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Genetic Variability in a Thelytokous Form of *Aphytis mytilaspidis* (Le Baron) (Hymenoptera: Aphelinidae)

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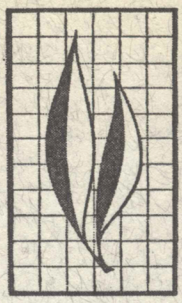

The possible mechanisms for generation of genetic variability in a thelytokous form of the parasitic wasp, *Aphytis mytilaspidis,* were explored. Through cytological studies the thelytokous form was found to retain the meiotic divisions in egg maturation. Restoration of diploidy is achieved through the fusion of two sister nuclei of the second meiotic division (terminal fusion). Heterozygosity can be retained for such loci as undergo crossing over. Males are produced by the thelytokous form in a low frequency; both thelytokous males and females are sexually functional; and sexual processes occur in the thelytokous population.

In zones of overlap, gene flow may occur between the thelytokous form and a related arrhenotokous form, thus adding to the genetic variability of the thelytokous form.

Since genetic variability can be generated in the thelytokous form through genetic recombinations in meiosis, through sexual processes within the population, and by introgression with the arrhenotokous form, thelytoky does not represent an evolutionary dead end.

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Genetic Variability in a Thelytokous Form of *Aphytis mytilaspidis* (Le Baron) (Hymenoptera: Aphelinidae)[1, 2]

INTRODUCTION

THELYTOKY — the parthenogenetic reproduction of females only—is common in the parasitic Hymenoptera, although not in all members of any one genus (Clausen, 1962). Our knowledge of its distribution in Hymenoptera is far from adequate, but it seems to be much more frequent than in other orders that include entomophagous species (Clausen, 1962). Foerester was apparently the first to note it, in 1850–1853, in the eulophid *Astichus arithmeticus* (Foerester) (Flanders, 1945*b*). Clausen (1962) lists about 30 genera containing thelytokous species, and DeBach (1969) found a surprisingly high incidence of thelytokous forms in the more thoroughly studied of those genera.

Thelytoky has been viewed by many as an "evolutionary dead end." Such a view was supported by the organism's apparent inability to generate and either show or preserve genetic variability because of the presumed absence of sexual processes and the modification or complete abandonment of the meiotic process (J. M. Smith, 1966; Sylvester-Bradley, 1959; Thoday, 1964; White, 1954, 1964).

In nature, however, and not only among the insects, a large number of thelytokous species or "forms" are widespread. The genus *Aphytis* (Howard) includes several such species. *A. chilensis* (Howard) and *A. chrysomphali* (Mercet) are widely distributed, and are adapted to the various conditions within their distribution zones. Other examples of apparently successful thelytokous species are in the *A. mytilaspidis* and *A. maculicornis* groups. Certain forms within these groups further complicate matters because they are morphologically identical, yet differ in biological properties (DeBach, 1964, and unpublished data; Hafez and Doutt, 1954).

In considering the cytological processes that lead to maintenance or restoration of diploidy, thelytoky can be subdivided into two main categories: apomixis and automixis. In apomixis, or ameiotic thelytoky, the meiotic divisions may be completely lacking. No reduction in chromosome numbers takes place. In automixis, or meiotic thelytoky, the meiotic process is at least in part retained, and diploidy is restored through various modifications of the process. (For a fuller discussion, see the Appendix, p. 165.) Progression toward homozygosis is, however, inevitable un-

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less strong heterotic effects exist, or the organism possesses an unusually high rate of nonlethal mutations, or the population displays more than one mechanism of diploidy restoration. Progression toward homozygosis will produce a highly polymorphic population, subdivided into homozygous clones that depend on mutation ratio for the capability to generate further variability.

Retention of sexual processes by a thelytokous form, even if sporadic and limited, may prevent such fixation in the population, and result in genetic variability. To achieve such variability requires (1) that females be capable of mating and executing the sexual processes; (2) that the occasional males in the thelytokous population be able to mate with and inseminate the females; and (3) that haploids be produced by the meiotic divisions. Thus females displaying either cleavage, central, or terminal fusion are natural candidates for sexual reproduction, and with the existence of sexual processes, a population displaying cleavage fusion might produce some genetic variability.

Thelytokous species have been able to diverge and attain differing biological properties of a magnitude similar to that in bisexual species, a fact that is opposed to the evolutionary dead end theory. In view of this, it seems curious that such a small amount of research has been conducted on thelytoky in the parasitic Hymenoptera. Many taxonomic studies merely mention in passing that thelytoky occurs, or that no males are known. Various authors have investigated the influence of environmental and nutritional factors on sex ratio in thelytokous species, since males usually occur in such species, if only rarely (Flanders, 1945 *a*; McCoy, 1967; Schlinger and Doutt, 1964; Wilson and Woodcock, 1960). These rare males were usually treated as "mistakes," and ignored. No thorough research was carried out to determine the significance of such males, nor were the relations between thelytokous and related arrhenotokous species investigated.

In research reported here, we explored the possible mechanisms for generation of genetic variability in a thelytokous form of the parasitic wasp, *Aphytis mytilaspidis* Le Baron. For both the cytological and genetic studies we used a thelytokous and an arrhenotokous form of the wasp. Both were collected by DeBach (1964) in Greece, and were shipped to California under the code numbers S&R 63-23-3 and S&R 63-63-4, respectively. Both cultures are maintained in the insectary of the Division of Biological Control, University of California, Riverside.

I. EGG MATURATION—CYTOLOGICAL STUDIES

The purpose of these studies was to determine the mechanism by which the thelytokous form retains diploidy in the absence of fertilization and to compare the process of egg maturation in the thelytokous and arrhenotokous forms. Unless otherwise specified, the material in this section refers always to the thelytokous form.

Material and Methods

Ovarian eggs. Adult females of *Aphytis mytilaspidis* were dissected in Clarke's physiological saline (Hale, 1965) under CO_2 anesthesia. Ovaries were removed by holding the female against the dissecting surface by her thorax and pulling the ovipositor away with a fine forceps. The ovaries were fixed in Carnoy's fluid (3:1 ethanol:glacial acetic acid), and stained according

to the method of Snow (1963). The detailed staining procedure is described below.

Deposited eggs. Cactus scale, *Diaspis echinocacti* (Bouche), is easily handled and was therefore used as the host for the wasps. The *Aphytis* female deposits eggs between the scale cover and scale body. Eggs were collected by removing the cover with a dissecting needle. They were transferred to a watchglass containing a solution of 5 per cent sodium citrate, for 10 to 20 minutes at 37°C, followed by a few seconds in 3 per cent sodium hypochlorite (until eggs became dumbbell-shaped). The eggs were next fixed in Carnoy's fluid for 40 minutes at 37°C, followed by two rinses of one-half hour each in 70 per cent ethanol. Staining was with Snow's carmin for 72 hours at room temperature, after which the eggs were rinsed with water until it ran clear. Specimens were mounted in Hoyer's medium and sealed with clear nail polish.

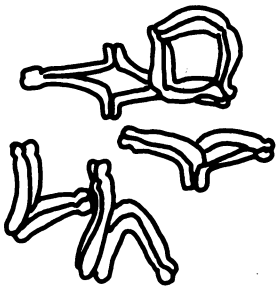
Preparations were examined and photographed with a Zeiss phase-contrast photomicroscope. Drawings were made either with a camera lucida or freehand.

Reproductive Systems of Thelytokous and Arrhenotokous Females

In the *Aphytis* females, two lateral ovaries, comprised of three ovarioles each, lead to lateral oviducts which end as a common oviduct opening at the base of the ovipositor. Two large, spherical organs (probably accessory glands) and a kidney-shaped spermatheca are connected with the common oviduct (fig. 3, B). The reproductive systems of the arrhenotokous and thelytokous females are similar in general appearance. In the ovariole of an adult female, four oocytes are found in various stages of yolk accumulation. Hence, at a given time, 24 eggs are in various stages of maturation. Each oocyte is accompanied by large nurse cells and is completely surrounded by a layer of follicle cells, one cell thick. That layer becomes very thin as the egg matures. Each unit of oocyte and nurse cells is separated from the adjacent unit by a plug of epithelial cells (fig. 3, A).

Chromosomes. Both arrhenotokous and thelytokous forms have a diploid number of 10 chromosomes, one metacentric and four subtelocentric pairs (figs. 1, A, C, D; 2, A; 4, C, D). Individual chromosomes at the second meiotic metaphase range in size from 3.5 to 6 μ, the metacentric chromosome being the largest.

Ovarian oocytes. Oogonial mitotic divisions can be found in the germarium. The earliest prophase stages of the meiotic division are not resolvable, and we could not recognize them. The germinal vesicle, however, shows clearly as a pale, spherical area near the anterior end of the oocyte (fig. 3, A). Diplotene, the earliest resolvable meiotic stage (fig. 3, C), and clear figures of diakinesis with chiasmata (figs. 1, A; 3, D), are readily recognizable among the more mature oocytes. The diakinetic figures are similar to those obtained in the arrhenotokous oocytes (fig. 2, A). Chiasma frequency was counted in 11 clear diakinetic figures. Sixty-one chiasmata were counted in 66 pairs of chromosomal arms. (A diploid nucleus with $2n = 10$ has six pairs of arms.) In cases of nonchiasmate arms, it was always one of the arms of the metacentric chromosome that lacked chiasmata. First meiotic metaphase (fig. 3, E) is the last stage reached by the mature egg while in the ovariole. At this point the nuclear elements are located in the anterior third of the egg. The spindle, in a preparation of whole eggs, tends to be parallel with the radial axis of the egg, but not pointing to the middle of the egg (fig. 3, F).



