A STUDY OF THE FACTORS AFFECTING FERTILITY OF LOZENGE FEMALES OF DROSOPHILA MELANOGASTER[1]*

RAY C. ANDERSON
University of Minnesota, Minneapolis

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THE reproductive structures and the related phenomena of copulation, fertilization, and oviposition have been widely studied in *Drosophila melanogaster* and related species. NONIDEZ (1920) described the morphology and histology of the various reproductive structures of *D. melanogaster* males and females and traced the course of events in the female between copulation and oviposition. Subsequent studies have been limited to certain structures or to aspects of reproduction.

The normal development of the reproductive structures of *D. melanogaster* has been described by DOBZHANSKY and BRIDGES (1928) and DOBZHANSKY (1930) in their investigations of intersexes. GLEICHAUF (1936) made a detailed study of the male reproductive structures and reported on the variability in shape and structure of various male and female structures. The relationship between the testes and reproductive ducts during development has been reported by STERN (1941) and STERN and HADORN (1939).

Numerous mutants of *Drosophila melanogaster* are known which have abnormal reproductive structures or decreased fertility. HYDE (1914) reported a defect in the female which prevented oviposition. MORGAN (1915) observed that the infertility of "rudimentary" females was due to the retention of eggs. Since that time many other investigators have described anomalies of the ovaries and eggs which were associated with certain mutant genes. Differences in the size and proportions of the reproductive structures in mutant females were reported by DOBZHANSKY (1924, 1927), SCHWAB (1940), and DOBZHANSKY and HOLZ (1943). Two mutant characters affecting the spermathecae have been described by WEXELSON (1928).

One of the manifestations of the mutant gene "lozenge" is infertility, almost sterility, of the homozygous females. Numerous alleles of lozenge have been discovered since BRIDGES in 1916 observed the original lozenge mutant (listed in BRIDGES and BREHME 1944, page 118). Fourteen of these alleles were studied by GOTTSCHEWSKI (1936), who used them in an investigation of qualitative differences between allelic genes. Although his study dealt primarily with the color, shape, and structure of the eyes, some mention was made relative to fertility. OLIVER and GREEN (1944) reported that three of the lozenge alleles showed low fecundity and fertility in homozygous females and that the parovaria and spermathecae were absent in those females. Compounds of those

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alleles also lacked the parovaria and spermathecae, but the females were more vigorous than were the homozygous females.

The existence of abnormalities of the reproductve structures combined with a decreased fertility suggested that valuable information relative to the cause of infertility of the females might be found by studies of the morphology of the ducts, the breeding behavior of the females, and the development of the reproductive structures.

MATERIALS AND METHODS

The lozenge (*lz*) gene is located on the first (X) chromosome at 27.7. Approximately twenty mutant alleles at this locus have been reported (BRIDGES and BREHME 1944), but about half of them have been either lost or discarded. All available lozenge stocks were obtained and used in this study. These included four that were mantained at the UNIVERSITY OF MINNESOTA (*lz^{g}Bx^{2}/ClB*, *lz^{s}/ClB*, *lz^{sB}/ClB*, and *ylz^{y4}/ClB*) and five that were received from the CALIFORNIA INSTITUTE OF TECHNOLOGY (*lz/ClB*, *lz^{3}/f*, *lz^{34k}/ClB*, *lz^{36c}/ClB*, and *lz^{37h}*). In addition, almondex, a lozenge-like gene assigned to the same approximate locus and considered by GOTTSCHEWSKI (1936) as a lozenge allele, was obtained from the CALIFORNIA INSTITUTE OF TECHNOLOGY (*amx/ClB*) and included in the study. Hereafter in this report the homozygous cultures will be referred to respectively as *lz^{g}*, *lz^{s}*, *lz^{sB}*, *lz^{y4}*, *lz*, *lz^{3}*, *lz^{34}*, *lz^{36}*, *lz^{37}*, and *amx*.

Flies were raised in mass cultures at a temperature of 23°±1°C, except where otherwise noted in the section on experiments. The food used was one of the standard corn meal-Karo-agar mixtures, seeded with yeast.

For the study of internal genitalia, etherized females were placed in drops of 0.7 per cent physiological salt solution on a microscope slide. Dissections were made under a 20× dissecting binocular. Sperm teased out of the female receptacles remained motile in the solution in many cases for more than four hours. A cover glass was added, and the fresh material was examined under both low (110×) and high (400×) powers of a compound microscope. Observations were also made under oil. Temporary slides stained with fast green were sometimes used.

Stained serial sections were used exclusively in studying the development of the reproductive ducts. In order to obtain larvae and pupae of desired ages, the life history details recorded by STRASBURGER (1935) were used—that is, larvae that made their way up the sides of the culture bottle and ceased all movements were considered as full-grown; the subsequent eversion of the anterior spiracles was considered to mark the beginning of the prepupal-pupal period. Early pupae were thus selected and isolated in separate vials at two-hour intervals; pupae were then removed and fixed at definite age intervals. In the preparation of pupae older than 12 hours a technique used by BEADLE (reported in D.I.S. 14) was utilized. A thin layer of glue was smeared on a microscope slide, after which the pupae were placed on the surface. After 10–20 minutes the pupal cases were securely fastened to the slide and could be split open by means of a sharp needle. Larvae and pupae were pricked with a fine needle to allow for rapid penetration of fixative. Fixation was made in hot

Bouin's, and the material was allowed to remain in the cooling solution overnight. After two to three hours in the fixative, the anterior one-third to one-half of the specimen was cut off to further facilitate penetration by fixative and succeeding fluids. The material was run through a series of alcohols, alcohol chloroform mixtures, and several changes of chloroform, and was then imbedded in rubber paraffin. Serial sections were cut at 10 microns. The majority of the slides were stained with Delafield's haematoxylin and eosin, but Heidenhain's haematoxylin was also used in some cases.

