

SELECTION FOR PARTHENOGENESIS IN *DROSOPHILA MERCATORUM*

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Received September 26, 1966

PARTHENOGENESIS, as a substitute for syngamy, is widespread in nature. This is especially true for thelytoky, a type of parthenogenesis in which virgin females produce almost exclusively female offspring. The evidence is overwhelming that this condition has arisen independently in dozens of species of organisms. Each case may indeed be regarded as a special evolutionary event in the sense that natural selection has favored the accumulation of genes which provide a genetic basis for parthenogenesis.

Little attention has been given to experimental study of the origin of the thelytokus state in the laboratory. This is probably due largely to the fact that most organisms cannot be reared sufficiently easily and in large enough numbers to make such a study feasible. After STALKER's (1954) discovery of low-rate parthenogenesis in *Drosophila* and his success in selecting for increased rate in *D. parthenogenetica* and *D. polymorpha*, the writer has sought a species of *Drosophila* well adapted to an extended study of this phenomenon. *Drosophila mercatorum* has met the requirements; several wild strains show very low unselected rates of parthenogenetic development. Impaternates are mostly diploid females; they are highly vigorous and viable and are fully fertile when crossed to sexually produced males. Selection has resulted in an approximately 60-fold increase in the rate of parthenogenesis.

MATERIALS AND METHODS

The observations and experiments were carried out on laboratory stocks derived from nine wild strains (Table 1). They will be hereafter referred to by the letter designations shown. All of these stocks belong to the widespread northern subspecies, *Drosophila m. mercatorum*. All have the same monomorphic sequential karyotype in the salivary gland chromosomes (WASSERMAN and WILSON 1957; WASSERMAN, 1962; CARSON 1965), being represented by the formula (relating to *D. repleta*) of X, 2detuvv³, 3bfh, 4, 5.

Discovery of a low level of thelytokous parthenogenesis in certain strains of *D. mercatorum* was made in April of 1961 (CARSON 1962). Measurements of the rate of parthenogenesis were made in two ways. In the case of all stocks in which the rate of parthenogenetic development of eggs is very low, virgin females were placed in groups of 30 in single 95mm × 25mm papered and heavily yeasted vials. Each group was changed to a new vial after 48 hours and this process was repeated until the flies had been through from 4 to 10 such changes. The empty vials containing the eggs laid by these virgins were then incubated and each adult fly which emerged was sexed and counted. Only an occasional male was obtained, and these have been invariably sterile. Smears of primary spermatocytes of such males showed them to be diploid and to lack a Y chromosome. The size of their wing cells is the same as that of normal fertile males.

TABLE 1

Laboratory strains of Drosophila mercatorum employed in the experiments

| Geographic origin of strain | Symbol used in this paper | Date of original collection | Collector and other data |
|-----------------------------|---------------------------|-----------------------------|-------------------------------|
| Rochester, New York | R | October 1957 | J. KRIVSHENKO |
| La Palma, El Salvador | S | August 1954 | W. B. HEED UTL-H62.60* |
| Guatemala | G | March 1944 | J. F. CROW UTL 1413.7 |
| Lima, Peru | L | January 1945 | C. PAVAN UTL 1529.3A |
| Manizales, Colombia | Man | October 1963 | A. HUNTER Man-11; isofemale |
| Hawaiian Islands | | | |
| Pupukea, Oahu | O | July 1963 | W. B. HEED C63 |
| Kokee, Kauai | K | July 1963 | H. L. CARSON C73 |
| Kamuela, Hawaii | H | July 1963 | H. L. CARSON C60.10 |
| Silversword Inn, Maui | M | July 1963 | H. L. CARSON C70.4; isofemale |

* UTL = University of Texas Laboratory number.

All females emerging were assumed to be of parthenogenetic origin. Repeated comparisons of the wing cell size of large numbers of impaternal females with diploid, bisexually produced specimens failed to reveal any polyploid individuals. In fact, no triploid, tetraploid or haploid individual of *D. mercatorum* has ever been found.

Estimates of low-level rates of parthenogenesis were made by isolating a sample of females from the strain being tested. Each female was provided with a vial of dark food and a small lump of yeast for 24 hours. The eggs laid during this period were then counted and an average number of eggs laid per female per 48-hour interval was computed. The total number of eggs screened for each strain was then estimated by multiplying (a) the mean number of eggs laid per female per 48 hours by (b) the mean number of females present in each 48-hour test group by (c) the number of 48-hour test periods (intervals of laying). The rate of parthenogenesis is then expressed in percent unfertilized eggs giving rise to impaternal offspring. Some of the lower rates are more easily understood if expressed in terms of offspring per million eggs (Table 2).

When the rates are higher, masses of females were not used. Aged females (usually 40 for each test) were placed singly in individual oviposition vials and left for 24 hours. Each female was then either discarded or allowed to oviposit for another 24 hours. The eggs were counted in each vial and the vials then incubated in order to determine the percent unfertilized eggs giving rise to adult offspring.

All rearing of *Drosophila mercatorum* has been carried out at $25^{\circ} \pm 1.0^{\circ}\text{C}$. In the earlier work, when rates were low, the females were carried in groups for about three weeks; except as mentioned later, egg counts were made on 10- to 15-day-old females. In the later tests, females were generally aged for from 7 to 8 days and tested at that time.