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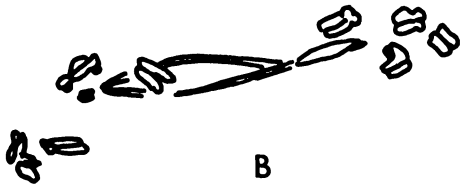

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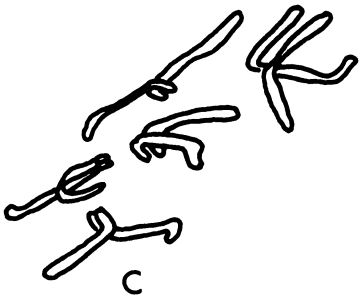

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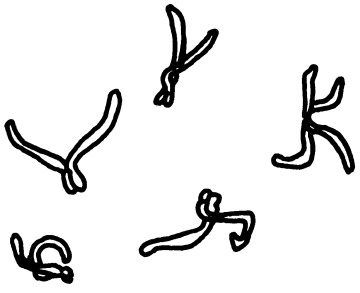

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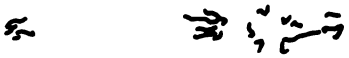

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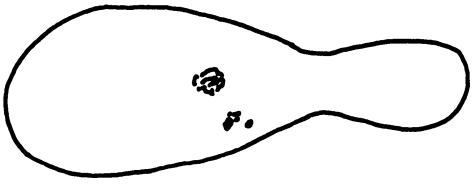

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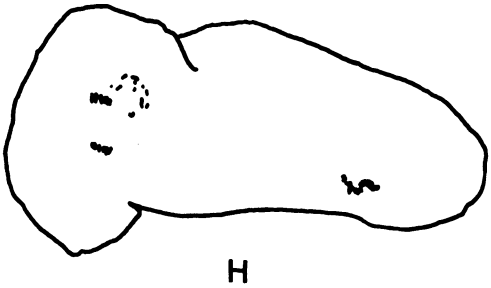

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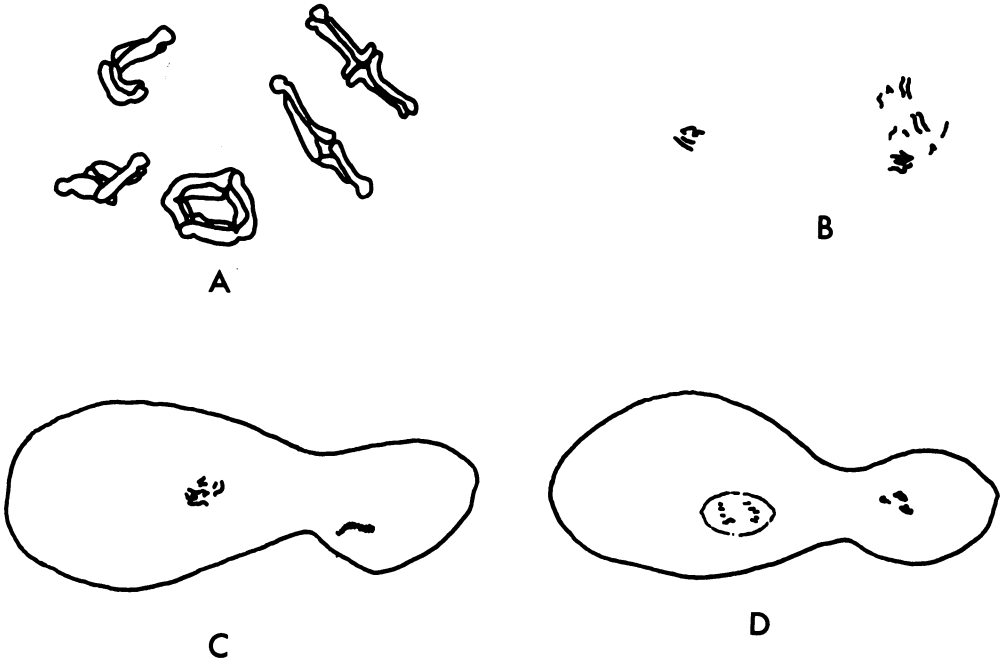


Fig. 2. Arrhenotokous female *Aphytis mytilaspidis*. *A*. First meiotic metaphase in an ovarian egg. *B*. Second meiotic anaphase in a deposited egg. *C*. First cleavage prophase. *D*. First cleavage anaphase, unfertilized egg. (*C* and *D* freehand drawing.)

The deposited egg. The first meiotic division enters anaphase only in the deposited egg (fig. 3, G). At 27°C, the meiotic division is completed within three hours following deposition.

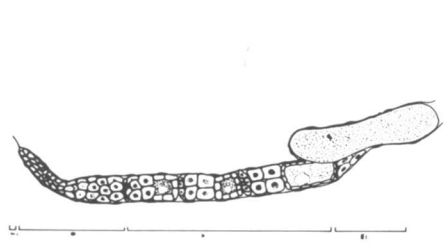
The first meiotic anaphase produces a peripheral and an inner nucleus; the latter, which is set deeper in the egg cytoplasm, is actually the first polar body (fig. 4, B). Five univalents, each divided into two widely spread chromatids connected only at the centromeres, are evident in each of the two nuclei (figs. 1, C, D; 4, B, C, D).

One egg in the thelytokous form showed an interesting configuration of the first meiotic anaphase (figs. 1, B; 3, H). Here a bridge and a chromosomal fragment are seen in what can be interpreted as a "four-strand double crossing over" in an inversion heterozygote. Since the occurrence of such events is highly improbable, it is presented here only as an uninterpreted curiosity.

A short "telophase-like" stage (figs. 1, E; 4, A) produces in the egg proper a peripheral nucleus with well-spread chromosomes and an inner nucleus, nearer the egg's stalk, with condensed and "entangled" chromosomes.

Each nucleus then continues the meiotic division independently at a different pace, the peripheral nucleus lagging. While the peripheral nucleus is in interphase, the inner nucleus proceeds to the second prematephase (fig. 4, B, C, D), and enters the second meta-

Fig. 1. Thelytokous female *Aphytis mytilaspidis*. *A*. First meiotic metaphase in a mature ovarian egg, one metacentric and four subtelocentric chromosome pairs. *B*. First meiotic anaphase in a deposited egg. A bridge caused by a "four-strand double crossing over" in an inversion heterozygote. *C*. Second meiotic metaphase of the inner nucleus. *D*. Interphase of the peripheral nucleus. *E*. First meiotic "telophase." *F*. First meiotic anaphase. *G*. First cleavage prophase. *H*. First cleavage anaphase. (*G* and *H* freehand drawing.) Egg size ranges from 0.15 to 0.20 mm.



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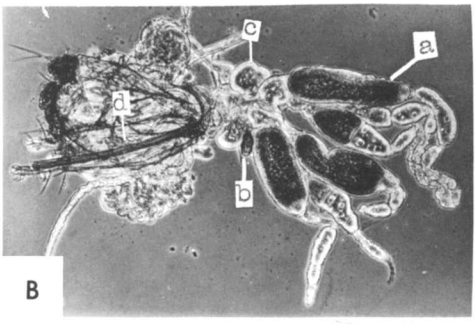

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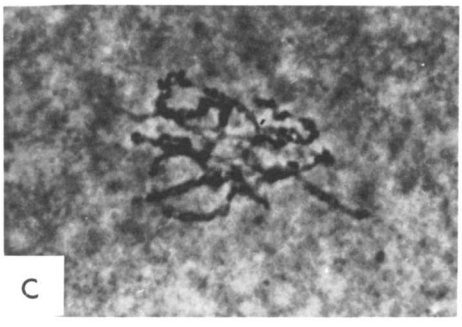

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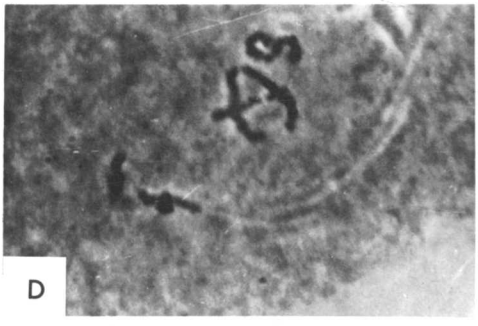

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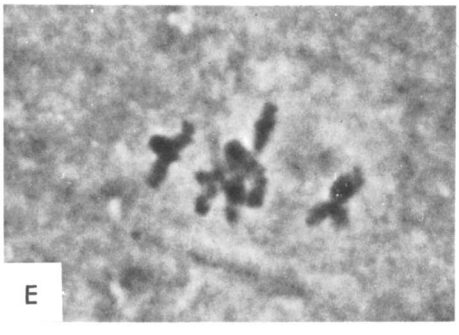

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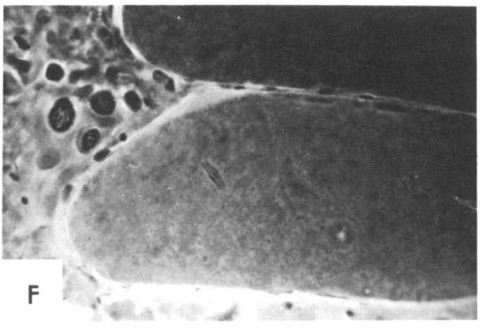

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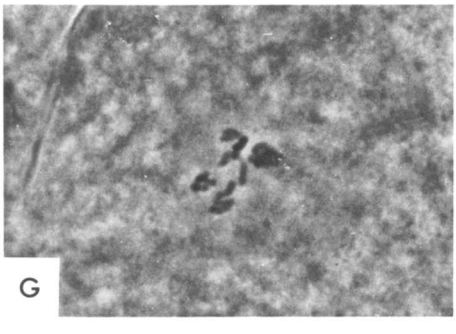

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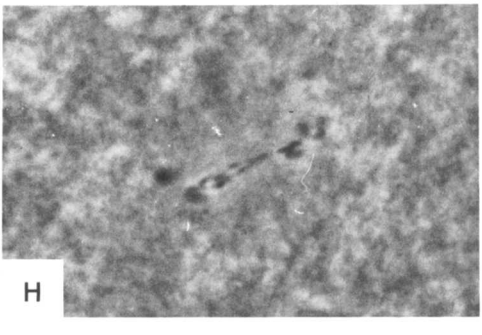


H

phase while the peripheral nucleus is still in late interphase. The inner nucleus then enters the second meiotic anaphase at the time that the peripheral nucleus enters second metaphase (fig. 4, E). There is a differential staining of the groups of the inner nucleus, the group nearer the peripheral nucleus becoming darker stained. Only later does the peripheral nucleus enter anaphase, at which time the inner nucleus almost completes anaphase (fig. 1, F). A third polar body is not produced, however, for the inner nucleus degenerates in anaphase. Up to that point, the thelytokous and arrhenotokous forms do not differ in meiosis (figs. 2, B; 5, C, D, E).