EXPERIMENTAL RESULTS

Morphology of the reproductive ducts

The internal genitalia of a normal *Drosophila melanogaster* female consist of two ovaries, paired oviducts joining to form an azygous oviduct, a uterus, a single ventral receptacle, two dorsal spermathecae, and two parovaria. These structures are shown in the photomicrographs on Plate 1 (fig. A and B). Structurally, the internal genitalia are as described by NONIDEZ (1920). The oviducts and uterus consist of cuboidal epithelium, lined by a thin cuticle, and surrounded by a layer of muscle. The proximal portion of the uterus, referred to as the vagina, has a thicker cuticular lining, while the distal portion, the uterus proper, has a thin lining and is capable of great distention. The ventral receptacle is a long cuticle-lined tube of cuboidal cells, with a lumen that is very narrow in the proximal end. The two dorsal spermathecae are mushroom-shaped bodies, connected by short narrow ducts to the uterus and lined by thick brown cuticles. The parovaria are large-celled, round to oval "glands" with short narrow ducts emptying into the uterus very close to the openings of the dorsal spermathecae.

OLIVER and GREEN (1944) found homozygous lz^g, lz, and lz^s females to lack the spermathecae and parovaria, although other structures were apparently normal. Females of these three genotypes have been re-examined, together with the other seven homozygous lozenge and almondex stocks and some of the combinations of the various alleles, including non-lozenge. In addition, males of each of the ten mutant genotypes were examined and were found to be normal with respect to their genitalia. In this study, cultures were raised at a temperature of $19° \pm 1°C$.

Thirty females homozygous for each lozenge allele were dissected, except that more homozygous lz^{37} and control (wild type) females were examined because of the variability observed with lz^{37} (see table 1). All the almondex and wild type females had normal internal genitalia. One homozygous lz^{37} female had normal genitalia, but the other 119 lacked at least the parovaria, and 66 of these lacked at least one spermatheca. In females homozygous for any of the other lozenge alleles, no parovaria or spermathecae were observed. In none of the groups were females found in which structures other than the spermathecae and parovaria were noticeably affected.

The appearances of the spermathecae and parovaria in the various females are given in table 1. The table is arranged to show only those parts of the geni-

talia which were present. Those in which the duct and the heavy brown capsule of the spermatheca were present are indicated by the symbol "S," although in many females the capsules were abnormal in shape or size; those having only spermathecal ducts or portions of ducts are indicated by "SD." The parovaria if present are classed as normal, "P," or abnormal, "AP," in

TABLE 1

Portions of the spermathecae and parovaria present in various lozenge females.

GENOTYPE	NO.	oo	1–2 SD	1SD +AP	1S	1S+P OR AP	1S+ 1SD	1S+1SD +P OR AP	2S	2S+AP	2S+P
"+"	100										100
$lz^{37}/+$	30										30
$lz^{34}/+$	30									8	22
$lz/+$	30									9	21
$lz^{g}/+$	30									11	19
$lz^{s}/+$	30									26	4
$lz^{3}/+$	30		1				4		25*		
$lz^{36}/+$	30					3*	1	7	2*	16*	1
$lz^{sB}/+$	30					15		3		3*	9*
$lz^{v4}/+$	30			1		4*	1	6	3*	15*	
$amx/+$	30										30
amx/amx	30										30
lz^{37}/lz^{37}	120	3	17		28*		21*		50*		1
lz^{37}/lz^{g}	30	16	7		5		2				
lz^{37}/lz^{s}	30	28	2								
lz^{37}/amx	20										20
lz^{34}/amx	20									6	14
lz/amx	20									7	13
lz^{g}/amx	20									6	14
lz^{s}/amx	20									18	2
lz^{3}/amx	20		5		2*		7*		6*		
lz^{36}/amx	20							3*		15*	2
lz^{sB}/amx	20					3		1	1	12*	3*
lz^{v4}/amx	20					2*			2*	15*	1
Others**											

* Many of the spermathecae included in this group were incompletely developed or abnormal in shape.

** The thirty females of each of the following genotypes were 100 per cent of the "oo" type: homozygous lz^{34}, lz, lz^{g}, lz^{s}, lz^{3}, lz^{36}, lz^{sB}, lz^{v4}, and the compounds lz^{34}/lz^{37}, lz/lz^{37}, lz^{36}/lz^{37}, lz^{sB}/lz^{37}, lz^{3}/lz^{37}, lz^{v4}/lz^{37}, and lz^{g}/lz^{s}.

"+" refers to wild type or wild type allele.

The symbols used to represent the genitalia are as follows: oo, complete absence of parovaria and spermathecae; SD, presence only of spermathecal ducts; S, presence of capsular portion and duct of spermatheca; P, presence of complete parovaria; AP, presence of abnormal parovaria.

which case only small or rudimentary glands or ducts were present. The symbol "oo" indicates the complete absence of spermathecae and parovaria.

The data in table 1 show that each of the mutant lozenge alleles, with the exception of lz^{37} and *amx*, when heterozygous with the normal allele (lz^{+}) had

some effect on the genitalia, and thus can be considered to be "semi-dominant," at the temperature used, with respect to its effects on the internal genitalia of the female. The lz^{37}, which was completely dominated by lz^{+}, differed from the other lozenge alleles in having variable structures in the homozygous condition and thus was the "weakest" member of the allelic series (where "weak" is used merely to signify a slight deviation from normal, and "strong," a marked deviation). In every tested compound between the various alleles the parovaria were absent and the spermathecae were abnormal or absent, only lz^{9}/lz^{37} and lz^{4}/lz^{37} showed the presence of ducts or spermathecae.

The almondex gene apparently has no visible effect on the female genitalia. Homozygous *amx* females were normal, and all lozenge/*amx* compounds were phenotypically no more extreme than the lozenge/lz^{+} females.

Dissections of the different lozenge genotypes revealed a great variability of the genital structures, even among females of the same genotype as shown in table 1. Perhaps the most striking feature was the wide range in spermatheca structure, varying from complete absence through rudimentary ducts, normal-sized ducts, rudimentary chitinous capsules, irregularly shaped capsules, up to normal and infrequently even accessory spermathecae. It is significant to note that the lozenge genes not only prevented the formation of certain structures, but in some lozenge/lz^{+} females so influenced development that "twin" spermathecae or accessory spermathecae were formed. The parovaria varied from complete absence up through rudimentary ducts, small club-shaped glands, long ducts, irregularly shaped glands, to normal parovaria. Representatives of various genitalia abnormalities are shown in Plate 1 (fig. C, D, E, F).

