Bull. Inst. Zool., Academia Sinica 13(2): 87-105 (1974)

DEVELOPMENTAL MORPHOLOGY OF THE FEMALE GENITAL DISC AND ITS ADULT DERIVATIVES UNDER SCANNING ELECTRON MICROSCOPE

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Received for publication, July 18, 1974

ABSTRACT

I. W. Tang (1974). *Developmental Morphology of the Female Genital Disc and Its Adult Derivatives Under Scanning Electron Microscope.* Bull. Inst. Zool. Academia Sinica 13(2): 87-105. The stereomorohology of genital disc and spermatheca in female *Drosophila melanogaster* wild-type and mutant *spermatheca* (*spt*) were examined by employing scanning electron microscopy. Homozygous mutant females exhibit temperature dependent phenotypic expressions when larvae are raised at different temperatures. The wild-type females always show the same phenotype when treated with different temperatnres, thus indicating a buffering capacity against environmental temperature variation. A lack of temperature buffering capacity as well as an abnormal spermatheca surface architecture in the mutant females under scan are suggestive of a more labile genital disc-field in the mutant larvae than that of the wild-type. An unknown ganglion just beneath the introvert portion of the spermatheca capsule was described and its possible functions were discussed.

Among eukaryotic developmental systems, the imaginal disc of holometabolous insects seems to be a useful system for analyzing gene action in determination and differentiation. It is a discrete tissue which can be readily isolated for experimentation and observation[32]. There is a temporal separation of determination and differentiation in imaginal disc development[16~18]. Determination of the discs occurs during the early stages of embryonic or larval life, while the cellular differentiation of the discs does not occur until metamorphosis[11,15,16]. Adult structures are formed by cellular differentiation of the discs, with each disc giving rise to specific adult derivatives (Fig. 1[8,16,31]).

Each insect imaginal cell passes through at least three stages of determination in going from a more general "prepattern", that is, a nonrandom distribution of certain chemical or physical factors within the cells, to more specific and definite pattern, with new structures in an orderly arrangement[23]. During the first twelve hours after fertilization, certain portions of the blastoderm and hypoderm are set aside for imaginal disc formation. Then from about thirty-six to fifty-two hours after fertilization, the cells destined to become imaginal structures form discrete and different types of imaginal discs. The cells in these discs remain undifferentiated, with typical embryonic cell morphology, that is a relatively spherical nucleus in a cell with little cytoplasm, few mitochondria, little or no secretory granules, few rough endoplasmic re-

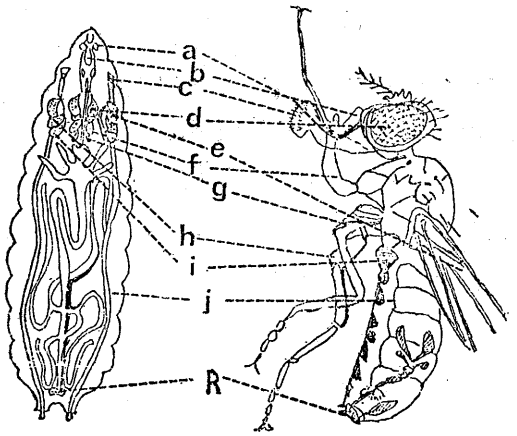

Fig. 1. Location of *Drosophila* imaginal discs and their adult derivatives. (modified from Nöthiger, 1972)

a=labrum	g=second leg
b=clypeo-labrium	h=third leg
c=humerus	i=haltere
d=eye-antenna	j=abdomen
e=wing-thorax	k=genital apparatus
f=first leg	

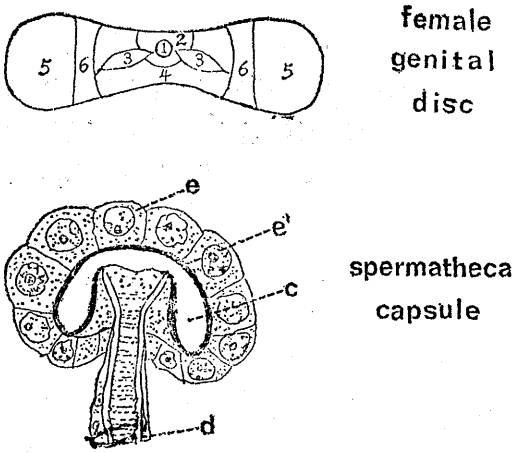

Fig. 2. (A) Female genital disc field potentials. (from Ursprung, 1957).
1=spermatheca
2=oviduct
3=paroviduct
4=uterus
5=dorsal & ventral plates
6=vaginal plate
(B) Adult spermatheca (from Nonidez, 1920).
c=cavity in the spermatheca capsule.
e=epithelial cell.
e'=cuticle.
d=spermathecal duct.

ticulum[22,33]. The second step in disc cell determination occurs sometime between the second and the third larval instar in *D. melanogaster*, that is about sixty to ninety-six hours after fertilization[6]. No gross morphological markers are visible to distinguish different types of disc cells until the final step of determination which usually takes place during the pupal-adult molt[9,16]. At the final step of disc cell determination, different regions of a given imaginal disc are determined as to the phenotypic expression of the cell type, the region and adult structure it will give rise to[9].

As a model system in studying the final step of disc cell determination, female *D. melanogaster* genital disc as well as its adult derivatives were used in this study. Some descriptive morphogenetic study of the *Drosophila* genital disc has been done (Fig. 2[13,14,24,30]). In the female as well as in the male, the unpaired, symmetrical genital disc is located in the last few segments of the larva[6]. All the adult reproductive duct system are formed from this single disc, with the exception of the gonads and the external genitalia (Fig. 3[1,6,7,16,18,24,28]).

Two things are expected to be delineated by this study, namely the timing of the duct branching out from the evolving disc and the possible temperature effect on the formation of the reproductive ducts. Two approaches were used, external topographical changes from late larval to early pupal stages as well as the adult spermatheca by scanning electron microscopy, as reported here and the gross cellular changes

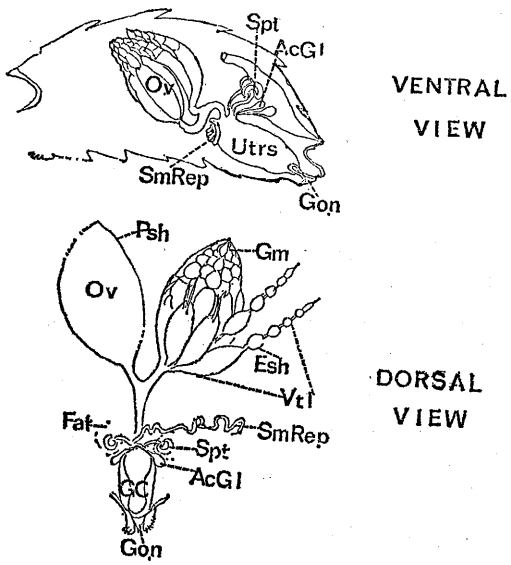

Fig. 3. Female reproductive system in *Drosophila melanogaster*. (from Demerec, 1950).
(A) Ventral View.
Spt=spermatheca.
AcGl=parovary.
SmRep=ventral receptacle, tubular receptacle, seminal receptacle.
Utrs=uterus.
Gon=gonopore.
(B) Dorsal View.
Spt=spermatheca.
AcGl=parovaria.
SmRep=ventral receptacle.
GC=genital chamber (uterus & vagina).
Gon=gonopore.
Ov=ovary.
Psh=peritoneal sheath.
Gm=germarium.
Esh=epithelial sheath.
Fat=fat tissue.
Vtl=vitellarium.

by light microscopy as reported elsewhere[25,26].

