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GONAD DEVELOPMENT AND GAMETOGENESIS IN *BOOPHILUS DECOLORATUS* (KOCH, 1844) (ACARINA : METASTRIATA : IXODIDAE)

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ABSTRACT

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Phases in the growth of the reproductive organs and gametogenesis were followed in both males and females of *Boophilus decoloratus* (Koch, 1844). Special attention was given to the timing of meiosis, the production of mature spermiophores, mating and spermiophore relocation in the female. The main growth phase of the male testes takes place during the nymphal stage while that of the accessory gland complex takes place during the 4 days after moulting and before mating. The main growth phase of female ovaries and oviducts takes place during the post-mating period on the host and the preoviposition period. Meiosis in males takes place only after the attachment of the newly-moulted ticks; spermatogenesis and spermiogenesis occur within a period of 4 days, after which pairing and spermatophore transfer take place. Meiosis was not observed in female ticks and it is concluded that this reduction division takes place only after spermiophore penetration of the oocytes and egg shell formation. Female ticks may be impregnated more than once, a maximum of 5 matings being deduced from counts of spermiophore capsules in the female seminal receptacle. Each spermatophore produced by male ticks yields either 1 but more commonly 2 spermiophore capsules. The reproduction of *B. decoloratus* is briefly discussed and compared with other Ixodoidea.

Résumé

LE DÉVELOPPEMENT DES GONADES ET LA GAMÈTOGÉNÈSE CHEZ LE TIQUE BOOPHILUS DÉCOLORATUS *(KOCH, 1844)*

Les auteurs ont suivi des phases de croissance des organes sexuels et la gamètogénèse de Boophilus décoloratus *(Koch, 1844) mâles et femelles. Ils ont surtout étudié le rythme de la meïose, la production de spermiophores en développement complet, l'accouplement et le dépôt des spermiophores dans la femelle. La phase principale de la croissance du testicule du mâle a lieu pendant le stade nymphal, tandis que celle des glandes accessoires pendant les 4 jours après la mue et avant l'accouplement. La phase principale de la croissance des ovaires et de la trompe utérine a lieu pendant la période qui suit l'accouplement sur l'hôte et pendant la période avant l'oviposition. La meïose chez le mâle n'a lieu qu'après les tiques, qui viennent de muer, se sont attachés; la spermatogénèse et la spermiogénèse se déroulent pendant 4 jours, suivies de l'accouplement et de la transposition des spermatophores. N'ayant pas pu mettre en évidence la meïose chez la femelle, les auteurs ont tiré la conclusion que cette division réductrice n'a lieu qu'après le spermiophore a pénétré l'ovocyte et après le développement de la coquille ovaire. L'impregnation de la femelle peut avoir lieu plusieures fois, un maximum de 5 accouplements a pu être constaté d'après le nombre de capsules des spermiophores rétrouvées dans le réceptacle séminal de la femelle. Chaque spermatophore produit par le mâle rend soit une ou plus fréquemment deux capsules. La reproduction de* B. décoloratus *est brièvement discutée et comparée avec d'autres Ixodoidea.*

INTRODUCTION

Ticks have an important role in the transmission of viral, protozoal and rickettsial pathogens (Neitz, 1956; Hoogstraal, 1966, 1967). Basic to an understanding of the transovarian passage of some of these pathogenic organisms is a knowledge of gonad development and gametogenesis (Khalil, 1969, 1970). Detailed descriptions of these processes have been published by a number of workers, including Khalil, 1969, 1970 and 1972 for *Argas* (*Persicargas*) *arboreus*, *Hyalomma* (*H.*) *anatolicum excavatum* and *Haemaphysalis* (*Kaiseriana*) *longicornis*, respectively, Oliver & Brinton, 1972 for *Dermacentor occidentalis* and Norval & Capitini, 1974 for *Amblyomma hebraeum*. Both Balashov (1972) and Oliver (1974) have given good reviews of the important literature dealing with reproduction in ticks. Little has been published on the genus *Boophilus*, however, whose members develop on 1 host individual. They are important because they have been held responsible for the spread of various pathogenic organisms such as *Babesia bigemina* and *B. bovis* (redwater or Texas-fever), *Anaplasma marginale* (gallsickness) and *Borrelia theileri* (spirochaetosis) in cattle (Theiler, 1962; Hoogstraal, 1956; Yeoman & Walker, 1967), and spirochaetosis in horses, sheep and goats (Hoogstraal, 1956). It is also suspected that ticks of this genus transmit *Babesia trautmanni* (porcine babesiosis) to pigs (Hoogstraal, 1956). Apart from their veterinary importance *Boophilus* species have been blamed, probably incorrectly, for the spread of the Q-fever pathogen, *Coxiella burneti*, in man (Hoogstraal, 1956; Theiler, 1962).

