TAXONOMY AND HYBRIDIZATION IN THE CARDINI GROUP OF DROSOPHILA

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INTRODUCTION

Recent studies on the taxonomy of the Drosophilidae have shown that in many cases species groups thought to be represented by one or a few species actually consist of many species, often so similar morphologically that they are readily and repeatedly confused. One such group, that to which *Drosophilia cardini* belongs, is of special interest, not only because it contains at least two species showing striking and clear-cut polymorphism in the abdominal banding pattern (a situation unusual for *Drosophila*), but also because at least two members of the group may occasionally reproduce by diploid parthenogenesis (Stalker, 1951 and unpublished). It is the purpose of this paper to describe two new North American members of the group, as well as to report some experimental hybridizations which have been carried out.

The discussion of the taxonomy will for the most part be limited to those species known from Central and North America and from the West Indies. Species so included are: *D. polymorpha* Dobzhansky and Pavan, *D. cardini* Sturtevant, *D. similis* Williston, *D. cardinoides* Dobzhansky and Pavan, *D. neocardini* Streisinger, and two species to be described below: *D. acutilabella* sp. nov. and *D. parthenogenetica* sp. nov. Of the species listed above, *D. neocardini* is reported only from Brazil, but is included here because its morphological similarity to *D. parthenogenetica* has been the cause of confusion in the past.

The *cardini* group is a taxonomically difficult one. Sturtevant (1921), in discussing the two members known at that time, *D. cardini* and *D. similis*, states that he could find no reliable criteria for separation of these two species in pinned specimens. Streisinger (1948) also emphasizes the difficulty of separation of the species on morphological criteria alone, and concludes that "the most convincing and conclusive method of classification proved to be their behavior in crosses." The workers in the University of Texas Laboratory have run into the same difficulty in their extensive collections of material from the southwest and Florida.

The members of the group show striking differences in abdominal banding pattern. However, although this taxonomic character is at times very useful, it must be used with caution as all the species considered here show some intra-specific variability in abdominal banding, and in the case of two, *D. polymorpha* and *D. acutilabella*, this variability is so striking as to make any separation based solely on banding pattern completely unreliable.

Although it is clear that morphological criteria for separating the members of this group will probably never be wholly satisfactory,

it is also clear that any separation which is possible on these grounds is extremely valuable, not only in sorting and saving newly collected material, but also in order that the pinned material available may be more fully exploited. Accordingly a key designed to distinguish the above-mentioned species is presented. This key does not include those members of the group (at present known only from South America) which have either divergent anterior scutellars or a comb of short, stout bristles on the anterior surface of the fore-femora.

KEY FOR NORTH AND CENTRAL AMERICAN MEMBERS OF THE CARDINI GROUP

1. Several well-differentiated bristles on posterior surface of fore-femur in addition to one or two bristles on proximal fourth. Fore-femur lightly haired on posterodorsal surface (as in fig. 1K)..... 3
   No well-differentiated bristles on posterior surface of fore-femur in addition to one or two on proximal fourth. Fore-femur densely haired distally on posterodorsal surface, at least in male (as in fig. 1J)..... 2
2. Scutellum somewhat pointed at posterior tip when viewed from above; scutellar dorsal surface convex when viewed from side. Largest of posteriorly directed heavy spine-like bristles on flexor surface of fore-femur with a length equal to or greater than the greatest diameter of the femur (fig. 1J). Apical index about 1.7.
   Male: Fore-femur densely haired distally on posterodorsal surface. Labellum when viewed in profile shows slight protuberance on ventral edge about two-fifths of the distance from the posterior margin; antero-ventral edge of labellum normally rounded (fig. 1A). Each clasper with parallel anterior and posterior row of heavy spine-like bristles (fig. 2E). (Brazil, Guatemala, Panama)..... **polymorpha** Dobzhansky and Pavan
   Scutellum flattened or gently rounded at posterior tip when viewed from above, dorsal surface of scutellum nearly flat when viewed from side. Largest of posteriorly directed heavy spine-like bristles on flexor surface of fore-femur with a length not more than two-thirds greatest diameter of femur, often about one-half greatest diameter. Apical index about 2.3. Fore-femur in both sexes densely haired distally on posterodorsal surface.
   Male: Labellum when viewed in profile shows no ventral protuberance, and smoothly rounded posteroventral edge, but forms almost a right angle at the anteroventral edge (fig. 1G). Clasper lacking the anterior row of heavy spine-like bristles (fig. 2F). (Mexico)..... **parthenogenetica** sp. nov.
3. Fourth-vein index about 1.5; crossveins dark but without clouds, palpi of both sexes roughly club-shaped with one or more well-differentiated bristles on anterolateral edge (figs. 2G, H). Last three abdominal segments in the female and last two in male more lightly pigmented than more anterior segments. (Brazil)..... **neocardini** Streisinger
   Fourth vein index about 1.7; crossveins dark but without clouds; at level of anterior crossvein L2 is usually closer to costa than to L3, or may be equidistant from them. At level of termination of L5 in posterior margin of wing, L2 is closer to costa than to L3. Palpi of both sexes very broad and rounded when viewed from below, and with hairs or weakly differentiated bristles on both medioventral and lateroventral surfaces (figs. 2 O, P, Q, R). In the male the palpi completely lacking any differentiated bristles, with anterior portion of ventral surface turned up so that it forms an anteroventral face. Last three abdominal segments in female and last two in male darker than more anterior segments. (Brazil, Central America)..... **cardinoides** Dobzhansky and Pavan
   Fourth-vein index about 1.7; crossveins with clouds. At level of anterior crossvein L2 is closer to L3 than it is to costa. At level of termination of L5 in posterior margin of wing L2 is equidistant from L3 and costa. Palpi of both sexes roughly club-shaped, with one or more well-differentiated bristles on anterolateral edge (as in figs. 2 I, J)..... 4
4. *Male:* Labellum when viewed in profile shows a prominent horn-like

protuberance on anteroventral edge (fig. 1 B). Anteromedial corner of anal plate with single long bristle which is strongly curved laterally. This bristle attached to prominent anterior process of anal plate (fig. 2 C). (Florida, Cuba) ..... **acutilabella** sp. nov.

