Title

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Authors

Hildreth, Philip E.
Lucchesi, John C.

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Philip E. Hildreth and John C. Lucchesi

September 1966

FERTILIZATION IN DROSOPHILA

III. A Reevaluation of the Role of Polyspermy in Development of the Mutant deep orange[1]

Philip E. Hildreth

Lawrence Radiation Laboratory and Department of Zoology, University of California, Berkeley, California[2]

and

John C. Lucchesi

Department of Zoology
University of North Carolina, Chapel Hill, North Carolina

INTRODUCTION

The effects of deep orange (dor), an X-linked (locus at 0.3) recessive mutant of Drosophila melanogaster, were first described by Merrell (1947). In addition to an alteration in the pigmentation of the eyes (which are orange in color), Merrell stated that dor causes a peculiar type of sterility: when mutant females are mated with mutant males all offspring die during the course of embryonic development (Fig. 1a); when mated with wildtype males (Fig. 1b) their daughters survive, their sons do not. However, when heterozygous females are mated with either mutant (Fig. 1c) or wildtype males (Fig. 1d) all of the expected progeny types survive. The results of these crosses suggest that the viability of the embryos depends on the maternal as well as the zygotic genotypes; dor zygotes survive only if the egg cytoplasm is produced by heterozygous mothers.

An extensive investigation of the effects of dor on embryonic development was carried out by Counce (1956). This author reported that when both parents are of mutant genotype, all presumptive daughters

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[2]Mailing address of senior author is Department of Zoology.

and some presumptive sons die during meiosis or the first cleavages (early lethals), but some males survive the early critical period and develop up to or beyond blastulation (late lethals). However, when the father is wildtype the frequency of late lethals is increased; in this case, both early and late lethals are exclusively male embryos, since the heterozygous daughters survive. Counce suggested that the higher frequency of late lethals in the latter cross could be due to the presence of accessory sperm in the egg cytoplasm. These sperm, bearing the wildtype allele of dor, were thought to alleviate the adverse developmental conditions imposed on the zygote by their mutant mothers. Thus, a larger proportion of dor/Y embryos would survive the early critical period if their father were wildtype than if it were mutant, the latter producing only Y- or dor-bearing sperm.

At the time of Counce's report the belief in the occurrence of polyspermy in Drosophila was widespread and her suggestion that accessory sperm may have a beneficial influence on development was considered to be exciting and important.

Our interest in the role of polyspermy in development was renewed when we observed that, contrary to reports by several authors, polyspermy is a rare rather than a frequent occurrence in Drosophila melanogaster (Hildreth and Lucchesi, 1963). Even in fertilized eggs from dor females, in which we had genetic grounds to expect polyspermy (based on the report by Counce), the frequency was low and never were more than two sperm observed in any egg. Coincidentally, we became aware of the fact that dor flies exhibit very limited mating drive. In light of our observations it was deemed worthwhile to

investigate more thoroughly certain aspects of mating behavior, fertilization, and early embryology of dor in order to reassess the influence of accessory sperm on development.

MATERIALS AND METHODS

Stocks

The homozygous dor females were obtained from four separate stocks. These stocks must be maintained in a heterozygous balanced condition, since the matings between dor females and dor males do not produce adult offspring. Females, heterozygous for dor and an X chromosome bearing inversions and genetic markers (see Table 1) were mated with dor males. The offspring included both dor males and females which were used in the experiments. Henceforth, the dor individuals of the first three stocks will be designated by the inversion chromosome balancer and those of the fourth stock by the date of origin (61e) as indicated in Table 1. The designation of the lines by these symbols is important, since the dor allele in the ClB, FM3, and FM4 lines had common origin from the original stock of Merrell, whereas the 61e dor allele arose as an independent mutation in our Laboratory. Since all lines have been maintained separately for several years, in mass cultures, there has been opportunity for the genetic backgrounds of the stocks to become diversified. In order to investigate the possibility that any deviations between our results and those of Counce could be caused by background differences it was deemed advisable to study more than just the original ClB line. It should be stressed that homozygous dor females were used exclusively in the experiments and

the designation refers only to the line from which they were obtained. Wildtype flies were collected from a Samarkand stock that has been mass-cultured for several years in our Laboratory.