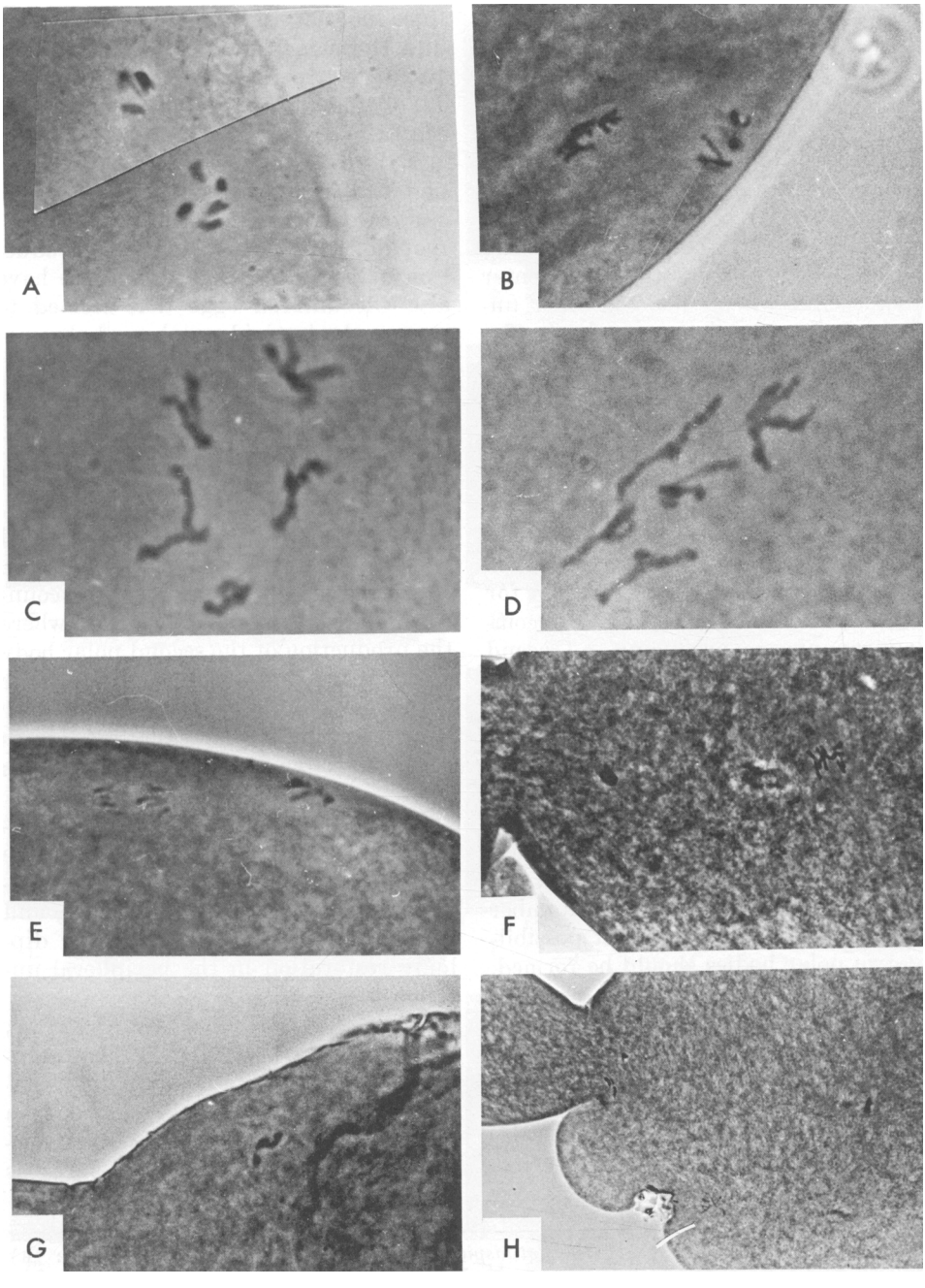
From that stage on, meiosis becomes very unclear in the thelytokous form. Two possible avenues may be open for the peripheral nucleus: (1) it may complete anaphase, producing a second polar body and a haploid egg nucleus. The egg nucleus may then double its chromosome number by endomitosis or by fusion of cleavage nuclei (See Appendix); or (2) the anaphase stage of the peripheral nucleus may or may not be completed, but the two anaphase groups of haploid products reunite to restore a diploid chromosome number (terminal fusion). In the first possibility, two polar bodies should be formed, and the egg nucleus should be completely homozygous. In the second possibility, one polar body should be formed (by the inner nucleus), and heterozygosity maintained for all loci that undergo an odd number of crossovers. The known ability of the thelytokous eggs to be fertilized and produce normal diploid adults (Rössler, and DeBach, 1972) shows that haploid products are available, and anaphase II of the peripheral nucleus is completed.

Evidence supporting the existence of terminal fusion is found in genetic studies, as will be discussed later, although figure 1, H, suggests the production of two polar bodies, and we have also encountered eggs that seemed to possess the haploid number of chromosomes in what looked like a first-cleavage metaphase (fig. 4, H). We were, however, unable to show unequivocally the completion of the peripheral anaphase and the production of the second polar body, nor were we able to show figures of endomitosis in a haploid product of such a peripheral nucleus. The same difficulties were also encountered in the arrhenotokous form, where the production of the second polar body from the peripheral nucleus was never observed. Thus, it remains unclear which of the two possible avenues is actually followed by the thelytokous form.

Figure 6 is an attempt to reconstruct the events during the meiotic divisions in the thelytokous egg as evident from the cytological studies, and to show the possible avenues for diploidy restoration in the peripheral nucleus.

During the course of these studies, we examined 78 ovarian eggs with resolvable meiotic figures and 124 deposited eggs (up to three hours from deposition) with resolvable figures. Distribution of the meiotic stages was as follows:

Fig. 3. Thelytokous female *Aphytis mytilaspidis*. *A*. ovariole. *B*. Reproductive system: (a) ovarioles, (b) spermatheca, (c) accessory glands, (d) ovipositor. *C*. Diplotene. Ovarian egg. Snow's carmin. *D*. Diakinesis. Ovarian egg. Snow's carmin. *E*. First meiotic anaphase. Ovarian egg. Snow's carmin. *F*. Mature ovarian egg. Snow's carmin. *G*. First meiotic anaphase. Deposited egg. Snow's carmin. *H*. First meiotic anaphase. Bridge caused by a "four-strand double crossing over" in an inversion heterozygote. Snow's carmin.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STAGE	NO. OF EGGS
Ovarian eggs	
Diplotene	5
Diakinesis	15
Metaphase I	58
Total	78
Deposited eggs	
Metaphase I	10
Anaphase I	10
Telophase I	2
Metaphase II with inner nucleus more advanced	21
Anaphase II (inner nucleus), metaphase II (peripheral)	14
Degenerating inner nucleus, anaphase II peripheral	8
Degenerating inner nucleus, haploid peripheral	8
Degenerating inner nucleus, prophase of peripheral	33
Degenerating chromatic spots, mitotic anaphase	1
Two cleavage prophases, chromatic spots	10
Two mitotic metaphases	5
Two mitotic anaphases	2
Total	124
Total no. of eggs examined	202

II. EGG MATURATION—GENETIC STUDIES

As it remained unclear whether terminal or cleavage fusion is responsible for the restoration of diploidy in the thelytokous form, and since each of these processes has different genetic consequences, genetic studies were conducted, utilizing available eye-color mutations, in an attempt to clarify the actual restoration process. Mutants with brown eyes and peach eyes were found in the arrhenotokous culture, and true breeding lines of these mutants were established through a series of crosses. Our investigations have shown the brown-eye color phenotype to be controlled by two different recessive mutations located on different chromosomes and designated by us *br* and *p*. It was also shown that *br* and *p* are complementary genes and that an individual either homozygous or hemizygous (in case of males) for both recessive alleles will show the peach eye color. The "wild type" wasps have a greenish-yellow eye color. Rössler and DeBach (1972) showed that thelytokous females can mate with arrhenotokous males and produce hybrid thelytokous offspring. Such hybrids, heterozygous for *br* and *p* loci, were obtained by the following crosses: (1) ♂ ♂ *br* arrhenotokous × ♀ ♀ wild type thelytokous; (2) ♂ ♂ *br/p* arrhenotokous × ♀ ♀ wild type thelytokous.

The F_1 hybrid females obtained from the above crosses were then set separ-

Fig. 4. Thelytokous female *Aphytis mytilaspidis*, deposited egg. *A*. First meiotic "telophase." *B*. second meiotic metaphase. *C*. Peripheral nucleus—interphase. *D*. Inner nucleus—premetaphase II. *E*. Second meiotic metaphase of peripheral nucleus and second meiotic anaphase of inner nucleus. *F*. Early second anaphase of peripheral nucleus. Degenerating inner nucleus. *G*. Degenerating inner nucleus. *H*. "First cleavage metaphase" with five distinct chromosomes. Degenerating spots near the egg's stalk. (All staining Snow's carmin.)