OBSERVATIONS

A. *Tests for parthenogenesis in unselected laboratory strains:* Virgin females from eight laboratory strains and from the F_1 between two of them were tested for capacity for parthenogenesis (Table 2). Only three of the strains tested manifested this capacity. Of these, the highest is the Salvador (S) strain in which approximately one egg in 1000 laid by virgin females completes development, giving almost exclusively diploid female offspring. The egg-laying capacity of the different strains varies greatly from an average of 2.6 per female per 48 hours in the Manizales strain to 150.9 per female per 48 hours in the F_1 of Rochester \times

TABLE 2
Rates of parthenogenesis in unselected laboratory strains of Drosophila mercatorum

| Strain | No. of ♀♀ used for egg counts | No. of hours of egg-laying | Total No. of eggs laid | Mean No. of eggs/♀/48 hr (a) | Total No. of females At beginning At end of tests | Mean No. of ♀♀ in each 48-hr test group (b) | No. of 48-hr test periods (c) | Est. No. of eggs screened a × b × c | No. of impatenate adults obtained | Rate: impatenate adults/10 ⁶ eggs |
|-------------------------------------|-------------------------------|----------------------------|------------------------|------------------------------|---|---|-------------------------------|-------------------------------------|-----------------------------------|--|
| Salvador | | | | | | | | | | |
| July 1961 | 20 | 497 | 477 | 46.1 | 300 269 | 28.5 | 70 | 91,970 | 89 | 967 |
| Oahu | | | | | | | | | | |
| Nov. 1963 | 20 | 480 | 204 | 20.4 | 300 277 | 28.9 | 115.5 | 68,094 | 9 | 132 |
| Rochester | | | | | | | | | | |
| July 1961 | 20 | 481 | 591 | 52.4 | 300 265 | 28.3 | 103 | 152,741 | 6 | 39 |
| F ₁ Rochester × Salvador | | | | | | | | | | |
| April 1961 | 15 | 362 | 1138 | 150.9 | 450 389 | 28.0 | 225 | 950,670 | 20 | 21 |
| Lima | | | | | | | | | | |
| July 1961 | 20 | 475 | 819 | 82.8 | 270 243 | 25.7 | 84 | 178,749 | 0 | 0 |
| Jan. 1964 | 20 | 480 | 1335 | 133.5 | 330 301 | 28.6 | 89.5 | 341,720 | 0 | 0 |
| Guatemala | | | | | | | | | | |
| July 1961 | 20 | 471 | 719 | 73.3 | 300 218 | 25.9 | 86 | 163,278 | 0 | 0 |
| Manizales | | | | | | | | | | |
| Jan. 1964 | 20 | 480 | 51 | 2.6 | 330 320 | 29.5 | 84.5 | 6,481 | 0 | 0 |
| Kauai | | | | | | | | | | |
| Jan. 1964 | 20 | 480 | 256 | 25.6 | 330 306 | 28.9 | 117.5 | 86,931 | 0 | 0 |
| Kamuela | | | | | | | | | | |
| Hawaii | | | | | | | | | | |
| Feb. 1964 | 20 | 480 | 1066 | 106.6 | 300 284 | 29.2 | 92.5 | 287,927 | 0 | 0 |

TABLE 3

*Selection for increased rate of parthenogenesis within the Salvador strain. First phase: In each cycle, parthenogenetic and bisexual reproduction are alternated**

| Cycle of selection | No. of ♀♀ used for egg counts | No. of hours of egg-laying | Total No. of eggs laid | Mean No. of eggs/♀/48 hr (a) | Total No. of females at beginning of tests | Mean No. of ♀♀ in each 48-hr test group (b) | No. of 48-hr test periods (c) | Estimated No. of eggs screened a X b X c | Actual No. of eggs counted | No. of impaternate adults obtained | Rate: impaternate adults/100 eggs |
|--------------------|-------------------------------|----------------------------|------------------------|------------------------------|--|---|-------------------------------|--|----------------------------|------------------------------------|-----------------------------------|
| S-0-Bi | 20 | 497 | 477 | 46.1 | 300 | 28.5 | 70 | 91,970 | ... | 89 | 0.0967 |
| July 1961 | | | | | | | | | | | |
| S-1-Bi | 20 | 480 | 806 | 80.6 | 270 | 29.4 | 36 | 85,307 | ... | 50 | 0.0586 |
| Sept. 1961 | | | | | | | | | | | |
| S-2-Bi | 20 | 480 | 597 | 59.7 | 270 | 29.5 | 36 | 63,401 | ... | 105 | 0.1656 |
| Oct. 1961 | | | | | | | | | | | |
| S-3-Bi | 20 | 480 | 512 | 51.2 | 300 | 29.7 | 48 | 72,991 | ... | 77 | 0.1055 |
| Jan. 1962 | | | | | | | | | | | |
| S-4-Bi | 20 | 480 | 525 | 52.5 | 240 | 28.4 | 32 | 47,485 | ... | 219 | 0.4612 |
| Feb. 1962 | | | | | | | | | | | |
| S-5-Bi | 20 | 480 | 248 | 24.8 | 120 | 28.0 | 19 | 13,194 | ... | 104 | 0.7882 |
| Mar. 1962 | | | | | | | | | | | |
| S-6-Bi | 20 | 480 | 861 | 86.1 | 210 | 28.9 | 28 | 69,672 | ... | 264 | 0.3789 |
| Apr. 1962 | | | | | | | | | | | |
| S-7-Bi | 20 | 480 | 480 | 48.0 | 180 | 29.5 | 24 | 33,984 | ... | 591 | 1.7391 |
| May 1962 | | | | | | | | | | | |
| S-8-Bi | 20 | 480 | 971 | 97.1 | 150 | 29.1 | 15 | 42,384 | ... | 246 | 0.5804 |
| June 1962 | 30 | 960 | 2325 | 116.3 | 50 | 10 | 20 | 23,250 | ... | 129 | 0.5548 |
| S-9-Bi | 20 | 960 | 1145 | 57.3 | 50 | 10 | 20 | 11,450 | 2325 | 15 | 0.6452 |
| July 1962 | | | | | | | | | | | |
| S-10-Bi | 20 | 960 | 1535 | 76.8 | 50 | 10 | 20 | 15,350 | 1145 | 65 | 0.5677 |
| Aug. 1962 | | | | | | | | | | 11 | 0.9607 |
| | | | | | | | | | | 92 | 0.5993 |
| | | | | | | | | | | 13 | 0.8469 |

* For further explanation, see text.

