of abdomen were boiled in 10% sodium hydroxide. This was done by transferring the genitalia to a drop of creosote. after clearing in a drop of phenol. The low power of a binocular dissection microscope was generally adequate, both for dissection and for observation of structure.

The genitalia under discussion are composed of four main parts, namely, male external genital apparatus, copulatory organs of male, ovipositor of female and spermathecae. It is necessary to distinguish minor parts of these structures in order to give detailed

descriptions of them and to discuss their comparative structure for the first time. The author used a compound microscope to check such minor parts at higher magnifications ($50\times$ to $400\times$).

Figure 1 shows the sclerotized organ of genitalia in a typical species of *cardini* subgroup, *Drosophila parthenogenetica* Stalker 1953.

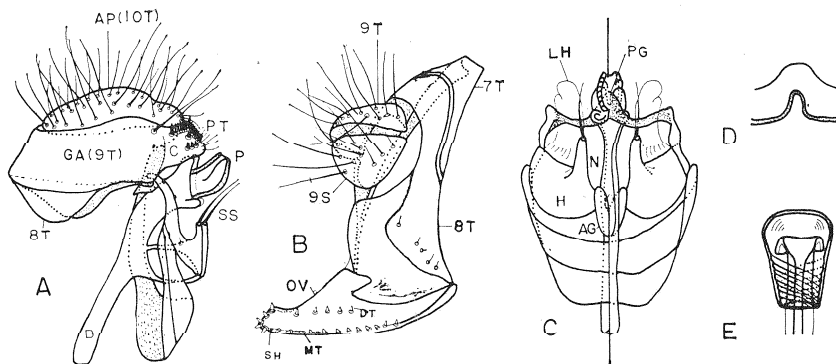


Fig. 1. Genitalia of *Drosophila parthenogenetica*. A, Lateral aspect (left side) of male genitalia. B, Lateral aspect (right side) of female genitalia. C, Ventral aspect of male copulatory organs. D, Bridge (decasternum) connecting the clasper. E, Spermatheca. AP, anal plate (10th tergite); GA, genital arch (9th tergite); 8T, 8th tergite; 7T, 7th tergite; 9S, 9th sternite; C, clasper; PT, primary teeth; SS, ^{para-}submedian spine; P, penis; B, basal apodeme of penis; AG, anterior gonapophysis; PG, posterior gonapophysis; LH, lobe of hypandrium; H, hypandrium; N, median notch; OV, ovipositor; DT, discal teeth; MT, marginal teeth; SH, subterminal hair.

DESCRIPTIONS

Drosophila parthenogenetica Stalker (Fig. 1).

External genital apparatus of male (Fig. 1 A):- Genital arch with a bristle above clasper, lower portion rather membranous; heel pronounced, posterior margin convex at middle. Anal plate separated from genital arch, kidney-shaped; rear angle slightly developed; pronounced tip with about 5 long bristles. Primary teeth of clasper about 8; secondary teeth in 3 groups, one along upper margin, about 7; one at center just above the primary teeth, 1; the third group on lower margin, about 3; marginal bristles about 8. Bridge connecting the clasper as shown in Figure 1 D.

Male copulatory organs (Fig. 1 A, C):- Partly figured by Heed (1962). Penis fused, slender, subapically with transparent sheath. Anterior gonapophyses small with a sensillum. Hypandrium semi-elliptical shaped, median notch deep; a pair of paramedian spines onto the protuberance of upper portion; transparent lobe of hypandrium present. Posterior gonapophyses separated from penis, W-shaped. Phallic formula= $ab'CdEfgHIkLmn$. Divergency index about=7.5. Phallosomal index about=1.3.

Female genitalia (Fig. 1 B):- Lower margin of 8th tergite with about 5 hairs. Lobe of ovipositor quadrate at tip, with about 17 marginal teeth and 5 discal teeth. Spermatheca as shown in Figure 1 E.

Drosophila neomorpha Heed and Wheeler (Fig. 2 A, D, G, J).