Male: Labellum when viewed in profile shows no protuberance on margin. Anal plate with one or two (rarely three) long, almost straight, anteriorly directed bristles on anteromedial corner (fig. 2 A). (West Indies, Florida, Central America, Mexico, Texas?) ..... **cardini** Sturtevant

There appears to be no simple and completely accurate way of separating females of *D. acutilabella* and *D. cardini* morphologically. However, since the light form of *D. acutilabella* (see description) is fairly common and since a comparable light form of *D. cardini* is not known, a mature female which keys to couplet 4 and is light is probably *D. acutilabella* rather than *D. cardini*.

Drosophila acutilabella sp. nov.

Male.—Arista exclusive of terminal fork with about five branches above and two below. Antenna light brown, third joint not noticeably darker. Front more than one-third width of head; front and vertex velvety golden-brown. Triangle and orbits slightly darker and somewhat shiny. Middle orbital about one-fifth other two. Second oral about four-fifths length of first. Carina large, broad below, not sulcate; flattened anterior surface somewhat pollinose. Face pale tan. Cheeks shining light brown, becoming darker anteriorly, their greatest width about one-fifth greatest diameter of the eye. Eyes with short pile which is golden ventrally, darker dorsally. Maxillary palpi pale yellowish-brown, with bristle at tip and two to three smaller bristles along lateral margin (see fig. 2 I). Labellum when viewed from side shows a very distinct anteroventrally directed horn at its lower anterior margin (see fig. 1 B).

Acrostichal hairs in six rows; no prescutellars. Anterior scutellars convergent. Mesonotum and scutellum shining yellowish-brown without a distinct pattern. Scutellum somewhat darker. Pleurae light brown. Anterior sternopleural about 0.4 length of posterior; median sternopleural varies from an undifferentiated hair to a bristle about 0.4 length of anterior. Halteres light brown with blackish areas on anterior surface of base and bulb. Legs light brown. Numerous recurved hairs on fore-tarsi. Fore-femora with several well differentiated bristles on distal two-thirds of posterior surface, in addition to one or two bristles on basal third. Largest of posteriorly directed, heavy spine-like bristles on flexor surface of fore-femur with a length nearly equal to the greatest width of the femur (fig. 1 K). Apical bristles on first and second tibiae, preapicals on all three.

Wings clear, crossveins slightly clouded. Two prominent bristles at apex of first costal section. Costal index about 3.3; fourth vein index about 1.7; 5x index about 1.2; 4c index about 0.8; apical index (length of third section of the costal vein divided by the length of its fourth section) about 1.7. Third costal section with heavy bristles on its basal two-fifths. Abdomen shining, yellowish-brown with black areas as described below.

Dark Form: First visible tergite with short, narrow posterior band restricted to median third of tergite and sometimes weakened in the

mid-line. Second tergite with longer band which may be produced anteriorly in the middle. Third tergite with posterior band showing notch in mid-line and with anteriorly produced lateral extensions which may or may not be broken free from body of band. Fourth tergite solid black except for lateral margins and clear area in mid-dorsal region. Tergites 5 and 6 solid black except for lateral margins.

Light Form: As above except that the black bands in tergites 1 and 2 are interrupted medially; band in tergite 3 is more deeply notched medially and lacks anterior processes. In tergite 4 lateral anterior processes are usually broken free from main band, and median clear area is usually open anteriorly. Tergite 5 usually has two clear spots on anterior half on either side of the mid-line. Tergite 6 solid black except at lateral margins.

Female.—Fewer recurved hairs on fore-tarsi. Labellum "normal" when viewed from side, that is, lacking antero-ventral horn (Fig. 1E). Abdomen shining, yellowish-brown, with black areas as described below.

Dark Form: First and second tergites as in male. Tergite 3 with posterior band showing anterior notch in mid-line and no anterior processes. Band in tergite 4 with median notch and prominent lateral anterior processes which may extend medially so as to enclose central clear area. Tergites 5 to 7 solid black except for lateral margins.

Light Form: Tergites 1 to 6 with narrow posterior band which is either greatly weakened or broken in the mid-line. Posterior band in tergite 6 very narrow. This tergite may have small median anterior black spot. Tergite 7 with median black area on either side of and above anal plates. The black bands do not reach the lateral margins of the tergites in any segment.

Length of body 2.3 mm; wing 2.3 mm.

The polymorphism in the abdominal pattern is apparently inherited in a simple Mendelian fashion, with the heterozygote phenotypically very close to the light form.

Internal Structures: A single anterior Malpighian tube, branches only at its distal sixth; posterior tubes with ends apposed without formation of continuous lumen. Testis light yellow, about six outer and three inner gyres. Vasa efferentia long slender and colorless. Sperm pump with two long diverticula. Spermathecae light brown, chitinized portion of bulb vase-shaped with the greatest diameter less than the height.

Eggs: with four long acuminate filaments.

Larva: The larvae skip.

Metaphase Chromosomes: 2 pairs of V's, 1 pair of rods and 1 pair of dots.

Puparium: reddish brown; pupal horns (including spiracle branches) about one-fifth length of body of puparium. About nine spiracular branches which are blackish proximally, becoming grayish-brown toward their tips. Stalk of pupal horn with black ring at base of spiracle branches; stalk about equal in length to longest branches.

Holotype male.—Holotype and paratype descendents of a single female from St. Petersburg, Florida: to be deposited in the American Museum of Natural History.

Drosophila parthenogenetica sp. nov.

