

**Implication of mating on oocyte development in red cotton bug,
Dysdercus koenigii (FABRICIUS, 1775) (Heteroptera: Pyrrhocoridae)**

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ABSTRACT. The ovaries of *Dysdercus koenigii* are consisted of meroistic telotrophic ovarioles. Each ovariole can be differentiated into tropharium and vitellarium. The tropharium contains stem line oogonia, newly formed oocytes, trophocytes, prefollicular cells and follicular cells. The vitellarium possesses 10-12 developing oocytes. The developing oocytes are connected to the trophocytes, present in the tropharium, by nutritive cords. During pre-mating period, the ovarioles change, resulting in increase the number of oocytes in the vitellarium. The developing oocytes in the initial stage of development are surrounded by columnar follicular cells, which are subsequently changed to cuboidal and squamous cells in a sequence. The process of vitellogenesis was initiated after 48 h of adult development with the appearance of perioocytic space. There was deposition of yolk material at the periphery of oocytes in the ovarioles of 72 h old females. The further development of oocytes and vitellogenesis remained suspended up to 12-14 days in the virgin females. On the other hand, mating stimulates the oocyte development and process of vitellogenesis. There were distinct morphometric and histological changes in the ovarioles as a consequence of mating; dimensions of vitellarium and oocytes of the mated female increased drastically. However, the size of tropharium and number of oocytes present in the vitellarium largely remained unchanged. The process of vitellogenesis also resumed followed by mating stimulus. Therefore, the oocytes were laden with yolk material; at this stage, the surrounded follicular layer is replaced by chorion.

KEY WORDS: *Dysdercus koenigii*, Ovariole, Tropharium, Vitellarium, Oocyte, Mating

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INTRODUCTION

Oocyte maturation and egg production are two functionally distinct, but closely related processes. It has been shown that either of the two processes could be modified by mating stimuli to evoke oviposition in the mated females. Therefore, the enhanced oviposition behaviour shown by the mated females may be a direct effect on laying the fully developed oocytes or an indirect effect on oocyte development. In the absence of mating, the females may retain the oocytes in their ovarioles and showed oosorption or lay unfertilized eggs. The insects, where females mate only after the maturation of their eggs, any increase in oviposition followed by mating is a direct effect, while in insects, where females mate either during or prior to egg maturation, the stimulatory effect of mating on oviposition could be coupled with initiation, and acceleration of oocyte growth (LEOPOLD 1976).

Dysdercus koenigii (FABRICIUS, 1775) is an agricultural pest of paramount economic importance. Both the nymphs and adults suck sap from the developing seeds and cause economic injury to the standing crops of cotton and other malvaceous plants. Both the males and the females meet special feats in order to reproduce. The distinctive features of the reproduction are pre-mating period of ca 3 days, multiple mating, and prolonged mating of about 72 h (GUPTA & SEHGAL 1995, 1997). Our earlier studies showed that mating had a profound effect on ovipositional behaviour of the female *D. koenigii* (GUPTA & SEHGAL 1995). This may be due to increased rate of oocyte development or stimulation of oviposition by mating. To understand the mechanism by which mating induced the oviposition was therefore, undertaken in the present research work. The processes of vitellogenesis and egg maturation as altered by mating were examined by morphometric and histological studies of the ovarioles of virgin and mated females.

MATERIALS AND METHODS

Present investigations were carried out on the adults of red cotton bug *D. koenigii*. A culture of this bug was maintained in the laboratory at a temperature 27.0 ± 2.0 °C relative humidity $70.0 \pm 5.0\%$, and 12 h light: 12 h dark, photoperiod regimen to obtain insects having sustained quality with respect to growth and adult viability. The insects were provided with cotton seeds which were previously washed in running water and dried on filter paper. Sterilized cotton swab soaked in water was kept in the rearing jar as a source of water to the insects. Individual mating pairs of the bugs were placed in small rearing jars (200 mL) and 5-10 mating pairs were kept in the jars of large size (500mL). Generally, the female lays eggs in batches beneath the cotton seeds. The eggs were separated within 12 hours of oviposition and incubated in small sterilized glass hatching vials (1x5 cm) in

a BOD incubator at high humidity (80% to 90%). Normally eggs hatched in 5-6 days. The newly emerged nymphs were transferred to glass jars containing food and water, and were reared as stock culture. For all the experiments, fifth instar nymphs were isolated from the stock culture. Male and female fifth instar nymphs were identified on the basis of their relative sizes, as female nymphs were broader and stouter than the male nymphs.