In the course of making dissections it was found that the phenotype of heterozygous lozenge females (lozenge/lz^{+}) could be modified considerably by varying the temperature at which the flies were raised. In order to measure this temperature effect quantitatively, the following procedure was carried out. Males of lz^{36} genotype were mated to wild type females. The females were allowed to lay eggs in culture bottles over 24-hour intervals, after which the bottles were placed in constant temperature chambers. One group was raised at 19°C; another, at 23°C. (At each temperature, the fluctuation was $\pm 1°$.) Two other groups were raised at 19° up to the pupal and mid-pupal stages and were then transferred to 23°, and vice versa for two other groups. In each group the spermathecae of 25 females were examined. Of those raised at the higher temperature, 24 had normal and one had abnormally shaped spermathecae; of those raised at the lower temperature, eight had normal, five had abnormally shaped spermathecae, and 12 had only one spermathecal capsule. In the groups raised at two different temperatures, only nine of those kept at 19° to the middle of the pupal period developed normal spermathecae, while 24 of those kept at 23° to the middle of the pupal period were normal. Of those kept at 19° to the beginning of the pupal period, 14 were normal; of those kept at 23°, 16 were normal. Although the data are meager, they do suggest that the lz^{36} gene has its major effect on its normal allele at the low temperature. It will be recalled that all the results in table 1 were obtained with flies that were raised at $19° \pm 1°$C.

Sperm motility and genitalia abnormalities

Abnormalities of spermathecae and parovaria in lozenge females suggested that their low fertility might be related to the sperm content in the female. OLIVER and GREEN (1944) found that homozygous *lz*^{g} females produced many eggs over an extended period of time, but that fertile eggs were few in number and appeared only during the first few days following copulation. They suggested that the phenomenon might be due to a possible loss of sperm viability in the ventral receptacles of the females.

In order to check on any irregularities in sperm transfer, observations of the sperm were made from the time of copulation to their storage in the tubular receptacle. Fifty-six virgin homozygous *lz*^{g} females and 27 virgin wild type females were aged 24 hours, and then each female was placed in an individual vial with five wild type males. Within three hours, 50 of the lozenge and 21 of the wild type females had copulated. In each case, the duration of copulation was carefully timed. This copulation period proved to be significantly shorter in the case of the lozenge females, being 15.1 ± 0.4 minutes as compared to 20.0 ± 0.8 minutes in the wild type females (p is less than 0.0001). Of the 50 lozenge females which did copulate, 14 failed to show any sperm upon being dissected; three of the wild type females which copulated also lacked sperm. This difference in frequency of insemination was not significant, p being equal to 0.19. In those females in which sperm were observed, the sperm could be observed moving in the uterus and in the other parts of the genitalia that were present. Sperm could be seen passing into and up the tubular receptacle.

Matings were then made to determine whether the sperm supply in the receptacles of the females was being depleted rapidly, or whether the sperm were becoming non-motile. Virgin females of the types used by OLIVER and GREEN (1944)—that is, *lz*$^{+}$, *lz*^{g}, *lz*^{s}, and *lz*^{g}/*lz*^{s}—were aged 48 hours and then mated to five wild type males in individual vials for 24 hours. At the end of definite time intervals in days, some of the females were dissected and the tubular receptacles examined for sperm content and sperm motility. Visual estimates were made of the number of sperm in the receptacles. Only a rough relative estimate was feasible, and this estimate concerned itself with the quantity of sperm in the ventral receptacle. An arbitrary scale from 0 to 100, with intervals of 10, was set up, with 0 denoting no sperm, and 100, a completely filled receptacle. Although this did not allow for a careful quantitative measurement, it became quite easy to grade the relative content to the closest 10 per cent. The results from these matings and dissections are shown in table 2.

The data in table 2 indicate that the initial amount of sperm stored in the tubular receptacles of lozenge females was less than in those of the control females. However, the most striking feature was the loss of sperm motility in a relatively short time in the lozenge females. Motile sperm were found in only two of the *lz*^{g} females that were examined after the fifth day. These two were found on the ninth day after copulation, but in both cases the motile sperm were in the distal end of the receptacle, whereas the proximal end was filled

with a mass of non-motile sperm. These females are "exceptional," insofar as motile sperm were not found in the receptacles of more than 100 other lozenge females (in this and other studies) that were dissected seven days or more after mating. In some females, the non-motile sperm appeared quite normal, except that no movement could be observed. In others, the sperm were twisted into a "rope," often "kinky," which sometimes occupied only the central part of the lumen proximally. Normal sperm and a "rope" of non-motile sperm from

TABLE 2

Sperm content and motility of sperm in tubular receptacles of females.