Salvador. The latter is much higher than either parent strain (52.4 and 46.1 respectively). The Lima strain showed a high egg-laying capacity both times that it was tested, although no parthenogenesis was found.

B. Selection for increased rate of parthenogenesis in laboratory strains.

1. *The Salvador strain. Selection procedure involving cycles of bisexual and unisexual reproduction within the Salvador strain:* As shown above, parthenogenetic offspring were originally obtained from the unselected Salvador strain by isolating virgin females at random and challenging them to reproduce without males. Of the 89 impaternal F_1 daughters, 52 were used in an attempt to found a parthenogenetic line. This line survived for seven months, or about 14 parthenogenetic generations; it finally declined to only two individuals and was discarded.

A new bisexual stock was established by crossing 30 of the F_1 impaternal daughters back to ordinary S strain males. The F_1 from this cross (Table 3) has been labelled S-1-Bi, in which the first letter stands for the strain designation, and the number set off by dashes indicates that the stock has had one generation of sexual outcrossing. "Bi" indicates the bisexual nature of the stock.

As a general rule, the procedure that was followed in the first phase of the experiments was to alternate unisexual and bisexual reproduction. Thus, for example, S-1-Bi females were isolated as virgins and challenged to reproduce parthenogenetically. The results of this and later selections for increased capacity for parthenogenetic development are given in Table 3. This table traces ten cycles of alternating unisexual and bisexual reproduction, extending for a little over one year. In each cycle, impaternal females were crossed to male sibs of their mothers, so that the amount of inbreeding is considerable.

At the eighth cycle (June 1962), an improved and more accurate method of obtaining egg counts and measuring rate of parthenogenesis was instituted. Egg counts were made from 20 females, 10 at a young age (about 3 days) and 10 at the age of about ten days. Vials containing eggs laid by individual virgin females were incubated and an additional direct measurement made of the number of impaternal offspring produced by each female. Thus, in the table, S-8-Bi was measured in three ways: (1) the older method, (2) the new method, and (3) the direct method (see Table 3). As the direct method is obviously the more accurate, it has been employed in all later (second phase) tests where the percent of parthenogenetic offspring is about one or above.

The data in the right-hand column of Table 3 indicate a leveling-off of the response of the Salvador strain at around the 1% level. Accordingly, selection within the strain was discontinued and S-10-Bi males were used in crosses to females from two other high-ranking selected lines (see Table 7).

Isolation of thelytokous substrains from the Salvador strain without outcrossing: During each cycle of selection, the impaternal females which were not used to cross to males were placed together in a half-pint culture bottle and were challenged to produce a unisexual sub-strain. The results are shown in Table 4. Although several of these substrains died out, most succeeded; five vigorous, easily maintained parthenogenetic strains are extant at the time of writing (Table 4). The rates of parthenogenesis in them were measured at various times after estab-

TABLE 4

Unisexual substrains derived from the Salvador (S) and Rochester (RS) strains without further outcrossing

| Impatinate substrain No. | Date of isolation | Date of test | No. of ♀ used for egg counts | No. hr of egg-laying | No. of eggs counted (or estimated) | Mean No. of eggs/♀/48 hr | No. of impatinate adults obtained (b) | Rate: impatinate adults/100 eggs (b/a) | Fate of substrain |
|-----------------------------|----------------------|-----------------|------------------------------------|-------------------------|--|-----------------------------|--|---|----------------------------|
| S-0-Im | Aug. 1961 | Aug. 1961 | 29 | 2011 | 2420 | 57.8 | 5 | 0.2066 | Died out Mar. 1962 |
| S-1-Im | Sept. 1961 | Mar. 1962 | 20 | 480 | 122 | 12.2 | 3 | 2.4590 | |
| S-2-Im | Oct. 1961 | Jan. 1963 | 56 | 3432 | 1493 | 20.9 | 19 | 1.2726 | Extant to date, Sept. 1966 |
| S-3-Im | Jan. 1962 | May 1962 | 20 | 648 | 595 | 44.1 | 3 | 0.5042 | Died out Aug. 1962 |
| S-4-Im | Mar. 1962 | May 1962 | 20 | 480 | 60 | 6.0 | 0 | 0.0000 | Discarded Nov. 1962 |
| S-5-Im | Apr. 1962 | Jan. 1962 | 30 | 1440 | 4782 | 159.4 | 6 | 0.6637 | |
| S-6-Im | May 1962 | Jan. 1963 | 40 | 1920 | 1894 | 47.3 | 48 | 1.0038 | Extant to date, Sept. 1966 |
| S-7-Im | June 1962 | Feb. 1966 | 40 | 1920 | 728 | 18.2 | 18 | 0.9504 | Discarded* May 1963 |
| S-8-Im | June 1962 | | | | | | 11 | 1.5109 | Extant to date, Sept. 1966 |
| S-9-Im | July 1962 | | | | | | | | Discarded* Jan. 1963 |
| S-10-Im | Sept. 1962 | | | | | | | | Died out Nov. 1962 |
| S-11-Im | Oct. 1962 | Nov. 1963 | 20 | 480 | 1140 | 114.0 | 4 | 0.3509 | Died out Nov. 1962 |
| RS-1-Im | May 1961 | July 1961 | 29 | 682 | 383 | 27.0 | 0 | 0.0000 | Died out Nov. 1962 |
| | | Mar. 1962 | 21 | 480 | (6387) | | 63 | 0.9864 | |
| | | Feb. 1962 | 21 | 504 | (2619) | 4.3 | 1 | 2.2356 | |
| RS-3-Im | Sept. 1961 | Feb. 1962 | 21 | 504 | 501 | 47.7 | 7 | 1.9473 | Died out Sept. 1962 |
| | | May 1962 | 41 | | (29,755) | 47.8 | 1000 | 3.3600 | |
| | | July 1962 | 20 | 960 | 6189 | | 41 | 0.6625 | |
| | | Aug. 1962 | 40 | 1920 | 1194 | | 8 | 0.6700 | |
| | | Nov. 1962 | 60 | 2880 | 1912 | 47.8 | 18 | 0.9414 | |
| | | Jan. 1963 | 40 | 960 | 4866 | 81.1 | 39 | 0.8015 | |
| | | Oct. 1963 | 40 | 1920 | 4844 | 242.2 | 61 | 1.2593 | |
| | | | 40 | 1920 | 3461 | 86.5 | 30 | 0.8668 | Extant to date, Sept. 1966 |

