THE POPULATION GENETICS OF PARTHENOGENETIC STRAINS OF *DROSOPHILA MERCATORUM*. II. THE CAPACITY FOR PARTHENOGENESIS IN A NATURAL, BISEXUAL POPULATION[1]

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Manuscript received July 18, 1975

ABSTRACT

Drosophila mercatorum is a bisexual species, but certain strains are capable of parthenogenetic reproduction in the laboratory. We investigated the parthenogenetic capacity of the virgin daughters of females captured from a natural, bisexual population in Hawaii. An isozyme survey indicated the natural population is polymorphic at about 50% of its loci, and its individuals heterozygous at 18% of their loci. The predominant mode of parthenogenesis in *D. mercatorum* causes homozygosity for all loci in a single generation. Despite this radical change in genetic state, 23% of the virgin female lines produced adult parthenogenetic progeny, and 16% produced parthenogenetic progeny themselves capable of parthenogenetic reproduction. The parthenogenetic rate as measured by the number of parthenogenetic progeny themselves capable of parthenogenesis divided by the number of eggs laid is around 10^{-5} for the virgin female lines. We argue that one of the major reasons for this low rate is that very few of the impaternate zygotes have a genotype that can survive and reproduce under the genetic conditions imposed by parthenogenetic reproduction. This intense selective bottleneck can be passed in a single generation if enough unfertilized eggs are laid, and once passed is accompanied by a large (perhaps a thousandfold) increase in the rate of parthenogenesis and by modifications in many phenotypic traits such as morphology and behavior.

PARTHENOGENESIS is a widespread phenomenon in the animal kingdom and in particular among the insects. Parthenogenesis must have evolved independently many times in the insects since it is common to find a single or just a few parthenogenetic species in an otherwise bisexual genus. For example, only one Drosophila, *Drosophila mangabeirai*, is known to have obligatory

[1] Supported by NSF Grant GB 41278 and NSF Grant BMS 74-17453.

[2] This work was partially carried out while TEMPLETON was a Junior Fellow in the Society of Fellows of the University of Michigan, whose support is gratefully acknowledged.

parthenogenesis in nature out of hundreds of species in this genus (MURDY and CARSON 1959). However, the work of STALKER (1954) and CARSON (1961, 1967a) has revealed that many Drosophila species have at least some capacity for parthenogenetic reproduction under laboratory conditions. Furthermore, the pronuclei in unfertilized eggs of *D. melanogaster* (a species from which no viable adults have yet been obtained parthenogenetically) undergo spontaneous fusions and duplications (DOANE 1960); events which can lead to parthenogenetic development (TEMPLETON and ROTHMAN 1973). It thus appears that many Drosophila are "preadapted" meiotically for parthenogenetic reproduction, at least under laboratory conditions. The question then arises as to what other factors limit or otherwise determine the evolutionary transition from bisexual to parthenogenetic reproduction in nature. A first step in answering this question is to see if females from wild, bisexual populations have any capacity for parthenogenetic reproduction. We have specifically investigated this capacity in a natural population of *Drosophila mercatorum*.

Drosophila mercatorum is a widespread, tropical bisexual species. No parthenogenesis has been recorded in nature in this species, but CARSON (1967a) has been able to isolate in the laboratory parthenogenetic strains stemming from three different geographical areas. For the most part, these strains were isolated from stocks that had been maintained in the laboratory for at least several months, often several years. Moreover, none of these strains were isolated directly as parthenogenetic stocks, but rather were isolated through an artificial selection scheme that involved "bridge cycles" in which sexual and parthenogenetic generations were alternated (CARSON 1967a). In this way CARSON obtained up to a 64-fold increase in the rate of parthenogenesis as measured by the number of viable impaternate adults produced from a given number of eggs laid by virgin females. These bisexual-unisexual "bridge" cycles were used because very few impaternate females were produced in the initial generations. Rather than risk losing the strain, these rare females were mated as soon as possible to genetically related males so that a large number of sexually produced daughters, isolated as virgins, could serve as the base for a new round of selection for parthenogenesis. One stock, S-0-Im, was isolated directly from a bisexual strain, but died out seven months later. All other previously isolated parthenogenetic strains were obtained using one or more bisexual bridge cycles.

The work described in this paper differs in two significant ways from the previous work. Firstly, the capacity for parthenogenetic reproduction is scored in the F_1 virgin female offspring of females caught in a natural population from Hawaii. Consequently, we are screening natural populations rather than laboratory populations. Secondly, we will also screen the initial impaternate progeny for their parthenogenetic capacity. In other words, we will establish parthenogenetic strains directly from the wild-caught females with no artificial selection or bridging. This screening procedure should measure parthenogenetic capacity in a fashion more directly related to the evolution of parthenogenetic populations in nature. Furthermore, such measures will yield insight into the factors which limit the evolution of parthenogenesis.

MATERIALS AND METHODS

On January 4 and 5, 1974, collections of *Drosophila mercatorum* were made at Kameula (Waimea), Hawaii (Big Island). Some specimens were caught in a horse paddock with banana mash bait in buckets, but most were caught at a nearby rubbish dump by sweeping with nets. Some 34 females and 54 males were captured in this manner. The males were run on vertical starch gel electrophoresis using the techniques given in BREWER (1970). The males were scored for 10 isozyme systems: α-GPDH, AcPh, ADH, XDH, Hexokinase, PGM, G-6-PD, 6-PGD and esterases A and B.