Culture Conditions

Initially the stocks were reared on standard fly medium (containing cornmeal, brewer's yeast, molasses, agar, and a mold inhibitor--tegosept) but the viability and fecundity of dor individuals from the FM3 and 61e lines was so poor that a banana medium (containing cornmeal, banana, agar, and tegosept) was tried. Viability was increased, but we were still unable to obtain sufficient eggs from the FM3 line for analysis. No attempt was made to quantitatively analyze the differences on the two types of medium. Wildtype flies were reared exclusively on molasses medium (M). Mutant flies either were cultured exclusively on this or were subsequently reared for two or more generations on banana medium (B). All flies were aged on the same type of medium as that on which they were reared. Unless otherwise indicated in the text or tables the flies were cultured on molasses medium.

Flies were etherized only at the time of collection and thereafter were shaken or aspirated into storage or mating vials in order not to reduce mating efficiency. Matings took place in vials that contained molasses medium.

Cytological Procedures

In order to determine, in certain cases if females had been inseminated, virgins were placed individually in culture vials with two males and left for several hours during which time the surface of the

medium was periodically checked for the presence of eggs. These were collected, gently squashed in Ringer's solution on a microscope slide, and examined. The first egg to be laid after an insemination usually exhibits a large plug of sperm adhering to the surface; other eggs, if fertilized, reveal the actively moving sperm within or near the anterior end.

For the study of developing embryos produced by mutant mothers, eggs were collected, following the method of Hildreth and Brunt (1962), fixed in Bradley-Carnoy solution (3 parts 100% ethanol:4 chloroform: 1 conc. glacial acetic acid), and stained by the Feulgen procedure of von Borstel and Lindsley (1959). Some were bulk stained and sectioned or prepared as whole-mounts; others were affixed to coverslips, fixed, and stained. Eggs were examined with a compound microscope at approximately 1000 magnifications.

RESULTS

Mating Behavior

Certain aspects of our interpretations depend on the frequencies of unfertilized eggs laid by dor females when mated with dor as opposed to wildtype males. Thus it was necessary to know the frequencies of copulations within each possible mating situation. Single virgin females of all lines except C1B and two males were placed in individual shell vials and observed at 5-minute intervals, and the number of copulations was recorded (see Table 2). The experimental design for the C1B line was different; a series of vials, each containing 12 dor females and 12 males, was set up and 24, 48, 72, and 96 hours later

females were dissected and their seminal receptacles examined for the presence of sperm (see Table 3). The dor males are greatly inferior to the wildtype in their mating propensities and are also less active in general. Thus, if virgin females were left with males, for a day or so, the females that had been with dor males would undoubtedly lay more unfertilized eggs than would those that had been with wildtype males. Males from the FM4 line mate more frequently and rapidly than males from the other lines but even they are much inferior to wildtype. The data also suggest that there is an inter-line difference between the females (see Table 2) in their receptivity to wildtype males.

Frequencies of Unfertilized Eggs from Inseminated Females

In order to determine the frequency of unfertilized eggs laid by inseminated dor females, and to ascertain if this frequency was influenced by the type of male used, eggs were collected from ClB and wildtype females known to have been inseminated by mutant or wildtype males. The eggs were prepared for cytological examination and scored for the presence of sperm or cleavage figures. The data are presented in Table 4. A homogeneity test of these data reveals that eggs from wildtype mothers have a greater chance of being fertilized than do those from dor mothers, but that sperm from the two types of male do not differ significantly in their ability to fertilize either type of eggs.