External genital apparatus of male (Fig. 2 A):- Briefly described by Heed and Wheeler (1957). Genital arch with a bristle at middle portion of posterior margin, highly sclerotized and broad below; heel pronounced. Anal plate with about 5 spine-like bristles at tip area; rear angle absent. Clasper rectangular, with 6 primary teeth; 6 secondary teeth at center of clasper and about 4 marginal bristles. Bridge connecting the clasper as shown in Figure 2 D.

Male copulatory organs (Fig. 2 G, I):- Partly figured by Heed, 1962. Penis slightly curved dorsad at tip in lateral view, with swelled transparent sheath. A pair of paramedian spines of hypandrium rather short. Phallic formula =aBCdEfgHIkLmn. Divergency index =7.0. Phallosomal index about 1.5.

Female genitalia:- Lower margin of 8th tergite with about 7 hairs. Lobe of ovipositor fusiform, with about 18 marginal and discal teeth. Spermathecae described by Heed and Wheeler (1957).

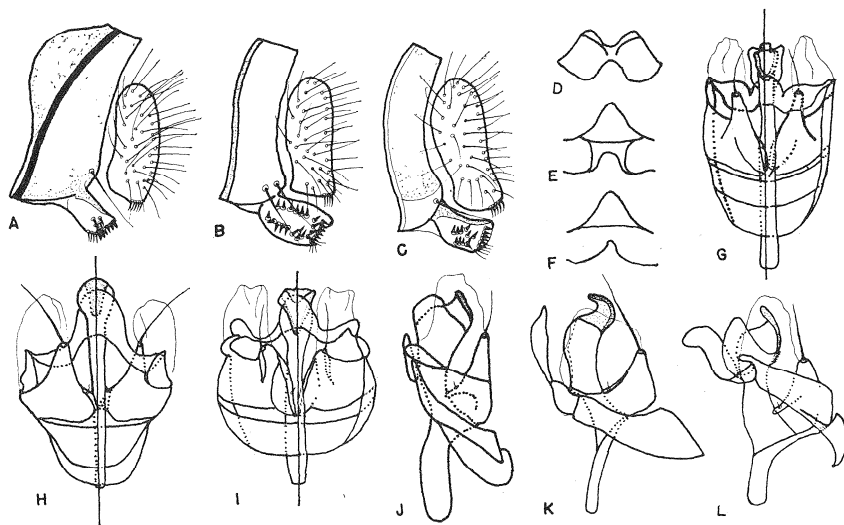


Fig. 2. Male genitalia of parental species and F_1 -hybrid. *Drosophila neomorpha*: A, external genital apparatus; D, bridge connecting the clasper; G, male copulatory organs in ventral aspect (left side) and dorsal aspect (right side); J, male copulatory organs in lateral aspect. B, E, H and K, *Drosophila polymorpha*; C, F, I and L, *D. neomorpha* ♀♀ × *D. polymorpha* ♂♂.

Drosophila polymorpha Dobzhansky and Pavan (Fig. 2 B, E, H, K).

External genital apparatus of male (Fig. 2 B):- Genital arch slightly narrow with 2 bristles above clasper. Anal plate with about 4 spine-like bristles at tip area; rear angle present. Clasper elliptical shaped, primary teeth about 7; secondary teeth in 3 groups, one along upper margin about 6; one at side by side above the primary teeth, about 4; the third group on lower margin, about 9; marginal bristles about 5. Bridge connecting the clasper as shown in Figure 2 E.

Male copulatory organs (Fig. 2 H, K):- Partly figured by Heed (1962). Penis fused, apically rounded in ventral view; strongly curved dorsad at tip in lateral view, with

indistinct sheath. A pair of paramedian spines of hypandrium long. Phallic formula =aBCdEfgHIkLmn. Divergency index=7.0. Phallosomal index about 1.5.

Female genitalia:- Lower margin of 8th tergite with about 5 hairs. Lobe of ovipositor narrowly pointed apically, with upper margin medially swollen. Spermathecae knob-like shaped, the inner duct narrow and the end of duct flared.

D. neomorpha ♀♀ × *D. polymorpha* ♂♂. Male hybrid (Fig. 2 C, F, I, L).

External genital apparatus (Fig. 2 C):- Genital arch narrow, heavily sclerotized in upper 4/5, with only one bristle just above the clasper. Rear angle of anal plate slightly pronounced. Clasper intermediate in shape; primary teeth 6; secondary teeth arranged in irregular row, 11; marginal bristles 5. Bridge connecting the clasper more similar to *polymorpha* than to *neomorpha*, as shown in Figure 2 F.

