# MORPHOMETRICAL AND HISTOLOGICAL STUDY OF THE REPRODUCTIVE SYSTEM OF DESERT LOCUST, SCHISTOCERCA GREGARIA (Forskal) ORTHOPTERA ACRIDIDAE

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The average total number of ovarioles per grasshopper was found to be 107.9, while the average number of ovarioles in right or left ovary was 53.9. The maximum difference between the number of ovarioles of the left and right ovaries was 7. The number of egg rudiments per ovariole varied from 13-20, with an average of 15 in the young females. The testicular follicles varied from 120-154, with an average of 132.9 per grasshopper.

The average length and breadth of the testes, testicular follicles, vas deferens between accessory glands and testicular follicles and ejaculatory duct was 7.65 mm. and 2.3 mm., 3.1 mm., and 0.24 mm., 15.5 mm. and 0.15 mm. and 1.6 mm. and 0.32 mm. respectively (Table 1).

The average length and breadth of spermatheca, spermathecal duct, lateral oviduct, common oviduct, and ovariole was 2.26 mm. and 0.91 mm., 12.5 mm. and 0.14 mm., 8.24 mm. and 1.29 mm., 5.0 mm. and 0.99 mm., 5.7 mm. and 0.19 mm. respectively. The length and breadth of the ovary was variable depending upon the development of eggs. The average length of uncoiled accessory gland was found to be 9.9 mm. in females about to lay eggs (Table 2).

### Introduction

Several authors have studied the reproductive system of different grasshoppers, but none of them investigated it thoroughly. Roonwal<sup>1</sup> recorded an abnormal structure in the female accessory glands of desert locust, Schistocerca gregaria. Chauvin<sup>2</sup> has given some histological data on the seminal vesicles and the accessory glands of the same species. Laird<sup>3</sup> has classified the testes of grasshoppers into "radiating," "fountain" and "intermediate" types. Gupta 4 described the development of female reproductive organs and their efferent ducts in Schistocerca gregaria. Albrecht<sup>5</sup> reported that the ovarioles in Nomadacris septemfasciata were considerably higher than those in Locusta migratoria. Waloff<sup>6</sup> gave the number of ovarioles found in Melanoplus bivittatus. The Morphometrical and histological studies of the reproductive system of Schistocerca gregaria have not been studied in detail. It was, therefore, decided to investigate the number of ovarioles and testicular follicles and make morphometric and histological studies of the reproductive system of desert locust, Schistocerca gregaria.

#### **Material and Method**

Locusts Schistocerca gregaria were bred in wooden cages of the size  $40'' \times 24'' \times 27''$ , which were fitted with two electric bulbs of 200 watt each, at the top of the cage to maintain  $35\pm 5^{\circ}$ C. temperature. Adults were fed on fresh cabbage (Brassica oleracea (L) leaves and nymphal stages were reared on artificial diet,7 consisting of glucose, yeast and wheat bran in the ratio of 2:1:2.

The reproductive organs of the preserved as well as fresh adults were dissected in water and measurements for morphometric studies were made from dorsal side. Measurements were made with vernier calipers. For minute structures ocular micrometer was used.

Permanant slides were prepared by passing the reproductive organs through glacial acetic acid, carbolic acid and xylol. The detailed study of cuticular part was facilitated by treating the specimens with 10% KOH to remove non-cuticular material. Borax carmine was used to stain the parmanant mounts. Preservation of adults was done in 70% alcohol.

For histological studies reproductive organs of one month old adults were used. Fixation was done in Bouin's fluid. Sections were cut at 5-6 micron, and stained with haematoxylin and eosin.

### **Result and Discussion**

The Male Reproductive Organs.—The male reproductive organs consist of paired compound testes (TES) paired lateral vasa deferentia (VD) paired accessory glands (AC.GL) and a median ejaculatory duct (DEG), Fig. 1. The vasadeferentia enlarge at the posterior end to become seminal vesicles (SMV). The ejaculatory duct opens into the median intromittent organ at its posterior end.

The Testes.—In sexually mature male the testes were paired yellowish rectangular bodies consisting of large number of long and narrow pear shaped follicles (TF). The testes occupied the fourth and fifth abdominal segments dorsal to the alimentary canal. The location of the testes is similar to that of *Locusta migratoria* as described by Oadri.<sup>8</sup> In relative position they were a little posterior in contrast to ovaries in female. The testes were much smaller than the ovaries, measuring approximately 8 mm.

