RARE PARTHENOGENESIS IN *DROSOPHILA ROBUSTA*

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Unmated females of certain animals, when held under domestication or in the laboratory, will produce large quantities of unfertilized eggs. This situation has made possible the discovery of an exceedingly low rate of natural parthenogenesis in forms which have previously been thought to reproduce wholly by sexual means (for example, Stalker, 1954, for various Drosophilidae; Yao and Olsen, 1955, for turkeys; Laven, 1957; Kitzmiller, 1959, for mosquitoes). Except for *D. parthenogenetica* (Stalker, 1954) and *D. mangabeirai* (Carson *et al.*, 1957), unmated Drosophila females of various species produce adult impaternate offspring only very rarely. Thus, Stalker screened somewhat less than 1,000,000 unfertilized eggs of 29 such species and obtained only three adults, two of *Drosophila polymorpha* and one of *Drosophila affinis*. The present account gives the results of extensive screenings of unfertilized eggs of *Drosophila robusta*.

MATERIAL AND METHODS

Over a period of three years, the writer performed an extensive series of selection experiments on *Drosophila robusta* (Carson, 1958). The technique calls for the isolation of females in groups of 50 of close to the same age. These flies are held on well-yeasted food for one week after which two females of the group of 50 were selected as parents and the remainder discarded. Approximately 13,800 females were handled in this way. Beginning in October, 1955, however, instead of discarding females eliminated by the selection procedure and their controls, the remaining flies were placed in a fresh bottle and retained. Each group of females was kept thereafter for four weeks; during this time each group of flies had a sojourn of one week in each of four culture bottles. Also, during this time ample fresh yeast was available to the flies and oviposition was continuous. After the females were removed from a bottle, a few males were added; these served to keep down growth of bacteria and moulds. After a week, these males were discarded. The bottles were then set aside and examined to see if any adult flies emerged. No bottle was discarded as negative until the youngest eggs therein were 21 days old. The time for development from egg to adult for *D. robusta* is 15–16 days at 25°C. which was the temperature maintained during these experiments.

The number of unfertilized eggs laid per bottle was not counted but has been roughly estimated in the following way. Unpublished data of the writer and Dr. H. D. Stalker indicate that a single unmated female of *D. robusta* under optimal conditions will lay from 67 to 91 eggs per day from the sixth to the 35th day of age. Accordingly, it is felt that under conditions of

moderate crowding, the number of eggs per female per day will be considerably less; 25/day (175/week) is taken as a moderate estimate. The average number of females per bottle was taken at approximately 30; each group started with 45 or 50 but dwindled during the four weeks due to deaths. Thus, 30 females laying 175 eggs per week in a bottle would produce 5250 eggs per bottle per week; in round numbers, then, each bottle may be said to contain about 5000 eggs. This is probably an underestimate, but the order of the magnitude is believed to be correct. Twelve strains were studied; each strain originated from a single pair of wild flies collected in nature. Ten of the strains originated from flies collected at Chadron, Nebraska (C6, C15, C39, C66, C72, C77, C79, C95, C102, C109), and two from flies collected at Steelville, Missouri (Sv135, Sv136). Further information on these strains will be found in Carson (1958).

Bottles were examined several times a week for the emergence of an adult fly. When such a fly was found, the following procedure was generally carried out. The fly was isolated, etherized and examined for sex and any morphological peculiarities. If it was reasonably freshly emerged, this fact could be recorded because it takes several days for a specimen of *D. robusta* to attain the dark body color characteristic of the adult. The food mass was systematically searched until the empty pupal case from which the fly emerged was found. The approximate time of development from egg to adult for such a fly was also calculated.

RESULTS

Between January, 1956, and March, 1957, a total of 14 parthenogenetically produced flies were found. A total of approximately 10,585,000 eggs was screened during this period, giving an overall rate of more than one adult female per million eggs (1.32×10^{-6}). Figure 1 shows diagrammatically the origin of each female. The open circles, containing strain numbers, represent a large number of unmated females which were tested from each original strain. Below the symbol is given the approximate number of unfertilized eggs screened. The solid circles represent impaternate females, and are numbered 1–14.

