HYBRID DYSGENESIS IN *DROSOPHILA MELANOGASTER*: MORPHOLOGICAL AND CYTOLOGICAL STUDIES OF OVARIAN DYSGENESIS[1]

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ABSTRACT

A type of intraspecific hybrid sterility, between two strains of *Drosophila melanogaster*, referred to as *GD* (gonadal dysgenesis) sterility, is observed when females from a type of strain called *M* are crossed with males from a second type called *P*. Absence of egg-laying is characteristic of female *GD* sterility and its manifestation is conditional on high developmental temperatures. Morphological and cytological studies of *GD* sterile females are described. These individuals were normal in body size and external appearance. No defects in sperm storage were observed. Both adult and larval ovaries were drastically reduced in size in comparison with control ovaries. This ovarian dysgenesis was sometimes unilateral, but more frequently it was bilateral, particularly in females developing at the highest test temperature. The ovarioles of dysgenic ovaries contained no vitellaria; the germaria lacked any cells resembling the cystocyte clusters of normal ovaries. It is concluded that sterility results from an early blockage in ovarian development, rather than from atrophy of previously developed structures. Possible mechanisms for this developmental arrest are discussed.

THE crossing of two different types of strains of *Drosophila melanogaster*, called *P* and *M*, results in varying degrees of nonreciprocal hybrid sterility (KIDWELL, KIDWELL and SVED 1977). This sterility is associated with male recombination and other dysgenic traits and is one manifestation of the syndrome of hybrid dysgenesis. Sterility occurs in hybrids of both sexes, but the frequency observed in females usually exceeds that of their brothers. (KIDWELL and KIDWELL 1975). Developmental temperature is critical for the manifestation of this type of sterility. For females, temperatures in excess of 24° tend to be restrictive; for males, the critical temperature range is slightly higher. The temperature-sensitive phase occurs in a period extending from late embryonic development to the second larval instar (KIDWELL and NOVY 1979). External morphology and mating ability appear to be normal, but sterile females do not lay eggs.

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This report describes morphological and cytological studies of the reproductive tract of sterile dysgenic females and compares the observed ovarian lesions with those caused by experimental manipulation and other genetic mechanisms.

MATERIALS AND METHODS

Flies were maintained on a standard corn meal-molasses-yeast-agar medium seeded with live yeast and containing propionic acid as a mold inhibitor. All temperatures were held constant within ±1°, unless stated otherwise.

Stocks used: The following wild-type stocks used in these experiments were maintained continuously in a 25° incubator: (1) Harwich: A strong *P* stock, which in previous experiments produced a high frequency of nonreciprocal, temperature-dependent F_1 female sterility when males were mated to Canton-S females (KIDWELL and NOVY 1979). (2) Canton-S: A strong *M* stock. (3) Oregon-RC: A standard laboratory *M* stock.

For further details of these three stocks, see KIDWELL, KIDWELL and SVED (1977). Consistent with previous usage, matings of Canton-S females with Harwich males are designated cross A. The progeny of such matings show high frequencies of sterility when raised at high temperatures (27–29°). The reciprocal cross is designated cross B. Cross B progeny are expected to have normal fertility.

Observation of adult ovaries: Flies were aged for two to three days after eclosion in order to allow any oocytes present to undergo full maturation. Ovaries were dissected into cold Drosophila Ringer's solution and Feulgen-stained according to a modification of the method of BRACHET and QUERTIER (1963) before whole-mounting in Canada balsam. In some cases, ovarioles were gently spread with tungsten needles prior to mounting. Observations were made with a compound microscope at magnifications ranging from 30 to 300 ×.

Observations of larval ovaries: Ovaries were examined in late third-instar larvae. They were sexed on the basis of size of gonads visible through the cuticle at 30× magnification. Individuals having large, conspicuous yellowish structures visible at the level of the 5th abdominal segment were considered to be males; the reliability of this criterion was confirmed several times by allowing presumptive males and females to complete development and checking the sex of the adults. Ovaries, along with a portion of surrounding fat body, were dissected under 40× magnification using fine tungsten needles and either observed in living condition at 40 to 70× magnification or stained and whole mounted. For staining, the organs were transferred to depression slides containing ice-cold Drosophila Ringer's solution. Just before staining, the ovaries were rinsed twice in ice-cold distilled water and then transferred to a drop of aceto-orcein (2% stain in 45% acetic acid) on a gelatinized slide over which a coverslip was placed. Twenty-four hours later the coverslip was removed and the ovaries dehydrated and mounted in Canada balsam. Ovarian size was measured using an ocular micrometer.