Mention should be made here, since it bears on the reliability of our cytological diagnosis, of some observations we made on eggs from uninseminated females (other than dor). The meiotic stages in these eggs were found to be cytologically similar to those in fertilized eggs. Polar body formation is also similar except that, occasionally,

a polar nucleus appears to fuse with the pronucleus.

Polyspermy

The fecundity of the FM3-line dor females was so low that no data on polyspermy or early embryology were available from these. Eggs were collected from C1B, FM4 and 61e females that were known to have copulated, and those eggs in meiotic stages through late telophase II were scored for the number of sperm present (see Table 5). Homogeneity tests show that chance deviations alone may easily account for the differences in frequencies among the four groups (X^2=5.3484, p = 0.1-0.2, d.f. = 3) as well as between those of the dor vs non-dor groups (X^2 = 0.7229, p = 0.3-0.5, d.f. = 1). Thus, polyspermy occurs infrequently (4.8% of 457 fertilized eggs), and very seldom is there more than one accessory sperm (1/457 = 0.2%).

Meiosis in Unfertilized Eggs

Prior to 1958 it was generally believed that the eggs of Drosophila melanogaster would not undergo meiosis unless fertilized. The publications by Doane (1958, 1960) have shown that, indeed, meiosis is completed in unfertilized eggs. It seemed possible that eggs from dor mothers might be exceptional in this regard since, apparently, they were exceptional in other respects. For the future discussion it was important to know whether meiosis is or is not completed in the absence of fertilization. From virgin dor (C1B) females eggs were collected over $\frac{1}{2}$-hour periods, aged for 1 hour from the end of each collection, fixed, and stained. Seventy-four of the 75 eggs examined had completed meiosis, one being arrested in telophase I. Two of the remaining 74 eggs were aberrant; even though the reduction divisions

had been completed one egg had chromosomes characteristic of telophase II and the other had nuclei characteristic of prophase of syngamy. The normal situation is represented by the 72 eggs that had completed the reduction divisions and then progressed through the interphase and prophase stages. These eggs had either one large aggregation of chromosomes which contained all four products of the reduction divisions, an aggregation of the contents from the three polar nuclei plus a separate group that represented the pronuclear chromosomes, or, in a few instances, chromosomes that were scattered throughout the cytoplasm. Thus, the observations on the completion of meiosis in unfertilized eggs from dor females are consistent with those of Doane on eggs from uninseminated wildtype females.

Early Development of Zygotes

For studies with the C1B line, since this was the line used by Counce, eggs were collected from inseminated females exclusively; in the other lines collections were made from females that were known to have copulated. Eggs collected over 20-minute periods were aged for 2, 4, or 6 hours, then fixed and stained. According to Rabinowitz (1941), eggs reared at 24°C should be in a stage from telophase II of the second maturation division to conjugation of the pronuclei within approximately 15 minutes after fertilization. The first cleavage occurs at about 24 minutes and there are then several rapid mitoses in the central oöplasm. At the 9th cleavage (about 99 min) the nuclei migrate to the periphery, forming the blasteme stage. After one or two additional mitoses the periphery is packed with nuclei (about 2 hr), and it is shortly after this time, when there are about 4000 nuclei, that cell walls form and the blastula stage is reached. At 25°C, gastrulation

occurs at about 3 hours, and between $3\frac{1}{2}$ and 3-3/4 hours the amnio-proctodael invagination forms, deepens, and extends posteriorly. In order to determine whether the 2- and 4-hour embryos, respectively, were in approximately the correct developmental stages, the blasteme-blastoderm and amnio-proctodael stages were used. No attempt was made to determine more precisely the time of death. The results are presented in Table 6. None of the zygotes died during meiosis ("early" early lethals) and, in fact, when aged for 6 hours, 96-100% of the embryos developed to or beyond the stage of amnio-proctodael invagination. The high frequencies of unfertilized eggs in the FM4 and 61e lines is a reflection of the technique used. Even though the females had copulated some were not inseminated. This may be due to testis abnormalities which have been observed in dor males (to be described in a future publication). In some males the abnormalities were so extreme that even though these individuals copulated there could not be a transfer of sperm to the storage organs of the females. Development appears to be slowest in the 61e line, but by 6 hours all individuals had developed to the amnio-proctodael stage.