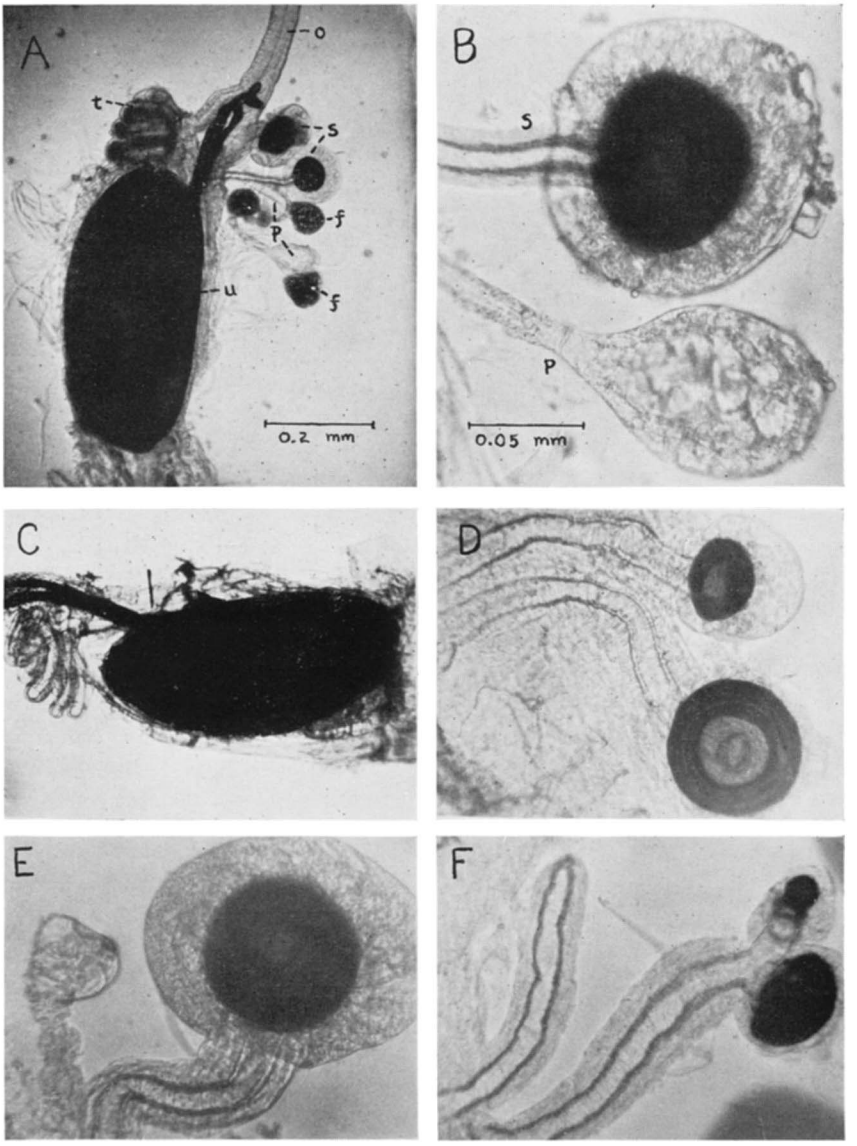
GENOTYPE	DAYS AFTER COPULATION	1*	5	7	9	11
lz^+	Total examined on each day	30	29	14	20	13
	Number having sperm	26	27	13	20	11
	Number having motile sperm	26	27	12	20	11
	Average sperm content**	98	82	68	57	42
lz^g	Total examined on each day	40	30	13	19	9
	Number having sperm	28	20	8	14	6
	Number having motile sperm	28	4	0	2	0
	Average sperm content	75	44	39	29	33
lz^s	Total examined on each day	30	29		15	
	Number having sperm	6	16		5	
	Number having motile sperm	6	4		0	
	Average sperm content	78	35		24	
lz^g/lz^s	Total examined on each day	30	31		20	
	Number having sperm	22	22		9	
	Number having motile sperm	22	2		0	
	Average sperm content	76	25		13	

* The first day represents the 24-hour mating period.

** The average sperm content is given in percentage, based upon visual estimates of the quantity of sperm in the tubular receptacle, with 0 denoting no sperm and 100 denoting a receptacle filled with sperm. The average sperm content is based only on females containing sperm.

a lozenge female are shown on Plate 2 (fig. A, B). The loss of sperm motility was observed in females of all three lozenge genotypes but not in the controls.

Day-to-day records were kept of the number of offspring produced by the females before they were dissected. The breeding results substantially corroborate the results reported by OLIVER and GREEN (1944) and are therefore not reported here. In general, offspring were produced by control (lz^+) females throughout the test, but most of the progeny of lozenge females appeared during the first three days. However, one offspring was produced by a homozygous lz^g female on the fifth day and one by a homozygous lz^s female on the fifth and also the sixth day. The breeding results parallel the decrease in sperm motility very strikingly.



Explanation of Plate 1

All the photomicrographs included on this plate were taken of unstained material. Figures A and C are of the same magnification, and figures B, D, E, and F are of the same magnification.

Figure A.—Reproductive tract, exclusive of ovaries, of wild type female. s indicates spermathecae; p, parovaria; o, oviduct; t, tubular receptacle; f, fat bodies; and u, uterus with egg inside.

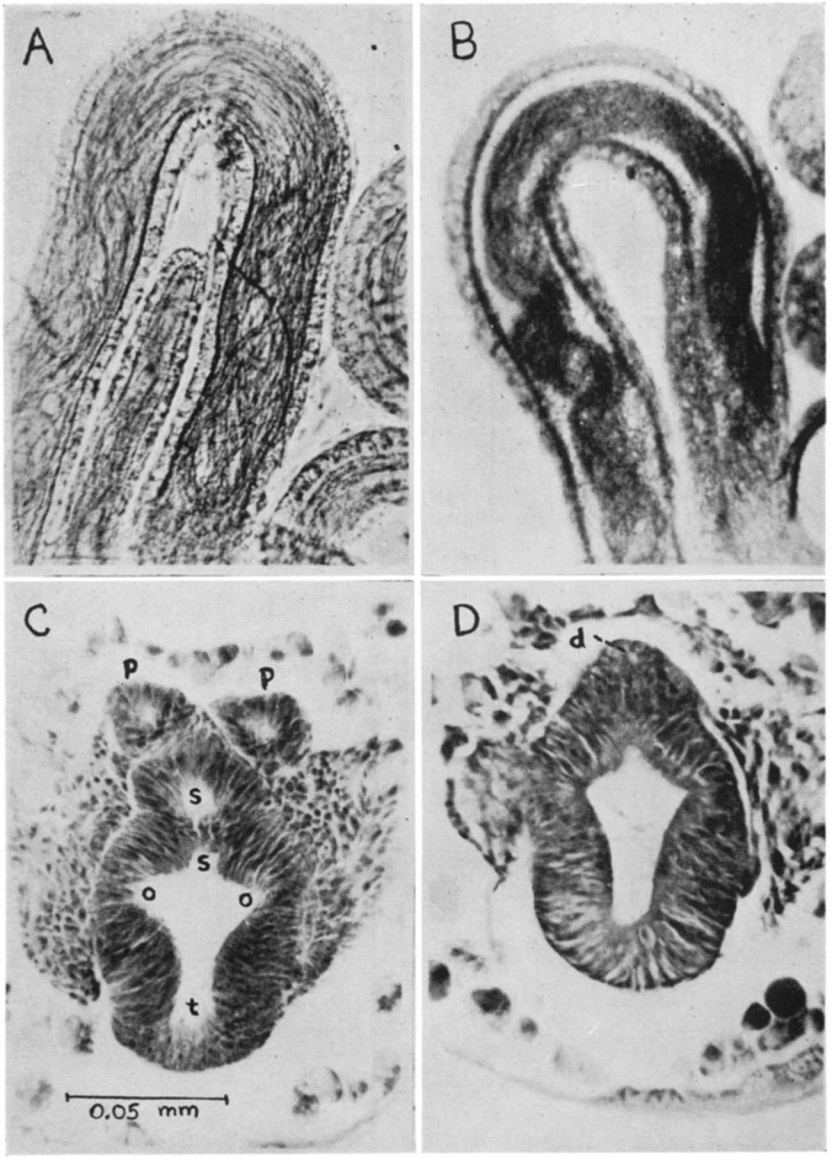
Figure B.—Spermatheca and parovarium of wild type female.