Observations of sperm storage: Sperm storage was observed by staining female storage organs. Seminal receptacles and spermathecae were dissected out, collected, washed and stained in the same manner as were larval ovaries except that pressure was gently applied to the coverslip. Twenty-four hours later the coverslip was removed by the dry ice method and the slide was immersed for 15 minutes in each of the following solutions: 70% ethanol, Ehrlich hematoxylin (counterstain), tap water, 70% ethanol. Dehydration was accomplished by two rapid changes in 100% ethanol before mounting in Canada balsam. Sperm heads were observed using phase contrast microscopy.

RESULTS

The relationships among temperature, ovarian size and sterility

In order to investigate the causes of the hybrid sterility associated with the *P-M* system, individual observations of body size, ovarian size and fertility were made. Cross A female progeny from Canton-S × Harwich matings were com-

pared with those of the reciprocal cross B and Harwich, Canton-S and Oregon-RC intrastrain matings. A set of these five matings was placed at each of the following temperatures: 18°, 21°, 25°, 27° and 29°. Parental flies were removed from the bottles one or two days before eclosion of progeny. Twenty-four hours after eclosion, female progeny were collected and mated individually to three Oregon-RC males in 95 mm shell vials kept in a 25° incubator. After five days, the flies were removed from the vials. Female body length and ovarian length and width of the F_1 progeny were measured, using an ocular micrometer. After 13 days, the progeny were counted.

The results are summarized in Table 1. No cuticular abnormalities were observed. There were no large differences in the overall body length of progeny of

TABLE 1

Observations of ovarian size and fertility of females raised at five temperatures

Parental mating	Temp.	No. of females	Mean ovarian length (in mm)	Mean ovarian width (in mm)	No. flies with unilateral dysgenic ovaries	No. flies with bilateral dysgenic ovaries	% Sterility	Mean no. progeny
Cross A	18°	25	1.21 ± 0.03	0.64 ± 0.02	0	1	4	102 ± 3
(Canton-S ♀ ×	21°	22	1.12 ± 0.04	0.60 ± 0.06	1	0	0	72 ± 3
Harwich ♂)	25°	37	1.01 ± 0.04	0.50 ± 0.02	4	2	5	47 ± 4
	27°	22	0.22 ± 0.01	0.11 ± 0.01	0	22	100	0
	29°	25	0.24 ± 0.01	0.12 ± 0.004	4*	21	100	0
Cross B	18°	24	1.13 ± 0.03	0.54 ± 0.02	1	0	0	97 ± 4
(Harwich ♀ ×	21°	24	1.09 ± 0.03	0.56 ± 0.01	0	0	0	77 ± 3
Canton-S ♂)	25°	37	1.10 ± 0.02	0.54 ± 0.02	0	0	3	75 ± 3
	27°	24	1.01 ± 0.03	0.52 ± 0.02	0	1	4	57 ± 3
	29°	24	0.97 ± 0.02	0.50 ± 0.01	0	0	4	49 ± 5
Harwich	18°	22	1.13 ± 0.02	0.58 ± 0.02	0	0	0	91 ± 5
	21°	19	1.06 ± 0.02	0.58 ± 0.02	0	0	0	62 ± 5
	25°	37	1.08 ± 0.02	0.60 ± 0.02	0	1	5	64 ± 3
	27°	22†	0.93 ± 0.02	0.44 ± 0.01	2	0	0	53 ± 4
	29°	25	0.94 ± 0.01	0.48 ± 0.01	0	0	0	52 ± 2
Canton-S	18°	25	1.15 ± 0.02	0.67 ± 0.02	0	0	0	86 ± 6
	21°	22	1.15 ± 0.02	0.59 ± 0.02	0	0	0	77 ± 4
	25°	37	1.16 ± 0.02	0.61 ± 0.02	0	0	0	65 ± 4
	27°	25	0.96 ± 0.02	0.44 ± 0.02	0	0	0	36 ± 5
	29°	23	1.00 ± 0.02	0.50 ± 0.02	0	0	9	36 ± 4
Oregon-RC	18°	25	1.09 ± 0.02	0.57 ± 0.02	0	0	0	69 ± 6
	21°	24	0.99 ± 0.03	0.51 ± 0.02	2	0	4	61 ± 4
	25°	34	1.15 ± 0.02	0.60 ± 0.02	0	0	6	53 ± 4
	27°	24	0.98 ± 0.01	0.49 ± 0.02	0	0	0	42 ± 3
	29°	24	0.98 ± 0.02	0.53 ± 0.04	0	0	21	25 ± 3

