GENETICS OF PARTHENOGENESIS IN *DROSOPHILA MELANOGASTER*. II. CHARACTERIZATION OF A GYNOGENETICALLY REPRODUCING STRAIN

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ABSTRACT

A strain of *Drosophila melanogaster*, named gyn-F9, can reproduce by gynogenesis. On mating with a male sterile mutant, *ms(3)K81*, gyn-F9 females produced impaternate progeny at a rate of about 15 flies per female, which was almost 2000 times as frequent as that of the control. When the females were mated with normally fertile males, the number of offspring varied extremely from parent to parent, with average fertility being much lower than that of normal females. Nearly one-third of these bisexual progeny were either triploid females or intersexes. Among the rest of the progeny, some were diploid impaternates having developed without syngamy. The gynogenetic property of gyn-F9 is primarily governed by a few genes, most likely two recessive genes, one each located on the second and third chromosomes. The impaternates were found to restore their diploidy by the fusion of two nonsister nuclei out of the four egg pronuclei which result from the second meiotic division (central fusion). Although nondisjunction occurs frequently in the meiosis of gyn-F9, this is unlikely to bring about an appreciable number of diploid gametes developing into impaternates. Possible mechanisms of the evolutionary origin of parthenogenesis are discussed in relation to these findings.

THE existence of parthenogenetic organisms has been challenging our concept of the adaptive significance of sexual reproduction. Many hypotheses proposed to explain the selective advantages of sexual over asexual reproduction are still in debate (for reviews, see WILLIAMS 1975; SUOMALINEN, SAURA and LOKKI 1976; CUELLAR 1977; MAYNARD SMITH 1978; BELL 1982; TEMPLETON 1983; BREMERMANN 1985). A flaw common to these arguments seems to be derived from our ignorance of genetic mechanisms governing parthenogenesis. An anticipation by WHITE (1978) remains unrealized, "At the present stage, it would seem more profitable to examine the genetic architecture of thelytokous species and the circumstances under which they have arisen than to engage in further model-building on the adaptive advantages or disadvantages of sexual reproduction."

An opportunity to elucidate the genetic mechanisms of parthenogenesis has arisen from the recent discovery of parthenogenesis in *Drosophila melanogaster* (FUYAMA 1984). The eggs that are inseminated by the sperm of a male sterile

mutant, *ms(3)K81,* initiate gynogenetic development, and a small fraction of them, by restoring diploidy, give rise to diploid impaternate adults. The mechanisms by which the impaternate zygotes restore diploidy are found to be indistinguishable from those prevailing among automictic thelytokous parthenogens, indicating that *D. melanogaster* is preadapted to parthenogenesis (FUYAMA 1986). In due course, a strain capable of reproducing by gynogenesis has been successfully established. This strain, named gyn-F9, was derived from the impaternate progeny which were produced by females from a meiotic mutant stock, *y mei-9^a/FM7c.* Each female of gyn-F9, on mating with *ms(3)K81* males, produces more than a dozen impaternate progeny—one or two thousand times as many as produced by ordinary strains (FUYAMA 1984, 1986). This strongly indicates that major genic mutations are involved in attaining the gynogenetic ability by gyn-F9. Characterization of this strain should provide an insight into possible genetic mechanisms underlying parthenogenesis and its evolutionary origin.

MATERIALS AND METHODS

Strains: *ms(3)K81* has been described (FUYAMA 1984). Briefly, the sperm produced by homozygous *ms(3)K81* males are defective in syngamy but are capable of activating eggs to develop parthenogenetically. The gynogenetic strain, gyn-F9, originated from a meiotic mutant stock, *y mei-9^a/FM7c* (BAKER and CARPENTER 1972), that was obtained from YUTAKA INOUE at the National Institute of Genetics, Misima (FUYAMA 1986). After its establishment, gyn-F9 has been maintained gynogenetically by mating with *ms(3)K81* males. In this stock, females homozygous for either *y mei-9* or *FM7c* are virtually absent. gyn-2-3-Bi is a bisexual strain, with second and third chromosomes that are derived from gyn-F9 and with sex chromosomes that are derived from a balancer strain, *SM1/Pm; TM3/Pr.* A wild-type strain, m361, was derived from a single gravid female collected at Oiso, Japan, in 1979. This strain was used as a standard wild type because of its excellent viability and fertility.

For all other markers and balancers, see LINDSLEY and GRELL (1968). Abbreviations for balancers: *FM7c = In(1)FM7, y^{31d} sc^8 w^a sn^{X2} v^{Of} B; SM1 = In(2LR)SM1, a1^2 Cy cn^2 sp^2; Pm = In(2LR)bw^{V1}; TM3 = In(3LR)TM3, y$^+$ ri p^p sep Sb bx^{34e} e^s Ser.*

Flies were reared on a cornmeal-yeast-glucose medium at 25°.