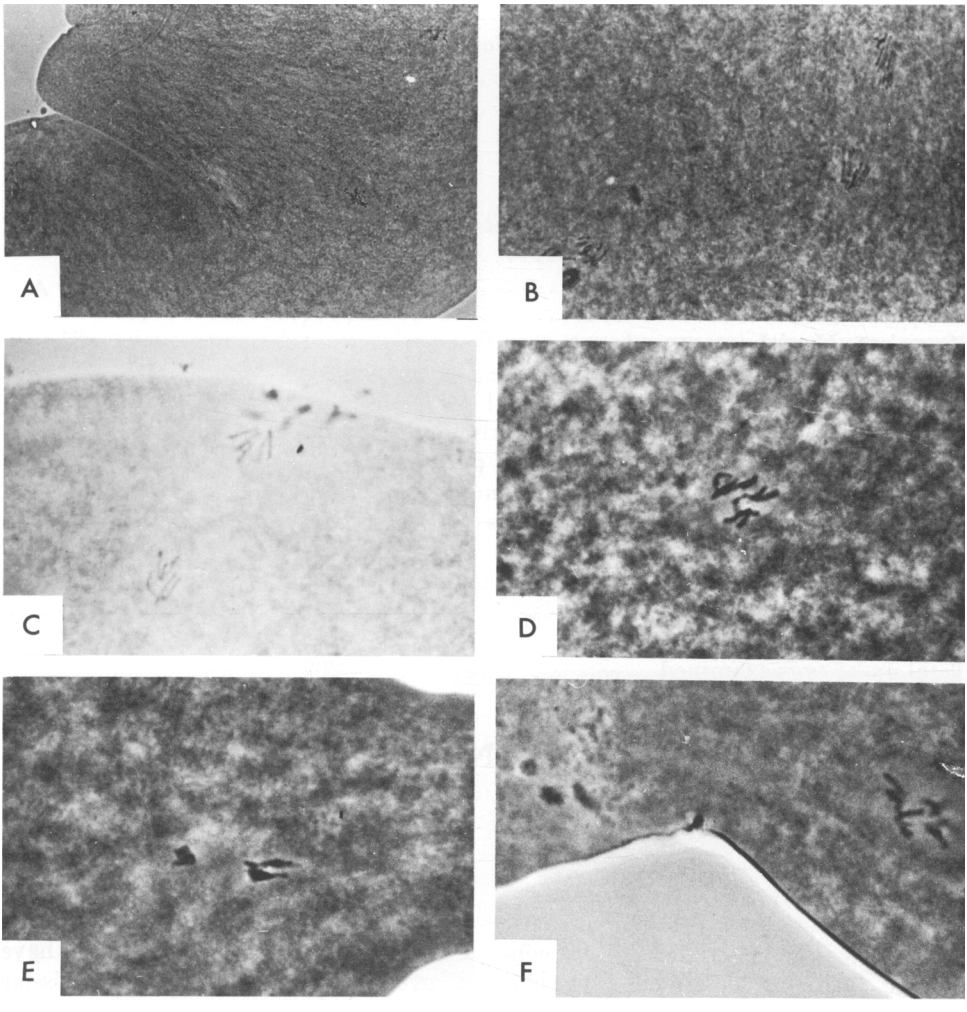

Fig. 5. Thelytokous female *Aphytis mytilaspidis,* deposited egg. *A.* Two cleavage metaphase plates, diploid. A degenerating chromatic spot. *B.* Anaphase, two diploid cleavage nuclei. Arrhenotokous female, deposited egg. *C.* Second meiotic metaphase and anaphase. *D.* Early second meiotic anaphase of the peripheral nucleus. *E.* Degenerating inner nucleus in the second meiotic anaphase. *F.* First cleavage anaphase and three degenerating chromatic spots. (All staining Snow's carmin.)

ately for oviposition. The appearance of brown-eyed and wild type progeny in the F_2 generation of cross (1), and of brown-eyed, peach-eyed, and wild type in cross (2) shows the heterozygosity of the F_1 hybrid female.

Each of the wild type F_2 females in cross (1) and each of the wild type and brown-eyed F_2 females in cross (2) were then set separately for oviposition to determine their genetic constitution through the segregation of the eye-color phenotypes in the F_3 generation. In order to increase the statistical reliability of the conclusions, only F_2 females with seven or more offspring were considered.

In cross (1), 66 wild type F_2 females were tested and three females were found to be heterozygous, producing

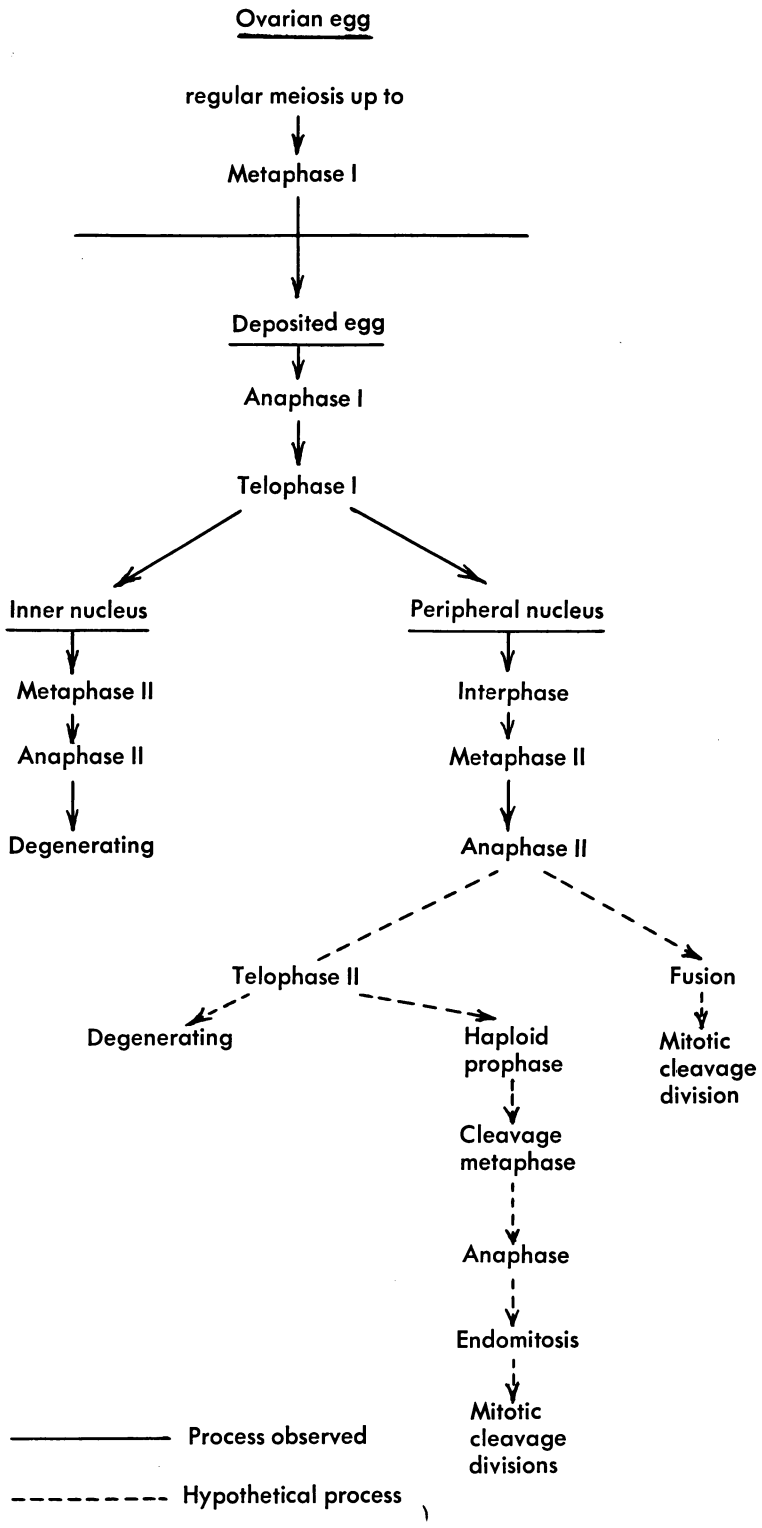

Fig. 6. Reconstruction of the meiotic division in a thelytokous egg.

both wild type and brown-eyed offspring.

In cross (2), 102 brown-eyed and 93 wild type F_2 females were tested; all brown-eyed females were found to be true-breeding homozygotes, four wild type females were found to be heterozygous, producing both wild type and brown-eyed offspring.

If terminal fusion prevails and *br* and *p* are not closely linked to their centromeres, some of the F_2 females should be heterozygous, and phenotypic segregation should appear in the F_3 offspring. If *br* and *p* are absolutely linked to their centromeres, all F_2 progeny should become homozygous, and no phenotypic segregation for *br* and *p* should be evident in the F_3 generation.

Cleavage fusion will render all F_2 females homozygous, with no segregation in the F_3.

Since seven of the 124 wild type F_2 females tested were found to be heterozygous for *br*, it is terminal fusion that prevails, although *br* must be in very close proximity to the centromere (about 5 map units).

III. SEXUAL PROCESSES IN THE THELYTOKOUS FORM

Maintenance of heterozygosity in individuals with terminal fusion depends upon the occurrence of crossing over. Although heterozygosity may be maintained, it cannot be enhanced, and an increase of homozygosity in such lines is inevitable. Eventually each female will become completely homozygous, and her descendants genetically identical. The thelytokous population will then become subdivided into numerous clones differing from each other, but with each clone comprised of genetically identical individuals.

Only the occurrence of sexual processes will allow gene flow between clones, produce new heterozygous combinations, and integrate the whole population into one flexible, evolutionary unit.

Since a thelytokous female will produce female progeny, regardless of whether or not fertilization has occurred, and because no visible mutations have been found in the thelytokous culture, it was impossible to use a direct approach to the study of sexuality in the thelytokous population. The following observations were made in an attempt to determine whether sexual processes might exist which would complement the normal thelytokous restoration processes, thus benefiting the population with the advantages of sexual reproduction.

Material and Methods

Counts of the sex ratio in the regular thelytokous culture maintained at 27°C were conducted over a period of three years. Sperm production by the males was determined by squash preparations of male testes. Functionality and haploidy of the sperm were determined through crosses of the males to virgin arrhenotokous females, some of them homozygous for recessive traits. The F_1 progeny were sexed, and where applicable, the F_2 male progeny were classed according to phenotype.

Results

Frequency of males in the thelytokous culture is summarized in table 1. Males are produced by the thelytokous culture at a low rate, but regularly. The results of March and August, 1969, are based on a small sample and do not necessarily prove the complete absence of males. These males have sperm pres-

ent in their testes (fig. 7).

When emerging males could be secured from the thelytokous culture, they were mated to arrhenotokous females. Twenty-eight crosses were made, and 24 females were successfully inseminated. These females produced a total of 496 females and 377 males. An average of 58.5 per cent (± 19.7) of the eggs were fertilized. These data were compared with data of reciprocal crosses and crosses concerning arrhenotokous ♂♂ × arrhenotokous ♀♀ (table 2).