Male.—Arista exclusive of terminal fork with about five branches above and two below. Antenna yellowish-brown, third joint not noticeably darker. Front over one third width of head; front and vertex light velvety brown. Triangle and orbits somewhat lighter and slightly shining. Middle orbital about one-fourth anterior which is four-fifths posterior. Second oral about four-fifths length of first.

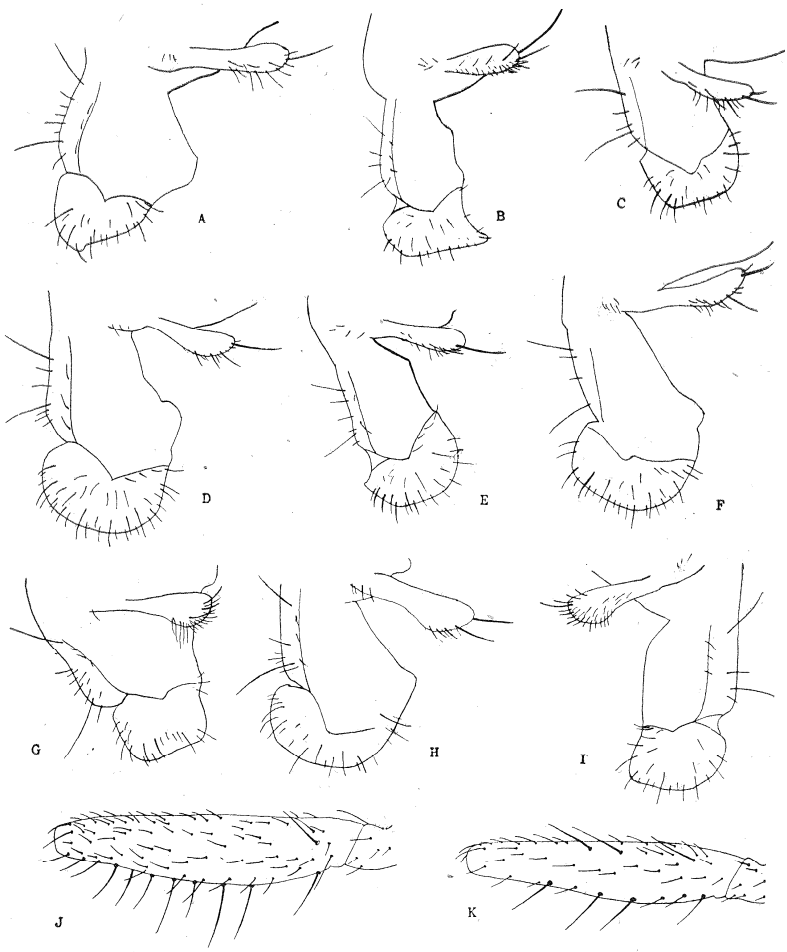

FIG. 1. Proboscis and palp, lateral view. A. *D. polymorpha* male. B. *D. acutilabella* male. C. *D. neocardini* male. D. *D. polymorpha* female. E. *D. acutilabella* female. F. *D. neocardini* female. G. *D. parthenogenetica* male. H. *D. parthenogenetica* female. I. Hybrid male (*D. acutilabella* female X *D. cardinoides* male).

Left fore-femur, posterior view. J. *D. polymorpha* male. K. *D. acutilabella* male.

Carina large, broad below, not sulcate, pollinose on flattened anterior surface. Face shining light brown. Cheeks shining yellowish-brown, their greatest width about one-sixth greatest diameter of the eye. Eyes with short, thick pile, blond below, darker above. Maxillary palpi light yellowish-brown with about five differentiated bristles on ventral anterolateral margin (fig. 2 K). Labellum when viewed from side with angular rather than rounded anteroventral edge (fig. 1 G).

Acrostichal hairs in six rows; no prescutellars. Anterior scutellars convergent. Mesonotum and scutellum shining yellowish brown without a distinct pattern. Pleurae light yellowish-brown. Anterior sternopleural about 0.4 length of posterior; median sternopleural form one-sixth to one-half length of anterior. Halteres light brown. Legs pale yellowish-brown, apicals on first and second tibiae, preapicals on all three. Fore-femur densely haired distally on posterodorsal surface as in *D. polymorpha* (as in fig. 1 J). Largest of posteriorly directed, heavy spine-like bristles on flexor surface of fore-femur with a length not more than two-thirds greatest diameter of femur, often equal to about one-half greatest diameter. No well differentiated bristles on posterior surface of fore-femur in addition to one or two on proximal third. Numerous recurved hairs on fore-tarsi.

Wings light brownish-gray, clear except for light clouds at cross-veins. Veins light brown. Two bristles at distal costal break, but ventral one very weakly differentiated. Heavy bristles on basal two-fifths of third costal section. Costal index about 3.4; fourth vein index about 1.5; 5x index about 1.1; 4c index about 0.5; apical index (length of third section of the costal vein divided by the length of its fourth section) about 2.3.

Abdomen shining light brownish yellow. Segments 2 to 6 with narrow black band on posterior edge, widely interrupted medially, and failing to reach lateral margin of tergite in any segment; bands are weak (or absent in young specimens) on tergites 5 and 6. Bands on all segments tend to show anterior processes laterally which never reach anterior margin. These processes are especially noticeable on segments 3, 4 and 5.

Females.—Anteroventral edge of labellum normally rounded when viewed from side, not angular as in male (fig. 1 H). Maxillary palpi not so broad when viewed from ventral surface, number of bristles on ventral anterolateral margin usually three to four (fig. 2 L). Fore-femur less densely haired on posterodorsal surface. Hairs on tibiae shorter, not recurved. Abdominal black bands show more strongly developed anterior processes at their lateral edges. Seventh tergite with median black spot above anal plates.

Length of body 2.1 mm; wing 2.0 mm.