Procedures: The number of adult impaternates produced per female parent was used to evaluate the capacity of gynogenesis. Virgin females to be tested were mated with homozygous *ms(3)K81* males and were allowed to oviposit for, as a rule, 16 days, with three to four transfers to new culture vials. Further details of experimental procedures are given below.

RESULTS

Productivity of gynogenetic and bisexual progeny by gyn-F9 females

Impaternate productivity: In an experiment testing the gynogenetic ability of meiotic mutants, a culture of *y mei-9^a/FM7c* produced an exceptionally large number of impaternate progeny (FUYAMA 1986). These impaternates were the progenitors of the gynogenetic strain named gyn-F9. The impaternate productivity of gyn-F9 females was first tested two generations after establishment; 75 female parents produced a total of 596 impaternates (7.95 per mother). After several generations of gynogenetic reproduction, the productivity seemed to have increased somewhat, probably due to unintended selection. At the

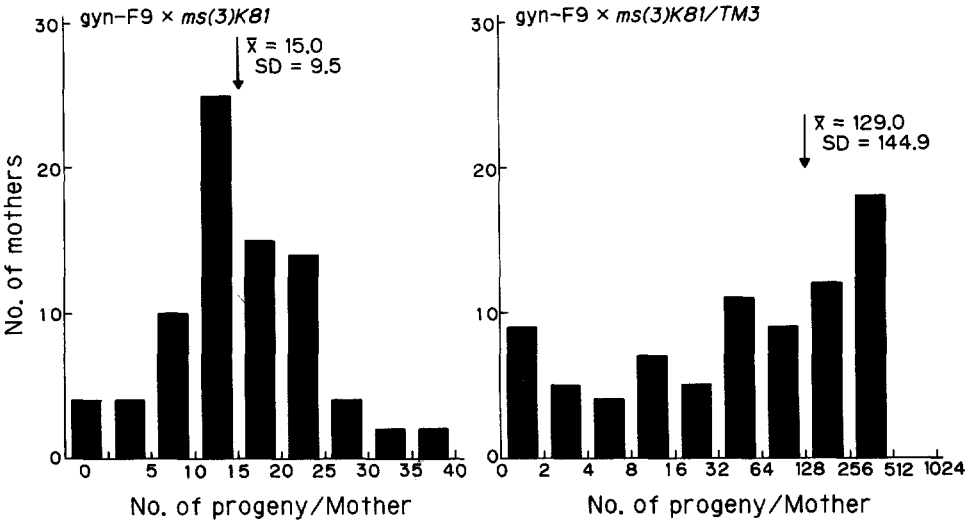

FIGURE 1.—Gynogenetic (*left*) and bisexual (*right*) productivities of progeny by gyn-F9 females. Virgin females were individually mated with either homozygous *ms(3)K81* males (gynogenetic) or *ms(3)K81/TM3* males (bisexual), and they were transferred to a new vial every 5 days, allowing them to oviposit for 16 days. Arrow indicates the average number of progeny per mother ($\bar{x}$) and the standard deviation (SD).

15th and 19th generations, 40 females from each were individually tested for their gynogenetic ability. The results, which were not significantly different between the two experiments, were pooled and are shown in Figure 1. Almost all the females tested (76 of 80) produced impaternate progeny, with an average of 15 offspring per mother. Of 1196 offspring produced in these experiments, 23 (1.9%) were *X0* males and five (0.4%) were gynandromorphs; the remainder were *y mei-9/FM7c* females.

Bisexual progeny: The productivity of bisexual progeny of gyn-F9 females that were inseminated by fertile *ms(3)K81/TM3* males were tested at the 17th and the 21st generations. The two tests gave similar results, and the pooled data are presented in Figure 1. In contrast to the impaternate productivity, the number of progeny varied extremely from parent to parent (note that the horizontal scale of the graph is logarithmic). Nearly 10% (seven of 80) of the tested females did not produce progeny, and 60% (48 of 80) produced less than 100 offspring, while some (four of 80) produced more than 400. The average of 129 offspring per female parent is very low compared with normal fertility of this species, which is likely to exceed several hundred under similar conditions (*e.g.*, ASHBURNER and THOMPSON 1978).