The 34 females were kept alive and placed on cornmeal-agar food sprinkled with live yeast, with each wild-caught female placed in a separate vial. Of these 34 females, 31 produced F_1 progeny. These progeny were approximately equal numbers of males and females, indicating that all F_1 progeny thus obtained in the lab were the result of sexual matings in nature. Some of the F_1 males and females were placed together in a vial as founders of isofemale bisexual stocks. Such stocks were given the designation K_x-0-Bi where K refers to Kamuela, x is the number assigned to that isofemale line (1 through 34), 0 refers to the number of bridge cycles (see CARSON 1967a) and in this case indicates no bridge cycles were made, and Bi designates this as a bisexual stock. Also, 15 to 38 F_1 females from each of the 31 isofemales producing F_1 progeny were isolated as virgins and placed together in a shell vial. The virgins were transferred to fresh vials every 5–7 days. In this way many unfertilized eggs would be laid in these vials. Each F_1 virgin female line was designated with the symbol K-x-F_1. At the end of an egg laying period lasting between 35 and 45 days after eclosion the surviving F_1 females were either frozen for possible later electrophoresis or crossed to certain laboratory stocks so that "coadaptation" experiments could be performed (TEMPLETON, SING and BROKAW 1976); however, these experiments are not the subject of this paper. These F_1 females were scored for the same 10 systems as the males and in addition the systems of NAD-dependent MDH and Fumarase. These were scored for the most part on starch gels using the techniques given in BREWER (1970), but occasionally the systems of EST-B, G-6-PD, XDH and ADH were scored on a polyacrylamide gel with .005 M histidine at pH 8.3 as the gel buffer and tric-citrate at pH 8.15 as the tray buffer, and otherwise using the techniques given in STEINER and JOHNSON (1973).

The vials containing the unfertilized eggs laid by the F_1 females were retained for 3 weeks after the F_1 females were transferred out of them. This allowed more than ample time for parthenogenetic larvae, pupae or adults to appear in these vials. Any parthenogenetic females emerging from these vials were put in fresh vials and allowed to lay unfertilized eggs for up to four weeks. In this way, parthenogenetic strains were established. Such stocks were designated by K_x-0-Im where Im refers to "impaternate". Some of these impaternate females did not produce any parthenogenetic offspring after 4 weeks of egg laying as virgins. Bisexual bridge matings were initiated with these females, although the fate of these stocks is not the subject of this paper.

In addition to this screening for parthenogenesis various egg-laying experiments were done on bisexual and impaternate virgin females. The first such experiment involved twenty K-0-Bi females drawn from several different iso-female lines. This group of females is hereafter referred to as "E-1". All twenty E-1 females eclosed on the same morning and were put in a common shell vial. This vial was examined with the aid of a binocular microscope every 24 hours thereafter. No eggs were observed until the fifth day after eclosion, at which time 217 eggs were counted (10.85/female). These eggs must have been laid between the fourth and fifth days after eclosion. Thereafter the females were periodically isolated into their own vials for 24-hour periods and the number of eggs laid by each individual female was counted. Nine such 24-hour egg-laying counts were performed, the last being on the 54th day after eclosion. During this period some of the females died, with only 13 of the original 20 still alive on the 54th day after eclosion. An additional egg-laying experiment involving three K-0-Bi females was performed and designated "E-2". Once again all females eclosed on the same morning, but unlike in the previous experiment they were always kept together in the same vial. All three females survived until the 50th day after eclosion, at which time this experiment was terminated. During these 50 days, twenty-three 24-hour egg-laying counts were made. In addition to these egg-laying experiments

with K-0-Bi flies, eighteen K_{28}-0-Im females were used in an egg-laying experiment of the same design as that for the E-1 females. These females were from the fourth parthenogenetic generation of this line, but as will be argued later they probably have genotypes identical to the original K_{28}-0-Im female. The vials in which the eggs were counted were retained and the numbers of pupae and emerging adults were counted for each vial. In this way, the rate of parthenogenesis in K_{28}-0-Im could be directly measured.

RESULTS

As mentioned above, the males collected from the natural population were scored for ten isozyme loci and proved to be polymorphic at five (*XDH*, *G-6-PD*, *6-PGD*, *Est-A*, *Est-B*). In addition to these males, 9 to 17 F_1 females from 29 of the 31 K-x-F_1 virgin female lines were scored after their egg-laying period and proved to be polymorphic at 7 of 12 systems (58%). These results indicate that this natural population of *Drosophila mercatorum* is polymorphic at about 50% of its loci—a figure comparable to other species of Drosophila.

The details of the isozyme survey of the K-x-F_1 females are given in Table 1. This table lists the polymorphic loci and indicates which isofemale lines showed segregation at a given locus. In all cases, the F_1 females showed both heterozygotes and homozygotes in the same isofemale line at the loci marked as segregating, indicating a mating type in nature of either $Aa \times aa$ or $Aa \times Aa$ (or $Aa \times a$ for the sex-linked loci). In either case, the resulting F_1 female progeny should be heterozygous with probability 1/2 at the loci marked as segregating. Consequently, the average number of loci at which an F_1 female in a given line is heterozygous is half the number of segregating loci. The percent loci at which the average individual is heterozygous is this latter number $\times$ 100 divided by 12 (or in those cases where some loci were not scored, 10 or 11). The percent loci heterozygous per F_1 female is given also in Table 1 for each isofemale line. The average heterozygosity over all lines of 18% is comparable to that estimated for other Drosophila species. In summary, this isozyme survey indicates that natural populations of *Drosophila mercatorum* are characterized by large amounts of polymorphism and high levels of individual heterozygosity.