* Abundant flies at time of discard.

TABLE 5

*Selection for increased rate of parthenogenesis within the Rochester strain. First phase: In each cycle, parthenogenetic and bisexual reproduction are alternated**

| Cycle of selection | No. of ♀♀ used for egg counts | No. of hours of egg-laying | Total No. of eggs laid | Mean No. of eggs/♀/48 hr (a) | Total No. of females at beginning of tests | Mean No. of ♀♀ in each 48-hr test group (b) | No. of 48-hr test periods (c) | Estimated No. of eggs screened aXbXc | Actual No. of eggs counted | No. of impaternate adults obtained | Rate: impaternate adults/100 eggs |
|--------------------|-------------------------------|----------------------------|------------------------|------------------------------|--|---|-------------------------------|--------------------------------------|----------------------------|------------------------------------|-----------------------------------|
| R-0-Bi | 20 | 481 | 591 | 52.4 | 300 | 28.3 | 103 | 152,741 | ... | 6 | 0.0039 |
| July 1961 | | | | | | | | | | | |
| R-1-Bi | 20 | 504 | 884 | 84.2 | 300 | 29.7 | 44 | 110,033 | ... | 6 | 0.0055 |
| Sept. 1961 | | | | | | | | | | | |
| R-2-Bi | 20 | 480 | 474 | 47.4 | 300 | 29.9 | 40 | 56,690 | ... | 22 | 0.0395 |
| Oct. 1961 | | | | | | | | | | | |
| R-3-Bi | 20 | 480 | 531 | 53.1 | 300 | 28.9 | 48 | 73,660 | ... | 22 | 0.0299 |
| Jan. 1962 | | | | | | | | | | | |
| R-4-Bi | 20 | 480 | 707 | 70.7 | 210 | 28.5 | 28 | 56,419 | ... | 42 | 0.0744 |
| Feb. 1962 | | | | | | | | | | | |
| R-5-Bi | 20 | 480 | 1245 | 124.5 | 150 | 27.4 | 23 | 78,460 | ... | 72 | 0.0918 |
| Mar. 1962 | | | | | | | | | | | |
| R-6-Bi | 20 | 480 | 880 | 88.0 | 270 | 28.2 | 45 | 111,672 | ... | 166 | 0.1486 |
| Apr. 1962 | | | | | | | | | | | |
| R-7-Bi | 20 | 480 | 795 | 79.5 | 180 | 28.9 | 24 | 55,141 | ... | 156 | 0.2829 |
| May 1962 | | | | | | | | | | | |
| R-8-Bi | 20 | 480 | 960 | 96.0 | 150 | 29.1 | 15 | 41,904 | ... | 87 | 0.2076 |
| June 1962 | | | | | | | | | | | |
| R-9-Bi | 20 | 960 | 1282 | 64.1 | 50 | 10 | 20 | 12,820 | ... | 24 | 0.1872 |
| July 1962 | | | | | | | | | | | |
| R-10-Bi | 20 | 960 | 1212 | 60.6 | 50 | 10 | 20 | 12,120 | ... | 25 | 0.2063 |
| Aug. 1962 | | | | | | | | | | | |
| R-10-Bi | 20 | 960 | 1814 | 90.7 | 50 | 10 | 16 | 14,512 | ... | 29 | 0.2000 |

* For further explanation see text.

ishment. Except for S-0-Im and S-11-Im, the rates do not differ significantly from the mean of 1.02% [$\chi^2_{(7)} = 8.6$; $p \cong .3$] S-11-Im, furthermore, when tested by the indirect method, gave 98 impaternalates from an estimated egg number of 11,400. This yields a rate of 0.860%, which is not far from the mean. These values do not differ much from those found from S-5-Bi and later cycles (Table 3). Accordingly, it may be concluded that selection has increased the rate of parthenogenesis approximately tenfold, from 0.1% to a plateau of about 1.0%.

2. *The Rochester strain:* Cyclic selection for parthenogenesis was also carried out with the Rochester strain in a manner similar to that used on the Salvador strain. The data for the latter strain are given in Table 5. The Rochester strain begins with a lower level of parthenogenesis than the Salvador strain (Table 2). Over about one year and ten cycles of selection, the rate of parthenogenesis in the Rochester strain was increased about 50-fold from 39 per million eggs to 0.2% (Table 5). A plateau was apparently attained at about the seventh cycle.