An interesting, but not unexpected, property of gyn-F9 is that the females inseminated by normally fertile males regularly produce triploid females and intersexes. By virtue of the visible markers—especially *Bar*, which is carried by the *X* chromosomes of gyn-F9—triploids could reliably be distinguished from diploids. Table 1 lists examples of the census of the offspring obtained by crossing gyn-F9 females with wild-type males. Triploids constituted approx-

TABLE 1

Composition of the bisexual progeny produced by the gyn-F9 females that were mated with wild-type males

Ploidy and sex	Mass mating		Pair mating	
	No.	%	No.	%
2n female	1165	41.5	1546	39.3
2n male	964	34.3	1158	29.5
3n female	436	15.5	714	18.2
3n intersex	243	8.7	512	13.0

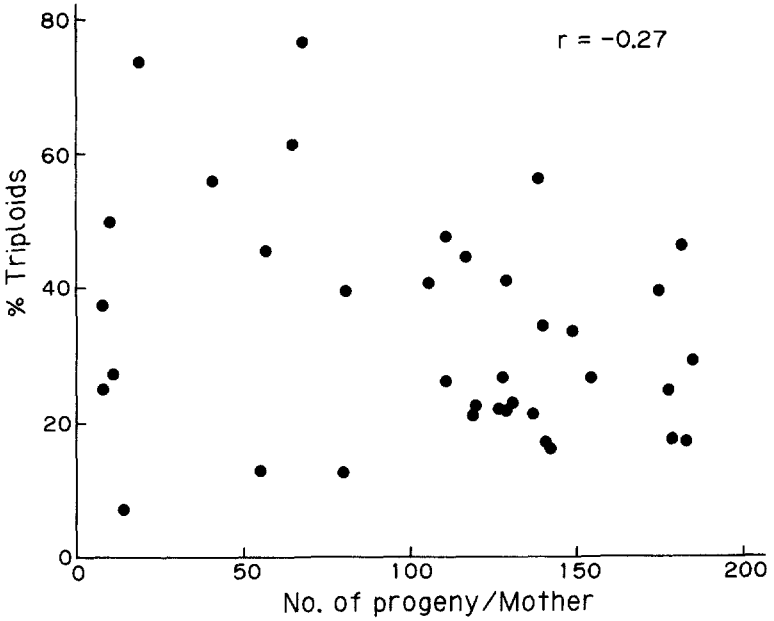

FIGURE 2.—The relationship between the progeny productivity and the proportion of triploids among the bisexual progeny of gyn-F9 females. Virgin females were individually mated with the males of a wild-type strain, m361, and were allowed to oviposit for a week. Triploid females, intersexes and meta-males were classified as triploids.

imately one-third of the bisexual progeny of gyn-F9. It is quite possible that the cytological mechanisms that bring about triploid zygotes are an essential part of gynogenesis, *i.e.*, the restoration of diploidy without syngamy.

A question that arises about the bisexual reproduction of gyn-F9 is whether the intra-ovum event that leads to the development of triploids is related to the unusually large variation of progeny number. To test this theory, the correlation between the number of progeny produced by each female and the proportion of triploids among the progeny was calculated and found to be very low (Figure 2). In fact, the correlation coefficient is not significantly different from zero, suggesting that departures from euploidy may not cause the inviability of the bisexual progeny.

TABLE 2

Impaternate productivity of the females having various chromosome constitutions

Genotypes	No. tested	Impaternates			Impaternates/ mother
		Female	Male	Gyn.[a]	
+/y mei-9; +'/Pm; +'/Pr	20	0	0	0	0.0
+/FM7; +'/Pm; +'/Pr	20	0	0	0	0.0
FM7/y mei-9; +'/Pm; +'/Pr	20	0	0	0	0.0
+/y mei-9; +'/+'; +'/Pr	25	10	0	0	0.40
+/FM7; +'/+'; +'/Pr	20	0	0	0	0.0
+/y mei-9; +'/Pm; +'/+'	20	2	0	0	0.10
+/FM7; +'/Pm; +'/+'	20	0	0	0	0.0
FM7/y mei-9; +'/+'; +'/Pr	25	39	2	0	1.64
FM7/y mei-9; +'/Pm; +'/+'	20	3	0	0	0.15
+/y mei-9; +'/+'; +'/+'	20	183	10	0	9.65
+/FM7; +'/+'; +'/+'	25	109	3	1	4.52
FM7/y mei-9; +'/+'; +'/+'	20	133	7	0	7.00

Chromosomes denoted by +' were derived from gyn-F9, and + chromosomes were from a balancer stock. For balancers and markers, see MATERIALS AND METHODS.

[a] Gynandromorph.