* These flies had only one ovary each.
† One of these flies had only one (normal) ovary.

the five parental matings raised at the same temperature. Body size and ovarian size tended to decrease with increasing developmental temperature in all groups. At 18° and 21°, there were no large differences in ovarian size among the five groups. At 25°, mean ovarian length, but not width of cross A, was slightly reduced compared to that of other groups. At 27° and 29°, all of the cross A females had ovarian dimensions that were between 20 and 25% of those of the control groups, with a corresponding drastic reduction in volume. Over all temperatures, very few flies in the control groups had ovaries this small. Such a marked reduction in ovarian size is now referred to as "ovarian dysgenesis." In this report, a dysgenic ovary is defined as one having dimensions reduced to at least 25% of normal.

As can be seen in Table 1, mean ovarian length and width are closely related to the frequency of dysgenic ovaries. Bilateral ovarian dysgenesis predominated in the 27° and 29° cross A females, but unilateral dysgenesis also occurred, notably in 25° females. Mean progeny production was calculated only for fertile flies. In general, progeny production in all groups tended to decrease with increasing developmental temperature. Progeny production of cross A females was approximately equal to that of the other groups of females at 18° and 21°, but at 25° it was slightly decreased relative to control flies. A very sharp increase in sterility of the cross A females occurred between 25° and 27°, which is not surprising since at 27° and 29°, 100% of the cross A ovaries were dysgenic. It is interesting to note that eight of the nine cross A females with one dysgenic ovary, which had been raised at low and moderate temperatures, were quite fertile; the remaining one produced three offspring, but was still technically classed as fertile. This result is consistent with BODENSTEIN's (1947) finding that experimental extirpation of one ovary results in an increase in the size of the remaining ovary. In contrast, there were instances of sterile (or near sterile) females in the control groups that had two apparently normal ovaries and laid eggs.

From the above observations, it is clear that the sterility observed in cross A females results from an abnormality in ovarian development. Consistent with previous findings (KIDWELL, KIDWELL and SVED 1977; KIDWELL and NOVY 1979), this arrest is conditional on a high developmental temperature.

Observations of adult ovaries

Living ovaries: Regardless of the temperature at which they were raised, the ovaries of the control group flies (Figure 1a) consisted of a number of parallel ovarioles whose vitellaria were swollen with maturing oocytes of progressively larger size in the basal region. The ovaries of control flies raised at higher temperatures tended to be slightly smaller than those raised at lower temperatures, but otherwise were almost invariably of normal appearance. In a few rare instances, a fly in one of the control groups was found to have one or a pair of abnormal, small ovaries.

In contrast, typical examples of unilateral and bilateral ovarian dysgenesis are illustrated in Figures 1b and 1c, respectively. The abnormally small ovaries