The testes were surrounded by adipose tissue and were intermediate type as the vasa deferentia ran along the whole length of the testicular follicles (Figs. 2 and 3). According to Laird<sup>3</sup> the number of follicles in 'intermediate' type of testes by the vas efferens (VE). The testes had no suspensory filaments (LG) as seen in the ovaries (Fig. 4). All the sperm tubes were held together by connective tissue and contained in a delicate peritoneal membrane. The total number of testicular follicles per testes varied from 120-154, with an average of 133. The maximum difference of the testicular follicle number among twenty adults was found to be 34. The total number of



Fig. 1. — Dorsal view of the male reproductive organs (Semi-Diagrammatic) 8  $\times$ 

Fig. 2. — Dorsal view of the testes—(Semi-diagrammatic)  $8 \times$ 

varied between 100-150. In Schistocerca gregaria they varied between 113-150. Laird placed Locusta migratoria and Dissosteira carolina in 'fountain type,' while the Schistocerca americana and other species of Schistocerca were placed in 'intermediate' type. He did not mention about Schistocerca gregaria.

The general structural plan of the testes was comparable to the ovaries. The sperm tubes arose at the anterior end of vas deferens attached

Fig. 3. — Lateral view of the testes—(Semi-diagrammatic) 8 ×,
 Fig. 4. — Dorsal view of the female reproductive organs (Semi-diagrammatic) 8 ×,

testicular follicles was greater than ovarioles in the ovaries. The average length and breadth of the testicular follicle was 3.1 mm. and 0.24 mm., respectively.

The testicular follicle was covered over a single layer of epithelial sheath (SH) resting on basement membrane (BMB) without clear cell walls and nuclei. Each testicular follicle contained a succession of zones in which the sex cells (SPI), were in different stages of development. The

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spermatogonia (SP 2) which later on became the spermatozoa (SP 3) were surrounded by the sperm cyst (CST). The testicular cysts were connected by strands of cells throughout the lumen of the sperm tube (Figs. 5 and 6).

All the stages of spermatogenesis were observed in these cysts. In the posterior end of the tubules the cysts degenerated, but the mature sperms remained in the form of groups.

The Vasa-Deferentia.—The paried non-convoluted vasa deferentia ran posteriorly encircling the alimentary canal united with the ejaculatory duct along with accessory gland to form common median ejaculatory duct in the ninth abdominal segment. The vasa deferentia ran very close and parallel in the region of testes. The average length of vas deferens between testes and accessory glands was 15.5 mm. The wall of the vas deferens consisted of single layer of cuboid epithelium (EPTH) limited by basement membrane. The circular muscle layer (CMCL) outer to the epithelium was very thin, discernable under high magnification (Fig. 7). The single layer of epithelium of vas deferens lacked distinct cell boundaries.

The Male Accessory Glands.- A pair of well developed coiled accessory glands were ventral to rectum between seventh to ninth abdominal segments. The two accessory glands opened separately, into the ejaculatory duct. Each accessory gland consisted of sixteen delicate tubules of different diameters and sizes coiled into a mass as in Nomadacris septemfasciata. The colour of these tubules varied from milky white to transparent one. The size of the two accessory glands might differ in certain individuals. The thin orange membrane in between the tubules was reported to be seminal vesicles by Albrecht<sup>5</sup> in Nomadacris. This membrane was also observed in Schistocerca gregaria. Qadri<sup>8</sup> has described the seminal vesicle as a paired orange yellow blind tubes in Locusta migratoria. The histology of accessory glands suggested that they were having a thin outer layer of circular muscles. The epithelium rested on basement membrane, with large squarish cells with clear round or oval nuclei, but the cell boundaries were not clear (Fig. 8.)

In sexually mature adults some tubules of the accessory glands in *Schistocerca gregaria* contained aggregates of spermatozoa, According to Else<sup>9</sup> such tubules in *Melanoplus differentialis* were considered as seminal vesicle, while the other tubules were ment for secretion of semen. It was difficult to demarcate these two types of coils externally.



Fig.	5	Longitudinal section of the testicular follicle.	$60 \times$
Fig.	6. —	Transverse section of the testicular follicle.	$60 \times$
Fig.	7. —	Transverse section of the vas deferens.	$60 \times$
Fig.	8. —	Transverse section of the male accessory gland.	$120 \times$
Fig.	9	Transverse section of the ejaculatory duct	$60 \times$

# STUDY OF THE REPRODUCTIVE SYSTEM OF DESERT LOCUST

The Ejaculatory Duct.—The ejaculatory duct was a short median whitish common exit tube of male genital system. It was broad muscular tubular structure and received the accessory gland on each side. The average length and breadth of the ejaculatory duct was 1.6 mm. and 0.32 mm., respectively. Its distal end was attached to the penis sac situated in a pit at the base of 9th abdominal segment. The distal end of ejaculatory duct immediately behind the intromittent organ was strongly musculated to form the ejaculatory sac<sup>10</sup> having slight striations on the ventral side.

The histology suggested that it was invaginated ectoderm layer at the posterior end of the ninthabdominal segment, as the single layer of columnar cells were lined by cuticle (Fig. 9).