Mapping of the factors responsible for gynogenetic ability of gyn-F9

Chromosomal allocation: In order to determine the contribution of the three major chromosomes to the capacity of gyn-F9 to produce gynogenetic progeny, females having various chromosome constitutions were produced by means of a conventional chromosome substitution technique using a balancer stock, *SM1/Pm; TM3/Pr,* and were tested for their productivity of impaternate progeny (Table 2). The results can be summarized as follows: First, the ability of an egg to develop gynogenetically is determined by maternal genotype. Second, the factors responsible for gynogenesis are recessive. These two conclusions are drawn from the fact that none of the F_1 hybrids between gyn-F9 and the balancer strain produced progeny. Third, the *X* chromosomes are relatively unimportant in determining the property of gyn-F9, because the females with *X* chromosome constitutions that were the same as those of gyn-F9 did not produce progeny if their autosomes were heterozygous. Some epistatic interaction between an *X* chromosome and one of the autosomes may be implicated, however, because those females which had a *y mei-9* chromosome produced an appreciable number of progeny when their second chromosomes were homozygous. Finally, factors located on the second and third chromosomes are of principal importance to the gynogenetic ability, and for full expression of the ability, both autosomes must be homozygous for these factors.

Mapping of the factors: Outcrossing is necessary to map the genes involved in the gynogenesis of gyn-F9 by use of conventional recombination techniques. For this, a bisexual strain, designated gyn-2-3-Bi, having the two major autosomes derived from gyn-F9 and the sex chromosomes from a balancer stock,

TABLE 3

Mapping of the factors responsible for gynogenetic ability on the second chromosome

Phenotype of mother	No. of mothers tested	No. of impaternates produced	Impaternates/ mother
S Sp Tft nw^D Pin^{Yt}	200	0	0.0
S Sp Tft nw^D +	100	0	0.0
S Sp Tft + +	180	7	0.039
S Sp + + +	100	31	0.310
S + + + +	100	46	0.460
+ + + + +	100	88	0.880
+ + + + Pin^{Yt}	100	55	0.550
+ + + nw^D Pin^{Yt}	180	12	0.067
+ + *Tft* nw^D Pin^{Yt}	140	0	0.0
+ *Sp Tft* nw^D Pin^{Yt}	100	1	0.010

was constructed. As expected, gyn-2-3-Bi females, when mated with *ms(3)K81,* produced impaternates at a rate similar to that of gyn-F9.

To map the putative locus on the second chromosome, the females of a multiple dominant marker stock, *S Sp Tft* nw^D Pin^{Yt}*/CyO,* were crossed to gyn-2-3-Bi males, and the resulting F_1 females heterozygous for the markers were backcrossed to gyn-2-3-Bi males. Various recombinant females emerged and were mated with *ms(3)K81* males, and the number of impaternates produced by these females was scored. With this procedure, one-quarter of the recombinant females are expected to have a second and third chromosome, both of which are homozygous for the genes of interest, and thus are expected to be capable of gynogenesis.

Table 3 shows the results. For as yet unknown reasons, the number of impaternate progeny obtained was less than expected. Nevertheless, the results clearly located a factor between the *Tft* locus (*Tuft,* 2-53.2) and *nw* locus (*narrow,* 2-79.3; for map position, see DOANE and CLARK 1984).

The same procedure was used to localize the putative locus on the third chromosome. Two independent experiments each employing different dominant marker stock were carried out, and the results are shown in Table 4. The first experiment, using *Gl Sb H* marker stock, located the locus between *Gl* (*Glued,* 3-41.4) and *Sb* (*Stubble,* 3-58.2). The second experiment, in which *Pr Dr* was used as the marker stock, indicated that the locus locates to the left of *Pr* (*Prickly,* 3-90.0), confirming the above conclusion.

For further pinpointing of these loci, more refined techniques apparently are required. However, the results obtained here, in conjunction with the observation that gyn-F9 produces more than a thousand times as many progeny as do normal flies, strongly indicate that mutations having occurred in these two loci play a crucial role in attaining gynogenetic ability.

The mechanism of diploidization in the gynogenesis of gyn-F9

In the mapping experiments described in the previous section, impaternate progeny having recombinant genotypes were regularly produced by mothers

TABLE 4

Mapping of the factors responsible for gynogenetic ability on the third chromosome

Phenotype of mother	No. of mothers tested	No. of impaternates produced	Impaternates/ mother
Gl Sb H	120	0	0.0
Gl Sb +	60	0	0.0
Gl + +	60	7	0.117
+ + +	60	121	2.017
+ + *H*	70	92	1.314
+ *Sb H*	70	16	0.229
Pr Dr	120	19	0.158
Pr +	60	2	0.033
+ +	60	107	1.783
+ *Dr*	60	48	0.800

that had two or more dominant markers. This suggests that meiotic recombination occurs in the eggs laid by gyn-F9 females. Furthermore, considering that the *X* chromosomes of gyn-F9 are permanently heterozygous for *y mei-9* and *FM7c*, and also that *FM7c* effectively suppresses recombination in the *X* chromosomes, the diploid chromosome number of gyn-F9 flies appears to be restored after the completion of meiosis.