Of the 31 F_1 virgin female lines established from wild-caught females, 7 (23%) produced one or more parthenogenetic offspring during the time allowed for egg laying. The lines that produced such impaternate offspring, as well as the actual number of offspring, are given in Table 2. Of these seven initial Im lines, five proved capable of parthenogenetic reproduction themselves (Table 2). In other words, about 16% of the wild-caught females produced daughters, some of which were capable of establishing a totally parthenogenetic line. Consequently, we conclude the capacity for parthenogenetic reproduction is quite high in the Kamuela population of *Drosophila mercatorum*.

It is also desirable to measure the rate of parthenogenesis by the number of emerging impaternate adults divided by the number of eggs laid. In order to measure this parthenogenetic rate, the number of eggs laid by the F_1 virgins must be estimated. This can be estimated from the data obtained from the E-1 and E-2 egg-laying experiments. Figure 1 gives a graphical summary of these data. As Figure 1 indicates, the number of eggs laid increases rapidly after the

TABLE 1

Results of the isozyme survey of the K-X-F_1 females for those loci that were polymorphic in the Kamuela population

Line	Locus†							Total no. of seg. loci	Ave. no. of heterozygous loci per ♀	% loci hetero. per ♀
	Est-A	*Est-B*	*Xdh*	*Acph*	*Adh*	*G-6-Pd*	*6-Pgd*			
K-1-F_1	seg	seg	—	—	—	*	*	2	1	10
K-4-F_1	seg	seg	seg	seg	—	seg	seg	6	3	25
K-5-F_1	seg	seg	seg	seg	seg	seg	—	6	3	25
K-6-F_1	seg	seg	seg	—	—	seg	seg	5	2.5	21
K-7-F_1	seg	seg	seg	seg	—	seg	seg	6	3	25
K-8-F_1	seg	seg	seg	seg	—	seg	seg	6	3	25
K-9-F_1	seg	seg	—	seg	—	—	—	3	1.5	13
K-10-F_1	seg	seg	—	—	—	seg	—	3	1.5	13
K-11-F_1	seg	—	seg	—	—	*	*	2	1	10
K-12-F_1	seg	seg	—	—	—	*	*	2	1	10
K-13-F_1	seg	seg	seg	—	seg	—	seg	5	2.5	21
K-14-F_1	seg	seg	seg	—	seg	*	*	4	2	20
K-15-F_1	seg	seg	seg	—	—	*	*	3	1.5	15
K-16-F_1	seg	seg	seg	seg	—	seg	seg	6	3	25
K-17-F_1	seg	seg	—	seg	—	*	*	3	1.5	15
K-18-F_1	seg	seg	—	—	—	*	*	2	1	10
K-19-F_1	seg	seg	seg	seg	—	seg	—	5	2.5	21
K-20-F_1	seg	—	—	—	—	*	*	1	0.5	05
K-21-F_1	seg	seg	seg	seg	—	*	—	4	2	18
K-22-F_1	seg	seg	—	seg	—	*	seg	4	2	18
K-23-F_1	seg	seg	seg	seg	seg	*	*	5	2.5	25
K-24-F_1	seg	seg	seg	seg	—	—	seg	5	2.5	21
K-25-F_1	seg	seg	seg	seg	—	*	*	4	2	20
K-28-F_1	seg	—	seg	seg	seg	seg	*	5	2.5	23
K-29-F_1	seg	seg	—	—	—	*	*	2	1	10
K-30-F_1	seg	seg	—	—	—	*	*	2	1	10
K-31-F_1	seg	seg	seg	seg	seg	seg	seg	7	3.5	29
K-32-F_1	seg	seg	seg	seg	—	*	*	4	2	20
K-33-F_1	seg	—	seg	seg	seg	seg	—	5	2.5	21
Percent loci heterozygous per female averaged over all lines:										18

* = not scored.
† *Est-A* has three alleles; all other loci have two alleles.
seg = segregating locus; both homozygotes and heterozygotes are found in the F_1.
— = all F_1 are homozygous and genetically identical at this locus.

fourth day after eclosion, plateaus around 50 eggs per female per day and then slowly declines. This steep rise after day four followed by a slow decline suggested an equation of the form:

$$y = a(t\text{-}4)^{.5} - b(t\text{-}4)^2$$

where y = the number of eggs laid by a female per day, t = the time in days since eclosion, and a and b are constants. The $a(t\text{-}4)^{.5}$ term will dominate the early part of the experiment and the $b(t\text{-}4)^2$ term would account for the decline that occurs later as t gets large. A least-squares regression was done on the E-1

TABLE 2

Capacity for parthenogenesis in the virgin F_1 female lines established from wild-caught females