From the 12 original strains, seven (Nos. 1–7, figure 1) impaternate females were obtained from about 7,500,000 eggs, a rate of approximately 0.93 flies per million eggs. Among the offspring of flies having a history of parthenogenesis in at least one ancestor, there were seven females (Nos. 8–14) obtained from approximately 3,085,000 eggs or a rate of 2.23 flies per million eggs. Each of the original seven flies came from a different strain. In most cases they were not held long as virgins but were crossed to males and their female progeny tested again in large numbers, if possible, for parthenogenesis. Notes on the characteristics and fertility of these females will be found in table 1. Only three of the seven flies from the original stocks appeared to have full fertility (Nos. 1, 4, and 7). Nos. 5 and 2 produced only female offspring. Parthenogenetic females 8–14 were pro-

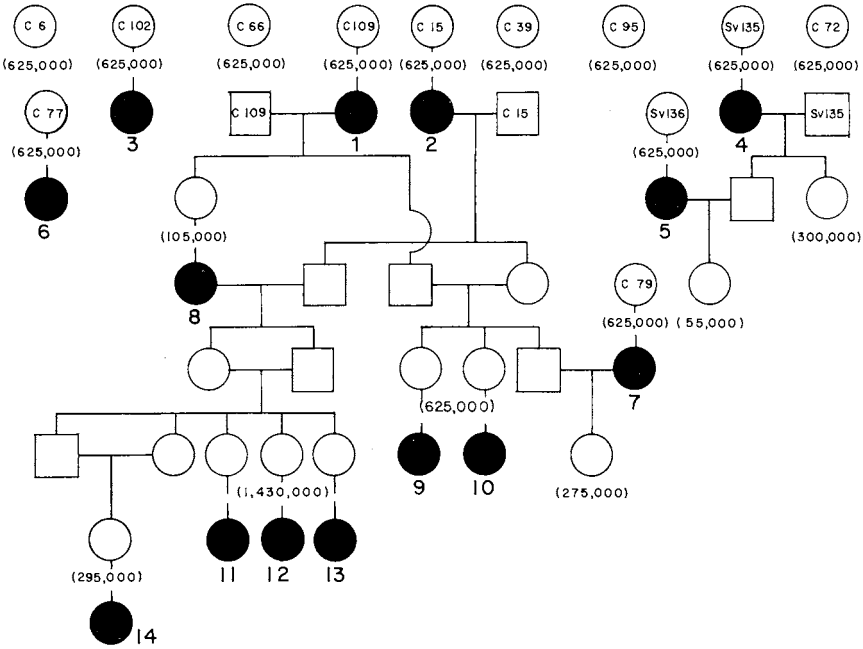

FIGURE 1. Pedigrees of impaternate females (Nos. 1–14) of *Drosophila robusta* (solid circles). The open circles in most cases represent sibships of unmated females; below these symbols is given the number of unfertilized eggs screened in tests for parthenogenesis. Each strain is numbered: (C = Chadron, Nebraska; Sv = Steelville, Missouri).

duced in lines which were descended from Nos. 1 and 2. Of these, only No. 8 gave offspring (figure 1, table 1).

DISCUSSION

In all likelihood, the parthenogenetic females found in this study arise through automictic fusion of meiotic nuclei in the cytoplasm of the unfertilized egg (see Stalker, 1954, 1956; Carson *et al.*, 1957; Murdy and Carson, 1959; Metz, 1959; Sprackling, 1960). It is not possible to say, however, whether aneuploidy or polyploidy is involved because in only one case was the diploid chromosome complement actually observed in an impaternate female. There seems little question, however, that the genetic state accompanying parthenogenesis is an unbalanced one; this is manifested by the rather considerable degree of sterility encountered among these females.

Capacity for rare parthenogenetic development was demonstrated to exist in seven different wild strains out of 12 studied; five of these strains originated from a single Nebraska locality and two came from a single Missouri locality. Such a finding does not preclude the possibility of the existence of strains with much higher rates, but nevertheless it is clear that the present finding is not due to a single, locally peculiar genetic situation.