To investigate the mechanisms by which diploidy is restored in the gynogenetic embryos, the genotypes of impaternate progeny produced by the females with *X* chromosomes that were multiply heterozygous for recessive markers were examined. The rationale of this method has been described (CARSON 1973; FUYAMA 1986). Females heterozygous for four recessive *X*-linked markers—*yellow* (*y*), *crossveinless* (*cv*), *vermillion* (*v*), and *forked* (*f*)—in coupling were produced according to the mating scheme shown in Figure 3. On average, one-quarter of these females are expected to be homozygous for both of the gynogenetic genes, thus to be capable of producing impaternate progeny.

Of 300 impaternates subjected to the progeny test, 231 produced a sufficient number of progeny to determine their genotypes. The genotypes thus determined were classified into 18 types and are listed in Table 5. With the exception of 11 individuals (4.8%) belonging to types 8 and 9, these impaternates were found to be heterozygous for one or more marker genes. Barring new mutation or failure of reductional division, such heterozygotes arise exclusively from the fusion of two of four egg pronuclei produced by meiosis II (TEMPLETON 1983). There are two sorts of postmeiotic pronuclear fusion, terminal fusion and central fusion, the genetic consequences of which are quite different from each other. With terminal fusion, a pair of sister-nuclei separated at meiosis II fuse to restore diploidy; therefore, centromeres are always homozygous and distally located loci tend to be heterozygous due to recombination occurring between the centromere and the locus. In contrast, central fusion, in which fusion occurs between nonsister nuclei of the second meiosis, brings

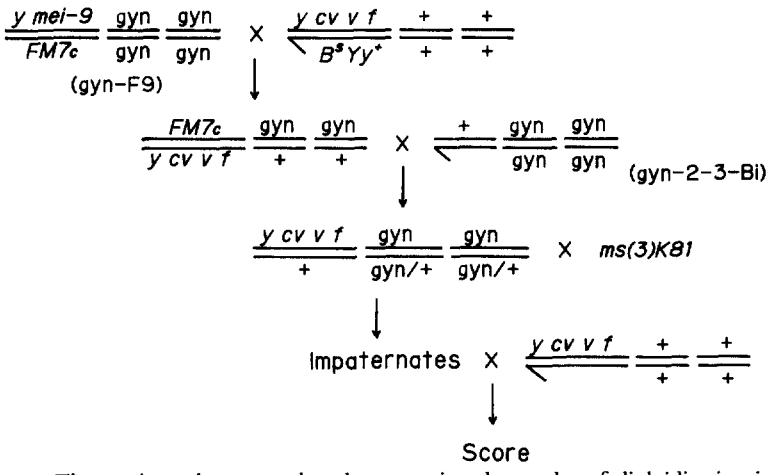

FIGURE 3.—The mating scheme employed to examine the modes of diploidization in the gynogenetic progeny of gyn-F9. The chromosomes designated by "gyn" are those derived from the gyn-F9 strain.

TABLE 5

Genotypes of the impaternates obtained from the females multiply heterozygous for sex-linked recessive markers

Type no.	Genotype of impaternates				No. produced
	y	*cv*	*v*	*f*	
1	+*m*	+*m*	+*m*	+*m*	129
2	++	+*m*	+*m*	+*m*	13
3	*mm*	+*m*	+*m*	+*m*	11
4	++	++	+*m*	+*m*	9
5	*mm*	*mm*	+*m*	+*m*	16
6	++	++	++	+*m*	15
7	*mm*	*mm*	*mm*	+*m*	9
8	++	++	++	++	9
9	*mm*	*mm*	*mm*	*mm*	2
10	+*m*	++	++	+*m*	2
11	+*m*	*mm*	*mm*	+*m*	4
12	+*m*	++	+*m*	+*m*	1
13	+*m*	*mm*	+*m*	+*m*	1
14	+*m*	+*m*	++	+*m*	2
15	+*m*	+*m*	*mm*	+*m*	3
16	+*m*	++	++	++	1
17	+*m*	*mm*	*mm*	*mm*	1
18	+*m*	+*m*	++	++	3
Heterozygotes	147	161	180	215	
Homozygotes	84	70	51	16	
% Heterozygotes	64	70	78	93	

++ and *mm*, respectively, indicate homozygote for wild-type allele and mutant allele; +*m* indicates heterozygote.

about permanent heterozygosity in the centromere, and heterozygosity decreases with increasing distance from the centromere (TEMPLETON 1983).