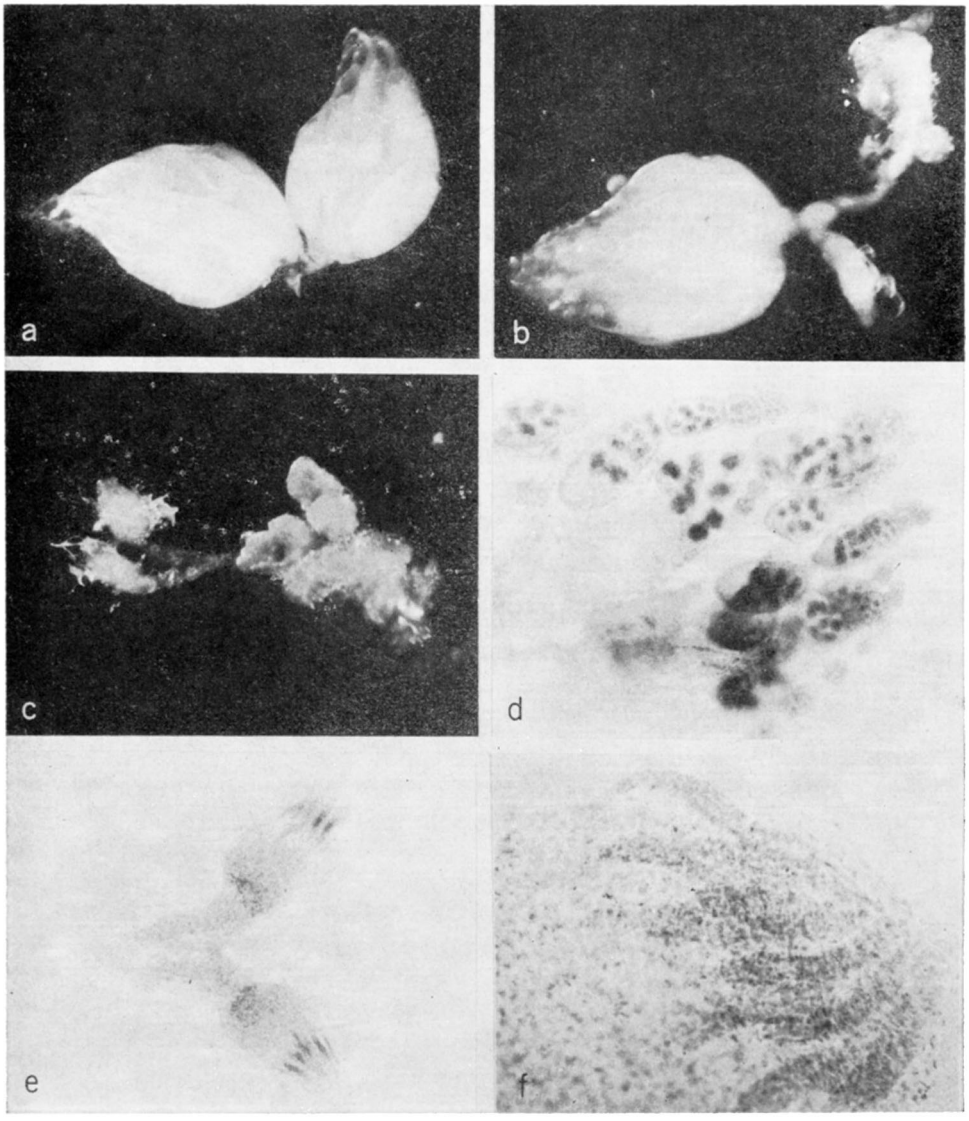

FIGURE 1.—(a) A pair of normal living ovaries from a 25° cross B adult (30×). (b) Unilateral ovarian dysgenesis in a 25° cross A adult. Bottom left: normal ovary; bottom right: dysgenic ovary; upper right: accessory organs (36×). (c) A pair of bilaterally dysgenic, living ovaries from a 27° cross A adult (36×). (d) Apical region of stained normal ovary from a 28° cross B adult (80×). (e) Stained pair of bilaterally dysgenic ovaries from a 27° cross A adult (80×). (f) Detail of apical region of a stained dysgenic ovary (300×).

had the appearance of small stubs of tissue that were covered with numerous white filamentous tracheoles, as were the normal ovaries. All accessory organs (glands, ducts, and external genitalia) seemed completely normal, except that none of these flies had eggs in their uteri.

Since cross A females exhibited normal courtship and mating behavior (KIDWELL and NOVY 1979), the capacity to store sperm was investigated by staining the storage organs of cross A females raised at 27°. The number and distribution of visible sperm heads were the same as for control flies raised at the same temperature.

Stained whole-mounted ovaries: The ovaries of control animals raised at all temperatures contained ovarioles filled with a chain of oocytes at successively advanced stages of maturation. Surrounding each oocyte/nurse cell complex was a monolayer of small follicular epithelial cells. The apices of the ovarioles (Figure 1d) contained stage I oocytes, revealed by intensely stained 16-cell clusters of nurse cell/oocyte complexes. Distal to these were large, intensely stained cells, which were presumably germ cells.