Studies on *Boophilus decoloratus* (Koch, 1844) and *B. microplus* (Canestrini, 1888) have shown that the life cycle of these ticks is very short when compared with that of 2 and 3 host species (Legg, 1930; Kitaoka & Yajima, 1958; Roberts, 1968; Arthur & Londt, 1973; Londt & Arthur, 1975). This is probably because both the pharate nymphal and pharate adult phases of the life cycle take place in close association with the host whose surface temperature is considerably higher than might be expected in the microclimatic situations off the host. This paper is devoted to the description of gross changes in the reproductive systems of male and female *B. decoloratus* throughout their development and to the timing of meiosis, spermiogenesis, mating and sperm relocation in the female. Some information on multiple mating, based on counts of spermiophore capsules in the female seminal receptacle, is also given.

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Materials and Methods

A Friesland calf was infested with *B. decoloratus* larvae reared from female ticks originally obtained from the farm "Three Breezes" near East London, Cape Province, South Africa. Samples of ticks were removed from the host daily throughout the parasitic phase of their life cycle and each tick was measured overall along an imaginary mid-dorsal line joining the tips of the palpi to the hind margin. At least 3 ticks of each sex were then dissected. When fully engorged adult females fell from the host, they were placed in a glass container in which a relative humidity of between 95–100% had been created by means of damp paper towelling, and kept in continuous darkness in an incubator maintained at 23 °C. Three of these females were removed from this container daily until the completion of oviposition and processed in the same manner as the ticks removed from the host. As the dissection of larvae proved difficult because of their small size, the study of the development of the reproductive systems commenced on Day 8 of the parasitic cycle, which corresponds with the first day on which nymphs were found to any great extent on the host (Arthur & Londt, 1973). During dissection, the reproductive organs were exposed and drawn with the aid of a camera lucida. Care was taken to arrange these organs so that they could be drawn in a single plane, thus making practicable subsequent measurements directly from the drawings. To ascertain the patterns of growth of the reproductive systems, the following measurements were taken: (i) Length of testis and vas deferens combined (N.B. these 2 regions were not treated separately as it was very difficult to differentiate them). (ii) Length of male accessory gland complex (measured mid-dorsally from a line joining the tips of the 2 "horns" of the dorso-median lobe to a line joining the tips of the 2 postero-ventral lobes). (iii) Length of both oviducts combined. (iv) Length of ovary. (v) Length of ovary and oviducts combined. Some measurements were made with a Map Measure (a device normally used to determine distances on geographical maps) and this enabled accurate measurements to be made of undulating organs such as ovaries, oviducts and testes. Once the reproductive system had been drawn accurately, the entire system was removed, placed on a glass slide, stained with either acetic-orcein (La Cour, 1941) or Janus Green B (Khalil, 1969), squashed, and studied under a compound microscope to determine the progress of gametogenesis. Information on mating and fertilization was also partly obtained by studying the contents of the seminal receptacle.