TABLE 1

FREQUENCY OF *APHYTIS MYTILASPIDIS* MALES IN SAMPLES DRAWN FROM THE THELYTOKOUS CULTURE OVER A THREE-YEAR PERIOD

Sampling period	No. in sample	Males	
		No.	Per cent
March, 1968	177	17	8.76
December, 1968 ...	1,006	171	14.60
March, 1969	190	0	0.00
August, 1969	288	0	0.00
May, 1970	1,058	11	1.00
September, 1970 ..	1,475	3	0.20
December, 1970 ...	422	7	1.63
March, 1971	656	5	0.76

The data in table 2 show no significant difference between crosses in percentage of egg fertilization. The cross "arrhenotokous ♂ × arrhenotokous ♀" produced a higher frequency of fertilized eggs than did the cross "thelytokous ♂ × arrhenotokous ♀" ($\chi^2 = 5.91$; $.01 < p < .02$). When we compare the frequency of fertilized eggs of the reciprocal crosses "arrhenotokous ♂ × thelytokous ♀," however, no significant difference is evident ($\chi^2 = .059$; $.80 < p < .90$).

The results show that thelytokous males are active and fertile and in no way inferior to arrhenotokous males. It should be noted, however, that the

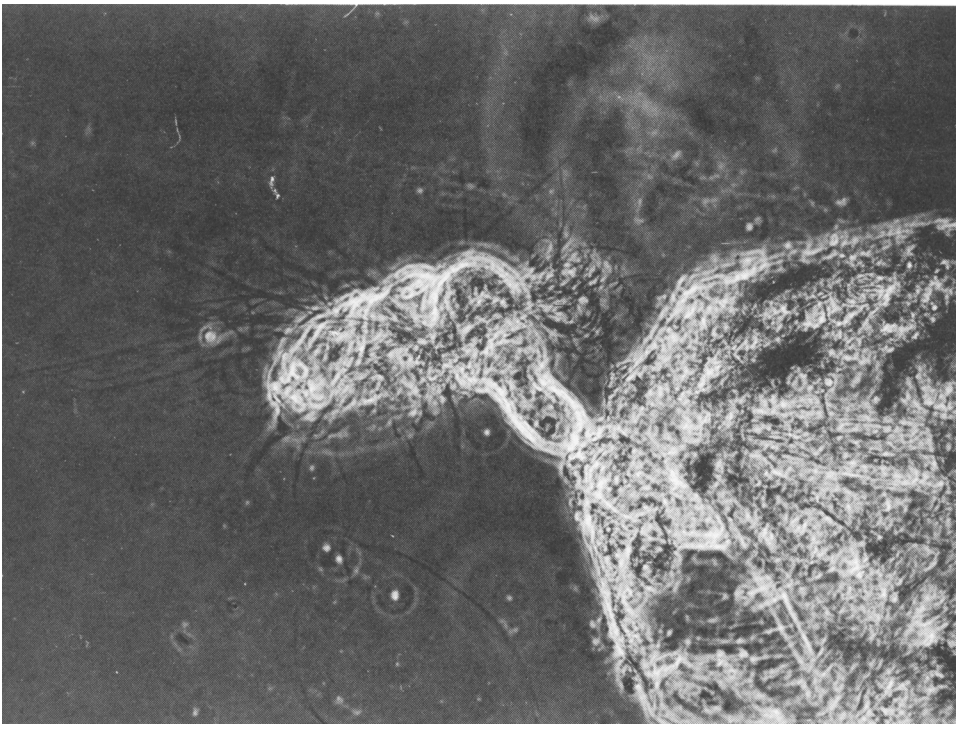

Fig. 7. Crushed testis of a male *Aphytis mytilaspidis* from the thelytokous culture, sperm present.

TABLE 2

EGG FERTILIZATION IN CROSSES INVOLVING THELYTOKOUS AND ARRHENOTOKOUS MALES AND FEMALES OF *APHYTIS MYTILASPIDIS*

Cross	Eggs*		
	No. fertilized	No. unfertilized	Per cent fertilized
Arrhen. ♂ × arrhen. ♀ (11 crosses)	411	240	65.7 ± 20.9
Thelyt. ♂ × arrhen. ♀ (24 crosses)	496	377	58.5 ± 19.7
Arrhen. ♂ × thelyt. ♀ (12 crosses)	119	86	57.5 ± 13.8

* In the crosses involving arrhenotokous females, fertilized eggs produced female progeny and unfertilized eggs produced male progeny. Thelytokous females produced only female progeny. In order to distinguish between fertilized and unfertilized eggs of the thelytokous female we have used in these crosses brown-eyed arrhenotokous males and wild type thelytokous females. The progeny produced by fertilized eggs were wild type but heterozygous (+/*br*). Their heterozygosity was determined through the eye-color phenotypic segregation in their offspring.

crosses using thelytokous males were not conducted simultaneously with the other crosses because of the scarcity of these males, and as a result, the statistical analysis suffers.

The possibility exists that the thelytokous male sperm might be viable but at the same time diploid. Fertilization would thus result in triploid individuals with an extremely reduced fertility. Consequently the males would have no practical value in the thelytokous population. To discern whether sperm are haploid, the following crosses were carried out: (1) wild type thelytokous ♂ × *br/br* arrhenotokous ♀, and (2) wild type thelytokous ♂ × *br/br; p/p* arrhenotokous ♀. Virgin F_1 females were obtained and set for oviposition on cactus scale as host.

If sperm are haploid, we would expect the F_1 females to be diploid and heterozygous (+/*br*) in the first cross and (+/*br; +/p*) in the second cross.

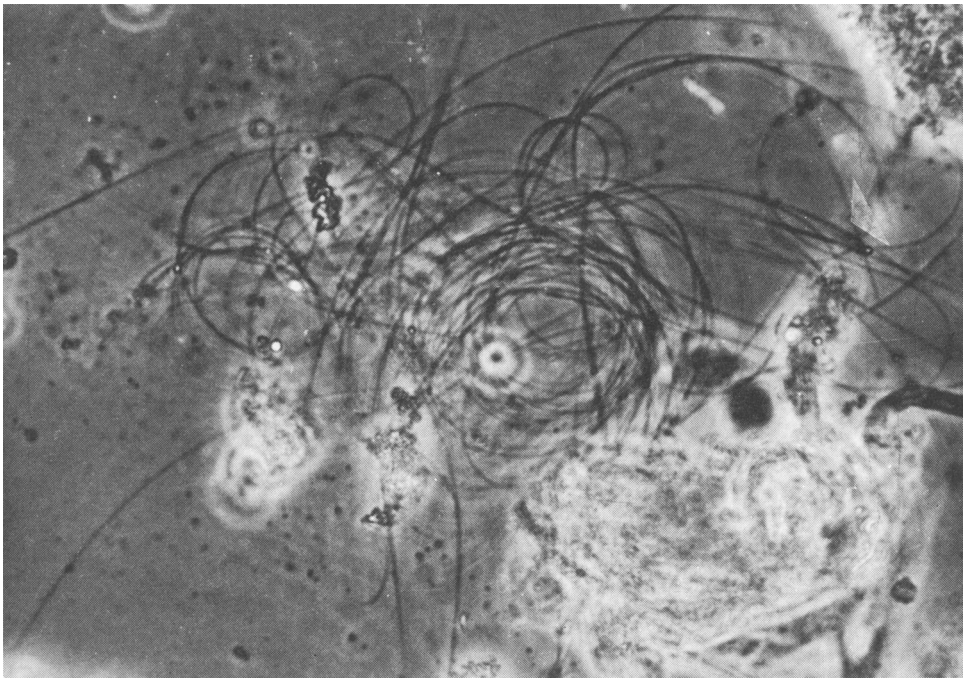

Fig. 8. Crushed spermatheca of a thelytokous female *Aphytis mytilaspidis*, sperm present.

The F_1 females will then produce F_2 males in the following phenotypic ratios: cross (1) 1:1 brown eyed:wild type; cross (2) 1:2:1 wild type:brown-eyed: peach-eyed.

If sperm are diploid, the F_1 females will be triploid and heterozygous (+/+/*br*) in the first cross and (+/+/*br*; +/+/*p*) in the second cross. The F_1 females will then produce F_2 male progeny in the following phenotypic ratios: cross (1) 2:1 wild type:brown-eyed; and cross (2) 4:4:1 wild type: brown-eyed:peach-eyed.

In the first cross, seven F_1 females were propagated to the F_2 and produced 233 wild type and 208 brown-eyed males. The phenotypic ratio is 1.12:1.00, and the deviation from a 1:1 ratio is not significant ($\chi^2 = 1.42$; $.20 < p < .30$).

In the second cross, 12 F_1 females were propagated to the F_2 and produced 212 wild type, 341 brown-eyed, and 195 peach-eyed males. The phenotypic ratios are 1.00:1.61:0.92, and the deviation from the expected ratio for diploidy is significant ($\chi^2 = 6.64$; $.01 < p < .02$), but even more so from the expected ratio for triploidy ($\chi^2 = 191.99$; $p \ll .01$). Rössler and DeBach (1972) have shown that such deviation may occur as a result of higher fitness of the wild type in a mixed thelytokous-arrhenotokous genome, and we may conclude that no triploidy of females resulted from the crosses to thelytokous males, and that sperm of thelytokous males are haploid.

That the thelytokous female is capable of sexual reproduction has already been demonstrated by Rössler and DeBach (1972) by use of arrhenotokous males carrying eye-color mutations. The thelytokous female can be inseminated, and the eggs can be fertilized and developed into diploid, viable, and fertile females. In samples drawn at random from the thelytokous culture, some inseminated females were found (fig. 8) whenever males were present, which shows that some insemination takes place within the thelytokous population. In the cytological studies, we often encountered sperm tails in the thelytokous eggs, but the frequency of such eggs was not determined.

DISCUSSION

In an ongoing revision of the genus *Aphytis* (Howard), an abundance of thelytokous species was recognized (DeBach, 1969). Unpublished data show at least six distinct thelytokous forms in the *A. mytilaspidis* species complex, some of which are sibling forms that can be distinguished only through their biological properties, such as host preference and the like. Since we have no information on the frequency and range of these forms in the field, we can only speculate on their origin or persistence. Followers of the evolutionary dead end theory would argue that these forms represent temporary variants of recent origin that will eventually disappear. Others will argue that these forms demonstrate the ability of a thelytokous form to diverge and adapt to more than one ecological niche. The main obstacle in these arguments lies in the question of whether all thelytokous forms in the *A. mytilaspidis* group have a common origin or whether they have appeared independently of each other.