Newly emerged females (0-1 h old) were isolated from the stock culture, sacrificed at stipulated ages of 0-1 h, 12 h, 24 h, 48 h and 72 h and their ovaries were taken out. To assess the influence of mating on ovarian development, the ovaries from the females mated for 24 h, 48 h and 72 h were taken out. The ovaries from the virgin females of corresponding ages were used for control. For morphometric analysis, length of ovariole, tropharium and vitellarium, number of developing oocytes in vitellarium and dimensions of oocytes were measured using software VImage 2014. All the experiments were replicated ten times. The data were analyzed statistically to one-way ANOVA followed by post hoc Tukey test using software SPSS version 19. Histological studies of the ovaries of above mentioned groups of females were carried out to understand the process of oocyte development as modified by mating. For histological studies, the ovaries were fixed in the Bouin's fixative and processed by standard histological technique. The thickness of the sections was preset at 5 μm . The sections were stained with hematoxylin and counterstained with 1% eosin prepared in 90% ethanol. The sections were observed under Nikon Eclipse E200 Upright microscope and photographed using a UCB500 camera. The morphometric analysis was done using VImage 2014 software.

RESULTS

Anatomy of ovary

The ovaries of *D. koenigii* are consisted of meroistic telotrophic ovarioles, each distinguishable into four well-defined parts. Anteriorly, the ovariole has a terminal filament, followed by a tropharium. The next region, largest of all, is vitellarium contains 10-12 developing oocytes. The vitellarium continues proximally as a pedicel which opens into the lateral oviduct (Fig. 1). The tropharium contains stem-line oogonia and the oocytes in the early stage of development, trophocytes and prefollicular tissue, (Fig. 2A, B). Both the trophocytes and oogonia appear to arise from the same type of cells, which became distinguishable in later stages of their development. The trophocytes in the beginning were actively dividing. They are mononucleate in the initial stage of development which later, fuse to form binucleate and multinucleate cells (Fig. 2B). The nuclei of the cells subsequently disintegrate and release their contents into the nutritive cord (Fig. 2B, C). The oogonia contain abundant cytoplasm and a dense nucleus and confined mainly to the

posterior part of tropharium (Fig. 2D). The prefollicular tissue occupies the part posterior to trophocytes in the tropharium (Fig. 2C, D). The older prefollicular cells surround the developing oocytes as they progress towards the vitellarium. These cells at this stage constitute a columnar follicular tissue. The young oocytes are found at the base of tropharium, distal to the trophocytes (Fig. 2D). Initially they were small and spherical, but as they grew and descended down into the vitellarium, orient in a straight line and became ovoid. The oocytes are constantly produced in the tropharium and moved down the vitellarium, where further growth and maturation takes place. During development, the oocytes in the tropharium remain small until the first batch of oocytes contained in vitellarium is ovulated. The oocytes in their early stage of development are surrounded by columnar follicle cells (Fig. 3A) which subsequently changed to cuboidal (Fig. 3C) due to increase in the size of oocyte. The cytoplasm of the oocyte in initial stage is clear and homogeneous (Fig. 3A, B, C). At the beginning of vitellogenesis, there appears a perioocytic space between the follicle cells and oocytes (Fig. 4B). In the peripheral part of the oocytes then appeared a number of small yolk spheres which fused to form larger sphere, that later moved towards the centre of oocyte (Fig. 3D). Different gradation of yolk deposition can be seen in the oocytes from anterior to posterior region. Further growth of oocyte resulted in transformation of follicle cells from cuboidal to squamous (Fig. 3D, E). After this the oocyte acquires the chorion.

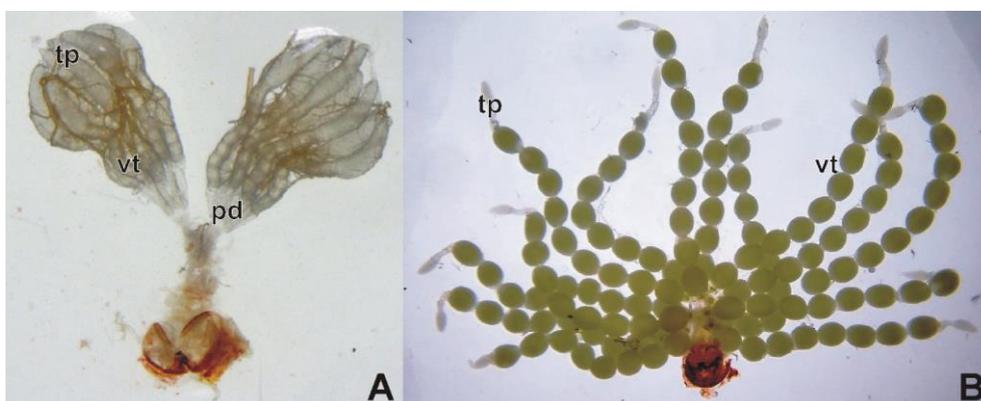


Fig. 1. Reproductive system of female *D. koenigii*: A – 24 h old female; B – 72 h mated female; tp – tropharium; vt – vitellarium; pd – pedicel.