Observations and Results

The general growth patterns based on overall measurements of body length of larvae, nymphs, adult males and adult females are shown in Fig. 1. The changes in overall length of engorged females during their preoviposition and oviposition periods are also represented diagramatically in Fig. 1. Larval ticks show little change over the first 24 hours on the host. In the ensuing 5 days, however, they grow rapidly, attaining their maximum length on Day 6 after infestation. Little change in length is observed over the following 2 days when the pharate nymph is undergoing development within the larval cuticle. On Day 7 (Fig. 1), the first few nymphs were found on the host. Nymphal growth followed a pattern very similar to that described for larvae. The mean maximum length for nymphs was recorded on Day 13 after infestation; thereafter little change in their mean length was observed. The first adult ticks were collected from the calf on Day 16 and, on both this and the following day, adult males were slightly longer than adult females. Very little difference in length between the sexes was observed until after mating had taken place. Mating was first recorded on Day 19 after infestation, but the majority of adults were not seen to be paired before Day 20. The mean length of adult males remained fairly constant throughout their parasitic period. Observations were terminated on Day 28 as males from a second infestation of the host had begun to emerge. Adult females grew rapidly after mating and the first fully fed females dropped from the host on Day 21 (Fig. 1). The mean length of females on Day 21 was calculated both from females removed from the host and those that had dropped. During the preoviposition period (Days 21–24), little change in the overall length of females took place. The first eggs were laid on Day 25 and egg-laying continued until Day 39 (i.e. oviposition lasted 15 days), the mean length of female ticks gradually decreasing during this period. The patterns of change in length shown in Fig. 1 are very similar to the changes in mass reported by Arthur & Londt (1973) for the same species. In the present study length was considered to be a more appropriate indicator of overall size changes as all assessments of growth of the reproductive organs were made in terms of length rather than mass.

General morphology of the reproductive systems

Male

The general morphology of the adult male reproductive system is shown in Fig. 2–5. There are 2 well-developed testes, joined posteriorly by a very fine filament which usually breaks during dissection. Each testis is widest posteriorly and gradually narrows towards the anterior end. It is S-shaped in lateral view and merges imperceptibly with the vas deferens. The vasa deferentia proceed anteriorly to a level just beyond the tip of the up-turned dorso-median lobe of the accessory gland complex before doubling back and entering the gland complex at a point between the dorso-median lobe and the dorso-lateral lobe (Fig. 4). The accessory gland system (Fig. 3–5) is composed of a number of tightly packed lobes. The names of these lobes have been derived from their positions relative to the complex as a whole and the terminology followed is that of Chinery (1965). The dorso-median lobe is the most conspicuous element of the gland complex, while the lateral and ventral lobes are somewhat less developed.

Female

The general morphology of the adult female reproductive system is shown in Fig. 6. There is a single tubular ovary which, because of the arrangement of the dorso-ventral idiosomal muscles, is often W-shaped when viewed dorsally. There are 2 convoluted oviducts which fuse into a common oviduct and this opens directly into the vagina. Just anterior to the seminal receptacle is a pair of tubular accessory glands which open into the vagina almost directly above the opening of the oviduct.