Recently, some scanning electron microscopic work has revealed the spatial orientation and the topography of the spermatheca in Coleoptera[29]. This has contributed to the understanding of the functional roles of the spermatheca and spermathecal gland in the granary weevils. It was felt that such a study on the female genital disc, of the wild type and some mutant such as *spermatheca* (*spt*[20]), would increase the understanding of the spermatheca in *D. melanogaster*.

MATERIALS AND METHODS

Late third larval instar and early pupal female genital discs and adult reproductive duct systems were dissected in cold Ringer's solution. To prevent the discs from collapsing, the specimens were fixed in 3% glutaraldehyde (EM grade, Fisher) in 0.2 M cacodylate buffer (pH 7.4) and rinsed three times in distilled water. Adult structures were found to be sufficiently rigid, and thus no fixative was employed. Tissues were mounted on stubs, quenched in liquid nitrogen for five minutes, and freeze-dried in a precooled Atmo-vac evaporator held at −80°C, between 50–100 mm mercury, overnight.

Freeze-dried specimens were vacuum coated with a gold platinum film of about 200 Å in thickness. A Cambridge MKII Stereoscan microscope was employed with an operating range between five and twenty kilovolts. Specimens of both the mutants *spt* and the wild type of various stages raised at 18°C, 25°C, and 28.5°C were examined.

RESULTS

Late third larval instar

A typical late larval femele genital disc is a symmetrical, lobular structure, with lateral stalks near the midline (Fig. 4).

0–4 hour pupa

By two hour post-spiracle eversion (2 hour pupa), the disc is about 1.5 times larger than the 120 hour larval disc. The mitotic zones remain, though fewer numbers of mitoses are seen (disc N=5 each in +/+ and *spt*; mitotic figures 2–4 per longitudinal section, dorsal half of the disc)[25,26]. The external shape reconstructed from the 4 hour pupal disc of +/+ is lobular with definite dorsal and ventral sides (Fig. 5 +/+, Fig. 22 *spt*). The dorsal portion

of the disc is oval in longitudinal section with a smooth, continuous dorsal surface. Ventrally, two lateral expansions of the disc give it the appearance that the disc is beginning to fold ventrally (Fig. 5). These expansions are probably due to the mitotic activity of the inner cell mass[25,26].

8-10 hour pupa

The size of the disc is about twice that of the 120 hour larval disc. The thickness of the disc also has increased (Fig. 6, +/+).

12 hour pupa

Dorsal central portion of the disc shows small buds and the distance between dorsal and ventral surface is farther apart (Fig. 7).

20 hour pupa

Buds are seen on the dorsal side of the disc (Fig. 23). The dorsal side of the +/+ disc shows from 3 to 4 small buds, and the ventral side begins to show budding or folding, while the *spt* disc only has 2 dorsal buds (Summarized in Table 1). These buds ultimately give rise to the female reproductive duct system. No branching of the ducts are seen at this stage and no rotation of primordia (buds) is found, in either the *spt* or the +/+ disc[25,26].

24 hour pupa

Disc cells show definits regions of grouping, the cellular layers as well as cell numbers are approximately doubled that of the 120 hour larval disc[25,26]. The overall size is about 2.5 times of the 120 hour larval disc. Definite ducts branching from the disc can be seen in the *spt* as well as +/+, (Fig. 8[25,26]).

48 hour pupa

Three to four distinct ducts are seen on the dorsal side in the wild-type (Fig. 9) and only two can be seen in the *spt*[25,26]. Three large buds beginning to form on the ventral side of the disc in both +/+ and *spt*.

Adult, posterior reproductive ducts

a) Wild-type (+/+)

The wild-type females develop chitinized spermatheca capsules and ducts by the time of eclosion[26]. In the posterior part of the adult abdomen there are two spermatheca ducts, with each a capsule at the distal end. The proximal end of the ducts are attached to the dorsal wall of the oviduct slightly anterior to the juncture of a pair of parovaria. Posterior to the parovaria, the oviduct thickens into the uterus which usually has enough space to house one egg at a time (Fig. 3).

The wild-type female has 2 spermathecae at 18°C, 25°C, and 28.5°C. Each spermatheca consists of a well developed capsule and a muscular duct, and is the mirror image of the other (Table 1). The spermatheca duct is lined with chitin, measures 10 μ across the lumen, and 20 μ across the outer diameter[25,26] Table 1 and 2). The size of the spermatheca duct remains about the same throughout the first seven days of adult development (Table 2).

There are two spermatheca capsules in each newly eclosed female which measures about 100×180 μ across the widest part of the

Fig. 4. Scanning electron micrograph of a normal (+/+) late larval disc (110 hours post oviposition). ST=lateral stalk, V=ventral side of disc. (475 ×).

Fig. 5. Pupal female genital disc, 4 hours after spiracle eversion. L=lateral portion, V=ventral portion, arrows pointing to anterior and posterior directions. (460 ×).

Fig. 6. Pupal female gemale genital disc, 8 hours after spiracle eversion, the size of disc has approximately doubled that of late larval disc. ST=stalks connecting disc to larval abdominal hypoderm. V= ventral side of disc. (285 ×).

Fig. 7. Pupal female genital disc, 12 hours after spiracle eversion (D) shows small buds, V=ventral portion of disc. (260 ×).

Fig. 8. Pupal female genital disc, 24 hours after spiracle eversion, ventral foldings (V) more prominent. (240 ×).

Fig. 9. Pupal female genital disc, 48 hours after spiracle eversion. Three large buds beginning to form on the ventral side (V), while three distinct ducts (D) are visible on the dorsal side (245 ×).