Strong circular muscles were outer to the longitudinal muscles (LMCL). The outer covering layer (CL) of circular muscles was clearly demarcated.

The Female Reproductive Organs.—The female reproductive organs consist of paired dorsal ovaries, paired lateral oviducts, a common ventral oviduct and spermatheca (Fig. 4).

The Ovaries.—The paired pear shaped yellowish ovaries were dorsolateral to the alimentary canal in the anterior abdominal region reaching up to the first abdominal segment or even up to thoracic region when eggs attained maximum size. The ovaries could attain the average length of 15 mm. in mature females.

Each ovary was united with the other middorsally and were covered over by adipose tissue (AT). The pear shaped ovarioles (OVL) arose in a longitudinal series along paired oviducts and their number varied in different individuals, <sup>11,12</sup> even in the right and left ovary. The maximum difference of ovarioles in right and left ovary was found to be 7.

The total number of such ovarioles per locust had a range between 101-117, with an average of 107. The number of ovarioles was greater in *Schistocerca gregaria* than *Locusta migratoria* as described by Boldyrev<sup>13</sup> to be from 82-111. The number of the externally visible and differentiated egg rudiments (DE) in the ovarioles might vary from 13-20 with an average of 15 in young females. The histology of the ovariole was found to be similar as found in other Acridids. The panoistic ovariole<sup>14</sup> had the most mature eggs (EG) at its proximal end adjacent to the oviduct. The epithelium surrounding the oocyte (OOC)



Fig. 10. — A portion of longitudinal section of anterior end of the ovariole  $120\times$ 



Fig. 11. — Transverse section of distal end of the ovarioles  $60 \times$ 

was single layered with large round to oval nuclei. The cell walls were not clear. The oocytes were surrounded by the chorion (CH) inner to the follicle cells (F.CL), while the follicle cells were covered over by peritoneum (G) (Figs. 10 and 11).

The Lateral Oviducts.—The paired white oviducts (OD) ran dorso-laterally to meet ventromesially beneath the alimentary canal in the 7th abdominal segment and emerged to form the median

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oviduct (Vagina, VAG), which ran along the eighth sternum. The average length of lateral oviducts in young female was 8.24 mm. The anterior end of lateral ducts expanded to form the calyx (CLX) into which opened the pedicels of the ovarioles. The anterior most extension of the lateral calvces beyond the first ovariole was the small much coiled tubular gland located in the anterior abdominal region. The two glands (T. GL) bent inward to touch its fellow of the other side. In the present study no such abnormality as observed by Roonwal<sup>12</sup> could be seen in the female accessory glands. The accessory glands were not found to be vestigeal as described by Gupta, 4 rather the glands acquired their maximum development shortly before oviposition. In uncoiled condition the length of glands varied between 3 to 12 mm. The average length of the uncoiled gland was found to be 9.9 mm. in females about to lay eggs.

The lateral oviducts had circular muscle fibres inner to the outer layer of longitudinal fibres (Fig. 12). The basement membrane supported the Cuboid-columnar epithelial layer thrown into many longitudinal folds to allow the expansion of lumen (LNM) of the oviduct. The epithelium had no distinct cell boundaries, but the round or oval nuclei with abundant chromatin granules were quite conspicuous.

The Median Oviduct.--The median oviduct ran dorsally along the sub-genital plate and opened



Fig. 12. — Transverse section of the oviduct.  $60 \times$ 

into the spacious genital chamber beneath the bases of the ventral and above the posterior margin of the 8th abdominal sternum. The average length of the median oviduct in young female was 5.0 mm. The paired glandular pouches on both sides of the vagina opened separately into the genital chamber. These pouches were the comstock-kellog glands as described by Slifer<sup>15</sup> in Nomadacris septemfasciata. These glands were colourless, with similar shape and size. Albrecht described these glands as brightly coloured in Nomadacris septemfasciata.

 TABLE I.—MORPHOMETRICAL STUDIES OF THE MALE REPRODUCTORY SYSTEM OF DESERT LOCUST,

 Schistocerca gregaria Forskal Based on Twenty Adults.

S. No.	Organs			Length (MM)	Average (MM)	Breadth (MM)	Average (MM)
1. 2. 3.(a) 3.(b) 4.	Testes Testicular follicle Vasa Deferentia Vasa Deferentia bearing t Ejaculatory duct	  esticular fol	  llicles	$\begin{array}{c} 6.6 & -8.6 \\ 2.7 & -3.5 \\ 12.6 - 19.5 \\ 2.5 & -3.9 \\ 1.2 & -2.0 \end{array}$	$\begin{array}{c} 7.65 \pm 0.95 \\ 3.1 \pm 0.4 \\ 15.5 \pm 4.0 \\ 3.3 \pm 0.6 \\ 1.6 \pm 0.4 \end{array}$	I.9 -2.9 0.2 -0.3 0.1 -0.2 0.25-0.4	$2.3 \pm 0.6 \\ 0.24 \pm 0.06 \\ 0.15 \pm 0.05 \\ 0.32 \pm 0.08$

TABLE 2.—MORPHOMETRICAL	STUDIES	OF THE F	EMALE F	REPR	ODUCTORY	System of .	DESERT	LOCUST,
Schistocerca	gregaria	Forskal	BASED	ON	Twenty	Adults.		