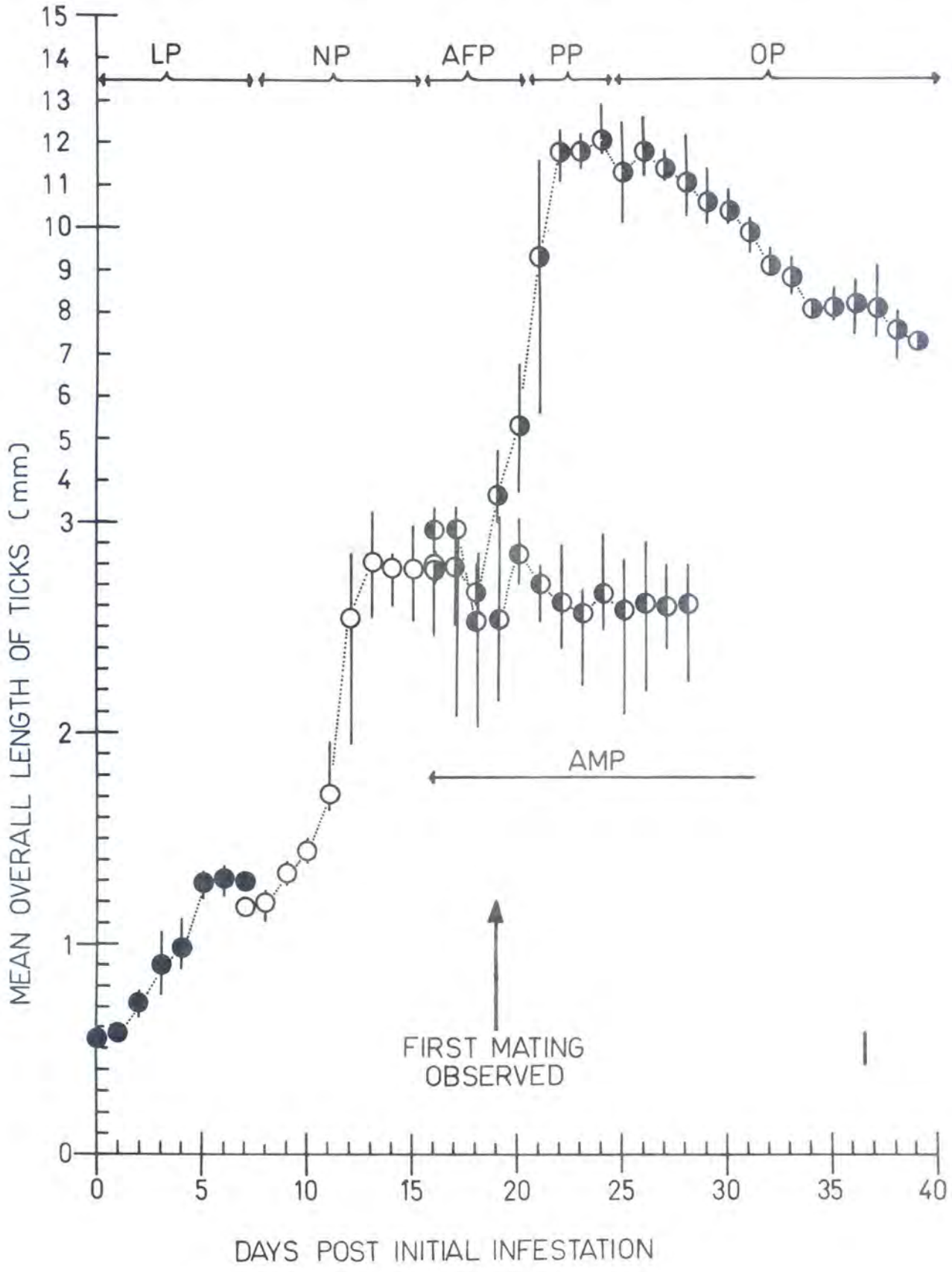


FIG. 1 Changes in the mean length of *Boophilus decoloratus* ticks throughout the parasitic phase of larvae (●), nymphs (○), adult males (◐) and adult females (◑) and the non-parasitic phase of the engorged female ticks. Vertical lines indicate range. AFP=Adult female parasitic phase; AMP=Adult male parasitic phase; LP=Larval parasitic phase; NP=Nymphal parasitic phase; OP=Oviposition period; PP=Preoviposition period