Line	No. of ♀♀ in line		Days* of egg laying	Estimated no. of eggs laid	No. of viable Im progeny	No. of Im progeny capable of parth. reproduction	Estimated "viable" rate	Estimated "true" rate
	Start	End						
K-1-F_1	17	15	37	6,887	0	0	0	0
K-2-F_1	30	24	43	13,984	1	1	0.715×10^{-4}	0.715×10^{-4}
K-4-F_1	30	27	37	12,267	0	0	0	0
K-5-F_1	30	29	35	11,791	1	0	0.848×10^{-4}	
K-6-F_1	38	34	35	14,389	0	0	0	0
K-7-F_1	36	32	35	13,590	0	0	0	0
K-8-F_1	35	29	35	12,790	0	0	0	0
K-9-F_1	30	30	35	11,991	0	0	0	0
K-10-F_1	38	30	35	13,590	0	0	0	0
K-11-F_1	30	26	40	13,306	0	0	0	0
K-12-F_1	17	12	37	6,241	0	0	0	0
K-13-F_1	33	23	37	12,052	0	0	0	0
K-14-F_1	19	16	37	7,532	0	0	0	0
K-15-F_1	30	24	43	13,984	0	0	0	0
K-16-F_1	35	32	37	14,419	1	0	0.693×10^{-4}	
K-17-F_1	30	26	43	14,502	0	0	0	0
K-18-F_1	28	27	38	12,252	0	0	0	0
K-19-F_1	35	34	37	14,849	0	0	0	0
K-20-F_1	28	26	38	12,030	0	0	0	0
K-21-F_1	31	24	35	10,992	0	0	0	0
K-22-F_1	30	28	35	11,591	0	0	0	0
K-23-F_1	31	30	43	15,797	3	2	1.899×10^{-4}	1.266×10^{-4}
K-24-F_1	31	26	37	12,267	0	0	0	0
K-25-F_1	30	25	45	14,987	5	1	3.336×10^{-4}	0.667×10^{-4}
K-26-F_1	31	25	40	13,306	0	0	0	0
K-28-F_1	33	31	39	14,735	1	1	0.678×10^{-4}	0.678×10^{-4}
K-29-F_1	15	8	37	4,950	0	0	0	0
K-30-F_1	27	23	38	11,138	0	0	0	0
K-31-F_1	30	24	37	11,621	3	1	2.581×10^{-4}	0.860×10^{-4}
K-32-F_1	31	27	43	15,020	0	0	0	0
K-33-F_1	35	25	35	11,991	0	0	0	0
Sum over all lines:				380,840	15	6	3.939×10^{-5}	1.575×10^{-5}

* The number of days is measured from the time of eclosion.

and E-2 data using this model, and the resulting curves are also plotted in Figure 1. As can be seen, both the E-1 ($a = 12.15$, $b = .023$) and E-2 ($a = 12.82$, $b = .026$) curves are very similar and give a reasonable description of the data. Since the E-1 design reduces the error variance more than the E-2 design, we will use the E-1 curve as a reasonable summary description of the egg-laying history of an individual K_x-0-Bi virgin female and thus hopefully of the original K-F_1 virgin females.

The F_1 females were kept in vials containing 15–38 females; so perhaps there were crowding effects on egg-laying ability. The similarity of the E-1 and E-2

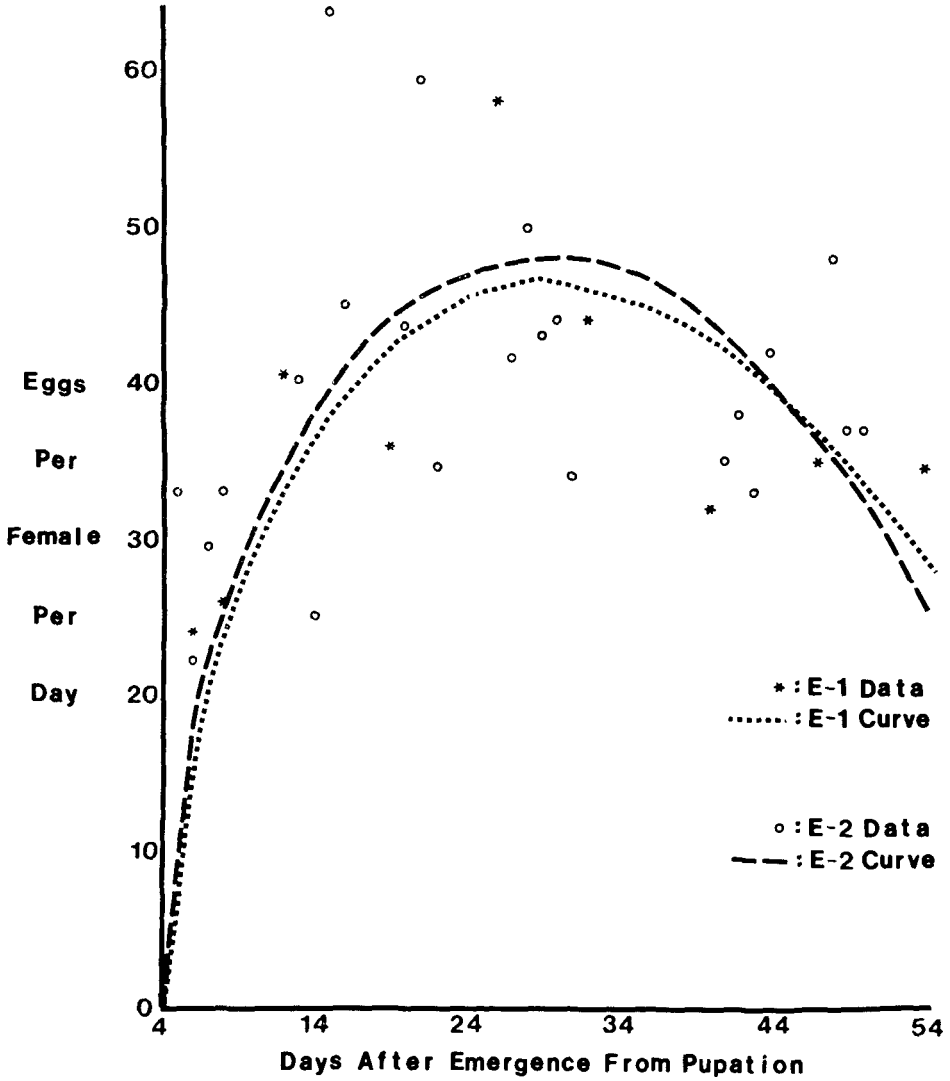

FIGURE 1.—The number of eggs laid daily by K_x-0-Bi virgin females as measured with two different experimental designs (1-E and E-2). The E-1 and E-2 curves were estimated from the data using least squares, as discussed in the text.

curves indicates there is no crowding effect whatsoever with 3 females/vial, but does not eliminate the possibility of such effects in vials with greater densities. Insight into this problem is provided by the day 5 data in which 3 females in the E-2 vial laid an average of 33 eggs/day/female while 20 female in the E-1 vial laid an average of 10.85 eggs/day/female. These values are significantly different (prob. $< .01$ under the hypothesis of homogeneity) and indicate that crowding may diminish the female egg-laying activity to 1/3 the isolated capacity.