Cross A ovaries from larvae raised at 27° and 29° consisted of uniformly small cells, presumably of mesodermal origin (Figures 1e and 1f). Staining of cells in the basal and peripheral regions was fairly light. Towards the apical region, intensely stained, parallel, finger-like structures about two-fifths as long as the ovary were seen. The ovaries were devoid of even immature oocytes and completely lacked any cells resembling the stage I cystocyte clusters seen in normal ovaries. The cells in the "fingers" appeared to be smaller than the germ cells seen in the Feulgen-stained normal ovaries and described by KING (1970) ABOIM (1945) and others as being larger than the mesodermally derived cells of the ovary. Nevertheless, the level of resolution afforded by whole mounted material does not make it possible to rule out the presence of oogonia. It seems more likely, however, that these structures correspond to the mesodermal elements of agametic ovarioles. ABOIM (1945) and BUCHER (1957) have observed the differentiation of all the mesodermal elements of the ovaries, including the darkly staining terminal filaments, in ovaries of flies in which the germ cells or their precursors had been experimentally destroyed.

Observations of larval ovaries: Late third-instar larval ovaries were observed in living females raised at 21, 25, 27 and 29°, and in whole-mounted preparations from females raised at 21, 25 and 27°. In order to reduce the possibility of experimental bias, larvae produced by the five parental matings were coded prior to dissection. The ovaries were located under 40 to 70× magnification by careful examination of the fat body at the level of the fifth abdominal segment, as described by BODENSTEIN (1950). Because they are minute in size and embedded within the fat body, it sometimes was impossible to find both ovaries in every animal, although at all four temperatures, both ovaries could be identified in 75 to 98% of the control animals examined. In contrast, at 25°, less than half the ovaries could be located in the cross A larvae; five out of 21 of these larvae possessed a pair of normal ovaries, seven of 21 possessed one normal ovary, and eight of 21 possessed no normal ovaries. In cross A females raised at 27° and 29°, only 2 to 3% of larval ovaries were visible under 70× magnification.

In many of the cross A larvae, the region of the fat body where the ovary is normally found had an unusual "pinched-in" appearance (Figure 2a) reminiscent of the arrangement of fat cells surrounding normal ovaries in control animals