During this selection period, eight attempts were made to establish unisexual strains in the manner previously described for the Salvador strain. All uniformly failed; even R-6-Im and R-11-Im, which were begun with 146 and 203 females respectively, declined and failed after three or four months.

3. *The bisexual bridge system:* When a high-ranking thelytokous strain is obtained, the problem arises as to how new genetic variability can be introduced into the strain without seriously diluting the genetic basis of its parthenogenesis. Ideally, nonrelated males from high-ranking mothers are desirable for making such an outcross. Accordingly, along with each high-ranking thelytokous strain, a bisexual strain ("the bridge") is maintained by a method first suggested by PROFESSOR H. D. STALKER. Each bridge is begun by crossing ten females from the thelytokous line to ten males from an unrelated strain. F_1 males (called "Bridge¹") are then crossed to ten more females from the thelytokous line; all other F_1 males and females are discarded. F_2 males are called "Bridge²" and the process is repeated. As the process of bridging is continued, the males in the bridge lines become genetically more and more like the thelytokous lines.

Various bridge stocks were used as a source of males in the most recent series of selections (see Tables 7 and 8). They carry, as the first two symbols, the letter and number of the impaternal stock to which they are bridged. The original sources of males used for starting the bridge stocks mentioned in Tables 7 and 8 are: RSB-4, sibling males; OB-2, Lima, Peru males; RSB-5, sibling males; RSS-17, Maui males; O-2, O-1-Bridge⁵ males; RSS-14, sibling males; O-1, sibling males; RSB-6, Kauai males.

4. *Selective advance in Rochester \times Salvador hybrid strains. First phase selections:* In March 1961, a mass mating was made between 30 Rochester females and 30 Salvador males. F_1 females were isolated as virgins; from about a little less than a million eggs screened, 20 adults were obtained (Table 2). These females were crossed en masse to a mixture of their sibs and double-cross hybrid males which were F_1 s from a double cross (Rochester $\text{♀♀} \times$ Salvador ♂♂) \times (Lima $\text{♀♀} \times$ Guatemala ♂♂). Thirty-nine females (RS-1-Im, Table 4) were obtained. The bisexual line from which virgins were isolated was called RS-1-Bi and cyclic

TABLE 6

*Selection for increased rate of parthenogenesis in Rochester × Salvador hybrid strains. First phase:
alternation of bisexual and unisexual reproduction*

| Cycle of selection | No. of ♀♀ used for egg counts | No. of hours of egg-laying | Total No. of eggs laid | Mean No. of eggs/♀/48 hr (a) | Total No. of females at beginning of tests | Mean No. of ♀♀ in each 48-hr test group (b) | No. of 48-hr test periods (c) | Estimated No. of eggs screened a X b X c | Actual No. of eggs counted | No. of impregnate adults obtained | Rate: impregnate adults/100 eggs |
|--------------------|-------------------------------|----------------------------|------------------------|------------------------------|--|---|-------------------------------|--|----------------------------|-----------------------------------|----------------------------------|
| RS-0-Bi | 15 | 362 | 1138 | 150.9 | 450 | 28.0 | 225 | 950,670 | ... | 20 | 0.0021 |
| May 1961 | | | | | | | | | | | |
| RS-1-Bi | 49 | 1194 | 2060 | 82.8 | 1410 | 28.5 | 329 | 776,374 | ... | 39 | 0.0050 |
| June 1961 | | | | | | | | | | | |
| RS-2-Bi | 20 | 471 | 689 | 70.2 | 960 | 29.5 | 121 | 250,579 | ... | 90 | 0.0359 |
| July 1961 | | | | | | | | | | | |
| RS-3-Bi | 20 | 480 | 1286 | 128.6 | 300 | 29.7 | 40 | 152,777 | ... | 29 | 0.0186 |
| Aug. 1961 | | | | | | | | | | | |
| RS-4-Bi | 20 | 480 | 305 | 30.5 | 300 | 29.4 | 40 | 35,868 | ... | 43 | 0.1194 |
| Oct. 1961 | | | | | | | | | | | |
| RS-5-Bi | 20 | 480 | 389 | 38.9 | 300 | 29.3 | 53 | 60,408 | ... | 104 | 0.1733 |
| Jan. 1962 | | | | | | | | | | | |
| RS-6-Bi | 20 | 480 | 532 | 53.2 | 240 | 29.7 | 24 | 37,921 | ... | 37 | 0.0974 |
| Mar. 1962 | | | | | | | | | | | |
| RS-7-Bi | 20 | 480 | 686 | 68.6 | 240 | 29.2 | 29 | 58,090 | ... | 69 | 0.1190 |
| Apr. 1962 | | | | | | | | | | | |
| RS-8-Bi | 20 | 480 | 782 | 78.2 | 210 | 28.1 | 32 | 70,317 | ... | 109 | 0.1557 |
| May 1962 | | | | | | | | | | | |
| RS-9-Bi | 20 | 480 | 1656 | 165.6 | 180 | 27.8 | 18 | 82,866 | ... | 96 | 0.1156 |
| June 1962 | | | | | | | | | | | |
| RS-10-Bi | 20 | 960 | 1649 | 82.5 | 50 | 10 | 20 | 16,490 | ... | 23 | 0.1395 |
| July 1962 | | | | | | | | | | | |
| RS-11-Bi | 20 | 960 | 906 | 45.3 | 50 | 10 | 20 | 9,060 | ... | 12 | 0.1325 |
| Aug. 1962 | | | | | | | | | | | |
| RS-12-Bi | 20 | 960 | 1627 | 90.8 | 50 | 10 | 22 | 19,978 | ... | 24 | 0.1201 |

selection was begun as in the previously described cases. The details are given in Table 6. A plateau was attained after about four cycles and the level remained steady at about 0.12% through the 12th cycle.