Consequently, the estimated number of eggs produced by N K_x-F_1 females during a period of T days after eclosion would be

$$N \cdot C \cdot \int_0^T [12.15(t\text{-}4)^{1/2} - .023(t\text{-}4)^2]dt = N[8.10(T\text{-}4)^{1.5} - .088(T\text{-}4)^3]/3 \quad (1)$$

where C = the crowding correction factor, which is 1/3. However, since some mortality is usually experienced during the time period T, we will substitute the average number of flies/vial = $N_a = (N_{\text{initial}} + N_{\text{final}})/2$ for N in equation (1). This average value is substituted because no detailed information on the time of mortality was gathered. However, it was noticed that most flies did die near the end of T, so that this procedure would tend to underestimate the number of eggs laid. These estimates are given in Table 2 and must obviously be regarded only as ballpark estimates. To test the validity of this estimation procedure, ten lines of 30 virgin females drawn from five of the K-x-Bi stocks were established during August, 1975. These ten lines were kept for 28 days after eclosion under conditions identical to those described above for the F_1 lines. However, all eggs laid were actually counted in this experiment. By direct count, an average of 8,361 eggs per line were laid during these 28 days. Equation (1) was also used to estimate the average number of eggs laid per line as 6,776. Hence, equation (1) does yield estimates that are of the right order of magnitude but that do tend to underestimate, as we previously claimed.

From these egg number estimates, the "viable" parthenogenetic rate (the number of viable Im progeny divided by the number of eggs, where "viable" means the fly survived to adulthood) and the "true" parthenogenetic rate (the number of viable Im progeny themselves capable of parthenogenetic reproduction within four weeks after eclosion divided by the number of eggs) may be estimated, if only crudely. These rates are also given in Table 2 for each K-x-F_1 line and for all lines pooled. As can be seen from this table, the viable rate ranges from 0 to 3.3×10^{-4} with a mean of 3.9×10^{-5}. Similarly, the true rate varies from 0 to 1.3×10^{-4} with a mean of 1.6×10^{-5}. Since the number of eggs laid per line was on the order of 10^3 to 10^4, we would expect many vials to show no Im progeny by chance alone even if all lines had the same inherent parthenogenetic rates somewhere near the overall means. To see if there are significant inter-line differences in parthenogenetic rates, the following tests were performed. First, assume each line has its own rate, λ_i. Let x_i = the number of viable Im adults produced in line i (or the number of "truly" parthenogenetic adults) and n_i = the number of eggs laid by line i. We assume x_i is distributed as a Poisson with mean $n_i\lambda_i$. The log likelihood for the entire data set of all 31 K-x-F_1 lines is thus:

$$\log(L_1) = l_1 = -\sum_{i=1}^{31} n_i\lambda_i + \sum_{i=1}^{31} x_i \ln(n_i\lambda_i) - \sum_{i=1}^{31} \ln(x_i!) \quad (2)$$

The maximum likelihood estimates of the λ_i are x_i/n_i for every i. Let l_0 be equation (2) under the hypothesis $\lambda_i = \lambda$ for all i. The maximum likelihood

estimate of λ is $\Sigma x_i/\Sigma n_i$. When evaluated at the maximum likelihood estimates. the quantity

$$2(l_1-l_0) = 2[\Sigma x_i \ln(x_i/n_i) - \Sigma x_i \ln(\Sigma x_i/\Sigma n_i)]$$

converges in law to chi square with 30 degrees of freedom (KENDALL and STUART 1973). Note that since this is a ratio of likelihoods, the resulting test statistic depends on $\hat{\lambda}_i/\hat{\lambda}$ and thus does *not* depend on the particular value of the crowding correction factor we used or on any other systematic bias in estimating the n_i. When this test was applied to the viable rates, the resulting chi-square was 47.03, which was significant at the .05 level. Hence, there were significant inter-line differences in the viable parthenogenetic rate. A glance at Table 2 reveals three lines had particularly high viable rates (K-23-F_1, K-25-F_1, and K-31-F_1). Deleting any one of these three lines from the test procedure yields a non-significant chi square (without K-23-F_1, chi square = 41.93; without K-25-F_1, 32.96; without K-31-F_1, 40.36—all have 29 degrees of freedom and the .05 significance region is above 42.56). Grouping these three high lines together yields a non-significant chi square of homogeneity (chi square = 0.62, d.f. = 2) and the remaining 28 lines also appear very homogeneous (chi square 14.58, d.f. = 27). These results imply that K-23-F_1, K-25-F_1 and K-31-F_1 are "hot" lines with a homogeneous viable rate around 10^{-4}, while the remaining 28 lines have a homogeneous rate of 10^{-5}.

When this homogeneity test is applied to the true rates, it yields a value of 21.95 with 30 degrees of freedom, which is not significant at the .05 level. Thus, with respect to the true parthenogenetic rates, all of the females caught in the Kamuela population produced daughters that seem to be homogeneous in their parthenogenetic capacities, with a true rate of around 10^{-5}.