Copulatory organs (Fig. 2 I, L):- Penis just intermediate between parental species; sheath much reduced *neomorpha* in shape, with small serration in ventral surface. Paramedian spines short. Protuberance of hypandrium rather shorter than the parental males. Phallic formula =ab'CdEfgHIkLmn. Divergency index=7.5. Phallosomal index about 1.9.

Drosophila belladunni Heed and Krishnamurthy (Fig. 3 A, D, G, J and Fig. 5 A, D).

External genital apparatus of male (Fig. 3 A):- Briefly described by Heed and Krishnamurthy (1959). Genital arch broad below, with a bristle above the clasper. Anal plate with 4 medium length bristles at tip area; rear angle absent. Clasper trapezoid, primary teeth about 6, first teeth stout and thick; secondary teeth in 2 groups, one along upper margin about 4; one on lower margin, 3; marginal bristles about 6. Bridge connecting the clasper as shown in Figure 3 D.

Male copulatory organs (Fig. 3 G, J):- Partly figured by Heed (1962). Penis fused, apically swollen in ventral view; transparent sheath serrated at posterior margin. Anterior gonapophyses rather large. Paramedian spines of hypandrium medium length and stout. Phallic formula =ab'CDEfgHIkLmn. Divergency index=6.5. Phallosomal index about 1.2.

Female genitalia (Fig. 5 A, D):- Lower margin of 8th tergite with about 7 hairs.

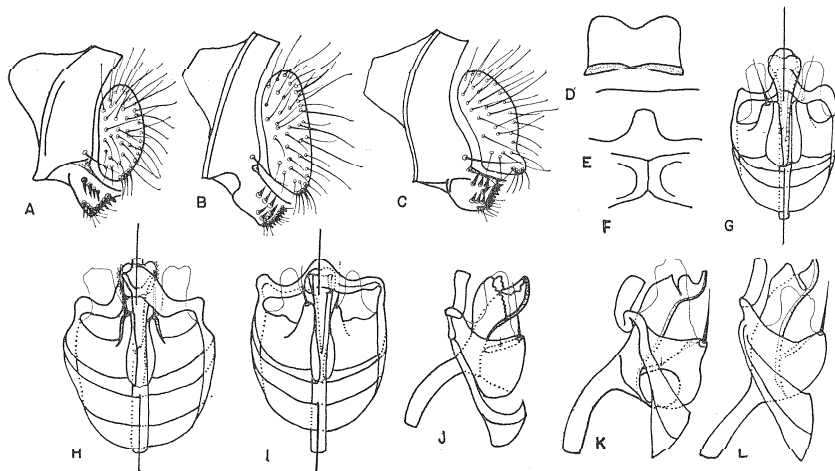


Fig. 3. Male genitalia of parental species and F_1 -hybrid. A, D, G and J, *Drosophila belladunni*; B, E, H and K, *Drosophila neocardini*; C, F, I, and L, *D. belladunni* ♀♀ × *D. neocardini* ♂♂.

Lobe of ovipositor exceedingly broaden at middle, and with about 14 marginal and about 4 discal teeth. Spermatheca as shown in Figure 5 D.

Drosophila neocardini Streisinger (Fig. 3 B, E, H, K and Fig. 5 B, E).

External genital apparatus of male (Fig. 3 B):- Described and figured by Hsu (1949). Clasper rectangular, primary teeth about 7; secondary teeth in 3 groups, one along upper margin, about 3; one at center, 1; the third group on lower margin, about 4; marginal bristles about 7. Anal plate with about 6 medium length bristles at tip area; rear angle rounded. Bridge connecting the clasper as shown in Figure 3 E.

Male copulatory organs (Fig. 3 H, K):- Partly figured by Heed (1962). Penis fused, curved dorsad at tip in lateral view, with a fissure subapically in lateral view; transparent sheath with serration at posterior margin. Paramedian spines of hypandrium stout. Phallic formula= $ab/CDEfgHikLmn$. Divergency index=6.5. Phallosomal index about 1.3.