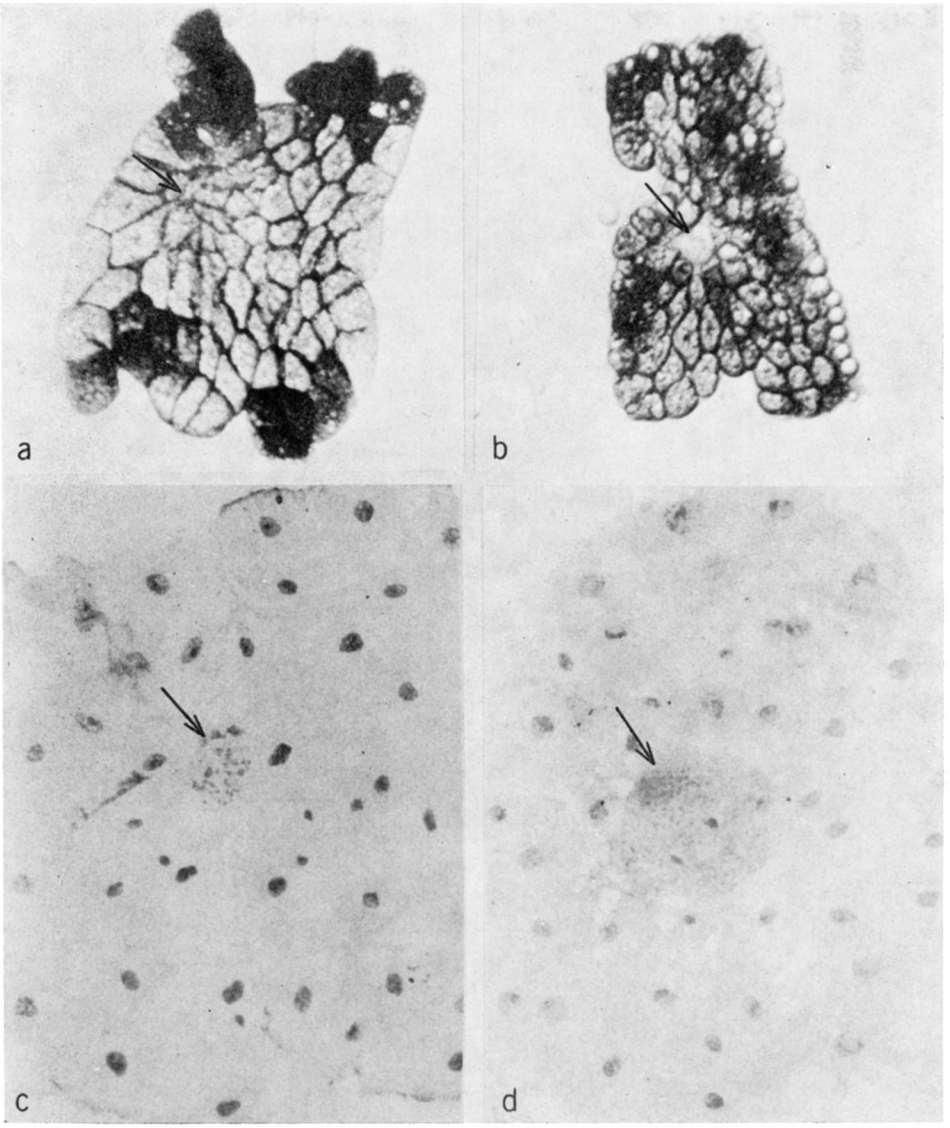

FIGURE 2.—(a) Fragment of unstained fat body containing ovary from 27° cross A late third instar larva (84×). Approximate position of ovary indicated by arrow. (b) Fragment of unstained fat body containing ovary from 27° cross B late third instar larva (60×). Ovary indicated by arrow. (c) Fragment of stained fat body containing dysgenic ovary from 27° cross A late third instar larva (140×). Ovary indicated by arrow. (d) Fragment of stained fat body containing normal ovary from 27° cross B late third instar larva (140×). Ovary indicated by arrow.

(Figure 2b). In order to examine this region of the fat body, a number of them were dissected out, stained and whole-mounted. Cross A ovaries from females raised at high temperature varied widely in severity of abnormality. The contrast

between a typical, severely affected, cross A ovary and a control ovary from a cross B female raised at high temperature can be seen in Figures 2c and 2d. The most prominent difference was a marked reduction in size (Table 2). Mean size of the stained whole mounted cross A ovaries from females raised at 27° was approximately half that of the ovaries of the other groups of larvae. Another difference was in staining properties of the affected ovaries. Some of these organs seemed to consist of only a few intensely stained and irregularly dispersed nuclei. In others, however, the number and compact arrangement of the nuclei more closely approximated the appearance of the control ovaries. There was less morphological disparity among the ovaries from cross A females raised at 25°, though some manifested the above-described severe abnormalities.

DISCUSSION

The ovarian abnormalities observed in cross A hybrid females have several important characteristics that must be taken into account when considering their possible etiology. The most conspicuous features are the small ovarian size and absence of vitellaria and immature egg chambers in both living or Feulgen-stained, whole-mounted ovaries from flies raised at high temperatures. At intermediate temperatures, a significant proportion of cross A females show these abnormalities unilaterally, with the contralateral ovary being in most cases perfectly normal. Bilateral ovarian dysgenesis results in total sterility. The number of flies with unilateral ovarian dysgenesis examined in this study was too small to assess the extent of any impairment in fecundity, but most of these flies seemed quite fertile. A few even produced more progeny than some of the control flies that had two normal ovaries.

Two observations indicate that the developmental block occurs well before eclosion of the adults and, thus, is truly a failure in development, which we call ovarian dysgenesis, rather than a degeneration of developed structures. First, temperature-shift experiments (KIDWELL and NOVY 1979) implicate the period beginning after the first third of embryogenesis and ending partway through the second instar, as the temperature-sensitive phase. Independent experiments by ENGELS and PRESTON (1979) are in reasonably good agreement with these results. Flies exposed to high developmental temperature before or after the temperature-sensitive period are able to complete normal oogenesis and produce progeny. Second, the larval ovaries in cross A flies exposed to high developmental temperatures are often so reduced in size that they must be stained and whole-mounted in order to be seen.

A wide variety of defects are known to result in female sterility in Drosophila. These include single gene mutations (for reviews, see KING 1970; KING and MOHLER 1975), non-Mendelian female sterility resulting from intra- and interspecific hybridization (*e.g.*, PICARD *et al.* 1977; BUCHETON and PICARD 1978; KERKIS 1933) and factors that affect germ cell determination and/or development (for a review, see COUNCE 1973).