DISCUSSION

It has been reported by Counce (1956) that when dor females are mated with dor/Y males the daughters die prior to nuclear migration (early lethals) and subsequent blastoderm formation; some sons die at this early stage but others develop to or beyond blastulation. When the father is dor^{+}/Y the frequency of "early" early lethals (during or prior to first cleavage) among dor/Y zygotes is reduced, the higher frequency of late lethals being accounted for by the presence and activity

of the dor$^+$-bearing accessory sperm in the cytoplasm. This is unusual, since the genes carried by these sperm do not become a part of the embryonic genome. Metabolic phases of the supposed supernumerary sperm nuclei may be different from those derived from fertilized nuclei. They may--hypothetically--never divide but be active at a time during which the major activities occurring in the egg are those leading up to fusion of the pronuclei in preparation for the first mitotic division. Furthermore, during the next hour or two (following syngamy) the metabolic phases of the nuclei must be relatively short, as there are at least ten more mitoses before blastoderm formation. During this time, the primary concern of the genome would appear to be the control of mitotic divisions, resulting in the production of a few thousand nuclei in a cytoplasmic syncytium.

It seems desirable to analyze in some detail the frequency of polyspermy necessary to account for the higher proportion of late lethals among dor/Y embryos, observed by Counce, when dor females mated with wildtype than when they mated with dor males. Among 164 eggs laid by dor females that had opportunity to mate only with dor males, Counce classified 109 as early lethals and 55 as late lethals; among 158 eggs laid by females that had opportunity to mate only with wildtype males, 27 were classified as early, 66 as late lethals, and in addition 65 adult daughters eclosed. If one assumes a 1:1 sex ratio and that all eggs were fertilized (assumptions made by Counce), then the frequency of late lethals among males in the former cross would be 67.1% (55/82) and in the latter 83.5% (66/79). This means that 16.4% (83.5 - 67.1, since totals are approximately equal) of the male zygotes, in the latter cross, would carry at least one dor$^+$ accessory sperm. Zygotes in our C1B

line never contained more than one accessory sperm, and there should be equal chances that this sperm would carry either an X or a Y chromosome. Therefore, if 16.4% of Counce's dor/Y zygotes have an accessory sperm that contains X^{dor^+} there should also be 16.4% that have a Y-bearing accessory sperm; thus the total frequency of dispermy should be 32.8%. We found 7.6% dispermy (5.5% for all dor lines combined) by cytological examination; the probability that chance alone could account for the difference between our observed frequency and the theoretically necessary frequency is much less than 0.0001 (X^2 = 25.38, d.f. = 1). Thus, the cytological evidence makes it appear extremely unlikely that polyspermy has the effect on development that has been proposed. At the time of Counce's publication it was believed that each fertilized egg contained five or six accessory sperm. This belief gave a sound basis for the above proposal on the influence of accessory sperm, since with the latter degrees of polyspermy there would be 31 chances in 32 $[1-(1/2)^5]$ or 63 chances in 64 $[1-(\frac{1}{2})^6]$ that an egg would have at least one dor^+-bearing accessory sperm.