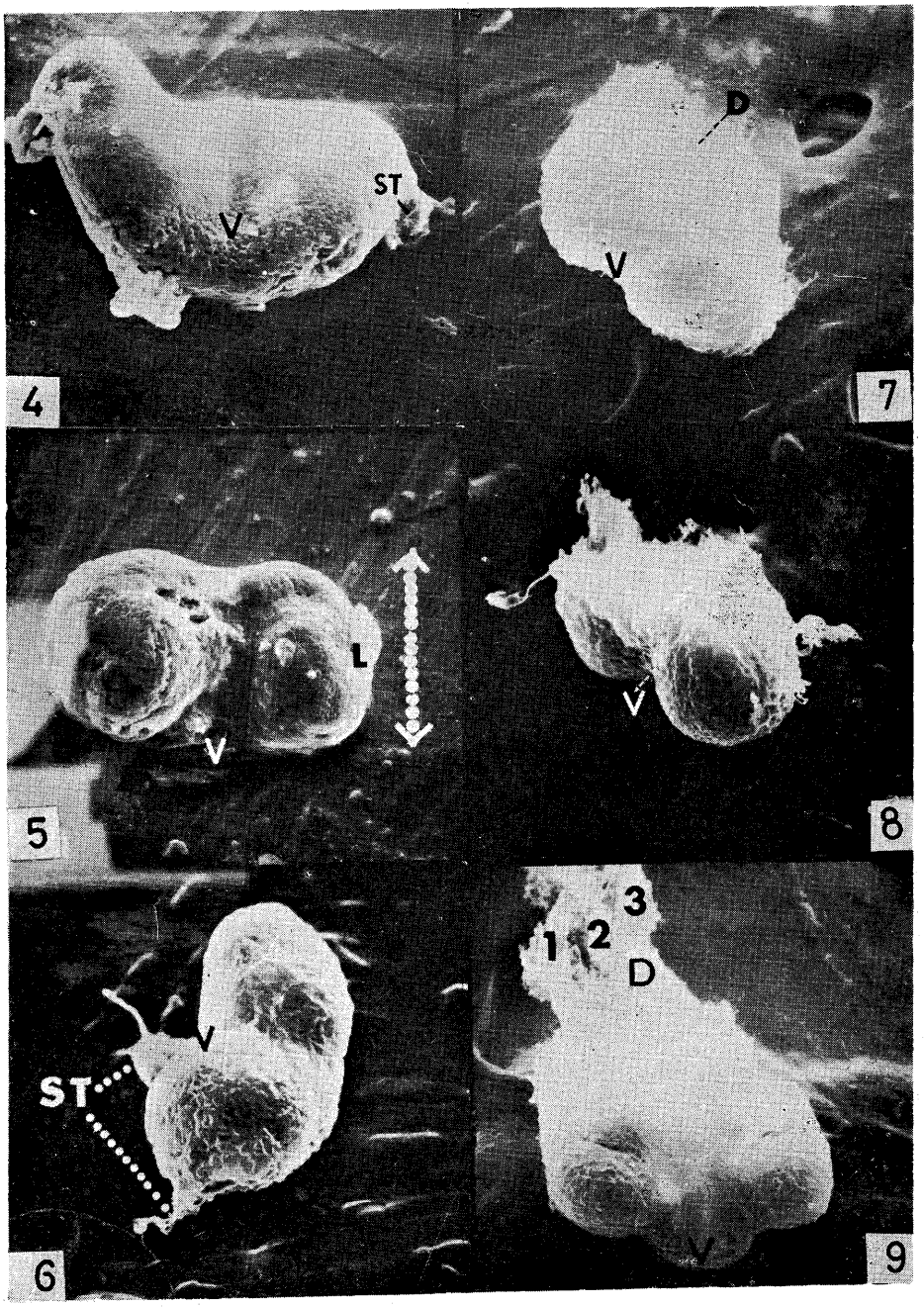
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TABLE 1
Scanning Electron Microscopy of Adult Spermatheca and Parovaria in *D. melanogaster* Wild Type and Mutant *spt* Female.

	18°C		25°C		28.5°C	
	+/+	Spt	+/+	Spt	+/+	Spt
Number of spermatheca capsule	2	2-4	2	1	2	1½, 2*
Number of spermatheca ducts	2	2-4	2	1	2	2-3
Number of parovaria	2	0	2	1	2	0-1
Number of lobes per spermatheca capsule	1	1	1	1-3	1	1-2
Number of ganglion near introvert, 7 day old.	1	1	1	1-3	1	1-2

* In wild type both capsule develop symmetrically; in mutant the size of capsule varies, in the 28.5°C mutant 1½ indicates little or no capsule at the end of one of the spermatheca duct, thus is asymmetric.

TABLE 2
Scanning Electron Microscopy of Adult Spermatheca in *D. melanogaster* Female Wild Type and Mutant *spt* reared at 25°C (N=20)

		1 day old		3 day old		7 day old	
		+/+	spt	+/+	spt	+/+	spt
Average size of capsue, in microns.	a) chitinized portion only	100×60	120×180	100×80	120×190	115×170	240×260*
	b) including the non-chitinized part	—	—	200×230	200×120	230×285	250×290
Size of the spermatheca duct, in microns.	a) lumen	11	12	13	12.5	14	13
	b) outer diameter	20	22	24.5	22	26.5	24.5
Size of spermatheca ganglion in microns		—**	—**	5×5	7×5	12×8	13×7

Note: * *spt* 7 day old spermatheca capsules vary from specimen to specimen, in size and shape, this is only a rough estimate.
** not detectable on day one, shortly after eclosion.

Fig. 10. Adult spermatheca, +/+, one day old virgin, 18°C. A spermatheca capsule is seen at the distal end of a spermatheca duct (D), the space seen where the duct joins the capsule is the introvert (IN). (525 ×).

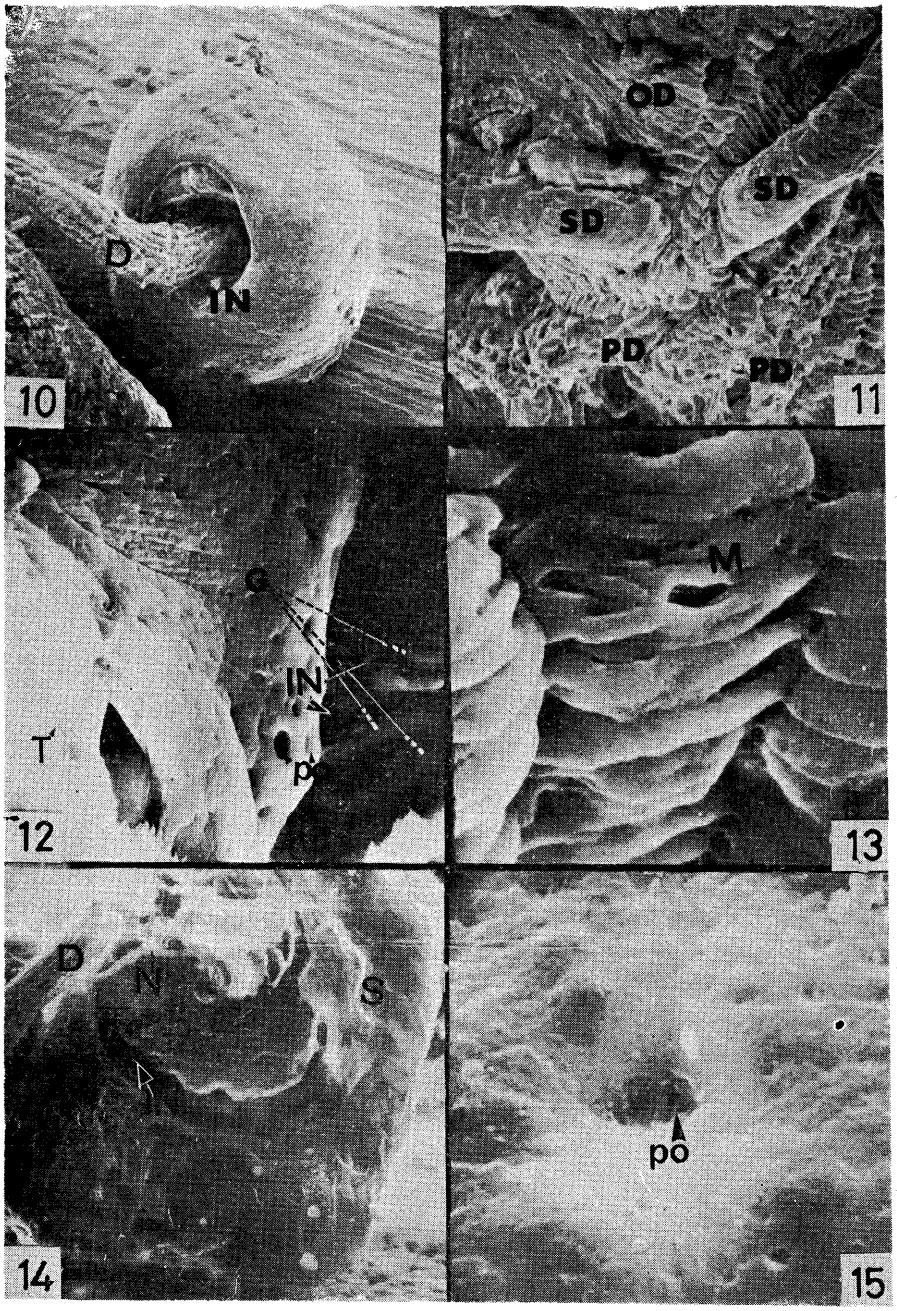
Fig. 11. Adult spermatheca, +/+, one day old virgin, 18°C. Close-up of the juncture on the dorsal side of oviduct (OD), showing a pair of spermatheca ducts (SD) just anterior to a pair of parovaria ducts (PD). (1054 ×).