TABLE 1

Fertility and other characteristics of impaternate females of *Drosophila robusta*

Female No.	Source of males used as mates	Fertility	Morphological abnormalities	Remarks
1	C109	Fertile; produced many offspring of both sexes	None	Laid eggs but produced no larvae or brown eggs as a virgin
2	C15	Partially fertile; produced only 2 offspring, both females	Slightly abnormal abdominal sternites	Became "plugged"; had large ovaries; had normal 2n chromosome group
3	C102	Sterile	Abnormal abdominal sternites	Laid eggs but produced no larvae or brown eggs as a virgin
4	Sv135	Fertile; produced many offspring of both sexes	None	
5	F_1 from No. 4	Partially fertile; produced 68 offspring, all females	None	Held in cool temperature prior to mating
6	C77	Sterile	None	
7	See fig. 1	Fertile; produced many offspring of both sexes	None	
8	See fig. 1	Partially fertile; several hundred offspring of both sexes	One deformed wing and abnormal abdominal sternites	
9	F_1 from No. 8	Sterile	None	
10	F_1 from No. 8	Sterile	None	
11			None	Not mated; held on food; died after 7 weeks
12			Irregular abdominal sternites	Not mated; died after 4 weeks
13			Grossly abnormal abdominal sternites and tergites	Not mated; died after 2 weeks
14	C66 stock	Sterile	Contained no ovaries	Lived 6 weeks with males

Calculation of rates must be done with caution because of the crude way in which the number of eggs has been estimated. Nevertheless, the method of estimation is at least internally consistent in these studies. The rate of parthenogenesis in the initial 12 strains has been calculated as .93 adult females per million eggs. This appears not to be inconsistent with the results of Stalker (1954) who found two dead embryos (but no adults) in approximately 11,000 eggs of *D. robusta* examined. The second seven parthenogenetic females were all found in lines descended from two of the original

ones (Nos. 1 and 2). The rate found in eggs laid by virgin females in whose ancestry is one or more parthenogenetic females is 2.2 adult females per million eggs, or about 2½ times the rate found in the original strains. Such an increase under such selection indicates that the parthenogenesis has a genetic basis and is not unexpected in view of the striking increases in rates obtained by Stalker (1954) by selection in *D. parthenogenetica* and *D. polymorpha*.

The existence of low rates of genetically-based parthenogenesis in many species of Drosophila is of particular interest in that a clear case has recently been found wherein the species concerned, *Drosophila mangabeirai*, reproduces wholly by obligatory thelytokous parthenogenesis (Carson *et al.*, 1957; Carson, 1961). Whereas there is no evidence that *D. parthenogenetica*, *D. polymorpha*, or *D. robusta* have any tendency to depend on parthenogenesis in natural populations, *D. mangabeirai* clearly has reached this stage in the evolution of parthenogenesis. Males are unknown in the laboratory and are apparently produced only as great rarities in nature.

SUMMARY

1. By screening large quantities of unfertilized eggs laid by unmated females in the laboratory, 14 parthenogenetically-produced individuals of *Drosophila robusta* were found. All were females. It is suggested that they arose through automictic fusion of haploid products of meiosis in the unfertilized egg.

2. Each of the initial seven females came from a different wild strain, five from Nebraska and two from Missouri. The rate of parthenogenesis in these strains was .93 adult females per million unfertilized eggs laid.

3. Virgin females with one or more parthenogenetic females in their ancestry show a rate of parthenogenesis about 2½ times that of the original strains.

4. A majority of the parthenogenetic females show reduced fertility, complete sterility and/or morphological abnormalities.

5. The evidence indicates that rare parthenogenesis such as is found in *D. robusta*, *D. parthenogenetica*, and *D. polymorpha* is genetically based and may serve as an example of an evolutionary stage through which the obligatory parthenogenetic species, *D. mangabeirai*, may have passed during the evolution of its parthenogenesis.

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