Figure C.—Reproductive tract of lz^{sB}/lz^{37} female. No parovaria or spermathecae are present.

Figure D.—Spermathecae of homozygous lz^{37} female, showing variation in size.

Figure E.—Abnormal parovarium and normal spermatheca of $lz^{36}/+$ female.

Figure F. "Twin" spermathecae and undeveloped spermatheca (duct only) of $lz^{3}/+$ female.



EXPLANATION OF PLATE 2

All the photomicrographs included on this plate are of the same magnification.

FIGURE A.—Portion of tubular receptacle of wild type female filled with sperm. The sperm were very motile at the time of dissection. Unstained preparation.

FIGURE B.—Portion of tubular receptacle of homozygous *lz*^{g} female nine days after copulation. The sperm were non-motile at the time of dissection, and as shown here, present a fuzzy "rope"-like appearance. Unstained preparation.

FIGURE C.—Transverse section through genital imaginal disc of 18-hour wild type female pupa. Note parovaria (p), spermathecae (s), and beginnings of tubular receptacle (t) and paired oviducts (o). Stained preparation.

FIGURE D.—Transverse section through genital imaginal disc of 18-hour homozygous *lz*^{g} female pupa. Compare with preceding figure, noting absence of parovaria and spermathecae. The dorsal layer of the disc shows some signs of differentiation (d). Stained preparation.

Relationship between degree of spermatheca abnormality and fertility

Although the internal genitalia are abnormal in many homozygous lz^{37} and lz^{37}/lz^{g} females, these females are often fertile. This fact suggested that some relationship might exist between fertility and structure of internal genitalia. As a test of this possibility, virgin lz^{37} and lz^{37}/lz^{g} females were aged 24 hours, kept with males for 24 hours, transferred daily into fresh vials for nine days, and then dissected. Counts were made of the offspring production for each day. Only females which contained sperm or produced offspring are included in the following data. In the lz^{37} group, one female which lacked both parovaria and spermathecae produced 165 offspring; ten females which lacked parovaria but had either one or two spermathecal ducts produced an average of 9.1 ± 8.4 offspring; and 71 females lacking parovaria but having one or two spremathecal ducts and capsules produced an average of 212.7 ± 10.5 offspring. The figures for the corresponding three groups of lz^{37}/lz^{g} were: 40 females with an average of 1.9 ± 1.1 offspring, 14 females with an average of 4.5 ± 2.3 offspring, and 21 females producing an average of 228.1 ± 21.6 offspring. A control group of 24 wild type females, with normal genitalia, produced an average of 451.1 ± 24.0 offspring.

In both lozenge genotypes those females in which spermathecae (capsules) were present showed a much higher fertility than did those lacking spermathecae or possessing ducts only. However, the average offspring production figures for all lozenge groups were significantly less than that for the control females. One lz^{37} female belonging to the group with spermathecae (capsules) produced 457 offspring, thus exceeding the mean for control females. The performance of the single lz^{37} female in the first group (lacking capsules and ducts) which produced 165 offspring appears almost to reverse the situation. However, an examination of the day-to-day record of this female showed that the offspring appeared only during the first three days after the mating period. This behavior is similar to that observed in some of the "highly-fertile" homozygous lz^{g} and lz^{s} females, in which upwards of 100 offspring are produced in the two to three days following copulation, although both of these genotypes lack parovaria and spermathecae. One lz^{37}/lz^{g} female which had a spermatheca has not been included in the above data because she apparently presents a special case. She had one spermatheca, but the duct leading to it was closed in the proximal portion. There were no sperm distal to this closure. This female produced no offspring.

The relationship between the type of anomaly of the internal genitalia and the length of the productive period of the females is shown in table 3. Only those females that lived for the nine days of the test are included. Parovaria were absent in all but the control females. The table is arranged so as to show how many of each type of female produced offspring only one day, two days, etc. As an example, 71 lz^{37} females had one or two spermathecal ducts with capsules, many of which were abnormal in shape or size. Of these females, one produced no offspring (although sperm were present in her ducts); two became sterile after two productive days; two more, after the third day; and so on,

with 13 being fertile on the ninth day. All the control females were fertile for from six to nine days. Many of the lz^{37} and lz^{37}/lz^{9} females with capsules and ducts were fertile into the second half of the test period. However, the females lacking spermathecal capsules were either sterile or were fertile only during the first two or three days of the test.

The data show that the presence of spermathecae, the capsular portion specifically, is accompanied by a prolongation of the fertile period following copulation and by increased fertility of the lozenge females. When the females were dissected after the ninth day, sperm in the ducts of the females with

TABLE 3

Relationship between the conditions of the spermathecae and the maximum productive period of females.