Isozyme work was done on some of the parthenogenetic lines as well, and is summarized in Table 3. Not all Im lines were surveyed because some died out and consisted of only one or a few individuals. Note that in all lines scored, all individuals are homozygous at all loci that were polymorphic in the natural population. Furthermore, a contrast of this table with Table 1 allows one to identify which loci were segregating in the bisexual ancestors of the Im lines and which are thus potentially heterozygous in the Im line. Pooling all Im lines, a total of 18 loci exist at which heterozygosity in the Im line is potentially possible. None of these loci is heterozygous. Previous work with *Drosophila mercatorum* (CARSON, WEI and NIEDERKORN 1969; CARSON 1973) has demonstrated that all previously isolated Im strains reproduce predominately through gamete or pronuclear duplication. Under this mechanism meiosis proceeds normally and produces a haploid pronucleus. This haploid nucleus then divides and the cleavage nuclei fuse to restore the diploid condition. This results in homozygosity for *all* loci. In addition to pronuclear duplication, diploidy may be restored *via* central or terminal fusion (CARSON 1973; TEMPLETON and ROTHMAN 1973). These fusion mechanisms have the potential for maintaining heterozygosity and, in particular, central fusion is more efficient in maintaining heterozygosity than sexual reproduction (CARSON 1967b). The fact that parthenogenetic flies are

TABLE 3

Allelic state of some of the K_x-0-Im stocks at those loci that were polymorphic in the Kamuela population

Stock	K_5-0-Im	K_{23}-0-Im	K_{25}-0-Im	K_{28}-0-Im
Parthenogenetic generation at time of survey	1st	3rd & after	2nd	2nd & after
Locus:				
Est-A	S†	S†	S†	S†
Est-B	M†	M†	M†	M
Xdh	S†	F†	S†	F†
Acph	*	S†	*	S†
Adh	S†	F†	F	F†
G-6-Pd	F†	*	F	F†
6-Pgd	*	*	*	*

† Known to be segregating in the K-X-F1 ancestors of the Im stock.
S = slow allele
M = medium allele
F = fast allele
* = not scored

homozygous for loci known to be segregating in their bisexual ancestors strongly implies that pronuclear duplication had occurred before the time of the isozyme survey. At the latest, the surveys were carried out on third-generation Im adults (Table 3), so pronuclear duplication must have occurred either during the initial bisexual-unisexual transition (e.g., as it most likely did in K_5-O-Im) or within the first two parthenogenetic generations. However, if the first Im generation had been produced by fusion and not duplication, we would expect meiotic segregation at the heterozygous loci such that even after duplication occurred in the line we should find flies homozygous for different alleles within the same Im strain (TEMPLETON and ROTHMAN 1973). However, all Im flies within a strain were genetically identical, implying that duplication most likely occurred during the initial bisexual-unisexual transition. These conclusions have been further strengthened for K_{28}-0-Im since subsequent isozyme studies on 17 loci have revealed total homozygosity and complete genetic identity between all individuals drawn from this line.

Of the parthenogenetic lines that were established, the one most easily maintained was K_{28}-0-Im, which was founded from a single Im female who in turn produced 16 second-generation Im offspring upon laying eggs for 20 days after eclosion. Because this line had so successfully made the bisexual-parthenogenetic transition, additional studies were done with it. The egg-laying profile of this strain was measured as described in the MATERIALS AND METHODS sections, and the results are given graphically in Figure 2. These experiments were done with the fourth Im generation, but since the evidence indicates these stocks reproduce by pronuclear duplication, these flies should be genetically identical to the original K_{28}-0-Im female. As can readily be seen by contrasting Figures 1 and 2, the Im females begin laying eggs two days sooner than their bisexual ancestors and

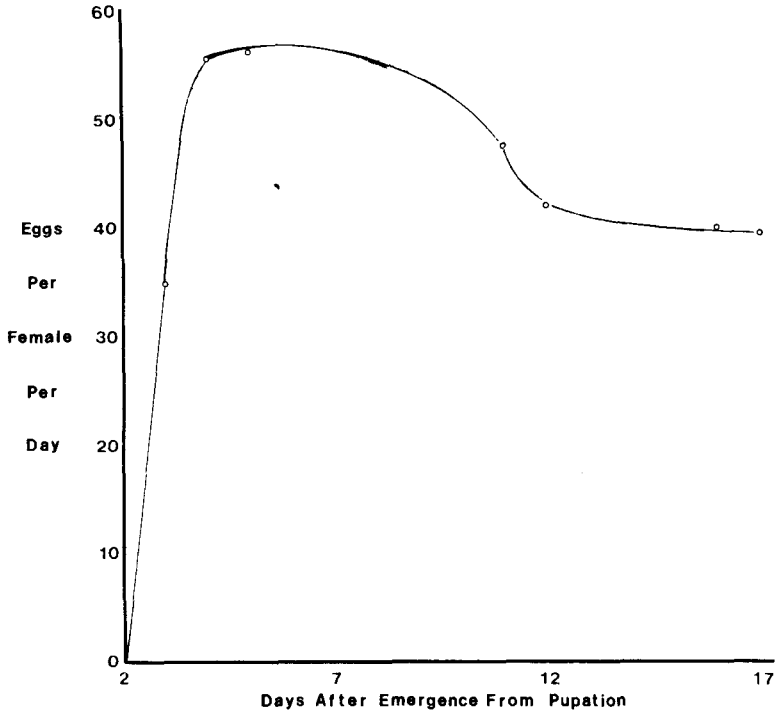

FIGURE 2.—The number of eggs laid daily by K_{28}-0-Im virgin females. Open circles indicate observed points, and a line is drawn to connect these empirical points.

reach their peak almost immediately, unlike the bisexual females, which peak around 25–30 days after eclosion, and then fall off in egg production to a level of about 40 eggs/female/day. Furthermore, the Im peak is higher than the bisexual peak. Consequently, it seems that part of the selection for parthenogenesis may be selection of flies with large egg-laying capacities.

