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The Genetic System in Parthenogenetic Strains of *Drosophila mercatorum*

(diploidization/pronuclear duplication/nuclear fusion/evolution)

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ABSTRACT **Five parthenogenetic laboratory strains of *Drosophila mercatorum* have been examined for their mode of diploidization in the eggs of females. Diploidization occurs either by pronuclear duplication leading to complete homozygosity or by nuclear fusion. If the latter occurs between pronuclei from different secondary oocytes, heterozygosity can be maintained. Each of the five strains shows some nuclear fusion and three show significantly different amounts, ranging from about 21% to less than 1%. Even in strains with a strong tendency towards homozygosity, it is argued that this dual system of nuclear reconstitution provides for genetic recombination; thus, the strains retain considerable evolutionary potential.**

The overwhelming majority of successful higher organisms reproduce sexually. This tenacious retention of a complicated system of genetic recombination (crossing-over, random segregation, syngamy) attests to the power of this genetic system to generate genetic variability and promote evolutionary adjustment. Without this variability, natural selection has no meaning and adaptive evolution ceases.

Curiously, however, some organisms have suffered severe restrictions on the very genetic system that enabled them to evolve in the first place. A striking example is the loss of the male sex in a diploid species, so that genetic continuity depends wholly on females reproducing by thelytokous parthenogenesis (1). Frequently, the loss of potential for recombination is complete, as in cases where an ameiotic system of retaining diploidy is substituted for meiosis and bisexual reproduction. Of special interest, however, are newly-developed diploid parthenogenetic systems that retain some potential for genetic recombination. This may occur if diploidization follows meiosis and results, at least in part, from pronuclear fusion of products of different secondary oocytes (2).

Drosophila mercatorum provides unique experimental material for the study of the genetical aspects of the incipient stages of parthenogenesis. Wild strains of this species are exclusively bisexual, but various vigorous thelytokous strains have been produced by selection in laboratories (3). These strains retain normal meiosis. Diploidy is restored in two ways: (*a*) by pronuclear duplication with resulting isogeny and loss of variability or (*b*) by pronuclear fusion of a type that retains recombinational heterozygosity (4). The present paper reports analytical studies of the manner in which these two modes of diploidization are balanced in the developing eggs of each of five parthenogenetic strains of this species.

MATERIAL AND METHODS

Details of the derivation of four of the five impaternate (Im) strains used have been published (3). Their origin stems from one or more of three wild strains, El Salvador, Rochester, N.Y., and Oahu, Hawaii. Dates of origin are as follows: RS-3-Im, September 1961; RSS-18-Im and RSB-7-Im, December 1964; and O-3-Im, May 1965. SFRSB-7-Im was established in 1970 from RSB-7-Im without outcrossing to males. A single female from the latter strain served as an original founder. For 10 subsequent generations, before August 1970, the strain was passed through single females in each generation. Tests of the diploidization patterns in all five strains were performed during 1971 and 1972. For each strain, a bisexual Bridge (Br) stock was prepared (4, 5).

TABLE 1. *Phenotypes of impaternate progeny arising from unmated females of D. mercatorum from five parthenogeneic strains*

Strain origin of females and genotype	Males from Bridge cycle number	No. of F_1 ♀♀ giving progeny	Phenotypes of impaternate progeny (F_1)				
			White	Spotless	Spotless white	Wild-type	Total
RSB-7-Im *sl* +/+ *w*	5	25	475	474	207	325	1481
RSS-18-Im *sl* +/+ *w*	10	56	788	757	274	345	2164
SFRSB-7-Im *sl* +/+ *w*	12	69	497	488	186	232	1403
O-3-Im + +/*sl w*	10	52	118	143	421	443	1125
RS-3-Im *sl* +/+ *w*	8	67	316	312	128	128	884

TABLE 2. *Impaternate progeny of doubly heterozygous females arising by nuclear fusion*

Strain	No. of wild type plus *sl w*	No. of wild type minus *sl w*	No. of wild-type progeny tested	No. heterozygous	Total tested	Total fusions	Percent fusions
RSB-7-Im	532	118	90	16	622	134	21.5
RSS-18-Im	619	71	263	34	882	105	11.9
SFRSB-7-Im	418	46	187	13	605	59	9.8
O-3-Im	—	—	86*	5	86	5	5.8
RS-3-Im	256	0	82	1	338	1	0.3

* In this strain, the spotless and the white phenotype were progeny-tested.

These were used to facilitate introduction of gene markers in order to monitor the type of diploidization occurring in the eggs. All of the impaternate stocks (except for O-3-Im) were homozygous for the sex-linked gene *sl* (spotless). Each Bridge stock was prepared by crossing a female from the Im stock to a male carrying the sex-linked recessive gene white (*w*). New tests of the linkage of *sl* and *w* have shown the distance between these two genes to be about 30 crossover units, somewhat less than the previously published figure (4). F_1 ♀ ♀ and ♂ ♂ are then crossed and a single *w* ♂ selected from the F_2. This male was then backcrossed to a single female taken at random from the Im stock. This initiates the second Bridge cycle.