Fig. 12. Adult spermatheca, +/+ one day old virgin 25°C. Details of introvert (IN) with pleated folds (G), large pores (po) and small pores, as tracheoles (T) can be seen. (2000 ×).

Fig. 13. Surface of wild type adult oviduct, same specimen as Fig. 16, each sarcomere measures about 0.1×0.55 micron, and has 2 to 4 roots. (10,000 ×).

Fig. 14. Wild type spermatheca, 7 day old, 25°C, fertilized. Ventral view of the spermatheca capsule (S), with duct (D), nerve (N) and introvert (IN), condensation on the outside of the large pores is visible. (425 ×).

Fig. 15. Wild type spermatheca capsular surface. A typical large pore (po) measures 0.4 micron across, with a longitudinal septum, specimen is one day old, fertilized, 25°C. (5400 ×).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umbrella shape structure (Fig. 2, 3). The chitinized portion of the capsule encloses a cavity which appears to be the expanded end of the chitin lined spermatheca duct[26]. No spermatheca ganglion was detected in the one day old +/+ female, however a nerve fiber was seen adjacent to the spermatheca duct near the entry of the introvert of the capsule (Fig. 20). The overall view of a spermatheca duct, introvert and the distal capsule of a virgin female +/+ is seen in Fig. 10. The close association of the two spermatheca ducts, the two parovaria ducts and the oviduct is shown in Fig. 11. A close-up of the surface structures just inside the introvert, with folds (G), some pores, and tracheoles is shown in Fig. 12. The muscular wall of the oviduct (Fig. 11) is magnified in Fig. 13. The ventral side of a mature seven day old female spermatheca capsule shows possible condensation droplets and surface pores (Fig. 14). The spatial relationship of the nerve fiber, spermatheca duct and the introvert is also demonstrated in Fig. 14. Unplugged large pores containing septa are often seen at intervals of 1.0 to 1.5 millimeter on the surface of the +/+ capsule, and numerous smaller pores are also visible (Fig. 15). The spatial aspects of the various part of the reproductive duct system in a newly eclosed +/+ is shown in Fig. 16, with the spermatheca arising dorsal to the oviduct, just anterior to the two parovaria.

The dorsal close-up of a +/+ unfertilized one day old spermatheca capsule, with a less expanded central portion (C) is seen in Fig. 17, while Fig. 18 shows a one day old fertilized spermatheca capsule, with a more expanded center portion. The expansion of the hollow center portion of the capsule was probably due to the sperm storage after mating. The capsules shown in Fig. 16, 17 and 18 represent mainly the chitinized portion of the capsule; the tissue surrounding the capsule which is termed the non-chitinized portion is practically non-existent in these newly eclosed females. However between day one and day seven, the non-chitinized portion of the spermatheca capsule undergoes a sizable expansion (Fig. 19). This is probably due to the hyperplasis (mitoses) as well as the hypertrophy (increase in cell size) of the smaller cells observed in light microscopy[25,26].

The ventral side of the capsule of the one day old (Fig. 20) and of the seven day old (Fig. 21) demonstrate the entry of the spermatheca duct into the introvert portion of the capsule, the introvert being the concave space just inside the capsule[28]. An innervation difference between the newly eclosed capsule and the mature seven day old capsule can be seen in the one day old capsule, whereas a large $5 \times 7\ \mu$ spermatheca ganglion is seen on the left side of the spermatheca duct in Fig. 21, both branches of the nerve fibers appear to be connected to a large nerve which enters into the wall of the oviduct.

Fig. 16. Adult female posterior reproductive ducts in a +/+ one day old unfertiized 18°C specimen. O=oviduct, S=capsule of spermatheca, D=spermatheca duct, P=parovaria, U=uterus, V=vagina. (200 ×).

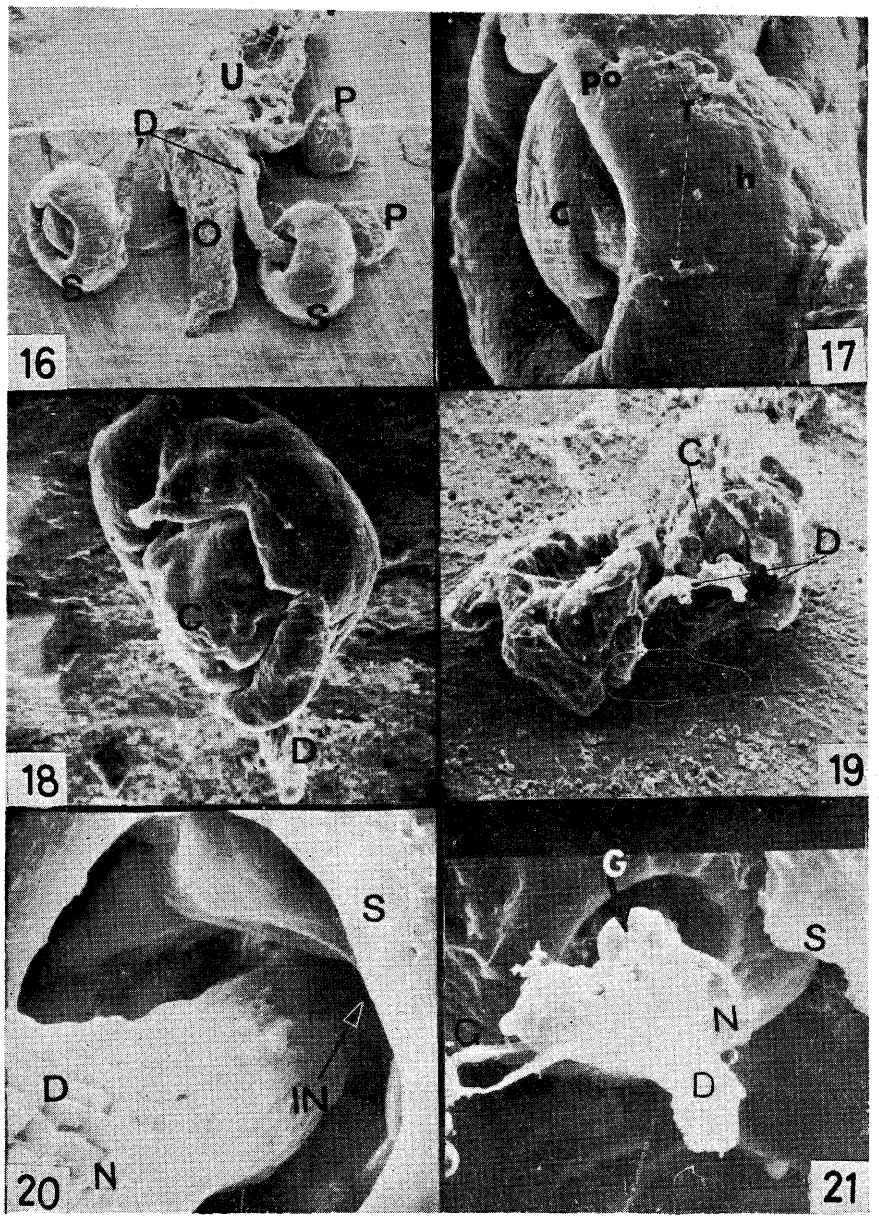
Fig. 17. Spermatheca capsule surface, +/+, see Fig. 21. Central portion of capsule (C) still unexpanded, po=large pore, unplugged, containing a septum (Fig. 20), h=large spot at regular intervals, T=tracheoles. (1040 ×).