The type of female sterility that most closely resembles ovarian dysgenesis is

TABLE 2

Mean length and width of late third-instar larval ovaries of females raised at three temperatures

Parental mating	No.	21° Mean length	Mean width	No.	25° Mean length	Mean width	No.	27° Mean length	Mean width
Cross A*	17	0.163 ± 0.006	0.182 ± 0.007	17	0.106 ± 0.009	0.130 ± 0.011	15	0.063 ± 0.009	0.073 ± 0.009
Cross B	12	0.143 ± 0.006	0.184 ± 0.013	23	0.117 ± 0.008	0.137 ± 0.011	12	0.129 ± 0.006	0.140 ± 0.010
Harwich	8	0.162 ± 0.011	0.184 ± 0.017	23	0.126 ± 0.006	0.145 ± 0.008	6	0.119 ± 0.011	0.131 ± 0.011
Canton-S	6	0.160 ± 0.009	0.180 ± 0.009	20	0.138 ± 0.006	0.165 ± 0.009	12	0.122 ± 0.007	0.138 ± 0.007
Oregon-RC	—	—	—	9	0.110 ± 0.008	0.126 ± 0.008	9	0.155 ± 0.011	0.155 ± 0.011

* Cross A: Canton-S ♀ × Harwich ♂; Cross B: Harwich ♀ × Canton-S ♂.

that which results from destruction of the germ cells or their determinants by experimental manipulation or genetic lesions. Puncture, centrifugation, microcautery, constriction and UV irradiation are some of the methods that have been used to destroy the primordial germ cells (pole cells) or their determinants in the polar plasm (COUNCE 1973). GEIGY (1931), ABOIM (1945), BUCHER (1957), POULSON and WATERHOUSE (1960), HATHAWAY and SELMAN (1961), JURA (1964) and OKADA, KLEINMAN and SCHNEIDERMAN (1974) used certain of these treatments, usually UV irradiation, on young embryos. FIELDING (1967) studied the embryology of the autosomal recessive mutant grandchildless (*gs*) of *D. subobscura*. This mutant is characterized by morphologically abnormal polar plasm and failure of pole-cell formation. Though these investigators pursued different aims, all reported observing unilaterally or bilaterally castrated flies in either the larval or adult stages. Several of these papers contain drawings or photographs of agametic ovaries that bear striking resemblances to the dysgenic ovaries seen in the present study. These resemblances provide further evidence to support the conclusion that germ line defects are implicated in ovarian dysgenesis, which manifest themselves long before maturation of the ovary in pupal or adult life.

Ovarian dysgenesis could be due to: (a) defective pole plasm, (b) nonformation of pole cells, (c) death of pole cells after formation, (d) inability of pole cells to divide initially, (e) retardation or total failure of pole cells to migrate, (f) migration to areas other than the normal site of the gonadal rudiment, (g) failure of germ cells to divide after inclusion in the forming gonad, (h) an indirect effect of abnormal gonadal structural development on oogenesis, or (i) a combination of some of the above. While the above list is by no means exhaustive, the experimental results make it possible to rule out at least some of these possibilities. Defective pole plasm, nonformation, death or nonmigration of the pole cells all seem unlikely because these events take place well before the onset of the temperature-sensitive period. Pole cells have been directly observed to form in cross A embryos raised at 29° at the same time that they form in reciprocal cross embryos raised at the same temperature (SCHAEFER, unpublished results). In addition, the defect probably is not due to a maternal effect causing the deposition of defective germ cell determinants in the egg because *M* strain females produce normally fertile progeny when mated to other *M* strain males. Furthermore, since cross A males are less sterile than females, any of the above defects would presumably affect them equally, since as far as is known (RABINOWITZ 1941; ABOIM 1945; SONNENBLICK 1950) events are the same in the two sexes up until inclusion of the pole cells in the developing gonads. Further experimentation is needed to investigate whether ovarian dysgenesis could be due to the inability of pole cells or later on, of germ cells to multiply, or to other abnormalities, possibly involving germ cell death.

Autoradiographic studies (ZALOKAR 1976) have revealed that less RNA synthesis occurs in the pole cells than in any other region of the embryo until these cells move into the midgut invagination during gastrulation, but that activation of translation systems begins somewhat earlier, at blastoderm formation. If

cytoplasm-chromosome interactions are responsible for the early germ cell defects seen in cross A embryos (KIDWELL and KIDWELL 1975; ENGELS 1979), it may be that a constituent of the *M* strain maternal cytoplasm (perhaps a substance pre-localized in the posterior polar region) is ineffective in *M/P* hybrids in activation of certain transcription processes that are necessary to ensure later functioning and proliferation of the germ cells.

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