The results obtained in this experiment suggest that diploidy was restored in most of the impaternates by central fusion, since the vast majority (93%) of them were heterozygous for the *forked* locus (*1*-56.7) which is centromere proximal, whereas the proportion of heterozygotes was lowest (64%) at the most distal locus, *yellow* (*1*-0.0). A small fraction (7%) of impaternates that were homozygous for *forked* could arise from a single recombination between the locus and the centromere. The map distance of the *forked* locus from its centromere seems enough to explain the observed heterozygosity. If we assume the map position of the centromere to be 65.0, and *forked* to be 56.7, on the assumption of central fusion and complete interference, we estimate heterozygosity to be 91.7%. The observed heterozygosity (93.2%) is higher than estimated, so the possibility of terminal fusion may be excluded.

Another possible mechanism of restoration is gamete duplication, where two identical haploid nuclei resulting from the first mitotic division fuse to bring about diploidy. The impaternates restored by this mechanism should be totally homozygous for entire genomes. Therefore, candidates for gamete duplication are limited to 11 impaternates belonging to types 8 and 9. However, the fact that these impaternates were either homozygous for all the wild-type alleles (type 8) or homozygous for all the mutant alleles (type 9) strongly indicates that these individuals were produced by central fusion after a recombination between the centromere and the *forked* locus.

These considerations lead us to conclude that, in the gynogenesis of gyn-F9, diploidy is restored obligatorily by central fusion or its genetic equivalent. This explains well why the *X* chromosomes of gyn-F9 have been kept heterozygous.

Gamete types produced by gyn-F9 females

As shown above, central fusion is probably the sole mode of diploidization in the gynogenesis of gyn-F9. However, it must be noted that central fusion is genetically equivalent to the suppression of meiosis I (see TEMPLETON 1983). Reductional nondisjunction at meiosis I, if it occurs at extremely high frequency, will bring about the diploid gametes that may be able to develop into the impaternates indistinguishable from those produced by central fusion. Although the low frequency of *X0* males found among the gynogenetic progeny suggests that such cases are rare, it is desirable to assess more directly the composition of gametes produced by gyn-F9 females. To accomplish this, the genotypes of F_1 progeny obtained from the cross between gyn-F9 females and *y cv v f*/$B^S Y y^+$ males were examined. Owing to the visible markers on the *X* chromosomes of gyn-F9, all possible genotypes ascribable to various kinds of gametes could be determined, except for meta-females and meta-males, which are expected to be very rare (Figure 4).

In the experiment, 80 gyn-F9 females produced a total of 10369 offspring; among them, 11 exceptionals were omitted, most of which were gynandromorphs. The remaining 10358 offspring were assigned, according to their phenotypes, to the respective types of gametes, and are listed in Figure 4.

Female gametes	Male gametes		
	X $\frac{y\ cv\ v\ f}{}$: A	Y $\frac{B^S Yy^+}{}$: A	0 Gynogenetic
A $\frac{FM7}{}$: A	AX: *y v B* (2nF) 2057	AY: *w sn B* (2nM) 1503	—
B $\frac{y}{}$: A	BX: *y* (2nF) 2035	BY: *B* (2nM) 1959	—
C $\frac{FM7}{y}$: AA	CX: *y B* (3nF) 1604	CY: *B* (3nIX) 732	CO: *y B* (2nF) 282
D $\frac{FM7}{}$: AA	DX: *y v B* (3nIX) 40	—	DO: *y w sn B* (2nM) 5
E $\frac{y}{}$: AA	EX: *y* (3nIX) 34	—	EO: *y* (2nM) 12
F $\frac{FM7}{y}$: A	—	FY: *B* (2nF) 66	—
G nullo : A	GX: *y cv v f* (2nM) 29	—	—

FM7 = *In(1)FM7*, $y^{31d}sc^{8}w^{a}sn^{X2}v^{01}B$

FIGURE 4.—The gamete types of gyn-F9 inferred from the cross between gyn-F9 females and *y cv v f*/$B^S Yy^+$ males. The top row of each box refers to zygote type; the middle row, the phenotype of zygote and, in parentheses, ploidy and sex of the zygote (F, female; M, male; IX, intersex); the bottom row, the number of progeny recovered. The boxes marked with a dash indicate that the corresponding zygotes will be inviable or very rare.