Chromosomes: 2 pairs of V's, 1 pair of rods (with strong secondary constriction), 1 pair of dots.

Puparium: Reddish brown; pupal horns (including spiracle branches about one-sixth length of body of puparium. About nine spiracle branches which are light grayish-brown in color. Stalk of pupal horn with black ring at base of spiracle branches; stalk about equal in length to longest branches.

Larva: The larvae skip.

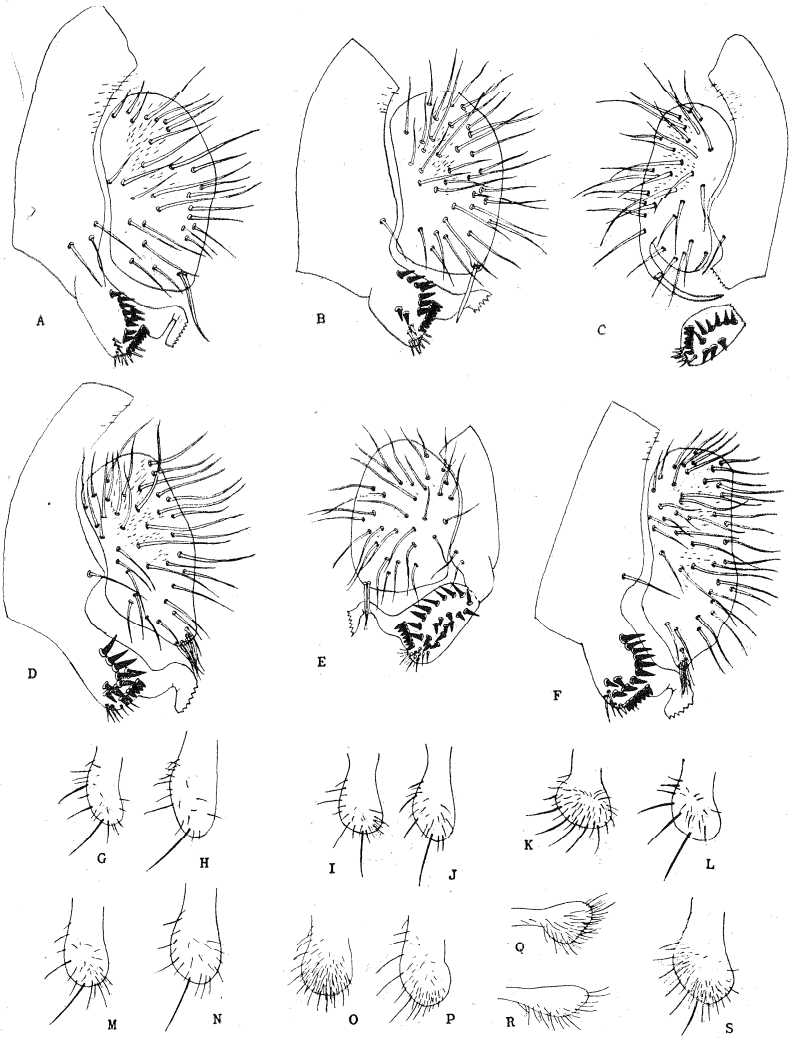

FIG. 2. Male external genitalia showing half genital arch, one anal plate and one clasper. A. *D. cardini*, Florida strain, left half. B. Hybrid, left half (*D. acutilabella* female X *D. cardini* male). C. *D. acutilabella*, right half. In this figure the clasper is shown removed from its normal attachment to the genital arch (indicated by the jagged line), and moved down in order to show more clearly the long, laterally curved anal plate bristle. D. *D. cardinoides*, Guatemala strain, left half. E. *D. polymorpha*, Brazil strain, right half. F. *D. parthenogenetica*, Atlixco strain, left half.

Ventral view of the left palpus, anterior end down in figure. G. *D. neocardini* male. H. *D. neocardini* female. I. *D. acutilabella* male. J. *D. acutilabella* female. K. *D. parthenogenetica* male. L. *D. parthenogenetica* female. M. *D. polymorpha* male. N. *D. polymorpha* female. O. *D. cardinoides* male. P. *D. cardinoides* female. S. Hybrid male (*D. acutilabella* female X *D. cardinoides* male).

Lateral view of right palpus. Q. *D. cardinoides* male. R. *D. cardinoides* female.

Eggs: With four long acuminate filaments.

Internal Structures: A single anterior Malpighian tube, branching only at its distal sixth; posterior tubes with ends apposed without formation of continuous lumen. Testis very pale yellow, about six outer and three inner gyres. Vasa efferentia long slender and colorless. Sperm pump with two long divericula. Spermathecae light brown, chitinized portion of bulb vase-shaped, with the greatest diameter less than the height. Long, thin ventral sperm receptacle with about 45 gyres.

Holotype male.—Holotype and paratype members of a laboratory strain from Atlixco, Mexico; to be deposited in the American Museum of Natural History.

THE EXTERNAL GENITALIA IN THE CARDINI GROUP

Studies of the ovipositor plates of the females in this group have shown little constant interspecific variability, and it appears that the female externalia are of little taxonomic value here. In the case of the males, Hsu (1949) in his survey of the Drosophilidae presented figures for *D. cardini*, *D. cardinoides* and *D. parthenogenetica*, (listed in his paper as *D. neocardini*). Figure 2 in this paper shows the external male genitalia of these species as well as those of *D. polymorpha* and *D. acutilabella*. Our figures for *D. parthenogenetica* and *D. cardinoides* agree fairly well with those of Hsu; however, the figure for *D. cardini* does not. In this species Hsu shows a fringe of short hairs on the antero-ventral edge of the anal plate, lateral to the long, anteriorly directed anal plate bristles. We have been unable to find any such fringe in *D. cardini*. Dr. Hsu has kindly re examined his preparations and finds that his original figure was in error.