At each cycle, attempts were made to isolate self-sustaining thelytokous strains without further outcrossing (Table 4). Although RS-1-Im succeeded for a while, it died out. At the third cycle RS-3-Im was established. It has been maintained easily in the laboratory without further crossing since its isolation from males in September 1961. Although in February 1962 an indirect measure indicated a rate of 3.4%, more recent direct measurements of its rate appear to be close to 1%.

Second phase selections: In October 1962, the second phase of selection was entered by outcrossing and then reselecting from F_2 or F_3 recombinants. Both of the strains mentioned previously, as well as the Oahu strain, were used in these crosses. Gene flow from a number of the other strains was provided by the use of the bisexual bridge stock system. These operations are detailed in Table 7. In this phase an outcross is made and then a new impaternate (Im) stock obtained and tested. Thus, for example, in the first line of Table 7, RS-12-Im females (daughters of virgin RS-12-Bi females, Table 6) are crossed to males from S-10-Bi (Table 3). These are run through two bisexual generations, after which F_2 virgins are isolated and a new impaternate stock (RSS-13-Im) is obtained and tested.

In the lower part of Table 7, a similar system is detailed wherein strain RS-3-Im females were crossed with either S-10-Bi or S-6-Bi males. From virgin F_1 flies of the latter cross, a high-ranking thelytokous strain (RSB-1-Im) was obtained and further outcrossings carried out as indicated in Table 7.

Of the various strains obtained in this manner, the last five in the upper part of Table 7 (RSS-17-Im through RSS-21-Im) appear to be the same, with a mean percent of about 4.5 [$\chi^2_{(4)} = 6.4$, $P = .20$]. In the lower half of Table 7, RSB-6-Im is significantly higher than RSB-5-Im [$\chi^2_{(1)} = 5.4$, $P = .02$]. RSB-6-Im, moreover, is significantly higher than the highest RSS group [$\chi^2_{(1)} = 10.1$, $P = .002$].

5. *First and second phase selections carried out on the Oahu strain and its hybrids with other strains:* In the original screening of the eggs of virgin females of the Oahu strain, nine impaternate females were obtained. The O-1-Im strain obtained from these, however, was weak but was kept with a bridge stock for four generations. When O-1-Im died out, O-2-Im was established from O-1-Bridge⁴ (Table 8). Selections within the Oahu stock were continued and a level of between 1.5 and 2.0% was obtained. Its level was thus higher than either of the other pure strain selections.

An outcross of O-1-Im to a high-ranking RSB strain produced OB-2-Im, a strain which has the highest measured rate (6.4%). It is not significantly higher, however, than RSB-6-Im [$\chi^2_{(1)} = 0.1$, $P = .7$].

Inheritance of the capacity for parthenogenesis: Despite the fact that extensive tests were made in 1961 and 1964, the Lima, Peru (L) stock of *D. mercatorum* has shown no parthenogenesis whatever (Table 2). To obtain an estimate of the

TABLE 7

*Selection for increased rate of parthenogenesis in Rochester × Salvador hybrid strains.
Second phase: Outcrossing with reselection from F₂ or F₃ segregants*