Female genitalia (Fig. 5 B, E):- Lower margin of the 8th tergite with about 5 hairs. Lobe of ovipositor pointed at tip and broaden at middle; marginal teeth about 18; discal teeth 3. Spermatheca as shown in Figure 5 E.

D. belladunni ♀♀ × *D. neocardini* ♂♂. Male hybrid (Fig. 3 C, F, I, L).

External genital apparatus (Fig. 3 C):- Genital arch with a bristle above the clasper, posterior margin concaved at middle. Anal plate with 5 medium length bristles at slightly pointed tip; rear angle slightly observed. Clasper rather circular, primary teeth 7; secondary teeth in 3 groups, one along upper margin, 3; one at center, 1; the third group

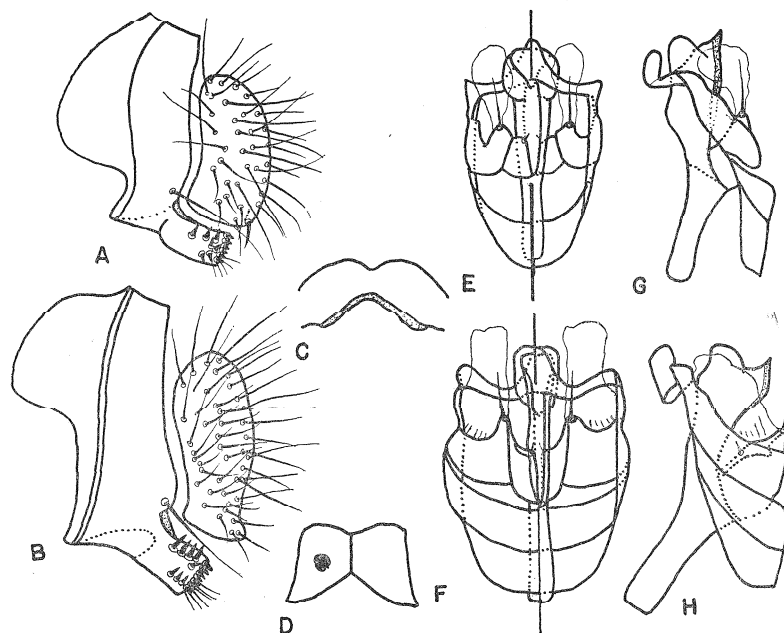


Fig. 4. Male genitalia of parental species and F_1 -hybrid. A, C, E and G, *Drosophila arawakana arawakana*; B, D, F and H, *D. a. arawakana* ♀♀ × *D. neocardini* ♂♂.

on lower margin, 3; marginal bristles 9. Bridge connecting the clasper as shown in Figure 3 F.

Copulatory organs (Fig. 3 I, L):- Penis slender, slightly curved dorsad at tip in lateral aspect, with a fissure subapically. Lobe to hypandrium rather small; paramedian spines rather short and weakened. Without sensilla at anterior gonapophyses. Phallic formula =ab'CDEfoghiklmn. Divergency index=7.5. Phallosomal index about 1.5.

Female hybrid (Fig. 5 C, F):- Lower margin of the 8th tergite with 7 hairs. Lobe of ovipositor intermediate in shape from parental females, apically more similar to *neocardini*; marginal teeth 18, discal teeth 3. Spermatheca as shown in Figure 5 F.

Drosophila arawakana arawakana Heed (Fig. 4 A, C, E, G and Fig. 5 B, E, H, K).

External genital apparatus of male (Fig. 4 A):- Genital arch with a bristle above the clasper. Anal plate large, rear angle present; with a stout bristle at tip. Clasper rectangular, primary teeth about 6; secondary teeth in 3 groups, one along upper margin, about 3; one at center 1-2; the third group on lower margin, 1-2; marginal bristles about 5. Bridge connecting the clasper as shown in Figure 4 C.

Male copulatory organs (Fig. 4 E, G):- Partly figured by Heed (1962), as *nigrodunni* complex. Penis fused, apically much dilated in ventral view; transparent sheath protruded dorsad. Hypandrium rather small; lobe of hypandrium knob-like shaped; Paramedian spines short. Anterior gonapophyses medium size. Phallic formula =aBCd'EfgHIkLmn. Divergency index=6.5. Phallosomal index about 1.2.

