SELECTION FOR PARTHENOGENESIS IN
DROSOPHILA MERCATORUM

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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 impaternate 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 impaternate 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° ± 1.0°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 ×

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 of tests	Total No. of females 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 impaternate adults obtained	Rate: impaternate adults/10^6 eggs
Salvador July 1961 Oahu	20	497	477	46.1	300	269	28.5	70	91,970	89	967
Nov. 1963 Rochester	20	480	204	20.4	300	277	28.9	115.5	68,094	9	132
July 1961 F₁ Rochester × Salvador	20	481	591	52.4	300.	265	28.3	103	152,741	6	39
April 1961 Lima	15	362	1138	150.9	450	389	28.0	225	950,670	20	21
July 1961	20	475	819	82.8	270	243	25.7	84	178,749	0	0
Jan. 1964 Guatemala	20	480	1335	133.5	330	301	28.6	89.5	341,720	0	0
July 1961 Manizales	20	471	719	73.3	300	218	25.9	86	163,278	0	0
Jan. 1964 Kauai	20	480	51	2.6	330	320	29.5	84.5	6,481	0	0
Jan. 1964 Kamuela Hawaii	20	480	256	25.6	330	306	28.9	117.5	86,931	0	0
Feb. 1964	20	480	1066	105.6	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	Total No. of females at end 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×b×c	Actual No. of eggs counted	No. impaternate adults obtained	Rate: impaternate adults/100 eggs
S-0-Bi July 1961	20	497	477	46.1	300	269	28.5	70	91,970	…	89	0.0967
S-1-Bi Sept. 1961	20	480	806	80.6	270	260	29.4	36	85,307	…	50	0.0586
S-2-Bi Oct. 1961	20	480	597	59.7	270	261	29.5	36	63,401	…	105	0.1656
S-3-Bi Jan. 1962	20	480	512	51.2	300	294	29.7	48	72,991	…	77	0.1055
S-4-Bi Feb. 1962	20	480	525	52.5	240	215	28.4	32	47,485	…	219	0.4612
S-5-Bi Mar. 1962	20	480	248	24.8	120	104	28.0	19	13,194	…	104	0.7882
S-6-Bi Apr. 1962	20	480	861	86.1	210	195	28.9	28	69,672	…	264	0.3789
S-7-Bi May 1962	20	480	480	48.0	180	174	29.5	24	33,984	…	591	1.7391
S-8-Bi June 1962	20	480	971	97.1	150	141	29.1	15	42,384	…	246	0.5804
	30	960	2325	116.3	50	50	10	20	23,250	…	129	0.5548
										2325	15	0.6452
S-9-Bi July 1962	20	960	1145	57.3	50	50	10	20	11,450	…	65	0.5677
										1145	11	0.9607
S-10-Bi Aug. 1962	20	960	1535	76.8	50	50	10	20	15,350	…	92	0.5993
										1535	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 impaternate 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 impaternate 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, impaternate 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 impaternate 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 impaternate 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 × Salvador (RS) strains without further outcrossing

Impaternate substrain No.	Date of isolation	Date of test	No. of ♀♀ used for egg counts	No. hr of egglaying	No. of eggs counted (or estimated) (a)	Mean No. of eggs/♀/48 hr	No. of impaternate adults obtained (b)	Rate: impaternate 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	......
		Jan. 1963	56	3432	1493	20.9	19	1.2726	Extant to date, Sept. 1966
S-2-Im	Oct. 1961	May 1962	20	648	595	44.1	3	0.5042	Died out Aug. 1962
S-3-Im	Jan. 1962	May 1962	20	480	60	6.0	0	0.0000	Discarded Nov. 1962
S-4-Im	Mar. 1962	June 1962	20	480	904	90.4	6	0.6637	......
		Jan. 1962	30	1440	4782	159.4	48	1.0038	Extant to date, Sept. 1966
S-5-Im	Apr. 1962	......	...	...	...	...	...	......	Discarded* May 1963
S-6-Im	May 1962	Jan. 1963	40	1920	1894	47.3	18	0.9504	Extant to date, Sept. 1966
S-7-Im	June 1962	Feb. 1966	40	1920	728	18.2	11	1.5109	Extant to date, Sept. 1966
S-8-Im	June 1962	......	...	...	...	...	...	......	Discarded* Jan. 1963
S-9-Im	July 1962	......	...	...	...	...	...	......	Died out Nov. 1962
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	......
			...	...	(6387)	...	63	0.9864	......
		Mar. 1962	21	480	43	4.3	1	2.2356	......
			...	...	(2619)	...	51	1.9473	Died out Sept. 1962
RS-3-Im	Sept. 1961	Feb. 1962	21	504	501	47.7	7	1.3972	......
			...	...	(29,755)	47.8	1000	3.3600	......
		May 1962	41	...	6189	...	41	0.6625	......
		July 1962	20	960	1194	...	8	0.6700	......
		Aug. 1962	40	1920	1912	47.8	18	0.9414	......
		Nov. 1962	60	2880	4866	81.1	39	0.8015	......
		Jan. 1963	40	960	4844	242.2	61	1.2593	......
		Oct. 1963	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		Mean No. of ♀♀ in each 48-hr test group (b)	No. of 48-hr test periods (c)	Estimated No. of eggs screened a×b×c	Actual No. of eggs counted	No. impaternate adults obtained	Rate: impaternate adults/100 eggs
					at beginning of tests	at end of tests						
R–0–Bi												
July 1961	20	481	591	52.4	300	265	28.3	103	152,741	…	6	0.0039
R–1–Bi												
Sept. 1961	20	504	884	84.2	300	294	29.7	44	110,033	…	6	0.0055
R–2–Bi												
Oct. 1961	20	480	474	47.4	300	297	29.9	40	56,690	…	22	0.0395
R–3–Bi												
Jan. 1962	20	480	531	53.1	300	279	28.9	48	73,660	…	22	0.0299
R–4–Bi												
Feb. 1962	20	480	707	70.7	210	189	28.5	28	56,419	…	42	0.0744
R–5–Bi												
Mar. 1962	20	480	1245	124.5	150	124	27.4	23	78,460	…	72	0.0918
R–6–Bi												
Apr. 1962	20	480	880	88.0	270	237	28.2	45	111,672	…	166	0.1486
R–7–Bi												
May 1962	20	480	795	79.5	180	167	28.9	24	55,141	…	156	0.2829
R–8–Bi												
June 1962	20	480	960	96.0	150	141	29.1	15	41,904	…	87	0.2076
	20	960	1282	64.1	50	50	10	20	12,820	…	24	0.1872
R–9–Bi												
July 1962	20	960	1212	60.6	50	50	10	20	12,120	…	25	0.2063
R–10–Bi												
Aug. 1962	20	960	1814	90.7	50	50	10	16	14,512	…	29	0.2000