In numerous organisms, the first recognizable effect of sperm penetration is the "activation" of the egg, which usually consists of a cortical reaction and a signal to complete meiosis. This is not the case in Drosophila, in which to date no fertilization reaction has been demonstrated and also in which meiosis is known to take place in the absence of fertilization (Doane, 1958, 1960). If unfertilized eggs laid by dor mothers also complete meiosis, Counce's data and interpretations would imply that meiosis is arrested in the majority of cases (21/34 eggs = 62.3% "early" early lethals) when fertilized with sperm from

dor/Y males; fewer eggs (5/24 eggs = 20.8%) fail to complete meiosis when penetrated by sperm from wildtype males. An alternative explanation would be that eggs from dor females are exceptional in that they do not complete meiosis unless fertilized. If this alternative were correct then dor-bearing sperm might initiate but not allow meiosis to proceed properly; a Y-bearing sperm might allow completion in some cases and dor^+- bearing accessory sperm in these eggs could give added impetus for more zygotes to develop to or beyond early cleavage stages. Since it is now known that eggs complete meiosis in the absence of fertilization, and that fertilized eggs develop through approximately twelve cleavages when they have a Y but no X chromosome (Poulson, 1940) or even when deprived completely of sex chromosomes (von Borstel and Rekemeyer, 1958), it seems extremely unlikely that an X-chromosomal mutant would inhibit either meiosis or early cleavages. It should be noted that the capacity for a zygote to undergo several cleavages probably depends on whether the cytoplasm has the necessary components to sustain mitotic activity as well as on the chromosomal makeup of the zygote. In oogenesis, multiplication of a cystoblast results in the production of 16 cells interconnected by canals (see King, 1964, for review). This permits the flow of molecules (including DNA) from the 15 nurse cells into the oocyte. When the oöplasm, built up in this fashion, has reached its final volume, the oocyte is sealed off from the degenerated nurse cells by the vitelline membrane. Ritossa and Spiegelman (1965) have recently demonstrated that the synthesis of ribosomal RNA is dependent upon the presence of and is directly proportional to the number of nucleolus-organizing regions in the genome. Since these regions are on

the sex chromosomes in D. melanogaster, zygotes lacking both sex chromosomes (such as those studied by von Borstel and Reckemeyer) must depend for their preblastula development on products (proteins or components of a protein-synthesis system such as ribosomes, long-lived messenger RNA, and appropriate enzymes) already present in the egg when it is fertilized. In the case of dor mothers these products would be synthesized by nurse cells and assimilated by presumptive oocytes, both dor/dor in genotype, and therefore might possibly be incapable of normal oöplasm production. Thus, dor mothers might not be able to supply cytoplasm that would support meiosis or early cleavages in zygotes deprived of sex chromosomes. It would be interesting to know if such is the case, but, there is, as yet, no evidence on development of nullo-X-Y zygotes from dor mothers.

The above considerations, added to our observation that the incidence of polyspermy in dor is low, led us to believe that a more plausible interpretation of Counce's data (1956) would be that

(a) unfertilized eggs complete meiosis and might be mistakenly identified as early lethals;

(b) dor females are inseminated less frequently by dor than by wildtype males, therefore, samples of eggs from the former would contain a higher frequency of unfertilized eggs than from the latter; and

(c) fertilized eggs complete meiosis and probably develop to the blastula stage at least, regardless of whether the sex chromosome of the male pronucleus is X^{dor} or Y; one might expect occasional exceptions due to cytoplasmic deficiencies of individual eggs.

Our cytological observations demonstrate that eggs from virgin dor females are not exceptional in their meiotic behavior. They complete meiosis (74/75 eggs), therefore insemination of the egg is not necessary to initiate maturation divisions (three eggs had chromosome configurations typical of presyngamy zygotes; these are discussed below). In her experiments Counce found that the first maturation division is apparently normal, but that eggs that cease development during the second maturation division are characterized by "large, distorted multipolar spindles on which the chromosomes are scattered helter-skelter." In 1960 von Borstel reported similar observations, but these were on postmeiotic activity in wildtype eggs that had been aged for periods ranging from 2 to 7¼ hours. The eggs were classified into four categories, which apparently reflect chronology, exhibiting (1) one to three normal appearing metaphases, (2) basket metaphases, (3) multipolar spindles, or (4) pycnotic metaphases. Although there are no figures in the report by von Borstel, the detailed descriptions of the basket metaphases and multipolar spindles closely fit the description (and figure) by Counce of the "early" early lethals that stop development before the first cleavage. Our samples from virgin females had some eggs with very clear metaphase spindles in the region where the first cleavage would occur. The chromosomes undergo replication and the diploid number of chromosomes could then be characteristic for metaphase of the first mitosis. Counce's descriptions of abnormalities beyond the first cleavage as having abnormal or multipolar, or polyploid spindles, clumped nuclei or nuclei of various sizes, and many