It will be noted that the male genitalia permit clear identification of three members of the group, *D. cardini*, *D. acutilabella* and *D. polymorpha*. The other three species (including *D. neocardini* which is not figured), are so similar that it is very difficult to obtain consistent separation on this basis.

THE METAPHASE CHROMOSOME CONFIGURATIONS

The members of the group treated in this paper fall into two general categories. *D. cardini* and *D. similis* resemble each other in having five pairs of rod-shaped chromosomes and one pair of dots, the determinations of Metz (1916) being based on strains from Cuba.

The remaining five species have two pairs of V-shaped chromosomes, one pair of rod-shaped chromosomes and a pair of dots. The descriptions of the chromosomes in *D. polymorpha*, *D. cardinoides*, and *D. neocardini* were based on Brazilian strains (Dobzhansky and Pavan 1943, Streisinger, 1946). The chromosomes of *D. acutilabella* have been very kindly examined by Dr. H. L. Carson, his determination being based on a St. Petersburg, Florida strain. The chromosomes of the Atlixco strain of *D. parthenogenetica* are reported by Wheeler (1949), and have been re-examined by Dr. Carson and by the author.

NOTES ON IDENTIFICATION AND DISTRIBUTION

Drosophila acutilabella, new species

Several strains of this species were collected from St. Petersburg,

Florida by the author in August of 1950 (type collection). Additional strains from Florida are as follows: Everglades (Texas workers), Winter Park (Mrs. H. H. Plough), Lake Placid (J. I. Townsend).

The Sturtevant collection contains specimens from Cristo and Herradura (C. W. Metz), Cuba. In addition female specimens from Lakeland, Miami and Tampa, Florida and from Panama may belong here since they have the light abdominal pattern characteristic of some *D. acutilabella*, and so far unknown in *D. cardini*. This species is readily confused with *D. cardini* in pinned material, but some relaxed males may be separated with certainty by the genitalia.[1]

Drosophila parthenogenetica, new species

This species, formerly confused with *D. neocardini* from Brazil, is known only from a single strain collected in Atlixco, Mexico (Patterson and Mainland, 1944), and generously provided by Dr. M. R. Wheeler.

Virgin females of this species will occasionally lay eggs which begin development; about 90% of the embryos die prior to hatching, the remainder may form normal fertile diploid females or (rarely) sterile diploid XO males (Stalker, 1951).

Drosophila cardini Sturtevant

Sturtevant (1921) lists this species from Cuba, Haiti, Porto Rico, Dominica, Costa Rica, Republic of Panama and Florida. Since *D. cardini* is liable to be confused with *D. acutilabella*, some of these records may actually represent the latter species. A separation of the two species is often possible in relaxed pinned males, and a survey of the material in the Sturtevant collection from the following localities indicated that it was clearly *not D. acutilabella*, and probably was *D. cardini:* Santiago de las Vegas, Havana (paratypes), Cuba; Miami, Florida; Republic of Panama.

In addition recently collected strains from St. Petersburg (H. D. Stalker), Lake Placid (J. I. Townsend), Everglades, Myakkas Head (Texas workers), Florida; Cuba (J. I. Townsend); Durango, Mexico City, Oaxaca, Monterey, Atlixco (Wheeler and Wagner) Mexico, belong here. The collections from Texas, New Mexico, Arizona, Guatemala and Costa Rica (Wheeler and Wagner, 1943) are not at present represented by living strains, and it is possible that some of these records may be for other members of the group.

Drosophila polymorpha Dobzhansky and Pavan

Dobzhansky and Pavan (1943) record this species from various localities in the State of São Paulo, and from the Federal District, Brazil. The Sturtevant collection contains specimens from these additional localities: Quirigua Guatemala (Th. Dobzhansky); Corumba, Matto Grosso, Brazil (R. G. Harris); Panama, Republic of Panama (A. H. Sturtevant).

In this species, as in *D. parthenogenetica*, virgin females will on rare occasions produce fertile diploid daughters.

[1]In a personal communication, Dr. M. R. Wheeler reports that out of 825 *D. cardini* group males trapped from ten localities in peninsular Florida by the Texas workers (June, 1953), fifty-one percent were *D. cardini* and the remaining forty-nine percent were *D. acutilabella*.

Drosophila neocardini Streisinger

Streisinger (1948) described this species from Teffe, Amazonas, Brazil. Wheeler (1949) in discussing the confusion in the group states that a strain collected from Atlixco, Mexico (Patterson and Mainland, 1944), and differing from Streisinger's type strain cytologically only in the shape of the Y-chromosome, also belonged to the species. The original Brazilian strain was not available at that time, but a more recent direct comparison of the Brazilian and the Mexican strains has shown that the latter represents a different species, described in this this paper as *D. parthenogenetica.*

Drosophila cardinoides Dobzhansky and Pavan

Dobzhansky and Pavan report this species from the State of São Paulo and from the Federal District of Brazil. Wheeler (1949) reports it from Guatemala City, Guatemala. He also states that the strain from Lake Okeechobee, Florida, examined cytologically by Wharton (1943), and considered to be *D. cardini* at that time, was probably *D. cardinoides* also. This strain was clearly not *D. cardini* as indicated by its metaphase chromosome configuration, but since the chromosome pattern would fit either *D. cardinoides* or *D. acutilabella*, and since the former species is unknown from Florida and the West Indies, it seems probable that Wharton was dealing with *D. acutilabella* rather than *cardinoides.*

The Sturtevant collection contains specimens from Port Limon, Costa Rica; and from Panama.