Fig. 18. Spermatheca capsule surface, +/+, same age as Fig. 22, fertilized. D=spermatheca duct, C=central portion of capsule, expanded. (430 ×).

Fig. 19. Mature wild type 7 day old fertilized spermathecae, 25°C. C=chitinized portion, covered by a thick layer of non-chitinized portion of the capsule, the spermatheca ducts (D) are cut. (182 ×).

Fig. 20. Ventral view of capsule-duct juncture, +/+ one day old unfertilized (Fig. 21). N=nerve fiber, no ganglion is visible, D=spermatheca duct, S=capsule, IN=introvert space; the rugged muscular surface becomes less rough inside the introvert. (1840 ×).

Fig. 21. Ventral view of capsule-duct juncture, +/+ 7 day old fertilized (Fig. 24). N=nerve fiber, G=ganglion, D=spermatheca duct, S=capsule. (915 ×).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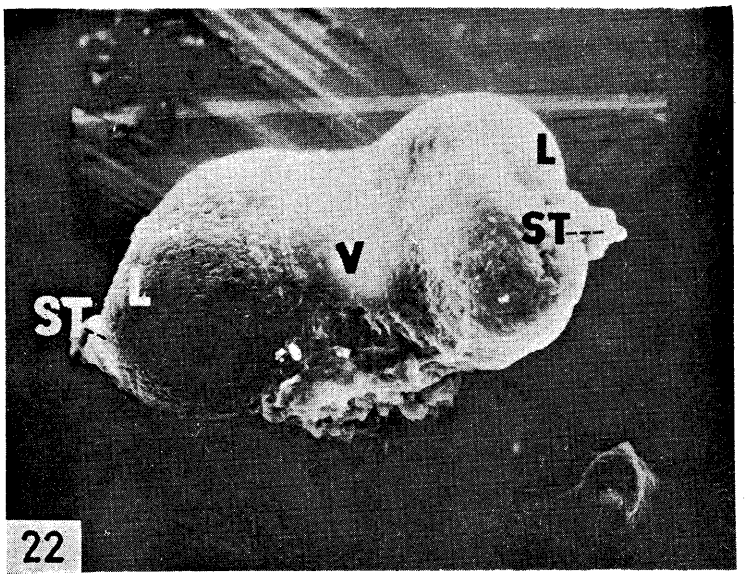


Fig. 22. Early pupal female genital disc, *spt*, 2 hr. 25°C. L=lateral portion, ST=stalks, V= ventral side, asymmetry of two lateral sides is visible. (485 ×).

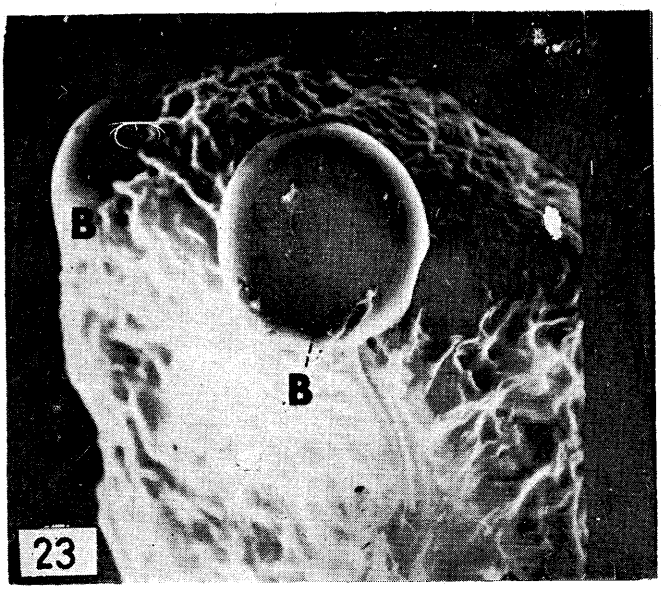


Fig. 23. Dorsal buds (B) of a 20 hour pupal disc *spt*, 25°C. (600 ×).

The non-chitinized portion of the capsule measures $200 \times 120\,\mu$ in the three day old fertilized female, and about $230 \times 285\,\mu$ in the seven day old fertilized +/+ female. In addition to the size increase, the toroidal shape of the capsule in the one day old female appears to expand into that of an elongated oblong shape in the mature seven day old fertilized female (compare Fig. 18 and 19).

Although the wild type female develops two spermathecae and two parovaria at the three temperatures examined in this study (18°C, 25°C and 28.5°C) such as shown in Fig. 16, and Table 1, the mutant *spt* female shows different phenotypes when the larvae are raised at different temperatures (Table 1).

b) Mutant *spermatheca* (*spt*)

The asymmetrical early pupal disc (Fig. 22) undergoes mitosis and gives rise to the adult female duet system, in similar position and orientation as in the wild-type female but with different numbers of ducts and capsules as well as different capsule and duct morphology (Table 1 and 2).

The *spermatheca* (*spt*) female develops two to four spermatheca capsules at 18°C (Fig. 24) with secondary fusions at the periphery of the capsules in some of the specimens. The two spermatheca ducts located closer to the oviduct and appear more muscular and thicker in diameter while the two ducts more posteriorly located appeared thinner and more membranous. At 28.5°C, the unfertilized one day old female develops $1\frac{1}{2}$ to 2 asymmetrical spermathecae with or without secondary fusions at the periphery of the capsule, and has one or two parovaria (Fig. 25). These 28.5°C *spt* females when fertilized continue the asymmetrical development into various shapes (Fig. 26, 27 and 29). Some of the specimens have a larger spermatheca on the left side of the oviduct (Fig. 26) while others have a larger spermatheca on the right side of the oviduct (Fig. 27, 29).