small cytoplasmic islands whether they contain one or several nuclei compare well with von Borstel's descriptions of polyploid spindles, and basket and pycnotic metaphases. Thus, it appears that unfertilized eggs were mistakenly identified as early lethals.

Our data show that during a given period of time (Tables 2 and 3) many more dor females copulate with wildtype than with dor males. For her egg collections, Counce used females that had been placed together with males on the previous day; since copulations were not individually observed, it is unlikely that all females copulated and even those who did were probably not all inseminated. Thus, it is very probable that there were unfertilized eggs in her samples and the frequency would be highest when females were together with dor rather than wildtype males. This difference in frequency persists even when precautions are taken to use only inseminated females (Table 4). If one assumes that the sex ratio among fertilized eggs is 1:1, as did Counce, but that eggs classified by her as early lethals were actually unfertilized, then her data could be reinterpreted as follows:

dor ♀♀ x dor^+ ♂♂ = 27 unfertilized (instead of early lethals), 66 late lethals (dor male embryos) and 65 dor/dor^+ daughters;

dor ♀♀ x dor ♂♂ = 109 unfertilized (instead of early lethals) and 55 late lethals. It should be noted that in the former cross the sex ratio of 66 lethal male embryos to 65 adult daughters is very close to the expected 1:1 ratio for fertilized eggs, and also that the frequency of unfertilized eggs (27/158 = approximately 17%) is much lower than from the females that might have mated with dor males (109/164 = approximately 66%).

Evidence gathered on embryonic development of dor individuals (Table 6) demonstrates that, if the egg is fertilized, meiosis is completed regardless of whether the sperm nucleus brings into the oöplasm a Y chromosome or a dor-bearing X chromosome. In fact, most fertilized eggs developed to the stage of amnio-proctodael invagination, which occurs at approximately $3\frac{1}{2}$ to 3-3/4 hours after fertilization. Accordingly, there are no early lethals in the sense used by Counce. The possibility exists that the sexes may differ with respect to the lethal phase which occurs after the time of amnio-proctodael invagination.

Since the initiation of our work, Dr. Counce-Nicklas has kindly consented to reexamine her slides and has indicated to us that some precleavage eggs were indeed unfertilized but that a few eggs, exhibiting meiotic figures, do contain a sperm. These eggs may be similar in nature to the three cases, in our material collected from virgin females, with chromosome configurations typical of presyngamy zygotes.

Another aspect to be considered in explaining the presence of what appear to be meiotic lethals is the time relationship between insemination and maturation divisions of the egg. If fusion of the pronuclei, which results in a diploid cleavage nucleus, is to occur, then not only must these pronuclei be in the proper place but also in the proper condition for union and subsequent cleavage. Since meiosis occurs regardless of insemination, there must be a regulative mechanism that, in most cases, accurately governs the time of sperm penetration and thus ensures the normal, coordinated sequence of events leading to syngamy. Insemination of the egg occurs in the uterus (Nonidez, 1920). Eggs in the ovarioles do not have metaphase I or later maturation