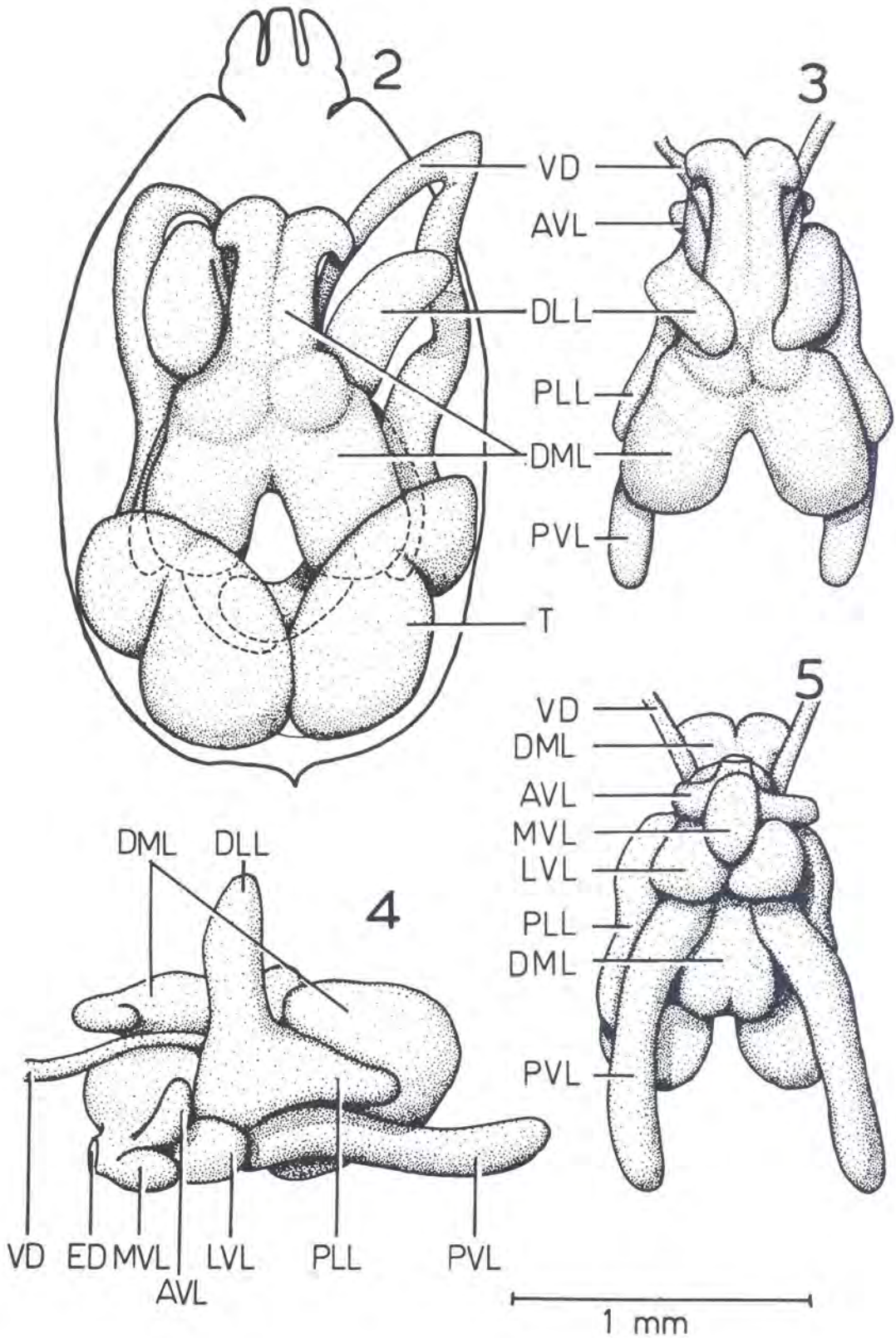


FIG. 2–5 *Boophilus decoloratus* male reproductive system. (2) Dorsal aspect of entire system of a mature male. (3–5) Accessory gland complex. (3) Dorsal aspect. (4) Lateral aspect. (5) Ventral aspect. AVL=antero-ventral lobe; DLL=dorso-lateral lobe; DML=dorso-median lobe; ED=ejaculatory duct; LVL=latero-ventral lobe; MVL=medio-ventral lobe; PLL=postero-lateral lobe; PVL=postero-ventral lobe; T=testis; VD=vas deferens