Both sides will agree that when a thelytokous variant appears with superior adaptive values it has a better chance to preserve those qualities than does an arrhenotokous variant, and will better exploit its ecological niche.

The possibility that thelytoky is of recent origin in Hymenoptera is supported by the fact that in no case do all species of a genus display thelytoky

(Clausen, 1962). On the other hand, species that are totally thelytokous are known over a wide distribution range and are apparently flexible enough to survive under various climatic and ecological conditions—for example, *Aphytis chilensis* and *A. chrysomphali*. Thus the question is not how far these forms can evolve, but rather, what evolutionary mechanisms are available to such forms.

Obviously, mutations serve as a primary source of genetic variability in a thelytokous as well as an arrhenotokous form. In the thelytokous form with which we are concerned, such mutations have a high probability of becoming homozygous immediately and thus being subjected to the test of natural selection upon appearance. Alleles at loci that are totally or partly linked to their centromeres will remain in a heterozygous state and add to the concealed genetic variability of the population. Maintenance of such genetic variability in a form that displays terminal fusion (such as the thelytokous form of *Aphytis mytilaspidis*) can be achieved only at a cost of high genetic load, which means a very low fitness of the homozygotes (Asher, 1970). For example, if we assume both homozygotes to have an equal fitness (=W) and a crossing over value of r for a given locus, then in order for that locus to remain continuously heterozygous: $W = \frac{r}{1-r}$ (when the fitness of the heterozygotes is 1). When a locus shows a crossing over value of 10 per cent, $W = 0.11$, and almost 90 per cent of the homozygotes will perish in each generation before maturity.

A far more efficient tool for the enhancement of genetic variability in a population is the sexual process. As already mentioned, the fact that the thelytokous forms need no sexual processes for reproduction has evoked the notion of the evolutionary dead end. Occurrence of sexual processes in an otherwise thelytokous form is obviously advantageous, as has been shown for cyclical parthenogenesis (Suomalainen, 1950; White, 1964). Parthenogenesis permits rapid increase in a population when it encounters favorable conditions, and enables a single individual to establish colonies. It also serves to preserve fit genotypes when they are produced. Sexual processes permit natural experimentation by formation of new genotypes that can be tested under natural conditions. When both processes (parthenogenesis and sexuality) are combined in a natural population, the advantages are tremendous.

The thelytokous form with which we are concerned is capable of sexual reproduction and apparently employs such processes. The occurrence of sexuality can only benefit the population since the individuals seem to be normal and produce viable and fertile offspring. S. G. Smith (1955), discussing the possibility of sexual processes in a thelytokous form of *Pristiphora erichsonii* (Htg.), has foreseen the production of predominantly triploid sterile individuals as a result of sexuality. In such a case the benefits of sexuality would be reduced by a high degree of genetic load and the loss of genetic material. The thelytokous form of *P. erichsonii* produces males which are able to mate with thelytokous females. According to Smith, four kinds of offspring are produced. Thelytokous offspring develop from unfertilized eggs and are either diploid females, if the restoration process took place in the eggs, or haploid males if they developed from eggs that failed to diploidize. Sexual offspring result from fertilized eggs. Most of these will be triploid and sterile, resulting from the fertilization of diploid eggs. A few diploid females will also be produced by eggs that failed to diploidize but were fertilized. Unfortunately no experimental verification of these assumptions is available to us. However, our

results seem to differ from Smith's. Fertilization of thelytokous eggs of *Aphytis mytilaspidis* resulted in normal diploid females. The rate of failure of diploidization is very low in that form (table 2). Nevertheless, when thelytokous females are inseminated, they produce over 50 per cent of diploid females that originate from fertilized eggs (Rössler and DeBach, 1972). When a sperm enters a thelytokous egg, it apparently competes successfully with the second polar body for conjugation with the egg nucleus, and triploid individuals (produced by simultaneous fertilization and terminal fusion) occur rarely, if at all.

In addition to the intrapopulational sexual process, introgressive processes between the thelytokous form and related arrhenotokous forms (Rössler and DeBach, 1972) may occur, allowing a certain amount of gene flow to prevail between the two forms.

The question arises as to what extent the aforementioned processes occur in nature. We do not wish to imply that such processes are frequent or that all thelytokous forms display sexuality. However, neither the thelytokous culture nor the related arrhenotokous culture in our experiments was chosen with a preconceived notion of possible conformity with the results. The cultures were brought to the laboratory because of the taxonomic complexity of the *Aphytis mytilaspidis* group, with the intention of conducting biosystematic studies without any knowledge of the occurrence of sexual processes within the thelytokous culture or of introgression with the arrhenotokous culture. The fact that these were found during our studies indicates that such processes may be more common in natural populations than was thought. Should a thorough investigtion of more hymenopteran species be carried out, we shall not be surprised to find that these processes prove to be very common in nature.

ACKNOWLEDGMENTS

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APPENDIX

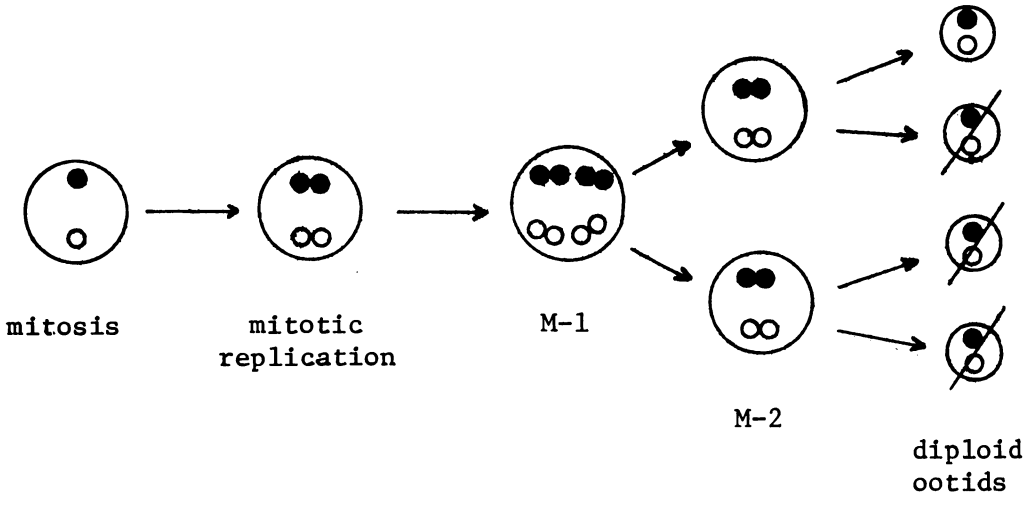
Apomixis

In apomixis with ameiotic thelytoky the meiotic divisions may be lacking completely. No reduction of the chromosome number takes place. According to Suomalainen (1950), the maturation divisions in some cases of apomixis differ from somatic mitotic divisions by a pairing of the chromosomes in various phases of the prophase, where some resemblance to the meiotic prophase was found, but without the formation of chiasmata.

Apart from the occurrence of mutation followed by somatic crossing over in the gonia, or of unequal crossing over, little visible variability can be generated, and all members of a clone tend to be phenotypically identical. Nevertheless, individuals may be highly heterozygous as a result of accumulation of recessive mutations that are not exposed to selection pressures in the usual way.

Automixis

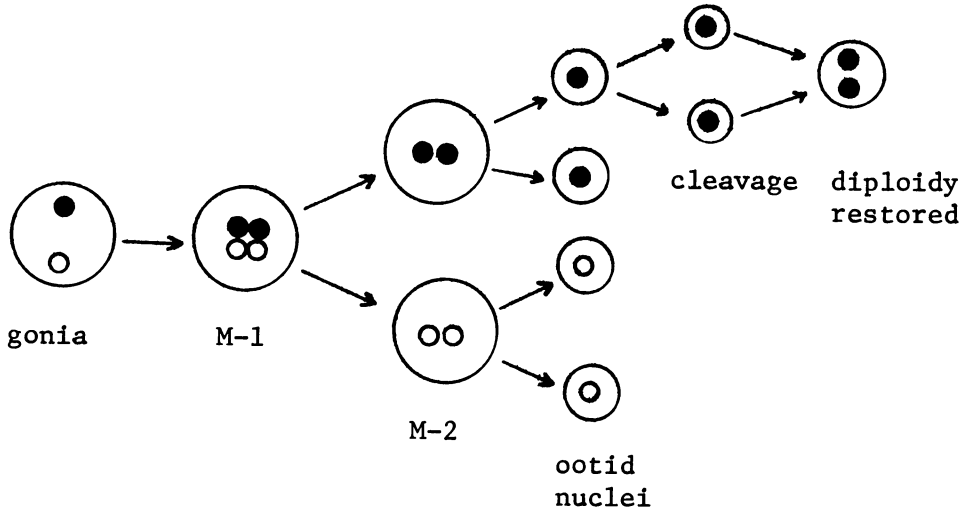
In meiotic thelytoky the meiotic process is retained, at least in part, and diploidy is restored through various modifications of the process, six of which are discussed below and shown in Appendix figures 1–6.



App. fig. 1. Restoration of diploidy in meiotic thelytokous parthenogenesis. Premeiotic replication of chromosomes. Normal meiotic division-1.

1. Premeiotic replication of the chromosomes, followed by a normal meiotic division-1 (fig. 1). White, Cheney, and Key (1963) have described the process in the grasshopper *Moraba virgo* Key. In that species, pairing of daughter chromosomes takes place during prophase-1, and heterozygosity, if present, is maintained. The genetic consequences are similar to those in apomixis, and no phenotypic variability can be generated except by mutation and gonial crossing over.