Drosophila similis Williston

No stocks of this species are now known to exist, and since the collections cited by Sturtevant (1921) this species has apparently not been knowingly collected anywhere. Furthermore none of the specimens at present in the Sturtevant pinned collection are known to belong here. Sturtevant has pointed out that although *D. similis* and *D. cardini* would not cross in the laboratory, they were morphologically so similar that he was unable to devise means of distinguishing pinned specimens of the two species. He lists *D. similis* from: Herradura, Havana, Santiago de las Vegas, Bartle and Cristo in Cuba; Porus and Port Antonio in Jamaica; St. Vincent (Williston type material), and Bay Mansion in Barbados. Sturtevant also states that he has examined specimens from Florida, Haiti, Trinidad, Panama, Honduras and Mexico, which he concluded might belong here, concluding " . . . the lack of any really satisfactory character to separate this species from pale specimens of *D. cardini* Sturtevant makes all these latter determinations doubtful."

Thus it appears that while *D. similis* was taken in the West Indies, the Florida records are uncertain. *D. cardini* appears in collections from both areas and *D. acutilabella* from Flordia and Cuba. The great similarity of *D. cardini* and *D. acutilabella*, plus the fact that certain individuals of the latter species are lighter than *D. cardini*, certainly suggests that *D. acutilabella* and *D. similis* might be synonyms. The one fact which prevents this interpretation is that in *D. similis*, Metz (1916) has reported the metaphase chromosomes as five pairs of rods

and a pair of dots, while in *D. acutilabella* there are two pairs of V's, a pair of rods and a pair of dots.

The University of Texas workers, in their keys, (Patterson and Wheeler, 1943; Wheeler, 1949), have indicated a possible separation of *D. simulis* from *D. cardini* on the basis of the anterior scutellars being divergent in the former species, convergent in the latter. Dr. Wheeler

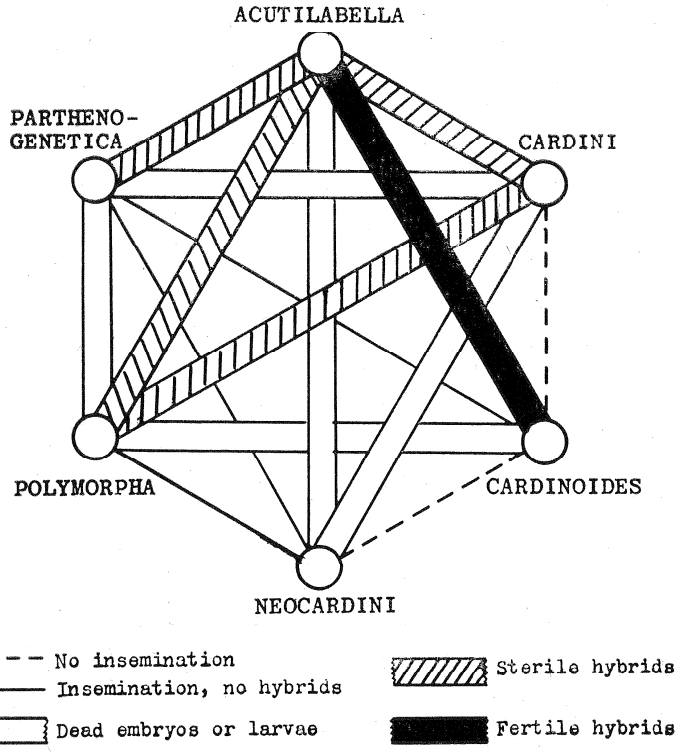

FIG. 3. Crossing Diagram for the *D. cardini* group. In this diagram the type of line or bar connecting any two species indicates the degree to which interspecific matings or hybrid production occur. Each connection shows the maximum crossability of the two species, considering both reciprocal crosses (except in the case of *D. parthenogenetica* where crosses using this species as the female are not included since large numbers of clearly impaternate (non-hybrid) progeny were always produced in such cases).

(personal communication) informs me that he no longer feels such a criterion reliable; thus the Texas records of *D. similis* from Florida, Texas and Mexico are open to question.

Thus, with the information available, one might expect that *D. similis* and *D. cardini* could be distinguished now only by cross-mating tests. Such tests between a group of strains assumed to be *D. cardini* might reveal one or more strains showing reproductive isolation from the

remainder if the group happened to include *D. similis*. A total of 80 such mating tests have been made between various members of the following 59 strains: Florida: St. Petersburg (18), Lake Placid (32), Myakkas Head (2), Everglades (1). Cuba (1), Mexico. Mexico City (1), Durango (1), Oaxaca (1), Monterey (1), Atlixco (1).

In all of these test crosses, mating occured promptly and larvae and adults were readily obtained. In the majority of crosses these adults proved to be fertile, in some cases they were not aged sufficiently to be tested for fertility. Although only certain crossing combinations were tried, it is assumed that in this case any two strains which cross readily with a third would cross with each other. Thus none of the 59 strains considered to be *D. cardini* showed any indication of cross-sterility with any of the others.

It is of course not certain whether the above strains actually belong to the species *D. cardini*, or whether they are all *D. similis*. However, since Sturtevant's records seem to indicate that *D. cardini* was the commoner of the two species, at least in Florida prior to 1916, it seems likely that the strains collected there in recent years, as well as those from Mexico actually are *D. cardini*. Recent collections of the group in the West Indies have apparently not been extensive, and it is possible that *D. similis* may be found in Cuba, where Sturtevant (1921) states that it was not uncommon around fallen fruit.

HYBRIDIZATION WITHIN THE CARDINI GROUP

Streisinger (1946) studied the crossing relationships within the group using the three species: *D. polymorpha*, *D. neocardini* and *D. cardinoides*. When interspecific matings of both reciprocal types and in all three combinations were made, he found insemination of females had occurred only in crosses between *D. cardinoides* and *D. polymorpha*, and even there no viable hybrids were produced.