| Parthenogenetic Stock No. | Bisexual origin | | No. of generations before isolation | | Date of establishment of parthenogenetic stock | Date of testing | No. of ♀♀ used for egg counts | No. of eggs counted | No. of impregnate adults obtained | Rate: impregnate adults/100 eggs |
|---------------------------|-----------------|-----------------------------|-------------------------------------|-----------|--|-----------------|-------------------------------|---------------------|-----------------------------------|----------------------------------|
| | ♀ parents | ♂ parents | bisexual | unisexual | | | | | | |
| RSS-13-Im | RS-12-Im | S-10-Bi | 2 | 1 | Oct. 1962 | Nov. 1962 | 20 | 1095 | 20 | 1.8265 |
| RSS-14-Im | RSS-13-Im | RSS-13-Bi | 2 | 1 | Jan. 1963 | Apr. 1963 | 40 | 4169 | 128 | 3.0702 |
| RSS-15-Im | RSS-14-Im | RSS-14-Bi | 3 | 1 | Aug. 1963 | Oct. 1963 | 40 | 3450 | 99 | 2.8696 |
| RSS-16-Im | RSS-15-Im | RSS-14-Bi | 3 | 1 | Dec. 1963 | May 1964 | 40 | 1373 | 32 | 2.3307 |
| RSS-17-Im | RSS-16-Im | RSB-4-Bridge ⁶ | 2 | 1 | July 1964 | Oct. 1964 | 49 | 2748 | 131 | 4.7671 |
| RSS-18-Im | RSS-17-Im | OB-2-Bridge ¹³ | 2 | 1 | Dec. 1964 | Feb. 1965 | 45 | 1270 | 67 | 5.2756 |
| RSS-19-Im | RSS-18-Im | RSB-5-Bridge ²⁰ | 2 | 1 | Apr. 1965 | Aug. 1965 | 45 | 1305 | 67 | 5.1341 |
| RSS-20-Im | RSS-19-Im | RSS-17-Bridge ²² | 2 | 1 | Oct. 1965 | Dec. 1965 | 40 | 2291 | 92 | 4.0157 |
| RSS-21-Im | RSS-18-Im | O-2-Bridge ³³ | 1 | 2 | Feb. 1966 | Feb. 1966 | 55 | 1866 | 72 | 3.8585 |
| RSA-1-Im | RS-3-Im | S-10-Bi | 2 | 1 | Oct. 1962 | Nov. 1962 | 20 | 1600 | 9 | 0.5625 |
| RSB-1-Im | RS-3-Im | S-6-Bi | 1 | 1 | July 1962 | July 1962 | 20 | 1107 | 9 | 0.8130 |
| RSB-2-Im | RSB-1-Im | RSA-1-Bi | 2 | 1 | Jan. 1963 | Nov. 1962 | 20 | 1178 | 23 | 1.9525 |
| RSB-3-Im | RSB-2-Im | RSS-14-Bi | 3 | 1 | June 1963 | Apr. 1963 | 30 | 3638 | 101 | 2.7763 |
| RSB-4-Im | RSB-3-Im | RSB-2-Bi | 3 | 1 | Dec. 1963 | Oct. 1963 | 40 | 2930 | 96 | 3.2765 |
| RSB-5-Im | RSB-4-Im | RSB-4-Bi | 3 | 1 | May 1964 | Feb. 1964 | 40 | 2505 | 89 | 3.5529 |
| RSB-6-Im | RSB-4-Im | RSS-14-Bridge ⁶ | 2 | 1 | July 1964 | May 1964 | 40 | 1293 | 43 | 3.3256 |
| RSB-7-Im | RSB-6-Im | RSB-5-Bridge ¹² | 2 | 1 | Dec. 1964 | Jan. 1965 | 40 | 1372 | 59 | 4.3003 |
| RSB-8-Im | RSB-5-Im | RSB-4-Bridge ²¹ | 2 | 1 | Mar. 1965 | Oct. 1964 | 50 | 2417 | 147 | 6.0819 |
| RSB-9-Im | RSB-6-Im | OB-2-Bridge ³² | 1 | 2 | Dec. 1965 | Mar. 1965 | 40 | 1988 | 63 | 3.1690 |
| | | | | | | Aug. 1965 | 40 | 2243 | 74 | 3.2992 |
| | | | | | | Dec. 1965 | 56 | 2150 | 52 | 2.4186 |
| | | | | | | Feb. 1966 | 40 | 2149 | 18 | 0.8376 |

RSS-17 through RSS-21 homogeneous: $\chi^2_{(6)} = 6.4$, $P = .20$.

TABLE 8

Selection for increased parthenogenesis in the Oahu strain and hybrids between it and other strains

| Parthenogenetic Stock No. | Bisexual origin | | No. of generations before isolation | | Date of establishment of parthenogenetic stock | Date of testing | No. of ♀♀ used for egg counts | No. of eggs counted | No. of impregnate adults obtained | Rate: impregnate adults/100 eggs |
|---------------------------|-----------------|----------------------------|-------------------------------------|-----------|--|-----------------|-------------------------------|---------------------|-----------------------------------|----------------------------------|
| | ♀ parents | ♂ parents | bisexual | unisexual | | | | | | |
| O-2-Im | O-1-Im | Bridge ⁴ | 4 | 1 | Jan. 1965 | Feb. 1965 | 19 | 1191 | 17 | 1.4274 |
| O-3-Im | O-2-Im | Bridge ¹⁸ | 2 | 1 | May 1965 | Oct. 1965 | 40 | 2191 | 46 | 2.0995 |
| O-4-Im | O-3-Im | Bridge ³¹ | 1 | 1 | Nov. 1965 | Feb. 1966 | 40 | 2012 | 31 | 1.5408 |
| OB-2-Im | O-1-Im | RSB-4-Bi | 2 | 1 | May 1964 | Jan. 1965 | 55 | 1413 | 90 | 6.3694 |
| OB-3-Im | OB-2-Im | RSB-6-Bridge ¹² | 2 | 1 | Mar. 1965 | Sept. 1965 | 40 | 2799 | 75 | 2.6795 |

TABLE 9

Inheritance of thelytokous parthenogenesis through the male and through the female parent

| Females tested | No. of ♀♀ used for egg counts | No. of hours of egg-laying | Total No. of eggs laid | Mean No. of eggs/48 hr (a) | Total No. of females At beginning of tests | At end of tests | Mean No. of ♀♀ in each 48-hr test group (b) | No. of 48-hr test periods (c) | Est. No. of eggs screened a × b × c | No. of impregnate adults obtained | Rate: impregnate adults/100 eggs |
|--|-------------------------------|----------------------------|------------------------|----------------------------|--|-----------------|---|-------------------------------|-------------------------------------|-----------------------------------|----------------------------------|
| F ₁ of L ♀♀ (0.0%, Table 2) × | | | | | | | | | | | |
| RSB-3-Bi ♂ ♂ (3.6%, Table 7) | 20 | 480 | 1495 | 149.5 | 120 | 114 | 29.3 | 48 | 210,257 | 3 | 0.0014 |
| F ₁ of impregnate ♀♀ (above) × | | | | | | | | | | | |
| RSB-3-Bi ♂ ♂ | 20 | 480 | 1953 | 195.2 | 330 | 322 | 29.6 | 42 | 242,673 | 223 | 0.0919 |
| F ₁ of RSB-3-Im ♀♀ (3.6%, Table 7) × | | | | | | | | | | | |
| L ♂ ♂ (0.0%, Table 2) | 20 | 480 | 1425 | 142.5 | 180 | 171 | 29.3 | 31 | 129,433 | 40 | 0.0309 |
| F ₁ of RSB-3-Im ♀♀ × ♂ ♂ from above cross | 20 | 480 | 1690 | 169.0 | 330 | 322 | 29.6 | 44 | 220,106 | 1638 | 0.7410 |