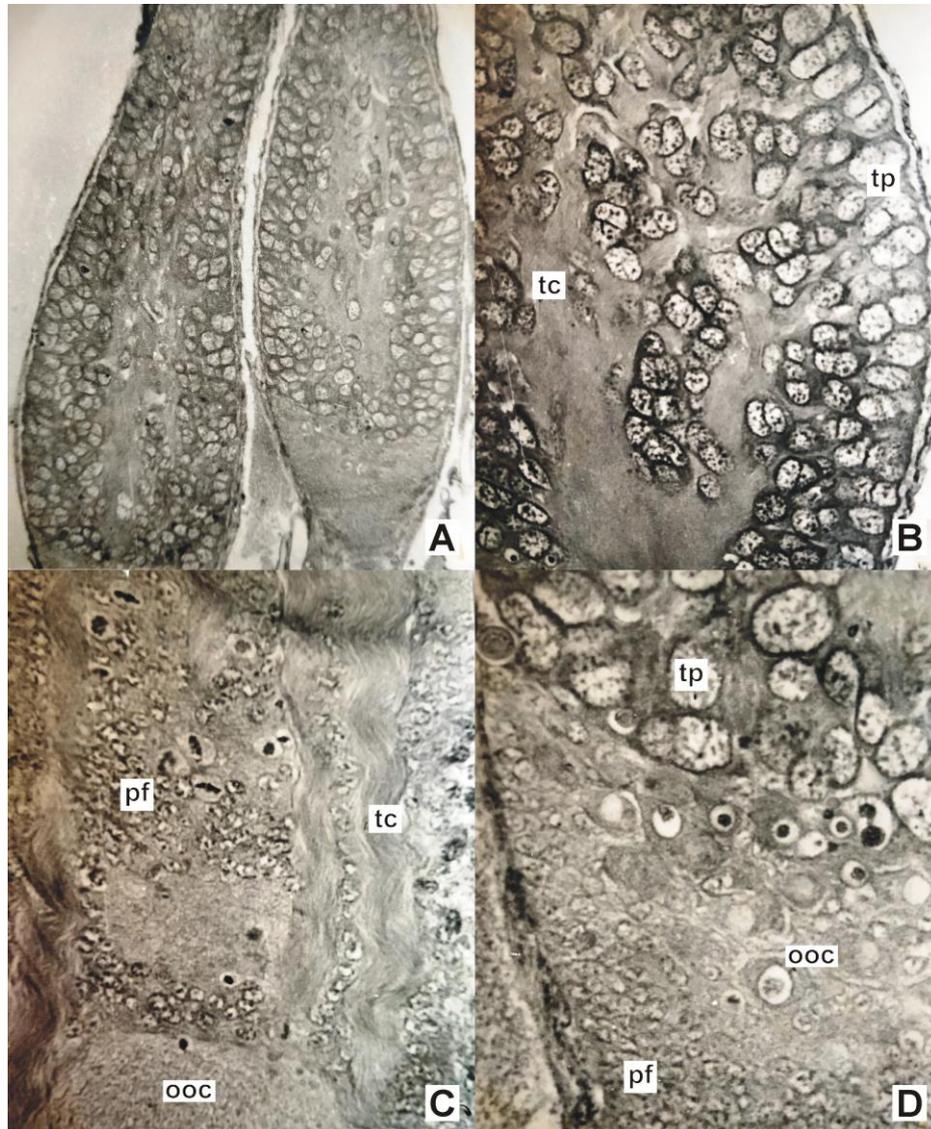


Fig. 2. L.S. of ovarioles of *D. koenigii* showing histological feature of tropharium: A – Gross morphology of tropharium; B – Mononucleate trophocytes, binucleate trophocytes and trophic core; C – Prefollicular cells ; D – Young oocytes and follicular cells. Abbreviations: ooc – Oocyte; pf – prefollicular cell; tc – trophic core; tp – trophocyte.

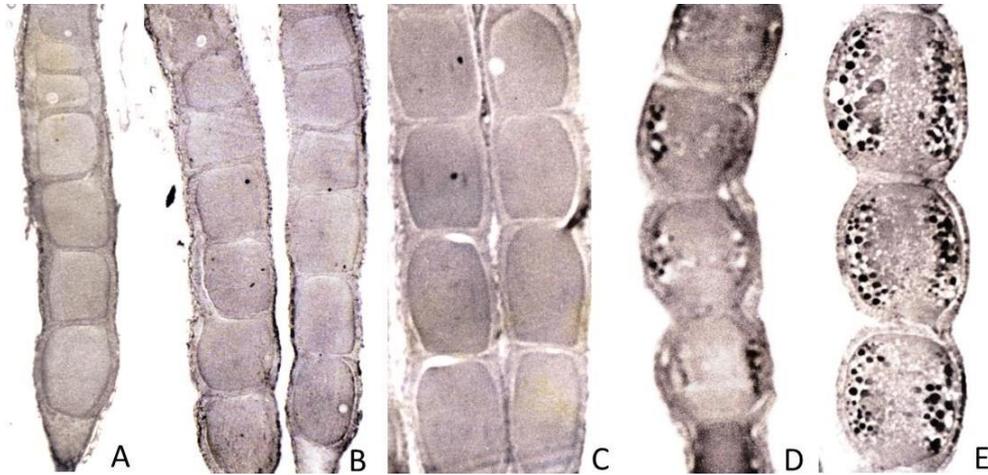


Fig. 3. Sections of ovarioles in vitellarium region showing various stage of oocyte development; the oocytes are surrounded by: A – Multilayered columnar follicular cells; B – Single layer of columnar follicle cells; C – Single layer of cuboidal follicle cells and appearance of perioocytic space; D – Single layer of cuboidal follicle cells with peripheral yolk globules; E – Single layer of squamous follicle cells and showing ooplasm with yolk material.