2. Normal meiotic division-2 followed by fusion of identical cleavage nuclei (fig. 2). Complete homozygosis is reached within one generation regardless of the genetic constitution of the mother. Recessive lethal genes are exposed and eliminated immediately, and



App. fig. 2. Restoration of diploidy in meiotic thelytokous parthenogenesis. Normal meiotic division. Fusion of cleavage nuclei.

the population carries no genetic load. A heterozygous mother may give rise to two or more types of homozygotes, and some short-term generation of variability may be expected. According to White (1970), such a mechanism is very rare and has been described so far only in scale insects and whiteflies (Homoptera). The frequently cited case of cleavage nuclei fusion (Seiler and Schaffer, 1941) in the psychid moth *Solenobia triquetrella* F.R. is now interpreted differently according to White.

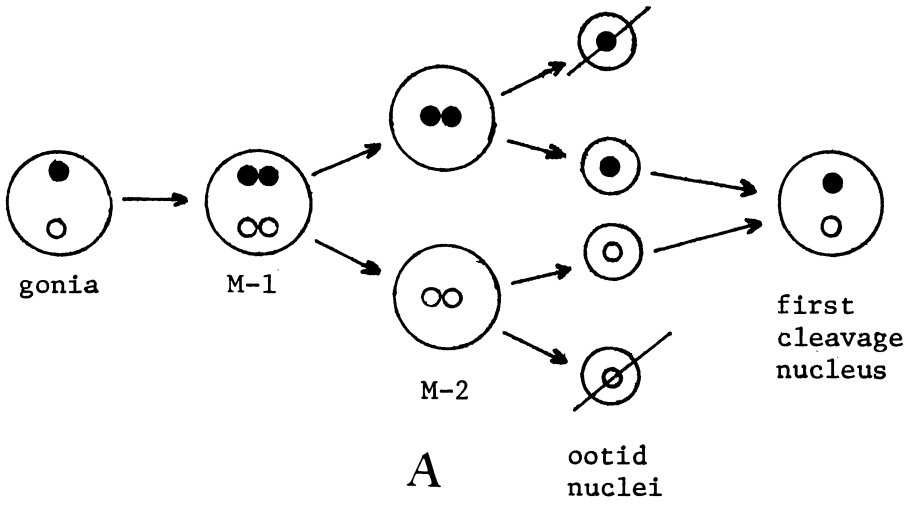
3. Abnormal meiotic division-1 followed by a normal meiotic division-2 (fig. 3, C, D), or normal meiotic divisions-1 and -2 followed by the fusion of haploid non-sister meiotic products (fig. 3, A, B) (central fusion—Asher, 1970). Permanent heterozygosis will be maintained for loci that are completely linked to their centromere, whereas loci that undergo crossing over will become homozygous. Given a female heterozygous for a locus (Aa) with a crossing over value of (r), the progeny of that female will consist of: ($r/4$) homozygotes (AA); ($1 - r/2$) heterozygotes (Aa); and ($r/4$) homozygotes (aa). Thus, genetic variability can be generated, and heterozygosis can be maintained at a cost of high genetic load (a lower fitness of the homozygotes—Asher, 1970). Central fusion was described by Stalker (1954) in *Drosophila parthenogenetica*. Abnormal meiotic division-1 followed by a normal meiotic division-2 (fig. 3, C, D) was described by Speicher, Speicher, and Roberts (1965) in the ichneumonid wasp *Devorgilla canescens* (Grav.).

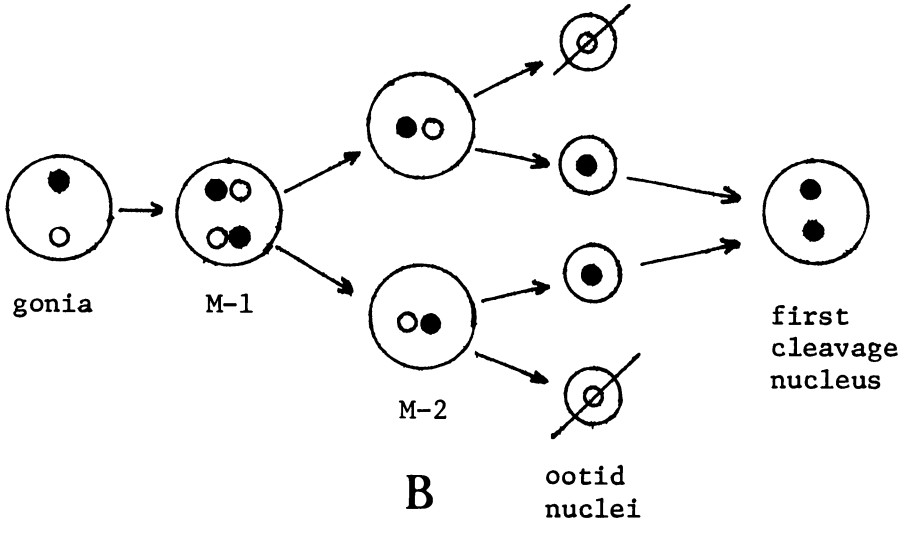
4. Normal meiotic division-1 followed by abnormal meiotic division-2 (fig. 4, C, D), or normal meiotic division-1 and -2 followed by a fusion of two haploid sister products of the meiotic divisions (fig. 4, A, B) (terminal fusion—Asher, 1970). Loci that are linked to their centromeres will become homozygous and maintain permanent homozygosis, whereas loci that undergo crossing over may maintain heterozygosis. Only loci that show complete independent segregation from the centromere will be permanently heterozygous. Given a heterozygous female for the locus (Aa) with a crossing over value of (r), her progeny will consist of: $\frac{(1-r)}{2}$ homozygotes (AA); (r) heterozygotes (Aa); and $\frac{(1-r)}{2}$ homozygotes (aa). Genetic variability can be generated and heterozygosity can be maintained at a very high cost of genetic load. Terminal fusion was described in *Drosophila parthenogenetica* by Stalker (1954). Abnormal meiotic division-2 (fig. 4, C, D) occurs in the coccid *Lecanium hesperidum* according to Suomalainen (1950).

5. Abnormal meiotic division-1 and -2 (fig. 5). One diploid egg nucleus and one polar body are produced. Given a heterozygous female for locus (Aa), her progeny will consist of: $\frac{1}{6}$ homozygotes (AA); $\frac{4}{6}$ heterozygotes (Aa); and $\frac{1}{6}$ homozygotes (aa). Genetic variability can be generated regardless of linkage, and heterozygosity can be maintained, provided the homozygotes are of low fitness. The psychid moth *Solenobia lichenella* L. possesses such a mechanism (Suomalainen, 1950).

6. Abnormal meiotic division-1 and utilization of the products of meiotic division-2 as cleavage nuclei (fig. 6, A, B). No polar bodies are produced. Individuals produced by heterozygous females will become mosaics for loci linked to the centromere (as in fig. 6, A) and will remain heterozygous for loci that undergo crossing over (as in fig. 6, B). Genetic variability can, therefore, be generated through the production of such mosaics. According to Suomalainen (1950) the gall wasp *Neuroterus baccarum* L. possesses such a mechanism.

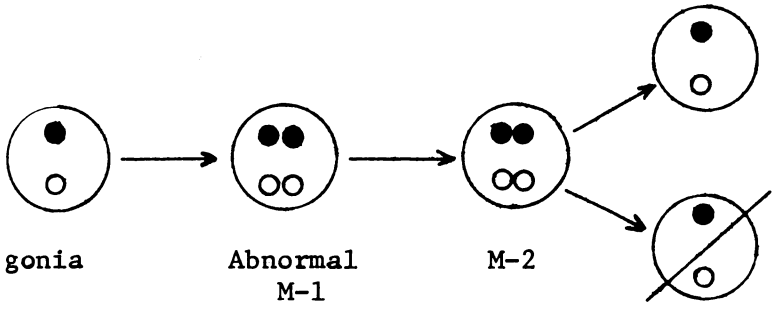
So long as an individual possesses





App. fig. 3. Restoration of diploidy in meiotic thelytokous parthenogenesis. *A*. Central fusion, no crossing over. *B*. Central fusion, with crossing over. Opposite page: *C*. Abnormal meiotic division-1, no crossing over. *D*. Abnormal meiotic division-1, with crossing over.