S. No.	Organs		*		Length (MM)	Average (MM)	Breadth (MM)	Average (MM)
Ι.	Ovary (Variable depend	ing	upon	the				
	development of eggs.)		,		12.0-16.0	13.8 ±2.2		
2.	Ovarioles				5.1 -6.4	$5.7 \pm 0.7$	0.13-0.25	$0.19 \pm 0.06$
3.	Lateral oviduct				7.0-10.0	$8.24 \pm 1.76$	0.9 -1.9	$1.29 \pm 0.61$
4.	Common oviduct			· · ·	3.4 -6.5	$5.0 \pm 1.5$	0.5 -1.5	$0.99 \pm 0.51$
5.	Spermatheca				1.8 -2.9	$2.25 \pm 0.64$	0.5 -I.I	$0.91 \pm 0.09$
6.	Spermathecal duct				7.2-14.9	12. $5 \pm 2.4$	0.1 -0.18	0.14±0.04
7.	Calyx and accessory gland				11.5-28.0	$21.06 \pm 6.94$	0.7 -1.8	1.14±0.66

The general colour and structure of median oviduct resembled the lateral oviducts, except that the epithelium was lined by intima. The vaginal epithelium (VAG. EPTH.) was with indistinct The nuclei were mostly ovoid cell bounadries. with clear chromatin granules. The presence of cuticle (CT) indicated the ectodermal origin of the epithelium.

The musculature consisted of an outer coat of circular muscle fibres surrounding the inner mass of well developed longitudinal muscle fibres, which occupied the spaces of the folds of the epithelium. A few longitudinal muscles were also present outer to the circular muscles.

The muscular coat was followed by the basement membrane on which rested the epithelium thrown into longitudinal folds (Fig. 13).



Fig. 13. — Transverse section of the median oviduct,  $120 \times$ 



The Spermatheca.-The spermatheca (SPT) was a single ovoid sac formed of yellowish white coiled tubules, in the 8th abdominal segment. The average length and breadth of the spermatheca in young female was 2.26 mm. and 0.91 mm., respectively. It was connected dorsally to the genital chamber by a convoluted muscular spermathecal duct (SPT.D). The expanded proximal end of the spermathecal duct was slightly sclerotised ventrally. The average length and breadth of the spermathecal duct was 12.5 mm. and 0.14 mm., respectively. Structurally the spermatheca consisted of a stratum of columnar epithelium resting on a basement membrane covered over externally by strong muscular coat.



#### 0.2mm



The muscular coat consisted of circular muscles outer to the inner layer of longitudinal muscles. The muscular coat was covered over by an interrupted covering layer (CL) (Fig. 14).

The histology of spermathecal duct suggested that the columnar epithelium with clear cells. and nuclei rested on basement membrane. The epithelium was lined over internally by cuticle and was covered over externally by a thin muscular coat (MUS) (Fig. 15).

### References

- Roonwal, Nature, 135, 394 (1935). Ι.
- R. Chauvin, Bull. Soc. Hist. Nat. Afe., 2. No. 28, 488 (1938).
- Laird, J. Morphol., 72, 477 (1943). 3.
- P. D. Gupta, Indian J. Entomol., 10, 75. 4. 1948).
- F. Albrecht, Anti. Locust Bulletin 1-9 (1956).
- N. Waloff, Physiol. Comp. Et. Oecol., 3, 6. 390 (1954).
- G. F. Hawden and P. H. Jones, Nature, 7. 182, 1627 (1958).
- Qadri, Trans. R. Entomol. Soc., London, 8. **90,** 121-175 (1940). E. L. Else, J. Morphol., **55,** 577 (1934).
- 9.
- R. E. Snodgrass (McGraw-Hill Book Com-IO. pany, 1935), p. 667.
- I. A. Rubtzor, Bull. Entomol. Res., 25, 339 II. (1934).
- 12. D. J. Nolte, J. Entomol. Soc., South Africa, 2, 196 (1939).
- 13. B. Boldyrev, Izv. Prinkl. Entomol., Leningrad **4(1)**, 189 (1929).
- A. D. Imms, A General Text Book of Entomology 14. (Mathew and Co., London), p. 886.
- 15. Slifer, J. Morphol., 65, 437 (1939).