Premating differentiation of oocyte

The characteristics of ovariole during development in pre-copulating stages are elucidated in Table 1. In a newly emerged female (0-1 h) the ovarioles were short, each measuring, on an average 1635 μm . The tropharium (1215 μm) contributed to a major part of the length of the ovariole and the vitellarium was very short measuring 420 μm ; it contained 2-4 oocytes of an average dimensions 102 x 83 μm to 134 x 115 μm (Fig. 4A). During successive stages of development the number of oocytes in the vitellarium increased; this resulted in its enlargement up to 2438 μm to house 9-11 oocytes in 72 h old females. The size of terminal oocyte in such ovariole increased to 269 x 250 μm and the oocytes were externally discernible to give beaded appearance to the ovarioles. Increase in the size of ovarioles was mainly due to increase in size of vitellarium which increased about six times to its size during 72 h of pre mating maturation (Fig. 5).

Histological studies indicated that oocytes were constantly produced in the tropharium and moved down to vitellarium during early stages of development. In 24 h old females the proximal oocytes were covered by single layer of columnar cells. The ooplasm of the oocytes was clear and homogeneous and devoid of yolk granules. The oocytes had

Table 1. Effect of mating on the development of ovariole of *D. koenigii*.

Parameter	Time after emergence				
	0-1 h	12 h	24 h	48 h	72 h
Length of ovariole [μm (Mean \pm SE)]	1635 ^a \pm 69	1922 ^b \pm 48	2646 ^c \pm 63	3382 ^d \pm 97	3686 ^d \pm 55
Length of tropharium [μm (Mean \pm SE)]	1215 ^a \pm 94	1271 ^{ab} \pm 35	1238 ^a \pm 40	1554 ^b \pm 47	1248 ^a \pm 40
Length of vitellarium [μm (Mean \pm SE)]	420 ^a \pm 28	651 ^b \pm 43	1408 ^c \pm 77	2128 ^d \pm 76	2438 ^c \pm 39
No. of oocytes present in the vitellarium (Mean \pm SE)	1.87 ^a \pm 0.37	3.60 ^b \pm 0.29	6.30 ^c \pm 0.24	9.10 ^d \pm 0.41	9.80 ^d \pm 0.34
Dimensions of first oocyte [μm (Mean \pm SE)]	102 ^a \pm 4 x 83 ^a \pm 3	117 ^b \pm 3 x 97 ^b \pm 3	189 ^c \pm 7 x 176 ^c \pm 7	215 ^d \pm 5 x 200 ^d \pm 4	233 ^d \pm 7 x 210 ^d \pm 7
Dimensions of last oocytes [μm (Mean \pm SE)]	134 ^a \pm 5 x 115 ^a \pm 6	141 ^a \pm 4 x 115 ^a \pm 4	269 ^b \pm 15 x 261 ^b \pm 17	276 ^b \pm 4 x 246 ^b \pm 3	268 ^b \pm 4 x 250 ^b \pm 6

Means followed by same letter in rows are not significantly different at $p \leq 0.05$ (Anova followed by Tukey test).

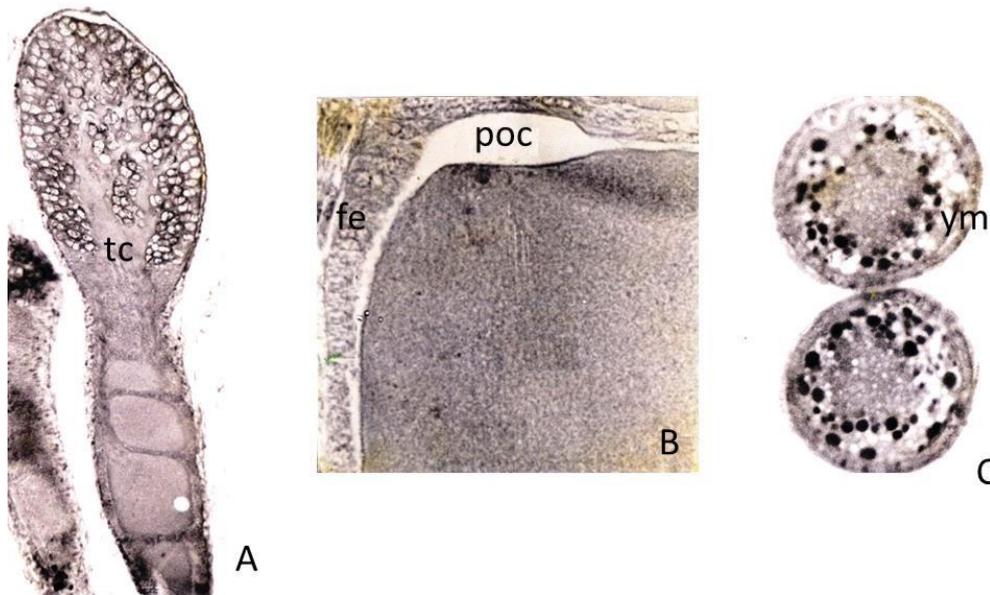


Fig. 4. Premating differentiation in the histological features of ovarioles of *D. koenigii* of age groups: A – 0-1 h old, showing a predominant tropharium and short vitellarium; B – 48 h old, showing appearance of perioocytic space and beginning of vitellogenesis; C – 72 h old, showing peripheral distribution of yolk material in the oocytes of distal region. Abbreviations: fe – follicular epithelial cells; poc – perioocytic space; tc – trophic core; ym – yolk material.