However, this is not the whole story. A total of 2850 eggs were counted directly in the K_{28}-0-Im egg-laying experiment, and these eggs gave rise to a total of 29 pupa cases and 27 viable adults, each capable of reproducing parthenogenetically. This means the "true" parthogenetic rate if 0.94%. Thus, the true rate has increased from 10^{-5} in the bisexual ancestral populations to 10^{-2}. This represents an increase of three orders of magnitude, and given that pronuclear duplication is the mode of reproduction, all of this increase must have occurred during the initial bisexual-unisexual transition.

The K_{28}-0-Im females differ from their bisexual ancestors in many other respects. Firstly, they are morphologically distinct in that they tend to be larger and much darker in color. The differences are such that the K_{28}-0-Im females are easily distinguished from K_x-0-Bi females under a low-power binocular scope. The K_{28}-0-Im females are also behaviorally *extremely* distinct from the K_x-0-Bi with respect to sexual responses to males, flight initiation behavior, geo- and photo-tactic responses, etc. Some of the details and evolutionary implications of

these behavioral modifications are the subject of another paper (CARSON, TERAMOTO and TEMPLETON 1977).

DISCUSSION

The work presented here shows that natural populations of *Drosophila mercatorum* do have a capacity for parthenogenetic reproduction. This capacity is low —around 10^{-5}—but this is sufficiently high to allow parthenogenetic strains to be established in a single generation from a relatively small sample of wild-caught females. Thus, parthenogenesis must be viewed as a true evolutionary potential in this species. This raises the question of why *D. mercatorum* has this potential in the first place and why it does not seem to utilize it in nature. In the introduction, we stated that the genus Drosophila in general seems to be "pre-adapted" meiotically to parthenogenetic reproduction. However, producing parthenogenetic "zygotes" is only one aspect of making the bisexual-unisexual transition. Once such "zygotes" are formed, they must first prove to be viable and then prove capable of parthenogenetic reproduction themselves. It will now be argued that a major limiting factor governing the bisexual-unisexual transition in *D. mercatorum* occurs *after* parthenogenetic zygotes are formed; that is, a selective bottleneck is operating upon the first parthenogenetic generation.

As mentioned in the RESULTS section, *D. mercatorum* reproduces parthenogenetically primarily by a mechanism called pronuclear duplication that results in total homozygosity in a single generation. Effectively, the bisexual-unisexual transition is a change from a diploid genetic environment with the potential for heterozygosity at a large portion of the loci to a pseudohaploid genetic environment with no heterozygosity possible at any locus. It is reasonable to assume such a drastic change in genetic environments would also be accompanied by very intense selection. Firstly, a large number of experiments with many species of Drosophila (KOSUDA 1972 and references therein) have shown intense selective forces accompanying inbreeding. Yet even the most severe inbreeding represents a less drastic and more gradual change in genetic environment than pronuclear duplication. Secondly, a number of experiments have shown that a great many "recessive lethals" exist in Drosophila populations (see LEWONTIN 1974 for a review), and all of these would have to be eliminated during the first parthenogenetic generation with pronuclear duplication or very shortly thereafter, with fusion followed by duplication. Finally, previous experiments with parthenogenetic strains of *D. mercatorum* (TEMPLETON, SING and BROKAW 1976; SING and TEMPLETON 1975) have indicated there is very intense selection favoring genomes coadapted at a multi-locus and even multi-chromosomal level to the genetic environment of total homozygosity. Thus, during the initial bisexual-unisexual transition selection should both eliminate recessive lethals (or lethal complexes) and favor those genomes best coadapted to the genetic environment of total homozygosity. It is felt that only rarely will a genome be completely devoid of recessive lethals and simultaneously be coadapted to this new genetic environment. Consequently, one of the major factors limiting the evolution of parthenogenetic reproduction is a selective bottleneck.

The importance of this selective bottleneck is shown by two observations which at first seem contradictory to this hypothesis. First, one might speculate that the Kamuela population of *D. mercatorum* has the capacity of parthenogenetic reproduction because it is characterized by a great deal of homozygosity which preadapts it to the genetic consequences of parthenogenesis. However, the isozyme survey reveals the Kamuela population is characterized by a large proportion of polymorphic loci and high levels of individual heterozygosity. Consequently, there is no evidence for "preadaption" to total homozygosity in the natural population.

The second apparent anomaly concerns the apparent ease with which parthenogenetic strains were isolated in this present work as opposed to previous work. In previous studies, most isofemale lines revealed no capacity for parthenogenesis at all, and no parthenogenetic lines were ever established without bisexual-unisexual bridge cycles. However, here 23% of the isofemale lines reproduced parthenogenetically and 16% could establish parthenogenetic strains without any bridging. Furthermore, the greatest previous increase in parthenogenetic rate was a 60-fold increase achieved only after artificial selection on bisexual-unisexual bridges followed by selection on parthenogenetic lines. However, the K_{28}-0-Im stock experienced a 1000-fold increase (as measured *vs.* the general Kamuela rate) or a 100-fold increase (as measured *vs.* the K-28-F_1 rate) in a single generation and with no bridge cycles. The major difference between this study and the previous ones is that our bisexual ancestors were drawn from a natural population and had high levels of heterozygosity. The previous studies had used laboratory stocks which had experienced some degree of inbreeding. Once again, we could speculate that inbred strains should have the greatest chance of making the bisexual-unisexual transition because inbreeding "preadapts" the flies toward total homozygosity. Yet just the opposite is true; the outcrossed, heterozygous flies have the greatest chance of producing truly parthenogenetic offspring. Consequently, we must conclude that inbreeding does not sufficiently mimic total homozygosity to produce such a predadaptation.