Female genitalia (Fig. 5 H, K):- Lower margin of the 8th tergite with 5 hairs. Lobe of ovipositor pointed at tip, broaden at middle; marginal teeth about 16, discal teeth about 4. Spermatheca as shown in Figure 5 K.

D. a. arawakana ♀♀ × *D. neocardini* ♂♂. Male hybrid (Fig. 4 B, D, F, H).

External genital apparatus (Fig. 4 B):- Genital arch broad below, with a bristle above the clasper. Anal plate with rear angle; tip slightly pointed, without prominent bristle. Clasper rectangular, primary teeth about 7; secondary teeth in 3 groups, one along upper margin, 4; one at center, 1; the third group on lower margin, 3; marginal bristles 6. Bridge connecting the clasper as shown in Figure 4 D.

Copulatory organs (Fig. 4 F, H):- Penis more similar to *arawakana* than to *neocardini*; apically rectangular in ventral aspect. Paramedian spines of hypandrium short. Phallic formula =aBCd'EfgHIkLmn. Divergency index=6.5. Phallosomal index about 1.5.

Drosophila acutilabella Stalker (Fig. 5 G, J).

External genital apparatus of male:- Briefly described by Heed and Krishnamurthy (1959). Genital arch with a bristle above the clasper, lower margin rounded. Anal plate with subapical extension bearing one exceptionally stout long curved spear-like spine at tip; rear angle present. Clasper quadrate, primary teeth about 6, small; secondary teeth in 3 groups, one along upper margin, 5; one at center, 1; the third group on lower margin, about 4.

Male copulatory organs:- Partly figured by Heed (1962). Penis fused, transparent sheath protruded dorsad in lateral view, apically rectangular in ventral aspect. Paramedian spines of hypandrium short. Sensillum of anterior gonapophyses absent. Phallic formula =aBCd'EfgHIkLmn. Divergency index=7.5. Phallosomal index about 1.2.

Female genitalia (Fig. 5 G, J):- Lower margin of the 8th tergite with about 6 hairs. Lobe of ovipositor exceedingly broaden at middle, marginal teeth about 15; discal teeth about 5. Spermatheca as shown in Figure 5 J.

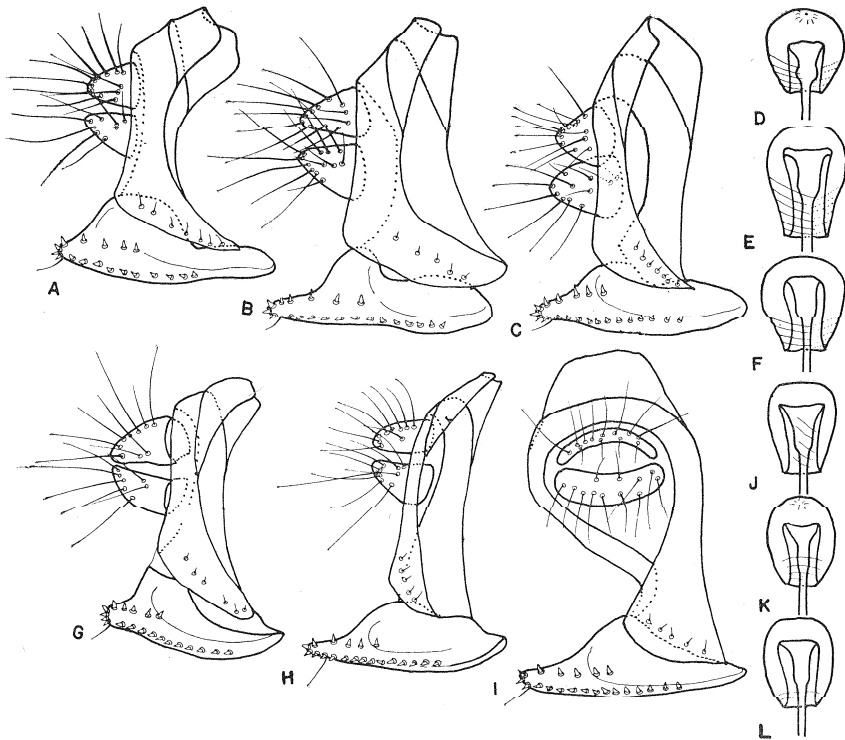


Fig. 5. Female genitalia of parental species and F_1 -hybrids. *Drosophila belladunni*: A, lateral aspect of female genitalia; D, spermatheca. B and E, *Drosophila neocardini*; C and F, *D. belladunni*♀♀×*D. neocardini*♂♂; G and J, *Drosophila acutilabella*; H and K, *Drosophila arawakana arawakana*; I and L, *D. acutilabella*♀♀×*D. a. arawakana*♂♂.