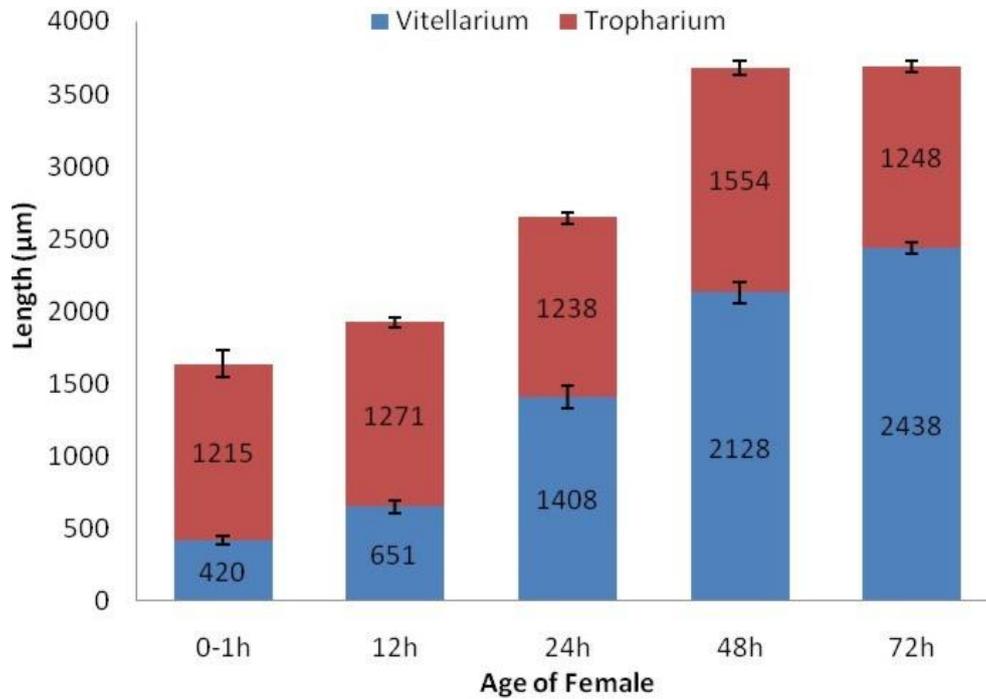


Fig. 5. Age specific changes in the morphometric characteristics of ovariole of female *D. koenigii* before mating.

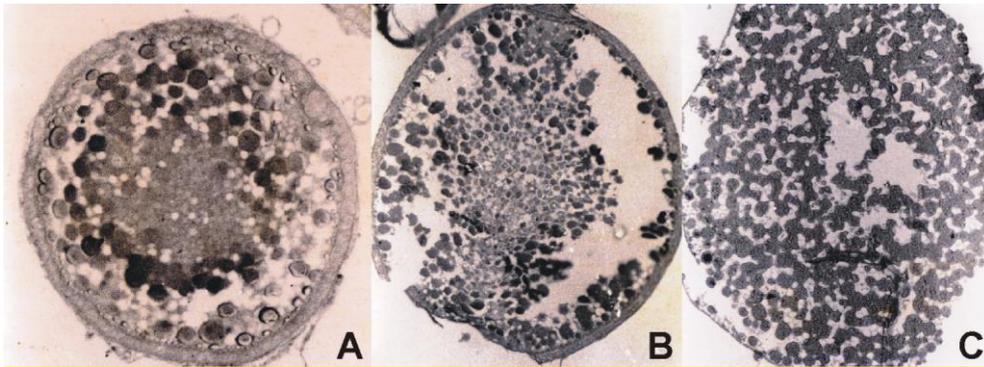


Fig. 6. Cross-section of oocytes of mated females; Female mated for: A – 24 h; B – 48 h and C – 72 h; showing rapid growth of oocyte and gradual increase in yolk contents during entire mating period.

peripheral nuclei and a connection with nutritive chord (Fig. 4A). The process of vitellogenesis was initiated on the second day with appearance of perioocytic space between follicle cell and oocyte (Fig. 4B). The oocytes of three day old female showed yolk material distributed at periphery (Fig. 4C). The process subsequently slowed down and arrested until the female received mating stimulus.

Table 2. Effect of mating on the development of ovariole of *D. koenigii*.