heterozygous loci that can segregate independently from the centromere, genetic variability can be generated through mechanisms 3, 4, 5, and 6.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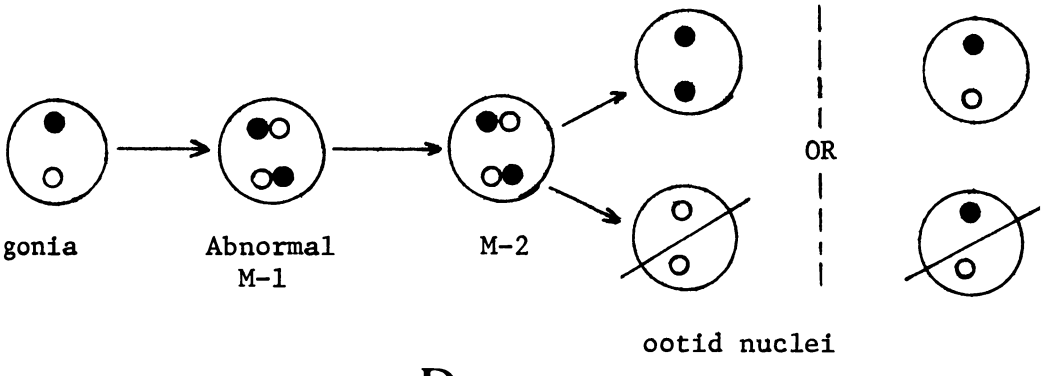
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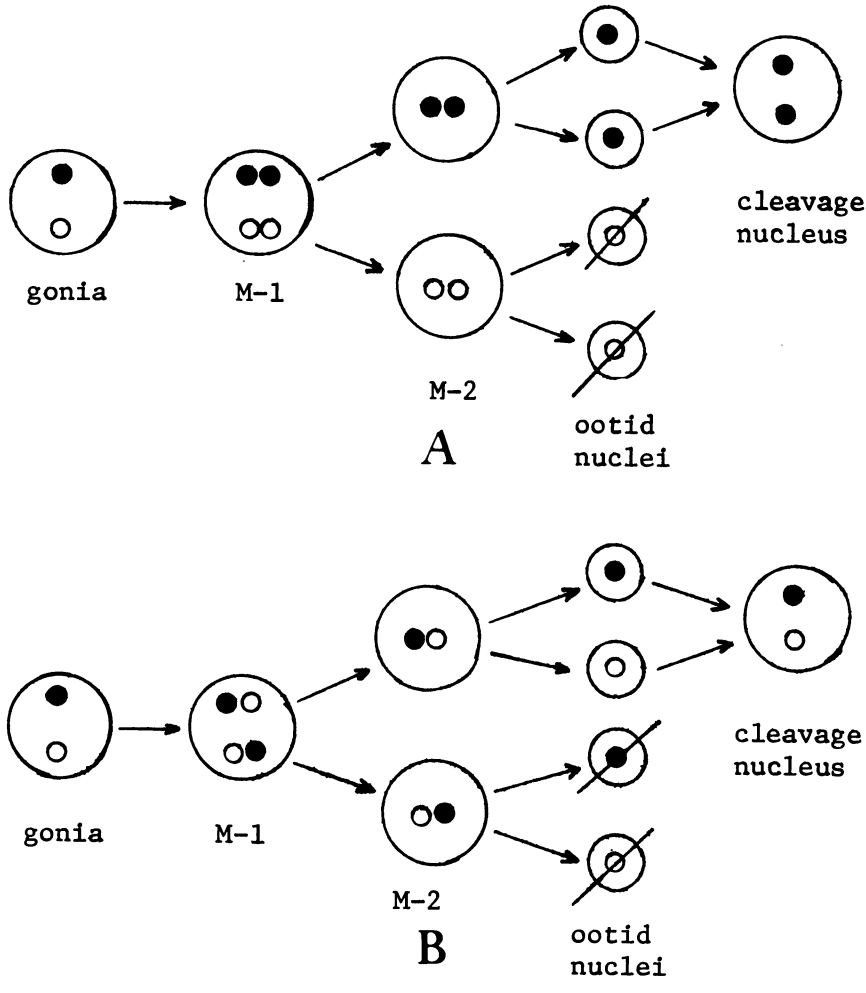
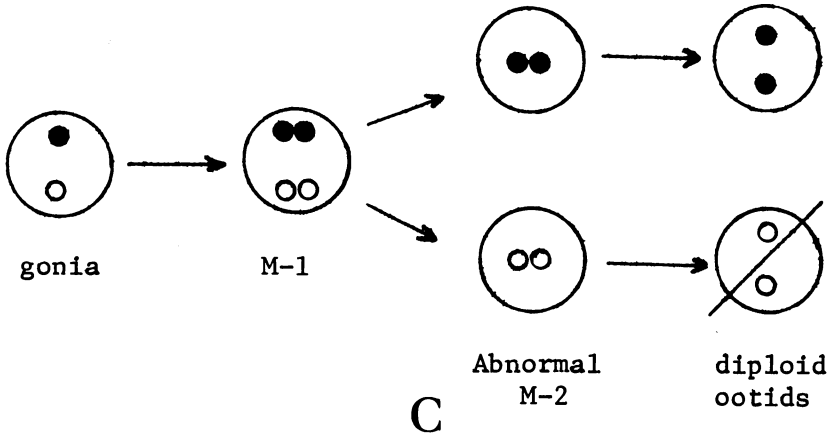


App. fig. 4. Restoration of diploidy in meiotic thelytokous parthenogenesis. *A*. Terminal fusion, no crossing over. *B*. Terminal fusion, with crossing over. Opposite page: *C*. Abnormal meiotic division-2, no crossing over. *D*. Abnormal meiotic division-2, with crossing over.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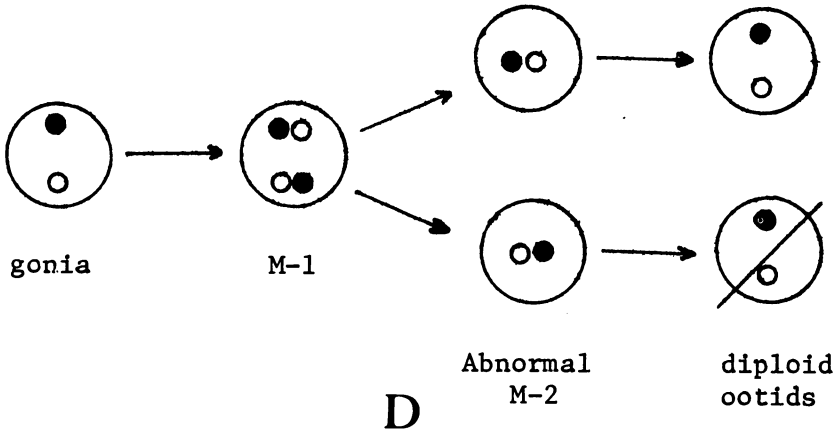
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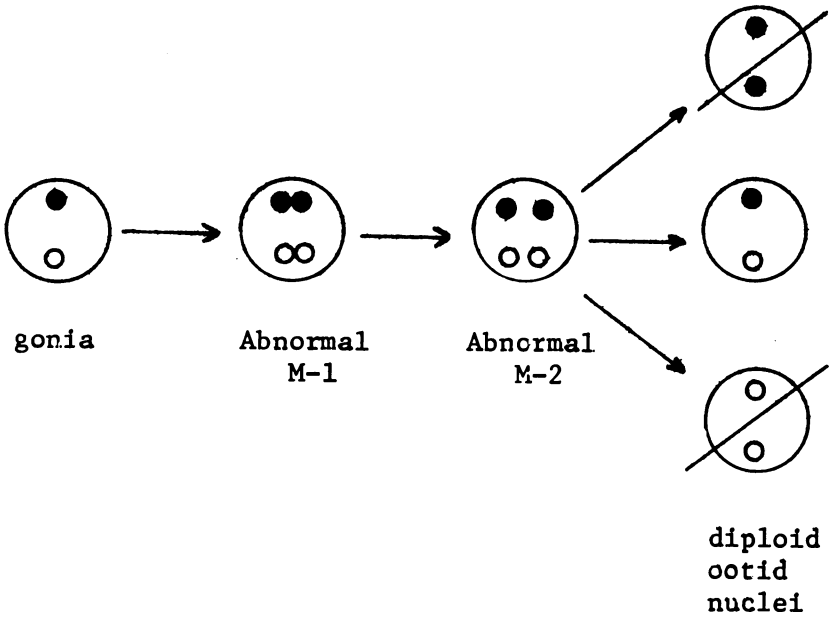
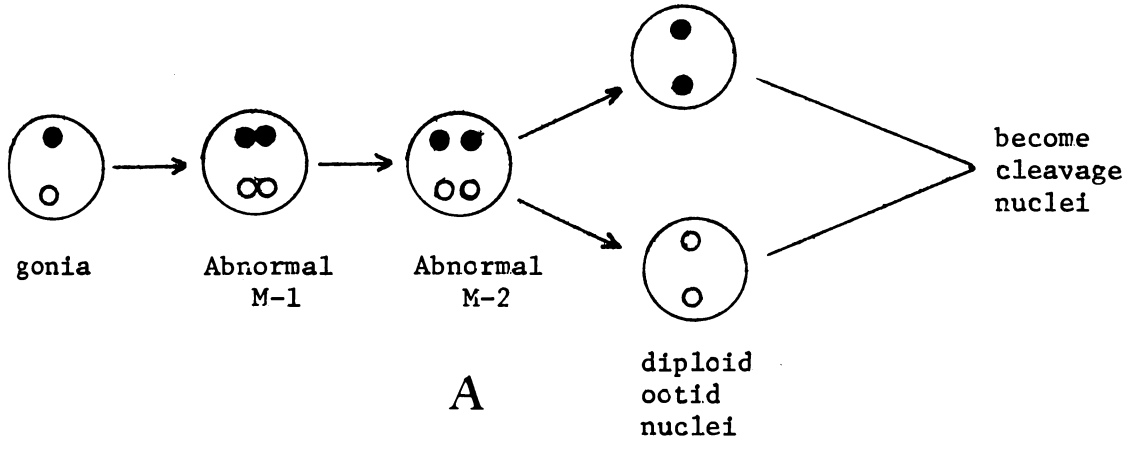
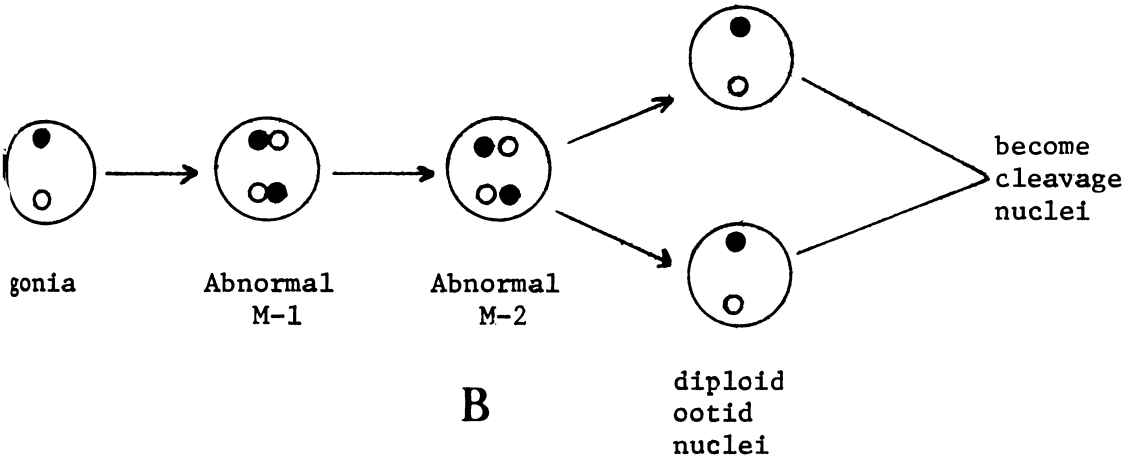


App. fig. 5. Restoration of diploidy in meiotic thelytokous parthenogenesis. Abnormal meiotic division-1 and -2. Heterozygous nuclei are four times as frequent as either homozygote.





App. fig. 6. Restoration of diploidy in meiotic thelytokous parthenogenesis. *A*. Abnormal meiotic division-1, no crossing over, no polar bodies produced. *B*. Abnormal meiotic division-1, with crossing over, no polar bodies produced.

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