With the discovery of the two new species described in this paper, and the availability of strains of *D. cardini*, it was felt that further tests of hybridization within the group would be worthwhile. These tests were carried out by placing equal numbers of 1- to 3-day-old males and females in vials, changing the food every second day, and following the pairs for thirty days. Since the adults were kept on such fresh food, there was very little accidental loss during the test period. The vials from which the adults had been transferred were examined daily for four consecutive days in order to detect the presence of dead embryos or larvae. Embryos which completed part of their development and died prior to hatching were detected by their brown color. When larvae appeared in any of the vials, if the food surface was felt to be unfavorable because of bacteria or molds, the larvae were transferred to fresh food, and in certain classes of hybrids it was found that such transfers permitted development to proceed to completion. At the end of the tests the females were dissected and the sperm receptacles examined for the presence of sperm. The results are summarized in Table I and in Fig. 3. In the table tests using strains from the same general locality are lumped. The strain of *D. polymorpha* used in these crosses was homozygous for the dark abdominal pattern gene. In *D. polymorpha* the dark and light pattern depend on a single pair of genes, with incomplete dominance (Cunha, 1949).

It will be noted that in only one category of crosses, those employing *D. neocardini* as females, was there any indication of complete sexual isolation. In the crosses between *D. cardinoides* and *D. polymorpha*

TABLE 1

SUMMARY OF INTERSPECIFIC CROSSES

Females	Males	Number of Pairs	Insem. Females	Sperm	Hybrids
D. cardinoides					
Brazil	*D. cardini*, Florida	24	0/23	.....	none
Guatemala	*D. cardini*, Florida	24	0/22	.....	none
Brazil	*D. acutilabella*, Florida	12	0/11	.....	none
Brazil	*D. parthenogenetica*	6	0/6	.....	none
Guatemala	*D. parthenogenetica*	12	5/12	*, s	none
Guatemala	*D. neocardini*, Brazil	18	0/16	.....	none
Guatemala	*D. polymorpha*, Brazil	18	15/17	*, s	d. e.
D. cardini					
Florida	*D. cardinoides*, Guatemala	6	0/6	.....	none
Florida-Cuba	*D. cardinoides*, Brazil	28	0/27	.....	none
Florida-Cuba	*D. acutilabella*, Florida	97	78/88	s, v	1 ♀
Florida-Cuba	*D. neocardini*, Brazil	36	32/33	s	d. e.
Mexico	*D. neocardini*, Brazil	36	23/34	.....	d. e.
Florida-Cuba	*D. polymorpha*, Brazil	72	61/64	*, s	5 ♂♂
Florida-Cuba	*D. parthenogenetica*	24	19/21	s	d. e. y.
Mexico	*D. parthenogenetica*	12	8/11	s, v	d. e. y.
D. acutilabella					
Florida	*D. cardinoides*, Guatemala	54	44/47	s, v	224 ♀♀, 2 ♂♂
Florida	*D. cardinoides*, Brazil	6	4/5	s, v	1 ♀
Florida	*D. cardini*, Florida	90	36/59	*, s	4 ♂♂
Florida	*D. parthenogenetica*	30	24/27	s	19 ♀♀
Florida	*D. polymorpha*, Brazil	72	59/62	s, v	20 ♀♀, 1 ♂
Florida	*D. neocardini*, Brazil	24	20/21	s	d. e.
D. neocardini					
Brazil	*D. cardinoides*, Guatemala	12	0/12	.....	none
Brazil	*D. cardini*, Florida	54	0/48	.....	none
Brazil	*D. acutilabella*, Florida	18	0/17	.....	none
Brazil	*D. parthenogenetica*	18	0/18	.....	none
Brazil	*D. polymorpha*, Brazil	24	0/22	.....	none
D. polymorpha					
Brazil	*D. cardinoides*, Guatemala	36	29/32	*, s	d. e.
Brazil	*D. cardini*, Florida-Cuba	66	49/55	s	d. e. l.
Brazil	*D. acutilabella*, Florida	30	25/27	s	d. e.
Brazil	*D. parthenogenetica*	42	32/39	s	d. e. l.
Brazil	*D. neocardini*, Brazil	48	1/44	s	none

*—Sperm grossly abnormal; s, sperm found in spermathecae; v, sperm found in ventral receptacles; d.c., death in eggs; d. e. l., death in eggs and larvae; d. e. y., death in eggs and young larvae.

(where Streisinger found insemination occurring very rarely), insemination by the end of the test period was the rule in these tests. The data agree very well with those of Streisinger in indicating a very high sexual

isolation between *D. neocardini* and the two species *D. cardinoides* and *D. polymorpha*.

Of the 19 crosses which actually resulted in insemination of the females, two showed no detectable production of hybrids, ten showed development of hybrids which died as embryos or young larvae, and the remaining seven resulted in the production of adult hybrid progeny.

The appearance of the sperm found in the spermathecae of the females was strikingly abnormal in a number of the crosses (marked by an asterisk in the table). In these cases, although occasional females were found in which the sperm appeared normal, in others it was thick, ropy and nonmotile. Since in all such individuals sperm was found only in the spermathecae, the ventral receptacle being empty, this may indicate that the sperm stored in the ventral receptacle was used up normally, and that stored in the spermathecae underwent degeneration.

The crosses carried out using *D. parthenogenetica* females and males of the other five species are not reported in the table. In every case a number of fertile female progeny were produced which were clearly impaternates rather than hybrids, as judged by their metaphase chromosomes, or their morphology and their breeding behavior. No clearly hybrid individuals have been obtained from *D. parthenogenetica* females.

Since in all the species included here some parthenogenesis has been observed, (with death prior to hatching in all species except *D. parthenogenetica* and *D. polymorpha*), the question arises as to whether or not the observed inviable embryos and young larvae produced in some of the crosses were indeed hybrids, or were simply impaternates. although the frequency of such inviable embryos was much higher in the crosses listed, than when virgin females are allowed to produce eggs in the absence of males, the possibility exists that insemination of a female might increase the rate of parthenogenesis. In the crosses listed this possibility could not be ruled out, although it seems improbable since tests using mated and unmated *D. parthenogenetica* females have indicated that mating with fertile conspecific males, or prolonged association with males of closely related species does not increase the rate of parthenogenesis (Stalker, unpublished).