However, these results do make sense when we regard a selective bottleneck as a major factor in the evolution of parthenogenesis. A female from an inbred line will produce during meiosis only a narrow spectrum of genome types so that when these are duplicated there is very little genetic variability among the offspring upon which selection can operate. Selection is only effective in these cases when coupled with bridging since the bridge technique is one way of increasing the spectrum of genotypes. On the other hand, a highly heterozygous female will produce a broad spectrum of genomes and thus the chance of her producing one of the rare genotypes that can survive the genetic consequences of parthenogenesis is greatly enhanced. Looked at in this fashion, we must change our emphasis from "preadaptation" to genetic variability since genetic variability is a limiting factor on the effectiveness of selection.

In this regard, it is interesting to re-examine the data given in Table 1. The 29 K-x-F_1 populations surveyed can now be divided into two groups—one group

consisting of 23 lines that produced no parthenogenetic progeny and a second group of 6 lines that did produce parthenogenetic progeny. The average level of heterozygosity per female over all lines was .18. For the 23 lines that produced no parthenogenetic progeny it was .16 (range: .05–.25) and for the 6 lines producing parthenogenetic progeny it was .25 (range: .20–.29). There is no significant difference between .16 and .25 with this small a sample, and the question remains as to how representative the 12 loci surveyed are of the general background. However, there is certainly no indication in these data that those flies most homozygous are more likely to produce parthenogenetic offspring; if anything, just the opposite seems to be true.

The observed parthenogenetic rate of 10^{-5} in the Kamuela population should reflect both the probability of a post-meiotic event occurring to restore diploidy in the eggs of the bisexual females as well as the chances of the resulting "zygote" having a genotype capable of developing and reproducing under the genetic conditions imposed by parthenogenesis. Once the initial selective bottleneck associated with the zygote's genotype is successfully passed, one would predict the parthenogenetic rate should increase dramatically since all subsequent parthenogenetic offspring are genetically identical to their parent except for mutations. This could explain the thousandfold increase seen in the K_{28}-0-Im stock. The fact that the parthenogenetic rate of K_{28}-0-Im after this selective bottleneck is passed is only 10^{-2} could be due to the elimination of deleterious mutants that arise each generation (but to explain the observed parthenogenetic rate solely by mutation implies a lethal mutation rate of .99/genome/generation—a rate that seems much too high [SLATKO and HIRAIZUMI 1973; WALLACE 1970; CROW and TEMIN 1964]) and to the failure of some unfertilized eggs to produce diploid nuclei even in a genetically homogeneous strain. Perhaps subsequent evolution in such parthenogenetic strains would operate on the meiotic and post-meiotic factors limiting the initiation of parthenogenetic development. This has apparently occurred in *Drosophila mangabeirai*, which has a parthenogenetic rate of about 6×10^{-1} and has a post-meiotic configuration of pronuclei unlike other Drosophila but that increases the chances for the restoration of diploidy (MURDY and CARSON 1959).

This intense selection for a genome capable of surviving and reproducing under total homozygosity would also explain the large morphological and behavioral modifications that occur in the bisexual-unisexual transition. It is a well established fact in plant and animal breeding that intense selection over a short period of time on one trait causes changes in many other traits due to pleiotrophic effects and to multi-locus disequilibria that form during the selective bottleneck and that are not given sufficient time to break up (e.g., MATHER and HARRISON 1949). In our case, we have all the selection operating over a single generation, and after it occurs the resulting genotypes are fixed except for mutation. Such conditions make modification of other phenotypic traits very likely.

It is reasonable to suppose that this selective bottleneck is even more difficult to pass in nature. Firstly, the number of potential parthenogenetic zygotes is

reduced because most eggs laid in nature are probably fertilized and the maximum egg-producing capacity of females is seldom expressed due to limited egg-laying sites. Secondly, once a parthenogenetic zygote is formed, it undoubtedly has a smaller chance of survival to adulthood in nature than the laboratory-reared zygotes, which are free from predation, environmental fluctuations and competition for food and space. Thirdly, once a parthenogenetic adult capable of parthenogenesis arises, it must not mate with males from the bisexual population in order to realize this capacity. Furthermore, such crosses to a bisexual stock are known to greatly reduce the parthenogenetic capacity of the female offspring (CARSON 1967a). With respect to this limiting factor, it is interesting to note that K_{28}-0-Im displays a very high degree of sexual isolation (CARSON, TERAMOTO and TEMPLETON 1977). Hence, this third limiting factor may sometimes be passed due to the pleiotrophic side-effects of the initial selective bottleneck operating upon parthenogenetic zygotes. Finally, once a sexually isolated parthenogenetic race is established, its continued existence depends upon its ability to compete with its bisexual progenitor. Since one of the prime determinants of an organism's niche is its behavior, the gross behavioral changes that accompany the bisexual-unisexual transition could result not only in sexual isolation, but also in a reduction of this "intraspecific" competition.

In conclusion, it seems probable that one of the major factors limiting the evolution of parthenogenesis in natural populations of *D. mercatorum* is selection for a rare genotype that can survive and reproduce under the genetic conditions imposed by parthenogenetic reproduction. However, this intense selective bottleneck can be passed in a single generation if enough unfertilized eggs are laid, and once passed is accompanied by a large (perhaps a 1000-fold) increase in the rate of parthenogenesis and by modifications in many phenotypic traits such as morphology and behavior.

The authors wish to express their gratitude to JOSEPH L. ANNEST and the two reviewers for critically reading an earlier draft of this paper.

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Corresponding editor: R. W. ALLARD