GENOTYPE	PHENOTYPE**	NUMBER OF FEMALES REMAINING FERTILE FOR DAYS AFTER MATING									
		NO. WITH SPERM	NO. STERILE	1*	2	3	4	5	6	7	8	9
lz^{37}/lz^{37}	Ducts and capsules (S)	71	1	70	70	68	66	59	41	27	15	13
lz^{37}/lz^{37}	Ducts only	10	4	6	5	2	0	0	0	0	0	0
lz^{37}/lz^{37}	No ducts	1	0	1	1	1	0	0	0	0	0	0
lz^{37}/lz^{9}	Ducts and capsules (S)	21	1	20	20	20	18	16	12	5	1	1
lz^{37}/lz^{9}	Ducts only	14	10	4	4	0	0	0	0	0	0	0
lz^{37}/lz^{9}	No ducts	40	32	8	8	0	0	0	0	0	0	0
"+"	Normal	24	0	24	24	24	24	24	24	23	22	20

* The first day represents the 24-hour mating period.

** All the females except "+" lacked parovaria.

capsules were usually motile, but those in females without capsules were non-motile in almost all cases.

Fertility of almondex females

Homozygous almondex females have been reported to be sterile. They differ, however, from the (other?) lozenge alleles in that their internal genitalia appear normal (see table 1). Therefore, the factors involved in this sterility and those in the case of lozenge females probably differ. Thirty virgin almondex females were aged 24 hours and mated individually for 24 hours to five wild type males. The females were transferred to fresh vials on the fourth and fifth days and were dissected after the sixth day.

Seven of the 30 almondex females were fertile. They produced 18 offspring, eight of which appeared during the first four days, one on the fifth day, and nine on the sixth day. These offspring consisted of 12 females which emerged, three females which failed to emerge, and three flies which failed to emerge and could not be identified as to sex. Numerous eggs were produced by almost all the females, and 24 of the 30 females had sperm (motile in all cases) in their ventral receptacles on the sixth day after mating. The amount of sperm re-

maining after six days was unusually high, averaging more than 80 per cent of capacity. The infertility of almondex females thus differs strikingly from that of lozenge females in that the very low fertility in almondex is not associated with loss of sperm motility. It appears that the infertility of almondex females may be due to egg defects or zygotic lethals, as indicated by the death of numerous pupae. The one-sided sex ratio (15 females to 0 males) suggests that the male zygotes die early in development. Studies on this problem are being continued.

Comparison of development of internal genitalia of lozenge and wild type females

The internal genitalia of Drosophila have a dual origin. The gonads arise from the early polar cells, but the remaining structures develop from an ectodermal disc and adjacent mesoderm. The ovaries of lozenge females are apparently normal and, therefore, were not studied.

In the study of the development of the reproductive ducts, serial sections were made of homozygous *lz*^{g} and wild type female full-grown larvae and pupae. The pupae were selected at six-hour age intervals, ranging from 0 to 36 hours, and also at 48, 72, and 96 hours. At the temperature used (23° ± 1°C), females of both types emerged at approximately 108 hours.

The development of the female genitalia in *Drosophila melanogaster* has been described briefly by DOBZHANSKY (1930), and in *D. virilis*, more completely by NEWBY (1942). Since the present report has as its primary interest the differences in development of *lz*^{g} as compared to normal wild type females, only those points will in the main be considered.

Up to the time the genital pore is formed, the development of the genital disc in *D. melanogaster* corresponds to that in *D. virilis*. However, the formation of the parovaria and spermathecae takes place somewhat differently from that described by NEWBY for *D. virilis*. In *D. melanogaster* a moderate dorsal evagination of the disc occurs. At about 12–15 hours of pupal life a pair of grooves appear in the posterior portion of the evagination and push out dorso-anteriorly as ducts. During this same pupal period two median ducts form from the anterior portion and push out dorso-anteriorly from the evagination, one ahead of the other. The posterior pair of ducts develop into parovaria, and the anterior pair become spermathecae. In the anterior portion of the lumen of the disc a ventral groove appears by the 18th hour and develops into the ventral receptacle. The anterior cavity proper develops as the oviduct, dividing into two branches at about 20 hours and reaching the ovaries at about 40 hours.

The imaginal disc of the full-grown *lz*^{g} female larva resembles that of the wild type female. Differences between the imaginal discs of *lz*^{g} and wild type females become evident early in pupal development. The beginning of the dorsal evagination appears clearly in the six-hour wild type female pupa, but in the *lz*^{g} pupa of the same age there is little if any dorsal differentiation of the disc. In the 12-hour pupae, the difference becomes more marked. The rudiments of the parovaria are recognizable in the wild type pupa at this time,

but no comparable structures are visible in the lz^g pupa. The developing parovaria and spermathecae become more distinct in the wild type 18-hour pupa; in the corresponding lz^g disc there are signs of a thickening or early differentiation of the dorsal layer (Plate 2, fig. C, D), but this apparent differentiation goes no farther, and no signs remain of it in the 48-hour lz^g pupa. The development of the oviducts and tubular receptacle in the lz^g pupa proceeds as in the wild type female. The development of the abnormal genitalia of lz^g females can thus be attributed to the failure of the dorsal layer of the imaginal disc to differentiate properly during the first 12 hours of pupal life.

DISCUSSION

The lozenge alleles have been studied as a group by only one worker, although they have been used in many diverse experiments. GOTTSCHEWSKI (1936) noted the great variability of the lozenge alleles and found it possible to break them up into three distinct groups on the basis of eye characters: (1) "blood-red" alleles, (2) brown alleles, and (3) almondex. The first group, with many representatives, he characterized as having rough, glossy, blood-red eyes; the second, also with many representatives, as having smooth, "rimmed," brown eyes; and the third group, represented only by almondex, as having small eyes with a brownish "shimmer." GOTTSCHEWSKI reported various degrees of infertility in the different groups. He believed that the infertility of the first two groups was not related, because females heterozygous for genes in the two groups proved to be fertile, although the homozygous females were sterile. GOTTSCHEWSKI also found differences between the first two groups with respect to their effects when in combination with other eye color genes. From his experimental data and from other data in the literature, GOTTSCHEWSKI concluded that qualitative differences in the end action of single phases of polyphenic alleles are due to qualitative differences of such alleles either in their whole nature or at least in some phases, and that consequently members of the same allelic series may behave as genes of different loci with respect to interaction.