Material	Days after emergence			
	4	5	6	7
I. Length of ovarioles [μm (Mean\pmSE)]				
Virgin ♀♀	3789 ^a \pm 107	3779 ^a \pm 66	4933 ^b \pm 102	4920 ^b \pm 116
Mated ♀♀	4056 ^a \pm 122	9675 ^b \pm 40	13347 ^c \pm 241	–
II. Length of tropharium [μm (Mean\pmSE)]				
Virgin ♀♀	1125 ^a \pm 49	1043 ^a \pm 43	1182 ^a \pm 26	1151 ^a \pm 46
Mated ♀♀	0956 ^a \pm 22	1087 ^b \pm 62	1372 ^b \pm 36	–
III. Length of Vitellarium [μm (Mean\pmSE)]				
Virgin ♀♀	2664 ^a \pm 79	2836 ^a \pm 59	3751 ^a \pm 93	3769 ^b \pm 105
Mated ♀♀	3100 ^a \pm 118	8588 ^b \pm 131	11755 ^c \pm 237	–
IV. Dimensions of first oocyte in vitellarium [μm (Mean\pmSE)]				
Virgin ♀♀	235 ^a \pm 7 X 212 ^a \pm 7	241 ^a \pm 7 x 210 ^a \pm 7	264 ^b \pm 10 x 248 ^b \pm 10	300 ^c \pm 12 x 258 ^b \pm 10
Mated ♀♀	243 ^a \pm 10 x 217 ^a \pm 11	651 ^b \pm 21 x 564 ^b \pm 24	920 ^c \pm 24 x 941 ^c \pm 24	–
V. Dimensions of last oocyte in vitellarium [μm (Mean\pmSE)]				
Virgin ♀♀	305 ^a \pm 9 x 264 ^a \pm 9	302 ^a \pm 10 x 264 ^a \pm 8	348 ^b \pm 15 x 294 ^{ab} \pm 15	389 ^b \pm 14 x 333 ^b \pm 14
Mated ♀♀	351 ^a \pm 19 x 312 ^a \pm 25	887 ^b \pm 23 x 715 ^b \pm 18	1061 ^c \pm 19 x 987 ^c \pm 27	–
VI. Number of oocytes in vitellarium (Mean\pmSE)				
Virgin ♀♀	10.60 ^a \pm 0.29	11.40 ^{ab} \pm 0.29	11.76 ^b \pm 0.34	10.40 ^a \pm 0.37
Mated ♀♀	11.30 ^a \pm 0.24	10.90 ^a \pm 0.26	10.90 ^a \pm 0.29	–

Means followed by same letter in a row are not significantly different at $p \leq 0.05$ (ANOVA followed by Tukey test).

Effect of mating on oocyte development

During prolonged mating of about 72 h, the ovarioles underwent a constant change and differed from those of virgin females. The size of ovarioles in mated females increased drastically i.e. almost four fold increase from 3.686 to 13.347 mm was observed in the size of ovarioles in 72 h of mating duration (Table 2). At this stage, the ovaries filled a major portion of the abdomen. In a virgin female, on the other hand, the increase the size of ovariole was insignificant during this period (Table 2). In the mated females, the increase in size of ovariole was mainly on account of increase in the size of vitellarium (Fig. 5), which

increased from 2.438 mm to 11.755 mm. This was in contrast to virgin females, where the vitellarium increased from 2.438 to 3.769 mm. The mating did not seem to affect rate of oocyte descent in the vitellarium as the number of oocytes in vitellarium of both the virgin and mated females were almost equal. However, a rapid increase in the dimensions of oocytes was observed in the mated females. The terminal oocyte increased about four times i.e. 262 x 250 μm to 1061 x 967 μm , in 72h of mating period. The virgin females of corresponding age had oocytes of dimensions 348 x 294 μm ; the size remained small until a prolonged preoviposition period of ca 12 days. Histological studies revealed that mating accelerated the process of vitellogenesis and enhanced the process of yolk deposition (Fig. 6A, B, C). A large amount of yolk spheres was accumulated in the oocytes during 72 h of mating, which then showed a lattice like yolk pattern in the oocytes (Fig. 6C). In the virgin females, the process of vitellogenesis remained halted and no predictable changes were seen in yolk granule pattern on 4, 5, 6 day of the life. The follicular cells surrounding the oocytes in mated females also showed a gradual transition from cuboidal to squamous cells and, finally, the chorion was laid down. At this time vitellogenesis stopped; the oocytes were now large, full of yolk spheres and ready to ovulate.