Among the seven possible gamete types of gyn-F9 (A–G) that would give rise to viable zygotes, two—*XX*; *A* (F) and *0*; *A* (G)—were most likely to be produced through reductional nondisjunction, suggesting that nondisjunction occurs at a considerable frequency in the meiosis of gyn-F9 females (about 1%). Nevertheless, the frequency of nondisjunction observed with the *X* chromosomes suggests that the possibility of occurrence of a diploid gamete by simultaneous nondisjunctions of the three major chromosomes is very low, as long as the autosomes fail to disjoin independently and at a frequency similar to that of *X* nondisjunction. The vast majority of the gynogenetic progeny of gyn-F9, therefore, seem to have restored their diploidy, after the completion of meiosis, by means of fusion of two centrally located egg pronuclei.

An unexpected result of this experiment is that a considerable number of eggs developed into diploid impaternate flies without syngamy, although they were inseminated by functional sperm (zygote types, C0, D0, and E0). The possibility that these impaternates might be derived from unfertilized eggs can be ruled out, because gyn-F9 females have never produced progeny unless

mated. A certain subset of eggs, therefore, seems to have a property of excluding sperm from syngamy. On the average, a female produced 3.7 such gynogenetic offspring. This figure is lower than the average number of impaternates being produced by mating with *ms(3)K81* males (Figure 1). However, considering that these impaternates among bisexuals were reared under more crowded conditions, the difference may not be meaningful. If so, another intra-ovum event besides the restoration of diploidy is indicated as being needed for an egg to develop gynogenetically; that is, two different cytological mechanisms *in toto* mimic syngamy.

DISCUSSION

On the evolutionary origin of parthenogenesis: There seems to be a consensus among authors that parthenogenetic ability is genetically controlled and that parthenogenetic organisms have evolved repeatedly from bisexual ancestors having a capacity of facultative parthenogenesis or tychoparthenogenesis (*e.g.*, SUOMALINEN 1950; WHITE 1973; CUELLAR 1974; SUOMALINEN, SAURA and LOKKI 1976). However, our present knowledge about the genetic mechanisms of parthenogenesis is scanty. In Drosophila, STALKER (1954) and CARSON (1967) have shown with three tychoparthenogenetic species that the productivity of parthenogenetic progeny can be increased by artificial selection. From these findings, they have suggested that parthenogenesis may be controlled by polygenes. The polygenic hypothesis of parthenogenesis, although widely accepted (*e.g.*, BELL 1982; LYNCH 1984), seems to raise a problem when considering the evolutionary origin of a parthenogenetic form from a bisexual species. A transition from sexual to asexual reproduction is necessarily accompanied with a radical reduction of additive genetic variance within the resultant asexual clone; consequently, this impedes the clone, further improving the capacity for parthenogenesis. Genetic variance may be restored by mating with bisexually produced males, but at the same time, the genetic ability of asexual reproduction will regress toward bisexuality. Therefore, if parthenogenesis is controlled by a polygenic system, highly successful parthenogens are unlikely to evolve by means of natural selection.

The present study has shown that mutations at a few loci cause a drastic increase in the capacity of parthenogenesis in *D. melanogaster*. The present case differs from the majority of thelytokous species in that gyn-F9 females never produce progeny unless inseminated. But, considering that autonomous egg activation without sperm entry is widespread among drosophilids (STALKER 1954), we may suppose that such mutations are actually involved in the evolutionary origin of parthenogenetic forms.

The impaternate productivity of gyn-F9 was found to be around 15 progeny per parent, which is sufficient to maintain this stock in the laboratory, but not to compete with bisexual *D. melanogaster* populations in nature. The situation, however, will be different for species for which the habitats are extremely patchy and where natural selection favors increased survival (*K*-selection) rather than higher reproductive ability (*r*-selection). We can find examples of such species in flower-breeding drosophilids; their fecundity is usually very low, and

some species lay only one egg a day (BRNCIC 1983). For females of such species, it may be possible to imagine that an abandonment of the efforts necessary to find their mates can compensate for the decrease of fecundity due to the attainment of parthenogenetic ability. In this regard, the characteristics of *D. mangabeirai*, the only known Drosophila that reproduces exclusively by parthenogenesis, seem to be suggestive. This species is distributed in Central and South America but is known to be very rare in its natural habitat, suggesting the utilization of very specialized breeding sites. The experiments carried out by CARSON, WHEELER and HEED (1957) showed that the females have poor fertility, with an estimation of 40 progeny per female after a 3-week laying period. Moreover, central fusion has been indicated to be a sole mode of diploidization in this species, judging from complete heterozygosity for inversions and also from a peculiar orientation of meiotic nuclei (CARSON, WHEELER and HEED 1957; MURDY and CARSON 1959).