The spermatheca duct to the right of the oviduct-uterus axis was severed to expose the cross section (Fig. 26) of spermatheca duct at its proximal end just prior to its joining into the oviduct (Fig. 28). The duct lumen measures 10 microns across, the overall duct diameter is about 24 microns. The internal wall of the duct shows a ribbed appearance (Fig. 28).

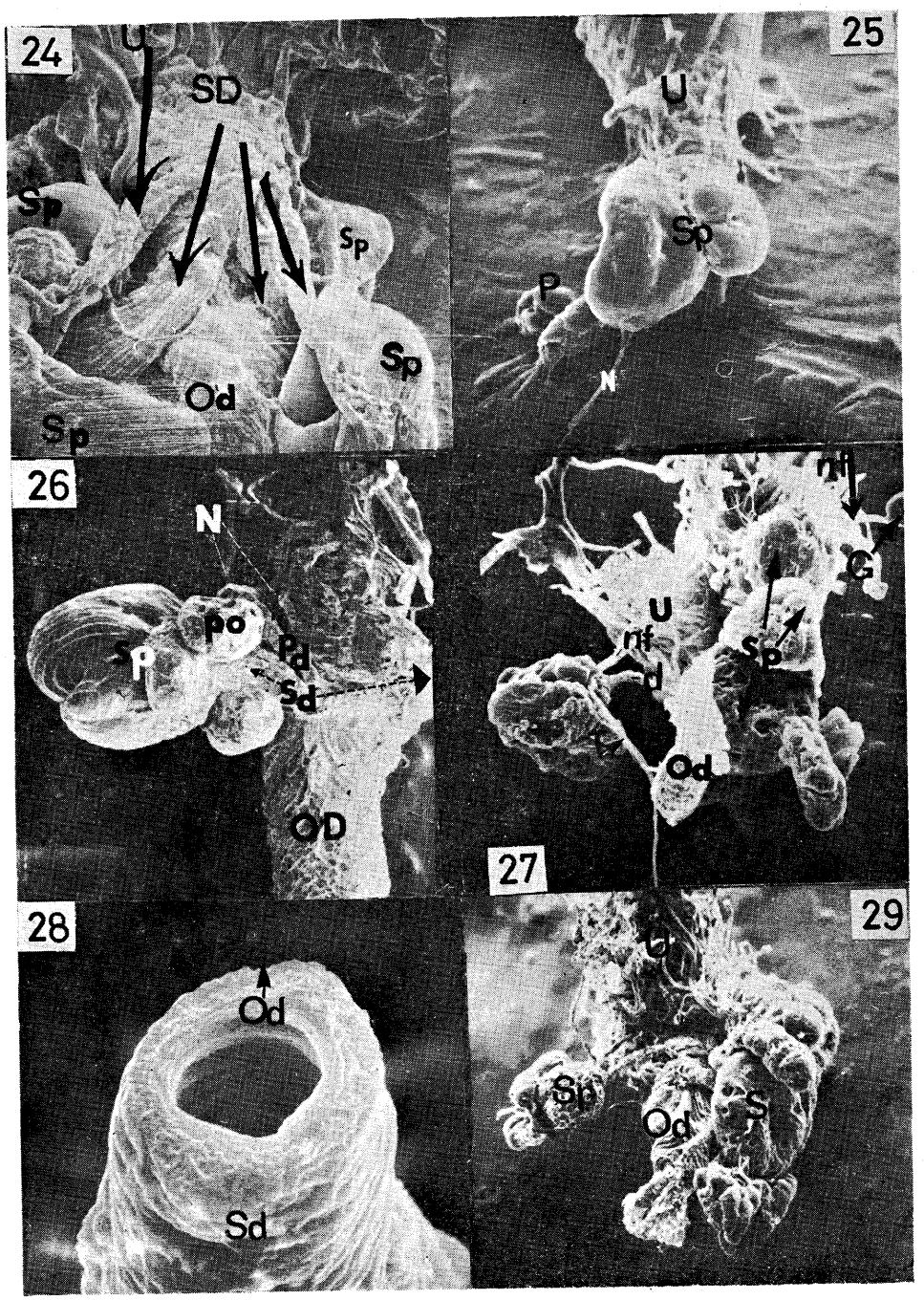
When then *spt* larvae are kept at 25°C, the disc gives rise to one spermatheca and one parovaria at eclosion (Fig. 30). If kept unfertilized, even after a prolonged period (Fig. 36), the capsules remain similar in size and shape to the one day old capsule. Changes that are found in the non-chitinized portion of the spermatheca capsule in the fertilized females are not found in the unfertilized females (Fig. 19, +/+; Fig. 32 through Fig. 35, *spt*). No ganglion near the entry to the introvert is seen in the unfertilized females, whether on day one or on day eight.

The mutant spermatheca capsule usually has a shallower introvert than in the wild type. This was confirmed by light microscopy on longitudinal sections of the capsule[25,26]. As well as by scanning electron microscopy (Fig. 20, +/+; Fig. 31, *spt*).

In addition to the difference in introvert between the wild type and the mutant, the mutant specimens usually exhibit a wide range of capsule shapes and sizes, with extra lobes (Fig. 32) or irregular growth (Fig. 33, 34 and 35), whereas the wild-type specimens usually exhibit uniformity in capsule morphology, as shown in Fig. 16.

DISCUSSION

The female genital disc appears to metamorphose later than the leg disc (120–128 hour), or wing disc (120–128 hour), or the male genital disc (104–110 hour) as reported by earlier investigators in this field[22,33,34]. The budding of the anterior-dorsal surface of the early pupal disc usually occurs at about twelve hours after spiracles eversion (142 hour post oviposition). The distinct spermatheca and parovaria ducts are seen usually at about twenty-four hours after spiracle eversion (154 hour post oviposition). Thus it appears that there is a lag between the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genital disc and the more anteriorly located discs with reference to the time of final differentiation. Also there appears to be a difference in the rate of differentiation between the male and the female reproductive ducts. But the difference in timing of larvae and pupae in various laboratories (Table 3) renders strict correlation of the data on different discs impossible.

Since the *spt* gene appears to affect only the spermatheca portion of the female duct system, this study placed on spermathecal topographical changes during development[11,20,25,26]. Some of the *spt* females at 25°C have only one each of parovaria and spermatheca. Earlier workers in field-mapping on female genital disc designated the central field as the origin for both the parovaria and the spermatheca (Fig. 2[15,30]). Thus it is not surprising that when the spermatheca is affected in the *spt* females, their parovaria is also affected. Such effect of the *spt* gene or some modifier gene in the *spt* stock has not been described before. Whether the fine structure of the mutant parovaria has also been affected would require further study.

The spermatheca capsule was seen to complete its final stage of differentiation after eclosion in both the mutant *spt* and the wild-type. Both non-chitinized portion (the outer portion) of the capsule and the unknown ganglion leading from the duct to the capsule show an increase in size after eclosion in the wild-type and the *spt* females which are fertilized.

The fertile female usually holds only one egg at a time in its oviduct-uterus. When the egg is released from the ovariole, it is housed in the uterus with its micropylar end slightly below the juncture of the ventral receptacle, parovaria, spermatheca and the uterus. Thus the release of a few sperms is sufficient to insure the fertilization of the newly arrived egg in the uterus. This evolutionary adaptation allows for a long period of continuous fertilized egg laying after a single mating as has been found earlier in +/+ and *spt* females[25,26].