Similarity in effects of lozenge alleles

The data in table 1 suggest that the nine lozenge alleles in their action on the internal genitalia of the females present a homogeneous series of genes. All of the alleles caused abnormalities of the reproductive structures. Females homozygous for each of the alleles except lz^{37} lacked any sign of the parovaria or spermathecae. All except one of 120 lz^{37} females lacked parovaria, and although 100 of them did have one or two spermathecae, the capsules in many were abnormal in size and shape. The interactions between lz^{37} and each of the alleles suggest a similarity in basic gene action with respect to the development of the reproductive structures. All but two of the alleles interacted in compound with lz^{37} to cause the absence of the spermathecae and parovaria. Two lz^{37}/lz^s females had spermathecal ducts, but 28 had no sign of the parovaria and spermathecae. Fourteen of 30 lz^{37}/lz^g females had spermathecal ducts or ducts and capsule. All the alleles except lz^{37} interacted in compound with

lz^+ to produce abnormalities of the genitalia in some of the females, although they differed somewhat in the degree of effect.

The three groups of alleles which GOTTSCHEWSKI formulated according to the action of the genes upon eye-color and eye-structure do not accurately separate them into groups with respect to their action upon the genitalia. Alleles lz^s and lz^{sB} are identical in the external structure and color of the eyes. However, they interact to different degrees with lz^+ (table 1) and to a lesser extent with lz^{37} in the development of the spermathecae. Alleles lz^s and lz^g belong to separate groups as determined by the characteristics of the eyes, yet they react more or less alike with lz^+ and with lz^{37} in the development of the spermathecae. Females homozygous for either lz^s or lz^g are also very infertile (GREEN and OLIVER 1941) and lack parovaria and spermathecae, and the sperm stored in the females lose their motility soon after copulation (table 2). The compound lz^g/lz^s also causes absence of parovaria and spermathecae and loss of sperm motility, but the fertility of the females is increased over either homozygote.

With respect to the almondex gene, the present work bears out GOTTSCHEWSKI'S consideration of it as a group separate from the other lozenges. However, whereas he found interaction between one of his "group 1" lozenges (lz^{35}) and almondex with respect to eye characteristics, the present work shows no similarity between any of the lozenges tested and almondex with respect to effects on genitalia and fertility. This would argue against the consideration of almondex as one of the lozenge alleles.

Semi-dominance of lozenge alleles

It is interesting to note that the lozenge alleles give observable effects on the genitalia of females having one lozenge and one normal gene (see table 1), although the eyes of these females are apparently normal. Only one allele, lz^{37}, was completely suppressed by the normal allele. Some of the females heterozygous for either lz^{34}, lz, lz^g, or lz^s had at least abnormal parovaria. The other four alleles had even stronger effects in heterozygous condition. With at least one of the alleles, the degree of effect of the lozenge gene upon the normal allele is dependent upon the temperature to which the flies are subjected. Flies with the genotype lz^{31}/lz^+, for example, generally have normal genitalia if they are raised at 23°C, but many have abnormal genitalia if the development occurs at 19°C. Thus, genes which have been considered previously as typical recessives actually act also as "semi-dominants" in one of their effects. So-called dominance and recessiveness are, of course, not always clear-cut and are greatly modified by genetic and environmental factors.

Causes of infertility of lozenge females

From the data presented on breeding behavior, it appears that the infertility of lozenge females is due to a complex of causes. Most apparent of these is the absence or abnormalities of spermathecae, which might conceivably represent a decrease in sperm-storing capacity. Likewise, the 25 per cent decrease in copulation time as observed in lz^g as compared to lz^+ and the lesser amount of

sperm initially stored in the tubular receptacle of the lozenge females shown for lz^g and lz^s in table 2 indicate some deviation from normal.

The most striking and probably the most important factor involved is the drop in motility of sperm in the ducts of the female. This loss of sperm motility in lz^g, lz^s, lz^g/lz^s and in lz^{37} females lacking capsules accounts very satisfactorily for the very brief period of fertility of lozenge females, a condition which at first seemed quite puzzling. At one time it was thought that egg failure might be responsible for this curtailment of fertility, but by a technique of double matings it was shown that this could not be the case (ANDERSON and OLIVER 1942).

In that study, homozygous lz^g females were mated to wild type males. After the females had lost their fertility, they were mated to males which carried $M\hat{e}/H$ on their third chromosomes. Many of the females which had become "sterile" regained their fertility with the second mating. In the present report, the sperm which are stored in the tubular receptacles of lz^g and lz^s females lose their motility within a few days after copulation (table 2). Sperm may still be found in the tubular receptacle after that time, but they are often clumped together. Only those in the lumen distal to the aperture will show any movement. The lz^g and lz^s females, it will be recalled, produce progeny only during the first two or three days after copulation.

Other factors are also evidently involved in the infertility, for females of similar phenotype with respect to the genitalia, but of different genotype, show significant differences in fertility (OLIVER and GREEN 1944). A number of cases of female sterility and infertility in various mutants have been reported in the literature (for example, CLANCY and BEADLE 1937), but none have involved sperm inviability. However, sperm inviability has been found by several workers to play a part in Drosophila interspecific crosses—namely, by D. D. MILLER (DOBZHANSKY 1941, page 271), and PATTERSON, STONE, and GRIFFIN (1942). In these cases only non-homologous sperm underwent degeneration, whereas in the present case of lozenge females all sperm (from lozenge or wild type males) were affected.

Significance of parovaria and spermathecae

The data on fertility of the lozenge females presented in this paper indicate the significance of various female reproductive structures. The parovaria have been a stumbling block to all would-be interpreters of Drosophila genitalia and are difficult to homologize with any of the structures of other insect groups (HEBERDEY 1931). NONIDEZ (1920) observed the gland-like structure and the refractile granules in its fluid and suggested that the parovarian secretion may activate the sperm or perhaps dissolve the thick portion of the ejaculate, but added that the function is obscure. Since then, workers have added little to these remarks. GOTTSCHEWSKI (1937) attributed his difficulty in artificially inseminating Drosophila females with sperm from the seminal vesicles of the males to the lack of parovarian secretion, although he was suc-

cessful in two cases (only three offspring, however). That both of these functions suggested by NONIDEZ are non-existent is shown in the present work in which females lacking parovaria (for example, lz^g and lz^s) contain active sperm during the first few days following copulation and are fertile during that period. Moreover, lz^{37} and lz^{37}/lz^g females with one or two spermathecae, but with no parovaria, produced approximately half as many offspring as did the wild type females (212.7 and 228.1 as compared to 451.1), and as shown in table 3, the sperm remained motile in the tubular receptacles for more than nine days. This may suggest that the parovaria are important in the control of fertility, but it must be remembered that the spermathecae of many of these females were small or abnormal in shape. Though the absence of parovaria may be a contributing factor, the evidence does not support the significance attributed by NONIDEZ to the parovaria in normal reproduction.