DISCUSSION

The ovarioles in *D. koenigii* are meroistic telotrophic type; such type of ovarioles has been described in many heteropteran (ŠTYS & BILIŃSKI 1990, HUMMEL 2006) and coleopteran insects (DE WILDE & DE LOOF 1973). These ovarioles are differentiated into tropharium and vitellarium. The tropharium is the main site of oocyte production. Besides, it also contains nurse cells i.e. trophocytes and prefollicular tissue. The trophocytes may be mononucleate, binucleate and multinucleate and showed various degree of nuclear disintegration, liberating their contents in the nutritive cord. The prefollicular cells surround the oocyte and it moved down into the vitellarium and form follicular epithelium. The vitellarium contains developing oocytes which retained connection with the tropharium via nutritive cord. Such an organization of the meroistic telotrophic ovarioles has been described by BUNNING (1994, 2005), LIU et al. (2014) and RUBSAM & BUNNING (2017). The contents of the trophocytes are moved to oocytes through the nutritive cords (DE WILDE & DE LOOF 1973, SZKLARZEWICZ 1997, SZKLARZEWICZ et al. 2009, RUBSAM & BUNNING 2017). The vitellarium contains the oocytes in a linear array, according to their development. Based on the yolk contents of the oocytes and morphology of the follicle cells, 7 types of such oocytes were described by BRUNT (1971). It was seen that while one set of oocytes underwent a rapid growth in vitellarium, the other sets remained quiescent at the base of tropharium. The growth of oocytes in the tropharium remained arrested till the

batch of oocytes contained in the vitellarium was ovulated.

Present study indicated that ovaries of *D. koenigii* undergo constant changes during 72 h of post-emergence development of the females. Significant changes in the size of vitellarium and oocytes were observed during development. The tropharium though did not show significant morphometric changes; indicated its complete growth during nymphal development. The oocytes were constantly produced and descend down into the vitellarium for their further growth. Consequently, the number of oocytes increased in the vitellarium. The egg maturation started in the adults like *Oncopeltus fasciatus* (DALLAS, 1852) (BONHAG & WICK 1953), *Dysdercus fasciatus* AUDINET-SERVILLE, 1835 (BRUNT 1971) and grasshopper *Romalea microptera* (PALISOT DE BEAUVOIS, 1817) (SUNDBERG 2001). The results were unlike to that in *Callosobruchus maculatus*, in which, WILSON & HILL (1989) reported, the egg maturation was completed prior to adult emergence. The oocytes of 24 h old females were small and enclosed in a mono-layered columnar follicular epithelium. BRUNT (1971) reported similar changes in the follicular cell morphology in relation to development of oocytes. These oocytes represented the first three stages described by BRUNT (1971). The ooplasm of such oocytes was homogeneous and devoid of yolk granules. During 48 h of development, there appeared the perioocytic space between the oocyte and follicular cells, this marked the beginning of vitellogenesis, and at this stage follicular epithelial cells become cuboidal (stage IV). Small yolk spheres, with peripheral distribution were seen in the oocytes of 72 h old females (stage V). The growth of oocyte after 72 h was arrested. Similar growth pattern of oocytes was reported in *Triatoma protracta* UHLER, 1894 (MUNDALL 1978). In oocytes, the yolk deposition began a few days after adult eclosion, continued till eight days and subsequently ceased. In *Harmonia axyridis* (PALLAS, 1773), OBATA (1988) reported that oocyte production starts after three days of emergence. On the seventh day of life there were many oocytes in the ovaries but without yolk. A sudden increase in the oocyte growth was seen and the oocytes were full of yolk by the tenth day of life. The process of vitellogenesis in all the oocytes present in the vitellarium was synchronous. However, the amount of yolk was lesser in the proximal oocytes than in the terminal as observed in *D. fasciatus*. In *Glossina pallidipes* AUSTEN, 1903, WALL (1989) reported that growth occurred first in the terminal oocyte which increased in a linear fashion for a few days and then the growth was arrested. In the second oocyte, the growth was slower initially but increased subsequently. In *Drosophila melanogaster* MEIGEN, 1830, the process of vitellogenesis and production of new eggs ceases in sexually mature virgin females (SOLLER 1999).

Our earlier study showed that the females were receptive for mating following 72 h of development (GUPTA & SEHGAL 1995, GUPTA 2004). The behaviour seems to be significant as by this time the vitellarium of each ovariole contained 10-12 oocytes and the process of vitellogenesis had commenced. Similar reports were obtained in *Harmonia*

axyridis (OBATA 1988), in which the mating behaviour was related with the ovarian development. In *Aedes aegypti* (LINNAEUS, 1762) also, preoviposition behaviour of the females was governed by egg maturation (KLOWDEN 1989).