Any part of the female duct system posterior to the entry of the egg into the uterus may play a role in forming some type of feedback stimuli to the prothoracic ganglion to stimulate for further eggs to be oviposited[12]. While spermatheca may not be the only part in the feedback circuit to such neural stimuli, it is a good candidate[25,26]. The increased oviposition[25,26] and the increased innervation (this paper) of the capsule of spermatheca in the *spt* females often exceed that of the wild-type females may indicate an egg releasing effect of the mutant gene *spt*.

The stalks connecting the female genital disc to the surrounding larval tissues were found to have tracheal and nervous supplies[25,26] (this paper). This finding is in agreement with the results on leg disc of Drosophila[22], also in the house fly[2]. Proper innervation by motor neurons is thought to be essential in the imaginal disc differentiation in the house fly[2,10]. Observations

Fig. 24. Posterior ducts juncture on dorsal side of oviduct (Od) before entering into uterus (U). *spt*, 2 day fertilized, 18°C. Sp=capsule of spermatheca, SD=ducts of spermatheca, no parovaria is seen, note peripheral portion of capsules are fused together. (425 ×).

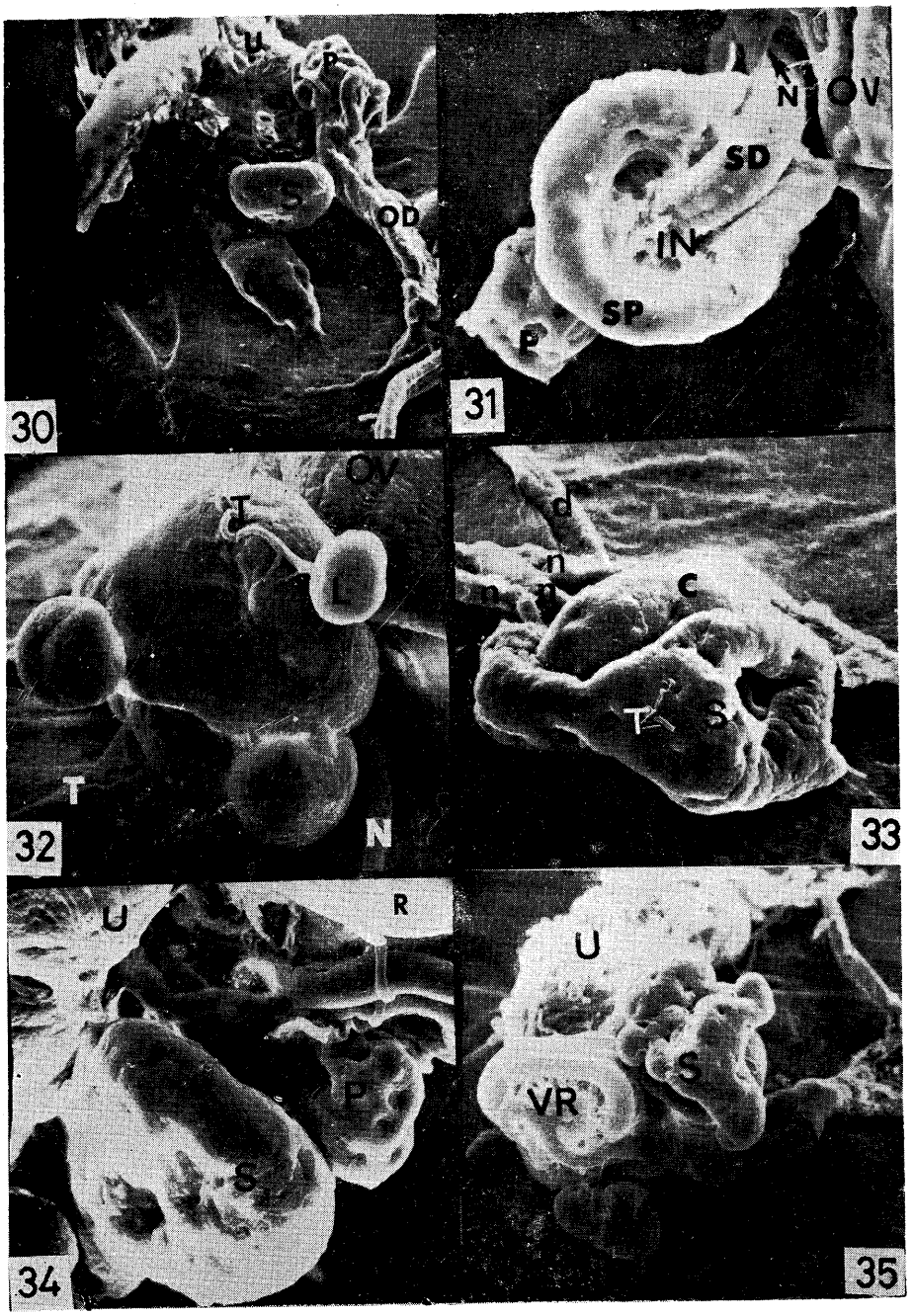
Fig. 25. One day old virgin *spt*, 28.5°C. An oversized spermatheca capsule (Sp) is supplied by a nerve (N), U=uterus, P=parovaria is not marked, just below oviduct (225 ×).

Fig. 26. Seven day old fertilized *spt*, 28.5°C. Only the left side of the oviduct (OD) is shown, Sp= hypertrophied spermatheca capsule, Sd=spermatheca duct, po=parovaria, Pd=parovaria duct, N=nerves. There is a very small spermatheca capsule, partially formed to the right of the oviduct not shown in this photo. (200 ×).

Fig. 27. Seven day old fertilized *spt*, 28.5°C. Asymmetrical development of the spermatheca capsule (Sp) is visible. nf=nerve fiber, d=spermatheca duct, U=uterus, Od=oviduct, G=ganglion. (200 ×).

Fig. 28. Spermatheca duct (Sd) cross section near the oviduct (Od), the proximal end. Duct lumen about 14 microns, the outer diameter of duct is 26 microns. (4300 ×).

Fig. 29. Seven day old fertilized *spt*, 28.5°C. Mature, asymmetrically developed spermatheca capsule (Sp). Od=oviduct, U=uterus. (146 ×).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made in this study support the above hypothesis. Further evidence may be gained from surface transplant of the female genital disc then check for any deviation in the adult structures derived from such surface transplants.

From the gross morphological results obtained in this study, a difference was found in the branching of the spermatheca ducts in the mutant and the wild-type females. It appears from the 18°C data that the extra branching (*spt*) of the ducts from the drosal surface of the pupal disc may have induced the extra spermatheca capsules to differentiate at the distal ends of the ducts. Some of the 28.5°C specimens show partially induced differentiation of capsules at the end of the ducts. Some of the specimens of *spt* show a lack or a deficiency in the number of parovaria with a concommitant increase in the number of spermatheca, thus it is conceivable that the presumptive parovarian duct has been converted into the spermatheca duct. Such conversion shows temperature sensitivity, that is to say at 28.5°C it is less complete; while at 18°C almost no parovaria is formed thus the conversion of parovaria into spermatheca is almost complete. However, the converted spermatheca may have secondary fusions at the periphery of the capsules. Thus it is still deviated from the wild-type spermatheca formation, since no secondary fusions have been found in the wild-type specimens during this study.