* For further explanation see text.

lishment. 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 impaternates 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 "Bridge[1]") 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 "Bridge[2]" 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 impaternate 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-Bridge[5] males; RSS-14, sibling males; O-1, sibling males; RSB-6, Kauai males.

4. *Selective advance in Rochester × 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_1s from a double cross (Rochester ♀ ♀ × Salvador ♂ ♂) × (Lima ♀ ♀ × 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	at end 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×b×c	Actual No. of eggs counted	No. impaternate adults obtained	Rate: impaternate adults/100 eggs
RS–0–Bi May 1961	15	362	1138	150.9	450	389	28.0	225	950,670	…	20	0.0021
RS–1–Bi June 1961	49	1194	2060	82.8	1410	1267	28.5	329	776,374	…	39	0.0050
RS–2–Bi July 1961	20	471	689	70.2	960	928	29.5	121	250,579	…	90	0.0359
RS–3–Bi Aug. 1961	20	480	1286	128.6	300	293	29.7	40	152,777	…	29	0.0186
RS–4–Bi Oct. 1961	20	480	305	30.5	300	288	29.4	40	35,868	…	43	0.1194
RS–5–Bi Jan. 1962	20	480	389	38.9	300	286	29.3	53	60,408	…	104	0.1733
RS–6–Bi Mar. 1962	20	480	532	53.2	240	235	29.7	24	37,921	…	37	0.0974
RS–7–Bi Apr. 1962	20	480	686	68.6	240	227	29.2	29	58,090	…	69	0.1190
RS–8–Bi May 1962	20	480	782	78.2	210	184	28.1	32	70,317	…	109	0.1557
RS–9–Bi June 1962	20	480	1656	165.6	180	153	27.8	18	82,866	…	96	0.1156
RS–10–Bi June 1962	20	960	1649	82.5	50	50	10	20	16,490	…	23	0.1395
RS–11–Bi July 1962	20	960	906	45.3	50	50	10	20	9,060	…	12	0.1325
RS–12–Bi Aug. 1962	20	960	1627	90.8	50	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 highranking 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 [$x^2_{(4)} = 6.4$, P = .20]. In the lower half of Table 7, RSB–6–Im is significantly higher than RSB–5–Im [$x^2_{(1)} = 5.4$, P = .02]. RSB–6–Im, moreover, is significantly higher than the highest RSS group [$x^2_{(1)} = 10.1$, 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–Bridge[4] (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 [$x^2_{(1)} = 0.1$, 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 F_2 or F_3 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 imparternate adults obtained	Rate: imparternate adults/100 eggs
	♀ parents	♂ parents	bisexual	unsexual						
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
						Nov. 1962	20	1178	23	1.9525
RSB-2-Im	RSB-1-Im	RSA-1-Bi	2	1	Jan. 1963	Apr. 1963	30	3638	101	2.7763
RSB-3-Im	RSB-2-Im	RSS-14-Bi	3	1	June 1963	Oct. 1963	40	2930	96	3.2765
RSB-4-Im	RSB-3-Im	RSB-2-Bi	3	1	Dec. 1963	Feb. 1964	40	2505	89	3.5529
						May 1964	40	1293	43	3.3256
RSB-5-Im	RSB-4-Im	RSB-4-Bi	3	1	May 1964	Jan. 1965	40	1372	59	4.3003
RSB-6-Im	RSB-4-Im	RSS-14-Bridge[6]	2	1	July 1964	Oct. 1964	50	2417	147	6.0819
RSB-7-Im	RSB-6-Im	RSB-5-Bridge[12]	2	1	Dec. 1964	Mar. 1965	40	1988	63	3.1690
RSB-8-Im	RSB-5-Im	RSB-4-Bridge[21]	2	1	Mar. 1965	Aug. 1965	40	2243	74	3.2992
RSB-9-Im	RSB-6-Im	OB-2-Bridge[32]	1	2	Dec. 1965	Dec. 1965	56	2150	52	2.4186
						Feb. 1966	40	2149	18	0.8376

RSS-17 through RSS-21 homogenous: $\chi^2_{(4)} = 6.4$, P = .20.

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

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	Total No. of females 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 impaternate adults obtained	Rate: impaternate 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 impaternate ♀♀ (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 × RS–2–Bi males). Bisexual F_1 virgin females from this cross were challenged to reproduce parthenogenetically and the impaternate 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%.

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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