Mating in *Dysdercus koenigii* lasts up to 72 h which enhances fecundity, fertility and organized oviposition behaviour and curtail preoviposition time (GUPTA et al 2019). Our studies indicate that mating had a pronounced effect on the ovarian growth and rate of vitellogenesis. There was an increase in the dimensions of ovarioles and the size of oocytes during 72 h of mating. The dimensions of ovarioles and oocytes in a virgin female, on the other hand, remained unchanged for prolonged time interval, indicating no predictable growth in the oocytes. This was similar to *Anthocoris* FALLÉN, 1814 (HORTON et al. 2005). In *Podisus nigrispinus* (DALLAS, 1851), SOARES et al. (2011) observed that the females started oogenesis and egg maturation soon after their emergence but mating was important to maintain egg production. In *Drosophila melanogaster* HEIFETZ et al. (2000, 2001) reported that mature oocytes in ovaries remained arrested at metaphase I of meiosis and ovulation triggered activation and release of *D. melanogaster* oocytes. In *Dysdercus koenigii*, the mating did not seem to affect the oocyte production but accelerated oocyte growth accounted for the increase in the size of vitellarium of mated females. In *Drosophila* FALLÉN, 1823 SOLLER et al. (1997) reported that mating enhanced rate of vitellogenesis and accumulation of yolk material in the oocytes. This was as a result of transfer of the male sex peptide into the female at the time of mating. Also, the mating in *Drosophila* induced increase in germline stem cells via the neuroendocrine system (SOLLER et al. 1997, AMEKU & NIWA 2016). Our studies also showed that in virgin females the oocyte growth remained arrested up to 12-14 days and the interval between two successive egg-batches laid by the virgin female was more (GUPTA & SEHGAL 1997, GUPTA 2004). Thus a few egg-batches were laid by virgin females and this accounts for the lower fecundity of these females. On the other hand, the inter-oviposition period was short in mated females and shorter in multiple mated females due to enhanced rate of oocyte growth, therefore mated females laid more egg batches; hence exhibited more fecundity (GUPTA & SEHGAL 1995). Similar observations were reported in *Aedes taeniorhynchus* by O'MEARA & EVANS (1976). In many species of crickets, the increased egg production was restricted to the mated females, and virgin females produced significantly lesser number of eggs in each batch (LOHER & EDSON 1973, BENTUR & MATHAD 1975). High rate of egg maturation was also shown in mated females of *Callosobruchus maculatus* (FABRICIUS, 1775) (WILSON & HILL 1989). Among the hemipterans, the influence of mating on egg maturation varies from species to species. In *Cimex lectularius* LINNAEUS, 1758 (DAVIS 1964), *Haematosiphon inodorus* (DUGES, 1892) (LEE 1954) and *Dindymus versicolor* (HERRICH-SCHAEFFER, 1853) (FRIEDEL 1974), the females generally mature no egg unless they are mated. In *Rhodnius prolixus* STÅL, 1859 (PRATT & DAVEY 1972, DAVEY 2007), *Oncopeltus fasciatus* (DALLAS,

1852) (GORDON & BANDAL 1967) and *Triatoma protracta* (MUNDALL 1978) though the virgin females mature and deposit some eggs, the process was significantly enhanced following copulation. The species-specific variations in the oocyte development was seen in *Dysdercus* AUDINET-SERVILLE, 1835 (ODHIAMBO & ARORA, 1973) i.e. in *D. cardinalis* GERSTÄCKER, 1873 there was no growth of oocyte in the virgin female and the females eventually laid eggs only after about five weeks. In *D. nigrofasciatus* STÅL, 1855 the rate of oocyte development in virgin females was extremely slow and the first egg batch was laid after 12 days of emergence. While in *D. fasciatus* there was not any significant difference in the rate of oocyte development in virgin and mated females. Similarly, in *Macrolophus caliginosus* WAGNER, 1951 (VANDEKERKHOLVE et al. 2006) and stink bug *Perillus bioculatus* (FABRICIUS, 1775) (ADAMS 2000, 2001) the ovarian development was not significantly affected by their mating status.

The histological studies indicated that the oocytes of the unmated female contained little yolk contents with a peripheral distribution; the process of vitellogenesis remained suspended. However, the oocytes show no sign of degeneration or resorption like those observed in *Dysdercus cardinatis* (ODHIAMBO & ARORA 1973). Similarly, In *Triatoma protracta*, MUNDALL (1978) reported the deposition of yolk begin in the terminal oocytes a few days after adult eclosion in unmated females. Subsequently, the process of vitellogenesis ceased and the terminal oocyte failed to mature and underwent resorption. The oocytes of mated females on the other hand showed increased yolk contents due to yolk deposition by the process of vitellogenesis during the entire period of mating. The small yolk spheres at the periphery fused and moved to the centre. As a result, in 72 h of mating, the oocytes were laden with full of yolk material.

Morphological changes in the follicular epithelium were also associated with mating. The oocytes in the vitellarium of 72 h old virgin female were enclosed in cuboidal follicular epithelium which was similar to stage IV of *Dysdercus fasciatus* described by BRUNT (1971). After 24 h of mating, the oocytes entered stage V. The follicular cells were still cuboidal but the yolk spheres increased in size and number. This caused the rapid growth of oocytes and transformation of follicle cells from cuboidal to squamous within 48h of mating. This represented the stage VI of the oocyte (BRUNT 1971). The squamous follicular cell secreted chorion by the termination of mating. The oocytes were then ready for ovulation. At the time the oocytes represent the stage VII. The follicle cells of virgin female of corresponding ages however did not undergo any predictable change. Changes in follicular cells, associated with mating, were also reported in *Acheta domesticus* (LINNAEUS, 1758) (DENNIS & BRADLEY 1989). Synthesis and secretion of proteins in the follicle cells took place throughout the period of vitellogenesis. In *Leucophaea maderae* (FABRICIUS, 1781), KOEPPE et al. (1981) reported similar results.

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Received: 5 July 2017

Accepted: 4 September 2019