At 25°C although only one spermatheca is found in the *spt* female it is usually hypertrophied, with larger capsule, or extra lobes. Thus a dosage compensation effect may be responsible for the apparently normal reproductive capacity found in the *spt* females. This dosage compensation hypothesis is further supported from the electrophoretic findings in that the amount of different enzymes assayed in the mutant and the wild-type are usually quite similar[25,26].

From the detailed analysis on the mutant *spermatheca* and the wild-type female genital disc, one sees that the control of the determination and differentiation in the female genital disc is rather complex. If the control circuits for determination and transdetermination were indeed similer to those in the bacteriophage lambda as suggested by Kauffman[19]. then the conversion of mutant phenotype in the *spermatheca* females from the 18°C and 25°C to that of the 28.5°C should be possible. Also by combining in proper proportions the disc cells from the 25°C with the 18°C disc cells, one should get the wild-type phenotype (two spermathecae per adult female).

From earlier studies[24,28,35] the function of spermatheca was thought to be for storage of sperm received from the male during mating in *Drosophila*. Some workers have also postulated a secretory role for the spermatheca in the maintenance of viability and fertility of sperm

Fig. 30. One day old *spt*, unfertilized, 25°C. One spermatheca capsule (S) and one parovaria (p) are found in this specimen. U=uterus, OD=oviduct, GT=gut. (212 ×).

Fig. 31. Eight day old *spt*, unfertilized, 25°C. To the left of the oviduct, a single spermatheca duct (SD) joins the capsule (SP); a very shallow introvert (IN) and a parovaria (P) as well as a nerve fiber (N) are shown. N ganglion is seen in this specimen. (525 ×).

Fig. 32. Two day old fertilized *spt*, 25°C. Extra lobes (L) with large dark spots well supplied with tracheoles (T) and nerve (N) can be seen. OV=oviduct. (1940 ×).

Fig. 33. Three day old fertilized *spt*, 25°C. A large spermatheca capsule (S), with expanded central portion (C), large and small pores, as well as tracheoles (T) and nerve fibers (2-3, from the left side of capsule), and an underdeveloped spermatheca duct (d) are shown. (485 ×).

Fig. 34. Three day old fertilized *spt*, 25°C. A single spermatheca capsule with no demarcation between the central and peripheral portion (S) is next to a parovaria (P), U=uterus, R=ventral receptacle. (370 ×).

Fig. 35. Seven day old fertilized *spt*, 25°C. An oversized spermatheca capsule (S) with many trcheoles on the surface, a parovaria (P), ventral receptacle (VR), U=uterus, and OV=oviduct are shown. (213 ×).

TABLE 3

Larval-pupal Timing in *Drosophila melanogaster***

Authors, years	Third Instar Larva			Pupa			
	Early, young	Middle, mature	Late, old, prepupupa.	Spiracle eversion, pale.	Tanning, sinks in water.	Bubbla, floats in H_2O.	Epicuticle, adult.
Bryant, 1970.	—	—	—	120	—	—	—
Fristrom & Heinz, 1968, DIS.				5 day (120–144)			
Garcia-Bellido & Merriam, 1971, Dev. Biol.	—	—	—	0–4	4–8	8–12	—
Poodry, Schneiderman, 1970.	—	—	—	124–126	—	144	156
Schubiger, 1968.	—	—	115	—	—	—	—
Shearn, et. al., 1971.	—	4 day (96 hr)	—	—	—	—	—
Tang (this study).	—	96–110	120–128	130–132	136–138	144–146	154–156
Wehman, 1969.	—	—	120–128	—	—	—	—
Ulrich, 1971.	—	—	115–120	—	—	—	—
Baker, 1967.	70–80	—	96	—	—	—	—
Chan & Gehring, 1971.	72–80	—	96–100	—	—	—	—
Doane, 1969, Fig. 4, Table 5, Park City Symp.	48–60	80–85	96–100	100	mid-pupa = 144 hr.		
Fristrom, D., 1969.	early	middle	late	—	—	—	—
	(hours not recorded)						
Fristrom, J. et al., 1969.	75–85	—	95–96	—	—	—	—
Garcia-Bellido,							
1966, Dev. Biol.		mature (hour not recorded)			—	—	—
1966, Exp. Cell Res.			96	96–100 (pupa formation)			
Gehring, 1966.	75–80	—	96–100	—	—	—	—
Mandaron, 1970.	—	—	95–96	—	—	—	—
Ursprung & Schabtach, 1968.	72	94	104	104 ± 15 minutes		—	—

* in hours.

stored in the female ducts in other insects[4,5,27,29] as well as in *Drosophila*[1,11]. Both functions are confirmed in *D. melanogaster*[26] (this paper). In addition, it is suggested here that the spermatheca may be a significant factor in controlling both the release of eggs from the ovariole as well as the oviposition event following fertilization with the timely release of sperm stored in the spermatheca. Further work may be done to isolate a spermatheca specific substance that would increase or decrease the rate of oviposition, or alter the pattern of neuronal firing in the head region[36].

Experiments using various hormones may be designed to observe the concommitant changes in the size of the non-chitinized portion of the spermatheca capsule and the size of the spermatheca ganglion along with changes in oviposition and/or other effects on the female reproductive physiology. This suggestion is based on models proposed by Ohtaki, *et. al.*[21] and by Grossfield and Sakri[12].

A parallel experimental series on parovaria would be indicated to study the factors affecting the conversion of parovaria-field into spermatheca-field in the female genital disc of mutant *spt*, as well as parovaria and spermatheca's separate contributions to the female *Drosophila* reproductive physiology.

SUMMARY

1. Mutant *spt* gene show temperature dependent phenotypic expressions when larvae are raised at 18°C, 25°C and 28.5°C, while the wild-type allele shows buffering capacity at the three temperatures examined, always having the same phenotype.
2. Homozygous mutant females exhibited a range of phenotypes at each of the three temperatures studied, including variation in the number of spermathecal capsules, the number of lobes per capsule, the size of the capsule, number of spermathecal ducts per specimen, presence or absence of a secondary fusion at the periphery of capsules in the specimens which possessed separate ducts, and the symmetry of spermathecal differentiation.
3. Scanning electron microscopy performed on the mutant and the wild-type revealed a difference in surface architectures, such as pore shape, pore size, pore frequency, tracheal and nerve supplies, between the two, as well as the number of spermatheca and parovaria. Thus it is suggestive of a more labile disc-field in the mutant female genital disc than in the wild-type.
4. Development of the organ spermatheca in *D. melanogaster* continues after eclosion from the puparium in both the mutant and the wild-type and is related to the mating status (Table 2).

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雌果蠅生殖腺蛻變前後之比較

魏 以 連

本文利用掃描式電子顯微鏡將雌果蠅 (*Drosophila melanogaster*) 之野生種與突變種 (spt) 之生殖腺在蛻變前後之形態加以研究比較，發現雌性生殖腺在蛻變後仍繼續生長與分化。突變種之生殖腺除在一般室內飼養溫度 (25°C) 時與野生種不同外，亦缺乏對環境溫度改變的緩衝性能，即在 18°C 與 28.5°C 時有多種不正常的表現型而野生種則在 18°C 與 28.5°C 之間始終呈一種表現型，由電子掃描時發現一神經細胞束位於貯精器球形體下端隨成蟲之年齡而增大，文中論及其可能功用。