heritability of the character through the male parent, L stock females were crossed with males from a high ranking bisexual line (F_1 from RSB-3-Im females \times RS-2-Bi males). Bisexual F_1 virgin females from this cross were challenged to reproduce parthenogenetically and the impaternal daughters produced were back-crossed to the same males. The bisexual virgin females produced from this latter cross were also tested for rate of parthenogenesis (Table 9, first two horizontal lines). These results may be compared with those obtained from the reciprocal cross (Table 9, lower two lines). It will be seen that high-ranking males transmit at least a small capacity for parthenogenesis to their F_1 daughters and that this rate is increased over 60 times by backcrossing. Likewise, males with no rank with respect to parthenogenesis strongly depress the rate of parthenogenesis among their daughters, although when F_1 males are backcrossed to high-ranking females the effect in reducing the rate of parthenogenesis is much less.

DISCUSSION

Following discovery of a low rate (not above 0.1%) of facultative diploid thelytoky in several wild strains of *Drosophila mercatorum*, it has been possible to increase the rate artificially about 60-fold, that is, to around 6%. Thelytokous laboratory strains so produced, some of which are more than five years old (130 generations), are highly vigorous and can be easily maintained without males in the laboratory. The slow attainment of the high levels and the fact that the selective advances follow interstrain hybridization, genetic recombination and reselection suggests that the character is influenced by a number of independent gene loci.

There is no evidence that the extremely low rate of parthenogenesis existing in wild strains serves any role in natural populations, that is, bisexual reproduction appears to be the normal mode for the species. Accordingly, it may be said that the directed evolution of a new mode of reproduction for this species has been accomplished.

A similar achievement has been made in *Drosophila parthenogenetica* by STALKER (1956), although in this case the situation is biologically less well balanced in that the mode of parthenogenesis results in quite a large proportion of triploids in addition to diploids. Although the level of parthenogenesis in *D. parthenogenetica* was close to that attained in the present experiments, all of STALKER's strains became infected with microsporidia and had to be discarded.

HAMILTON (1953) reared the normally bisexual grasshopper *Schistocerca gregaria* for four generations by parthenogenesis. No measurements of change in parthenogenetic rate were made. ROTH and WILLIS (1956, 1961) have described facultative parthenogenesis in five species of cockroaches. *Periplaneta americana* was reared for two generations without males, but the lines apparently died out owing to the presence of developmental abnormalities. This recalls the condition in *Drosophila robusta* (CARSON 1961).

In addition to the above cases, low rates of parthenogenesis are found rather widely among animals, e.g. *Drosophila* (STALKER 1954), mosquitoes (KITZMILLER 1959), bees (TUCKER 1958) and turkeys (YAO and OLSEN 1955).

Under suitable conditions such as periodic shortages of males in small local populations (STALKER 1956), low-frequency rates of parthenogenesis might serve as a basis on which natural selection could build parthenogenesis into a major mode of reproduction in the species. The success of the present experiments suggests that such an interpretation is correct. Compared with *Drosophila mangabeirai* (CARSON, WHEELER and HEED 1957), the only known species of *Drosophila* that reproduces wholly by thelytokous means, *D. mercatorum* is far less efficient. Thus, in *D. mangabeirai*, about 60% of the eggs yield viable adults whereas in the most efficient stock of *D. mercatorum*, the level is around 6%.

I am most grateful, as always, to PROFESSOR H. D. STALKER for his interest and advice in many discussions. Much of the work was done in Australia while the writer held a Fulbright Fellowship at the University of Melbourne, and many thanks are due to PROFESSOR M. J. D. WHITE. DR. VINCENT STRANGIO made some key transfers while the writer was away from the laboratory. I am indebted to the Genetics Foundation, University of Texas, for supplying some stocks, as well as to DRs. M. WASSERMAN and J. KRIVSHENKO for supplying others. MRS. BEVERLY GRALNICK, MISS CHRISTINE STEINER, MR. JOHN A. NIEDERKORN, JR. and MISS IRENE WEI all provided valuable technical assistance. The work has been supported by grants G-20107 and GB 3147 from the National Science Foundation.

SUMMARY

Three wild strains show very low rates of thelytokous parthenogenesis, the highest being only about one adult female per 1000 unfertilized eggs. Artificial selection involving cycles of alternating bisexual and unisexual reproduction within the strain from El Salvador resulted in about a tenfold increase; similar selections within the Rochester, New York strain raised the rate from 39 offspring per million eggs to about 0.2%. Artificial selection practiced on hybrids between Rochester and Salvador, in which a special bridge system for obtaining high-ranking males was used, resulted in the production of a strain showing a level of 6.1%. Selection within an Oahu, Hawaii strain resulted in a level of between 1.5 and 2.0%. When outcrossed to other selected strains, another strain demonstrating a level of 6.4% was obtained. These highest levels are more than 60 times the highest of the unselected levels. The character can be transmitted through the male parent. It is suggested that the capacity for parthenogenesis in *D. mercatorum* is polygenic. The experiments directly demonstrate that low-level facultative parthenogenesis can be built into a major mode of reproduction by selection. The data presented and the mode of successful selection suggest the correctness of H. D. STALKER's model for the origin of parthenogenetic strains. Thus, periodic shortages of males in small isolated populations could institute cycles of bisexual and unisexual reproduction of the type used experimentally in these studies. At some point in time, a parthenogenetic strain so built can become capable of self-support without further outcrossing and reselection.

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