The two dorsal spermathecae have been described as sperm-storing structures (NONIDEZ 1920) which also function as secretory structures, the secretion serving in the maintenance of sperm viability (HEBERDEY 1931). In his phylogenetic study of the reproductive structures of the Insecta, HEBERDEY regarded these two dorsal spermathecae of Drosophila as true seminal receptacles. The data on lz^{37} and lz^{37}/lz^g females bear this out. Those females having no ducts or having ducts but not capsules were with only a few exceptions either sterile or very infertile. They produced fewer than ten offspring on the average and produced no progeny after the third day following copulation (table 3). Females with ducts and capsules retained their fertility for a longer period of time. Some of them remained fertile for the nine days of the test, although the proportion of such fertile females was much less than the proportion of control females which remained fertile for that period of time. As was mentioned, the lz^{37} and lz^{37}/lz^g females were less fertile than the control females. The dorsal spermathecae evidently function in sperm maintenance through the elaboration of some secretion or by some similar means. It is very difficult to ascertain the exact manner in which they function, but it is quite evident that they are necessary for any degree of normal fertility.

HEBERDEY did not believe the ventral tubular receptacle of various Diptera (including Drosophila) to be a primary seminal receptacle, but instead considered it to be simply an outpocketing of the vagina (similar to a *bursa copulatrix*) which in many cases has taken over the functions of a seminal receptacle. The data on lozenge females fit this explanation very well also, for the tubular receptacle in the absence of the dorsal spermathecae and parovaria functions in sperm storage, but fails to maintain the sperm over an extended period of time. In females which lack the capsular part of the spermathecae, the sperm stored in the tubular receptacles clump into a non-motile mass.

Although the present work gives some evidence of the significance of the various female reproductive structures, a complete picture remains yet to be drawn. It will take additional studies on various mutants as well as studies on experimentally altered females before various dubious points can be cleared up. However, the extensive use of Drosophila in genetic and evolutionary

studies calls for a clearer interpretation of the reproductive phenomena both in normal and mutant stocks.

Effect of the lz^g allele on the development of genitalia

The developmental study reported upon in this paper was carried out with the purpose of determining the manner in which the abnormality of lozenge female genitalia arose. The absence of parovaria and dorsal spermathecae in the homozygous lz^g female and probably in females homozygous for the other lozenge alleles is due to irregular development of the genital imaginal disc. This deviation from normal is first apparent in the six-hour pupa, but very likely is present in even earlier stages. In lz^g females, the dorsal cells of the imaginal disc fail to differentiate into genital ducts. A dorsal evagination appears in lz^+ females by the sixth hour of pupal life and continues to develop into the parovaria and spermathecae. Although a feeble and abortive differentiation of the dorsal cells in the genital disc of the lz^g pupa occurs between the twelfth and twenty-fourth hours, all signs of this differentiation disappear by the forty-eighth hour. The dorsal portion of the imaginal disc in lz^g females, therefore, remains undeveloped. The presence of a temperature-sensitive period near the time of pupation as shown in $lz^{36}/+$ may fit in with the developmental picture in that the temperature-sensitive period occurs at about the time of the expected differentiation of the dorsal evagination.

It is of interest to compare the irregularity in lz^g genitalia development with the irregularity in eye development reported by WADDINGTON and PILKINGTON (1943) for one of the lozenge mutants. They found that the abnormal adult eye of lz^s flies resulted from a failure of the cells of the middle layer of the eye to penetrate between the cells of the outer layer in early pupal life, with a later failure of the retinulae to penetrate the basal layer. Although the anomalies of the genitalia seem also to be related to the failure of the cells to differentiate normally, it seems unwise at present to speculate on the relationship between these developmental irregularities in eyes and genitalia.

SUMMARY

Parts of the female genitalia, parovaria and spermathecae, have been found to be absent or abnormal in nine different lozenge alleles of *Drosophila melanogaster*, and in all except lz^{37} the lozenge genes have been found to behave as "semi-dominants." The male genitalia are not affected. The lozenge alleles show interaction effects on the genitalia of the females, with evidence of incomplete dominance between some alleles.

The gene almondex (*amx*) has no effect on the genitalia, and has no modifying effect on females that are heterozygous lozenge/*amx*. Homozygous almondex females are very infertile, and the progeny which occur are all females. The infertility of almondex females does not resemble that of lozenge females.

The genotype lz^{36}/lz^+ is very sensitive to temperature changes, with the sensitive period occurring at about the time of pupation.

The infertility of lz^g females (and probably other lozenges) is apparently due to a number of factors, including a shorter copulatory time, smaller initial

storage of sperm, possibly a smaller sperm-storing capacity, and degeneration of sperm within the tubular receptacles of the females.

The dorsal spermathecae are evidently necessary for high offspring production and extended sperm viability, as demonstrated by differences in fertility and in sperm motility between females with and without dorsal spermathecae but of similar genotype.

Females lacking parovaria but having the capsular portions of the spermathecae are able to produce great numbers of offspring over an extended period of time, indicating only minor or supplementary functions on the part of the parovaria in sperm viability and in fertility of female.

The tubular receptacle is not able to maintain sperm over an extended period of time in the absence of the spermathecae and parovaria.

Abnormal differentiation of the genital imaginal disc in the *lz*^{a} female becomes apparent within the first six hours of pupal life. Cells of the dorsal layer fail to differentiate properly, and the parovaria and spermathecae which normally develop from this layer are absent.

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