Most authors who have argued about the adaptive significance of sexual reproduction have assumed reproductive advantages for parthenogens over sexuals and have sought conditions by which sexual reproduction could compensate the cost of producing males or the cost of meiosis (*e.g.*, WILLIAMS 1975; MAYNARD SMITH 1978). This supposition might be a fallacy so far as automixis is concerned, if it results from a small number of mutations. At least one such mutation will probably perturb meiosis. As is known in many meiotic mutants of Drosophila, genetic perturbation of meiosis is often associated with a severe reduction of fertility not only due to aneuploidy but also due to as yet unknown reasons (BAKER and HALL 1977, and literature therein). The impaired fertility might thus be an inevitable cost of attaining automixis, or the "cost of not producing a male." Indeed, a survey of the literature has suggested that most parthenogenetic animals show much lower hatchability of eggs when compared with bisexuals (LAMB and WILLEY 1979). Moreover, automictic parthenogenesis usually brings about a rapid increase in homozygosity that will impose a considerable amount of genetic load upon an incipient parthenogen. Such disadvantages accompanying automixis may explain why automicts are relatively rare and apomicts, which are freed from these costs by abandonment of meiosis, are flourishing (BELL 1982). From these considerations, we may suppose that automicts will be frequent among *K*-strategists, whereas apomicts, although originating from automicts, will evolve to *r*-strategists.

On the origin of reproductive isolation: Another intriguing problem is how a newly arisen parthenogenetic form can establish reproductive isolation from the ancestral bisexual species. Without such isolation, the genetic integrity that enabled asexual reproduction will be easily broken up by genetic contamination from bisexual males (LYNCH 1984). Some authors have claimed the importance of physical isolation. For instance, CUELLAR (1977) states that parthenogenetic forms, by exploiting previously unoccupied habitats, can exist in isolation from their sexual relatives. The geographic distribution of parthenogenetic animals known as the rule of "geographic parthenogenesis" is considered to support this idea. Alternatively, some authors have considered that asexual forms can

coexist with sexuals by developing sexual isolating mechanisms. Some degree of sexual isolation has been demonstrated in the parthenogenetic strains of *D. mercatorum* to arise spontaneously or by means of artificial selection (IKEDA and CARSON 1973; TEMPLETON 1983). This indicates the existence of genetic variability with respect to sexual isolation, but the selective force in nature that intensifies the isolation to the level sufficient to prevent gene flow from sexuals is unknown.

Recently, LYNCH (1984) suggested that the genetic disruption resulting from the hybridization between a newly arisen parthenogen and its sexual relatives (destabilizing hybridization) might be the major selective force leading to an establishment of reproductive isolating mechanisms between them. His exhaustive survey of the literature has shown that the maladaptedness of the hybrids between sexuals and asexuals, which may be caused by various mechanisms inherent to the genetic system of asexual reproduction, is a widespread phenomenon. The properties of gyn-F9 seem to be in accord with the destabilizing hybridization hypothesis, suggesting a possible mechanism of establishing reproductive isolation. When gyn-F9 females were mated with normal males, fecundity varied extremely from parent to parent. As a result, the average progeny number per female was much less than normal. There still remains a possibility that this adverse effect on fecundity was caused by genes not related to parthenogenetic ability, but, as stated above, mutations affecting meiosis are likely to bring about inviable eggs even if inseminated by normal sperm. Moreover, nearly one-third of the progeny were either triploid female or intersex; the former produce very few progeny and the latter are completely sterile. Such inviability and infertility of progeny, if common among the hybrids between parthenogenetic females and bisexual males, will enforce isolation between them.

A more striking finding about the bisexual progeny of gyn-F9 is that a small fraction of them were impaternates produced without syngamy. These impaternates themselves, of course, can reproduce gynogenetically. This implies that a parthenogenetic clone can coexist with bisexuals in a population even though matings take place. When such a population encounters a temporal shortage of males, the parthenogenetic clone will be successfully established in the habitats being made available by elimination of bisexuals. Cases of the coexistence of parthenogenetic forms with their sexual ancestors have amply been documented (BELL 1982; LYNCH 1984). More careful examination may reveal that some of these asexual clones have been maintained in such a way.

Other remarks: It has been shown that triploid flies constitute nearly one-third of bisexual progeny of gyn-F9. Triploid Drosophila have been useful for various fields of genetics, such as sex determination, gene dosage compensation, and others (LAUGE 1980; STEWART and MERRIAM 1980). Previously existing triploid stocks of *D. melanogaster* usually have attached *X* chromosomes; consequently, *X*-linked alleles are not easy to introduce into the stock. The present system, being devoid of such difficulty, provides an efficient means to obtain a large number of triploids with desirable genotypes.

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