division figures (see King, Rubinson, and Smith, 1956 for oocyte development), therefore at some time after the egg leaves the ovariole meiosis is initiated, and this normally would be at or very close to the time of sperm entrance. It is probable that syngamy will occur only if the sperm penetrates the egg during a rather precise period, and the correct timing is regulated by the oocyte's being in such condition, when it leaves the ovariole, that it will normally start its maturation division at the time the sperm penetrates. If penetration is too late the sperm may not differentiate nor form a pronucleus in time for syngamy to occur. In our fertilized eggs, fixed immediately after collection, we have seen a few examples to support this view. These eggs were in second maturation divisions but the sperm were characteristic in morphology and position of those normally observed when the egg chromosomes were in anaphase I. This phenomenon cannot be ascribed to an inherent effect of the maternal dor genotype on the oöplasm constituents, since it has also been observed by Schneider-Minder (1966) among eggs collected from a Berlin wildtype strain. In approximately 5% of the eggs collected and aged for 3 hours prior to fixation, this author reported that meiosis had begun (as evidenced by the presence of meiotic chromosomal configurations), yet sperm tail fragments and occasionally sperm heads were visible.

Several years elapsed between the time of the original experiments and that of our work. It is possible that changes in the dor (C1B) stock during this period may be responsible for the discrepancies between the two sets of results. We believe this not to be the case, since all of our stocks showed uniform results in regard to monospermy frequencies and early embryological development; in addition, the

descriptions of early lethals bear a striking resemblance to post-meiotic chromosomal configurations in unfertilized eggs. A reinterpretation of the original data appears, therefore, to be warranted.

We do not wish to detract, in any way, from Counce's fine embryological studies and interpretations of the data on the post-blasteme developmental stages, nor do we wish to detract from the insight that was shown in the interpretation of results from earlier stages. Based on beliefs accepted by biologists at the time, these interpretations also were accepted as valid.

SUMMARY

Earlier studies on the mutant dor had indicated that, among dor zygotes from mutant mothers, there were two stages of lethality. Mutant daughters died, when the father was also mutant, during meiosis or first cleavages (early lethals); some mutant sons died during these early stages while others survived to or beyond the blastula stage (late lethals). If the father was wildtype there appeared to be an increased frequency of late as opposed to early lethals among the dor/Y embryos. It was suggested that the presence of wildtype accessory sperm ($\underline{X}^{dor^+}$) in the cytoplasm of the developing zygote alleviated the adverse influence imposed on the cytoplasm by the dor/dor genotype of the mother.

Our results indicate that

(1) the frequency of polyspermy is not high enough to account for the effect on development that has been proposed,

(2) probably many unfertilized eggs were previously misclassified as early lethals, since it was not known until recently that unfertilized eggs complete meiosis,

(3) both fertilized and unfertilized eggs from dor females complete meiosis, and

(4) fertilized eggs, even when the father is mutant, usually develop to or beyond the early stages of gastrulation, and thus early lethals do not exist typically in the dor stocks.

The bearing of precocious meiosis on what appear to be exceptional early lethals is discussed.

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	(a)	(b)
P	dor/dor x dor/Y	dor/dor x dor$^+$/Y
F_1	[dor/dor] (dor/Y)	dor/dor$^+$ (dor/Y)
	all die	live die

	(c)	(d)
P	dor/dor$^+$ x dor/Y	dor/dor$^+$ x dor$^+$/Y
F_1	[dor/dor] (dor/Y)	dor/dor$^+$ (dor/Y)
	dor/dor$^+$ dor$^+$/Y	dor$^+$/dor$^+$ dor$^+$/Y
	all live	all live

Fig. 1. Embryonic survival as influenced by the interaction of maternal and embryonic genotypes. Within brackets are indentical F_1 dor ♀ genotypes and within parentheses identical F_1 dor ♂ genotypes.

TABLE 1

Stocks from which dor females and males were obtained. See Bridges and Brehme (1944) for the descriptions of the genetic markers and C1B inversions, and Mislove and Lewis (1954) for descriptions of the FM3 and FM4 inversions.