Description of the Hybrids

D. acutilabella ♀♀ *X D. cardini* ♂♂. *Four males*, sterile when tested against females of both parental species. Bristles minute. Testes rudimentary. Genitalia intermediate, see figure 2 B.

D. acutilabella ♀♀ *X D. parthenogenetica* ♂♂. *Nineteen Sterile females*, all from homozygous light *D. acutilabella* mothers; showed very light abdominal pattern characteristic of *D. parthenogenetica*. Fore-femora showed the thick distal hairyness characteristic of *D. parthenogenetica*, but with an occasional bristle (2 femora out of 36 examined) on the distal posterodorsal surface as in *D. acutilabella*. In one female the last three abdominal tergites were only partially fused in the mid-dorsal line.

D. acutilabella ♀♀ *X D. polymorpha* ♂♂. *Twenty females*, almost completely sterile. Produced one daughter when backcrossed to *D. polymorpha* males. All hybrids developed abnormally black palpi and third antennal segments as they aged. Fore-femora with thick distal hairyness characteristic of *D. polymorpha* but with one or two bristles on the distal postero-dorsal surface, as in *D. acutilabella*. The *D. acutilabella* mothers were all homozygous light, the *D. polymorpha* fathers homozygous dark, and the hybrids showed an intermediate pattern midway between light *D. acutilabella* and dark *D. polymorpha*, and resembling the heterozygous pattern of *D. acutilabella*.

One male. Labellum intermediate, showing a short anterior horn, as in *D. acutilabella* and a very slight ventral protuberance as in *D. polymorpha*. Genitalia with second row of bristles on clasper weakly developed, anteromedial corners of anal plates without anterior process of *D. acutilabella*, but bearing two bristles intermediate in length between those of *D. acutilabella* and *D. polymorpha*. Fore-femora with thick distal hairyness of *D. polymorpha* and one distal posterodorsal bristle as in *D. acutilabella*.

D. acutilabella ♀♀ *X D. cardinoides* ♂♂. *Two hundred twenty-five females.* Fertile, but with daily egg production about one-fourth normal. Hind-gut just anterior to rectum showing typical whitish yellow of *D. acutilabella* rather than strong orange of *D. cardinoides*. Produced backcross progeny of both sexes when mated to males of either parental species. In the original crosses in which the *D. acutilabella* parents were homozygous light, the abdominal banding of the F_1 hybrids was intermediate, resembling heterozygous light in *D. acutilabella*. Where the *D. acutilabella* parent was homozygous dark the hybrid females were also dark, resembling homozygous dark *D. acutilabella*, or *D. cardinoides*.

Two males. Sterile when tested with females of both parental species. Palpi and labellum were intermediate between those of parental species, (see figs. 1 I and 2 S). Testes rudimentary. Genitalia with anterior process on anteromedial corner of anal plates, but with two moderately long straight bristles, rather than the many short straight ones of *D. cardinoides* or the single long curved one of *D. acutilabella*.

D. cardini ♀♀ *X D. acutilabella* ♂♂. *One female.* In this case, despite the fact that the *D. acutilabella* male parent was homozygous light, the abdominal patterns of the hybrid was dark, resembling the *D. cardini* mother or homozygous dark *D. acutilabella*. This female was normal in appearance, and produced many normal looking eggs but failed to mate with males of either of the parental species.

D. cardini ♀♀ *X D. polymorpha* ♂♂. *Five males.* All sterile when tested with both types of females; testes rudimentary. In their genitalia these males were *D. cardini*-like, having 2 to 3 well developed bristles at anteromedial corners of the anal plates and lacking the well developed secondary or anterior row of bristles on the clasper as in *D. polymorpha*. The labellum was intermediate, showing a weakly developed *D. polymorpha*-like ventral protuberance; the fore-femora showed the thick hairyness of *D. polymorpha* but also a single bristle on the distal posterodorsal surface, characteristic of *D. cardini*.

DISCUSSION

Taking into account the known geographical distribution, it will be seen that the six species treated above are completely isolated genetically, in that the two forms which produce fertile hybrids, *D. acutilabella* and *D. cardinoides*, are not sympatric, the former being known from Cuba and Florida, the latter unrecorded from either Florida or the West Indies. With the possible exception of the allopatric pair, *D. acutilabella* and *D. polymorpha*, (see Description of the Hybrids, above), all the other species combinations produced sterile hybrids or none at all. Thus, either complete reproductive isolation or geographic isolation obtains throughout the group. Among the isolating mechanisms, sexual or psychological isolation appears (in the laboratory) to be rather inefficient, as evidenced by the high frequency of cross insemination observed. On the other hand, inviability or sterility of the hybrids forms an effective genetic barrier in the great majority of interspecific crosses.

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SUMMARY

1. The taxonomic status and known geographic distribution of the North and Central American members of the *Drosophilia cardini* group is reviewed.

2. Two new species, *D. acutilabella* from Cuba and Florida and *D. parthenogenetica* from Mexico are described.

3. An illustrated key for the identification of pinned specimens is presented.

4. Hybridization tests between six of the species of the group show complete reproductive isolation in all crosses except that between the allopatric species *D. acutilabella* and *D. cardinoides*. *D. acutilabella* females when mated to *D. cardinoides* males will produce fertile hybrid females and rare sterile hybrid males. The hybrid females will produce progeny of both sexes when backcrossed to males of either parental species.

5. Where reproductive isolation occurs it depends on sexual (psychological) isolation, hybrid sterility and/or hybrid inviability.

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