D. acutilabella♀♀×*D. a. arawakana*♂♂. Female hybrid (Fig. 5 I, L).

External genitalia:— Lower margin of the 8th tergite with 5 hairs. Lobe of ovipositor exceedingly broaden at middle, apically slender; marginal teeth 16; discal teeth 5. Spermatheca as shown in Figure 5 L.

DISCUSSION

So far as the author is aware, there is not one instance in *Drosophila* taxonomy where closely related species cannot be differentiated by genitalia. A good case in point is the taxonomically difficult *repleta* species group in North, Central and South America which consists of 67 described species and several undescribed forms. The shape and coloration of the body are so similar in many instances that identification is possible only by dissecting out the genitalia. Accordingly, there are no taxonomic difficulties with the *cardini* species group, at least in morphology of the male genitalia, even though almost all specimens look superficially very similar in size, shape and general coloration.

The *melanogaster-simulans* cross will be considered first, since it represents the first known case of hybridization in the genus *Drosophila*. It was analyzed by Sturtevant (1929 a, b). These two species have the same metaphase chromosome pattern and are strikingly similar morphologically. They show a few minor differences, but the only satisfactory characters by which they may be separated is the external male genital apparatus. The posterior process of the genital arch appears like a clamshell in *simulans*, while it is like a small hook in *melanogaster*. It was found that all hybrids had small undeveloped gonads, and that they were completely sterile. Their genital apparatus was intermediate in shape.

Futch (1962) has hybridized many newly obtained specimens in the *cardini* species group. According to his data, five hybrids which are dealt with in this study were completely sterile; except in the case of the female hybrid of *acutilabella*♀ × *a. arawakana*♂, only female offspring are produced.

Therefore, the author has confirmed the fact that the rear angle of anal plate of male hybrids is slightly like that of the parental male, as the tip of anal plate becomes pointed, the clasper appears to become intermediate in shape from both parental species, the secondary teeth arranged in irregular rows, the bridge connecting the clasper more similar to the parental male, the penis just intermediate in shape and the protuberance of hypandrium rather shorter than in the parental male.

On the other hand, the lobe of the ovipositor of female hybrids was rather broad or nearly intermediate in shape at the middle portion from both parental species and the spermathecae were just intermediate in shape.

SUMMARY

This paper describes some phenotypical characters of genitalia useful for species separation in 7 species of the *cardini* species group and in 5 F₁-hybrids between four members of the *cardini* subgroup and two members of the *dunni* subgroup.

In the following are summarized F₁-characters observed in *cardini* hybrids:

- 1) Genital arch—Male hybrid of *neomorpha* × *polymorpha*, for instance, heavily sclerotized in upper 4/5. The genital arch is wholly sclerotized in *neomorpha* and weakened in *polymorpha*. Other cases seem to be intermediate in shape.
- 2) Anal plate—The rear angle of anal plate of male hybrids is somewhat like that of the parental male. The tip of anal plate becomes pointed from the parental species.
- 3) Clasper—It appears to be intermediate in shape. The secondary teeth are arranged in irregular rows.
- 4) Bridge connecting the clasper—It is rather similar to the parental male.
- 5) Penis—It is just intermediate in shape between those of the parental species.
- 6) Anterior gonapophyses—Male hybrids of *belladunni* × *neocardini*, for example, lack sensillum at anterior gonapophyses.
- 7) Protuberance of hypandrium—It is rather shorter than in the parental male.

- 8) The lobe of ovipositor of female hybrids was rather broad or nearly intermediate in shape at the middle portion in comparison with parental species.
- 9) Spermathecae were just intermediate in shape.

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