After 5–12 such cycles, the mode of diploidization in the Im stock was tested by crossing a female from the original Im stock to a white male from the Bridge stock. F_1 ♀ ♀, which carry the two recessives in repulsion (*sl* +/+ *w*), are then allowed to produce progeny without males. Four gametic types (apparently four functional pronuclei) are produced; two are straights (*sl* + and + *w*) and two crossovers (+ + and *sl* w). The diploid female progeny arise either from duplication of one of these four meiotic products or from fusion between two of them. The double recessive *sl w*/*sl w* is of special significance in that it cannot arise from any sort of pronuclear fusion (excluding rare 4-strand double crossovers). Rather, it must arise by pronuclear duplication (or possible fusion of identical haploid cleavage nuclei). Accordingly, the frequency of spotless white females among the progeny of doubly heterozygous mothers gives a direct measure of the amount of pronuclear duplication. An equal number of wild type are presumed to have arisen in the same way and will be expected to be homozygous + +/+ +. Previous studies have shown that the viability of *sl w*/*sl w* and + +/+ + is very similar (4). Accordingly, any excess of the wild type over the *sl w* class may be judged to be due to pronuclear fusion. The mode of diploidization was checked in another way by test-crossing wild-type impaternate females to *sl w* males. Those shown to be carrying either of the recessives must have arisen by nuclear fusion rather than duplication.

One stock, O-3-Im, is phenotypically wild type. Accordingly, in the preparation of the Bridge, *sl w* males were used. When tested, females thus carried the genes in the coupling phase (*sl w*/+ +). From such a genotype, the impaternate spotless white progeny can arise by either fusion or duplication; therefore, the ratio of this class cannot be used to estimate the frequency of duplication, as in the repulsion case. Therefore, all estimates of the frequency of duplication in this stock were made from test crosses of crossover products (females phenotypically either spotless or white). As before, homozygotes must arise by pronuclear duplication.

RESULTS

The phenotypic ratios observed among the impaternate daughters of doubly heterozygous virgin females from each strain are given in Table 1. Rather than "F_2", such progeny are referred to as "F_i" (impaternate filial generation). Homogeneity chi-squares were calculated for the replicate tests of each strain. In none were the differences close to significance, and the data for each strain have been pooled. The percent of nuclear fusion occurring in the eggs of females of each strain is given in Table 2. For the four strains having the markers in repulsion, fusion was estimated by the excess number of wild type over the *sl w* class, as explained previously. This figure, divided by the sum of the two crossover classes, gives the percent fusion. Results of test crosses of wild-type progeny are also shown. Within all four strains, homogeneity tests revealed no significant difference between these two types of measurement. Accordingly, the data were pooled and a percent fusion was calculated from the totals. In O-3-Im, measurement of fusion is based solely on progeny tests. Although RSS-18-Im and SFRSB-7-Im do not differ statistically ($\chi_1^2 = 1.6$, $P = 0.20$–0.30), each of the other strains are significantly different from these two and from each other.

DISCUSSION

Fusion rates vary among the strains, ranging from about 21% to less than 1%. These variations apparently represent permanent consistent genetic differences between the strains. These differences have apparently been fixed in these strains at or shortly after the time of their initial isolation from sexual reproduction 5–10 years earlier (130–260 generations).

Accordingly, these strains retain the capacity for genetic recombination even though they reproduce unisexually. Whether this capacity serves them in the actual retention of the genetic variability that could make recombination meaningful is a question that cannot be answered definitely by the data presented here.

An indication, however, that genetic variability can be retained for some time is provided by the following facts. RSB-7-Im is a strain in which about 21% of the impaternates arise by pronuclear fusion. Strain SFRSB-7-Im was initiated from a single female of RSB-7-Im after it had been in existence for 5 years (1970). It was further propagated through single females for each of 10 generations. When tested, this strain manifests only about half as much fusion as its progenitor. It

is suggested that this change may have been brought about by stochastic affects during the period of derivation. Implicit in these observations is the existence, in early 1970, of genetic variability for the fusion trait in the strain RSB-7-Im 5 years (130 generations) after isolation from sexual reproduction.

Intuitively, it may seem unlikely that genetic variability could be maintained by a system in which the tendency to pronuclear duplication, and therefore homozygosity, is so strong. Such a conclusion, however, may be premature. The complex mathematical properties of the population genetics of this system are now under examination (6, 7). The outcome of these studies promises to permit an experimental design that will determine the possible effects of selection in maintaining heterozygosity and genetic variance under this genetic system.

The conclusion may be drawn that, despite inbreeding of a most vigorous sort, the capacity for genetic recombination is retained to some degree in all five strains. Such a diploid thelytokous system, if it were to exist in nature, has several features that enable it to retain evolutionary potential.

In the first place, the nuclear fusion mechanism generates a variety of heterozygotes. The system thus provides for the exploitation of their fitnesses under different environments. This heterozygosity, moreover, is not fixed in the manner characteristic of some parthenogenetic systems (8). Whereas a fixed heterozygote would not be expected to change except to accumulate recessive mutants, the present system has greater flexibility. Thus, deleterious genes can be effectively weeded out of the heterozygous states and eliminated by the pronuclear duplication system. This elimination can occur in each parthenogenetic generation.

Secondly, if the way can be kept open to an occasional sexual outcross, the enormous recombination potential of meiosis can still function efficiently within the single sex. A powerful feature of the system is the production by the pronuclear duplication system of an array of novel, completely homozygous states. Many can be synthesized by recombination in a single parthenogenetic generation after an outcross. These homozygous genotypes can thus be immediately exposed to selection along with the heterozygotes. Some of them might be highly favored in some environments. The system, then, has even more powerful features than those found in some facultatively outcrossing plants, which cannot achieve new homozygosity so rapidly after an occasional outcross (9).

Accordingly, the view expressed here contrasts with the traditional one that holds that parthenogenetic systems lead an organism into obligatory and uniform homozygosity, rigidly fixed heterozygosity, or some other evolutionary dead end.

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