Line	Genotype		Origin of dor	Source of stock
C1B	In(1)C1, sc t^2 v sl B/dor	x dor	Merrell (1947)	S. Counce-Nicklas
FM4	Ins(1)FM4, y^{31d} sc^8 dm B/dor	x dor	Merrell (1947)	S. Counce-Nicklas
FM3	Ins(1)FM3, y^{31d} sc^8 dm B l/dor	x dor	Merrell (1947)	C. W. Clancy
61e	Ins(1)sc^{S1} dl -49 sc^8, y w^a v B/dor	x dor	Hildreth (1963)	P. E. Hildreth

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TABLE 2

Frequencies of females that mated within specified times.

P females	Medium	Days aged ♀♀	Days aged ♂♂	Hours together	Number of ♀♀ set up	% mated with ♂♂ dor	% mated with ♂♂ dor$^+$
61e	M, B	5-7	5-7	½	72	0	58.5
				4	70	1.4	68.5
FM4	M, B	5-6	5-6	½	230	13.4	76.9
				4	152	48.6	91.4
FM3	M	2-3	2-3	½	29	0	25.7
				4	136	0	47.7

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TABLE 3

Numbers of dor females inseminated while with males for specified periods of time. The mutant flies were from the C1B line. The numbers in parentheses are precentages.

	Hours			
♂♂	24	48	72	96
dor	0/12 (0)	1/10 (10.0)	1/12 (7.7)	0/10 (0)
dor^+	6/10 (60.0)	6/10 (60.0)	9/12 (75.0)	

TABLE 4

Frequencies of unfertilized eggs laid by inseminated females. The *dor* individuals are from the Cl B line. The difference between females is highly significant ($X^2 = 12.38$, $p < 0.0005$) while the differences between males are not: total *dor* vs *dor*$^+$ - $X^2 = 2.1281$, $p = 0.1 - 0.2$; *dor* ♀♀ x *dor* vs *dor*$^+$ males $-X^2 = 0.7199$, $p = 0.3-0.5$; *dor*$^+$ ♀♀ x *dor* vs *dor*$^+$ males $p = 0.2-0.3$ (Fisher exact method; see Fisher, 1958).

P ♀♀	P ♂♂	No. fertilized	No. unfertilized	% unfertilized	Total %
dor	*dor*	133	13	8.90	7.66
	dor$^+$	108	7	6.09	
dor$^+$	*dor*	133	3	2.20	1.43
	dor$^+$	143	1	0.69	

TABLE 5

Frequencies of dispermic eggs from females that had mated with wildtype males. The numbers in parentheses are totals for all dor lines. Data for y w^a v B are from Hildreth and Lucchesi, 1963. One egg, in group designated by *, was trispermic.

♀♀	Medium	Number of eggs		
		Monospermic	Dispermic	%
dor (Cl B)	M	110	9	7.6
dor (FM4)	M, B	101	2	1.9
dor (61e)	M, B	46	4*	8.0
		(257)	(15)	(5.5)
y w^a v B	M	178	7	3.8

TABLE 6

Developmental stages of embryos fixed at various ages. Parents were dor x dor. Numbers in parentheses are percentages.

Line	Medium	Aged (hr)	Eggs	Unfertilized	Zygotes	Preblasteme cleavages	Blasteme-blastoderm	Amnio-proctodael invagination
61e	B	2	22	9(40.9)	13	6(46.2)	7(53.8)	0
		4	61	16(26.2)	45	3(6.7)	37(82.2)	5(11.1)
		6	20	5(25.0)	15	0	0	15(100)
Total			103	30(29.1)	73			
FM4	M	2	22	3(13.6)	19	0	16(84.2)	3(15.8)
		4	23	0	23	0	4(17.4)	19(82.6)
		6	26	1(4.0)	25	0	1(4.0)	24(96.0)
Total			71	4(5.6)	67			
C1B	M	2	256	5(1.9)	251	2(0.8)	227(90.4)	22(8.8)
		4	65	3(4.6)	62	1(1.6)	16(25.8)	45(72.6)
		6	107	1(0.9)	106	1(0.9)	1(0.9)	104(98.2)
Total			428	9(2.1)	419			

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