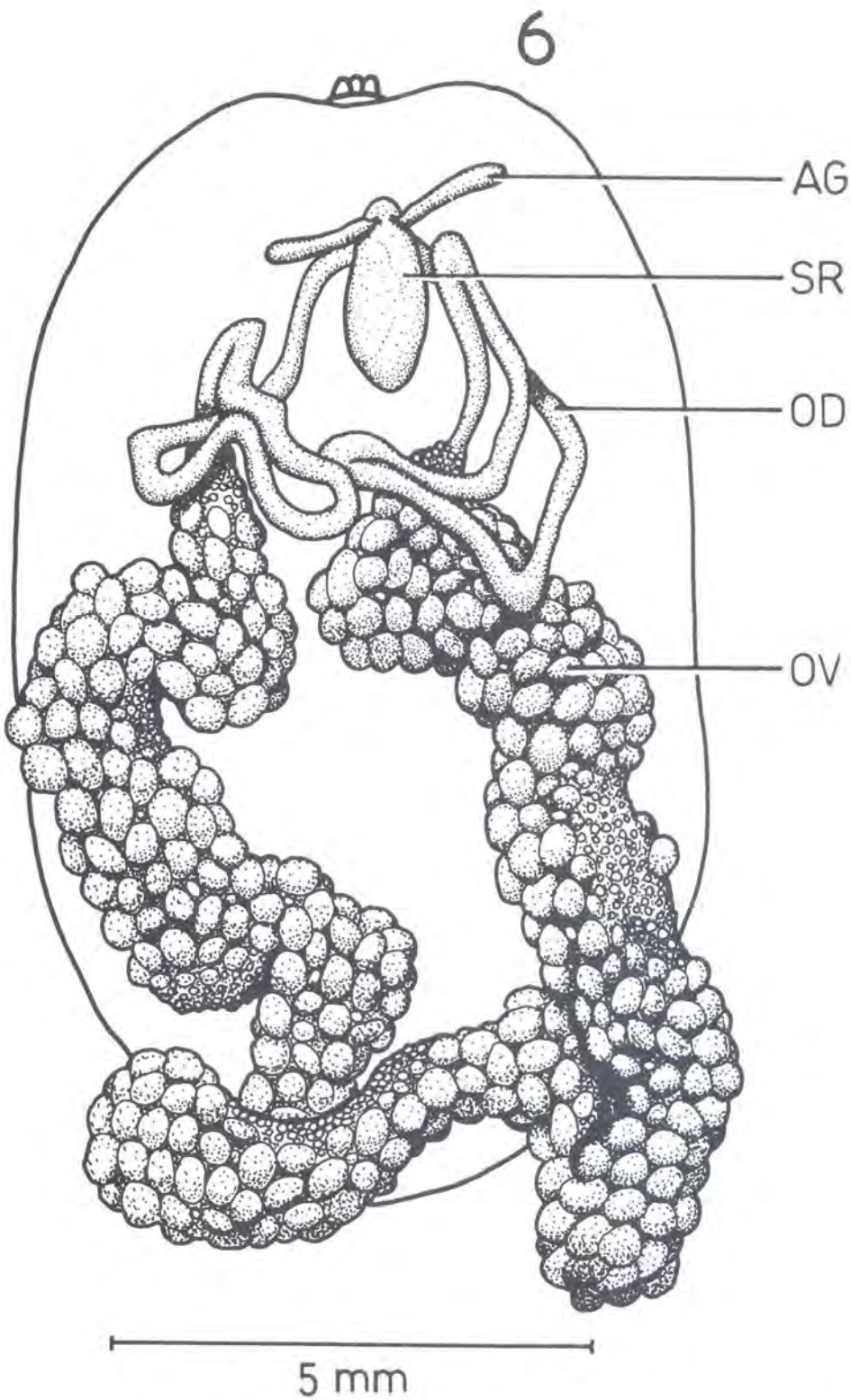


FIG. 6 *Boophilus decoloratus* adult female reproductive system. AG=accessory gland; OD=oviduct; OV=ovary; SR=seminal receptacle

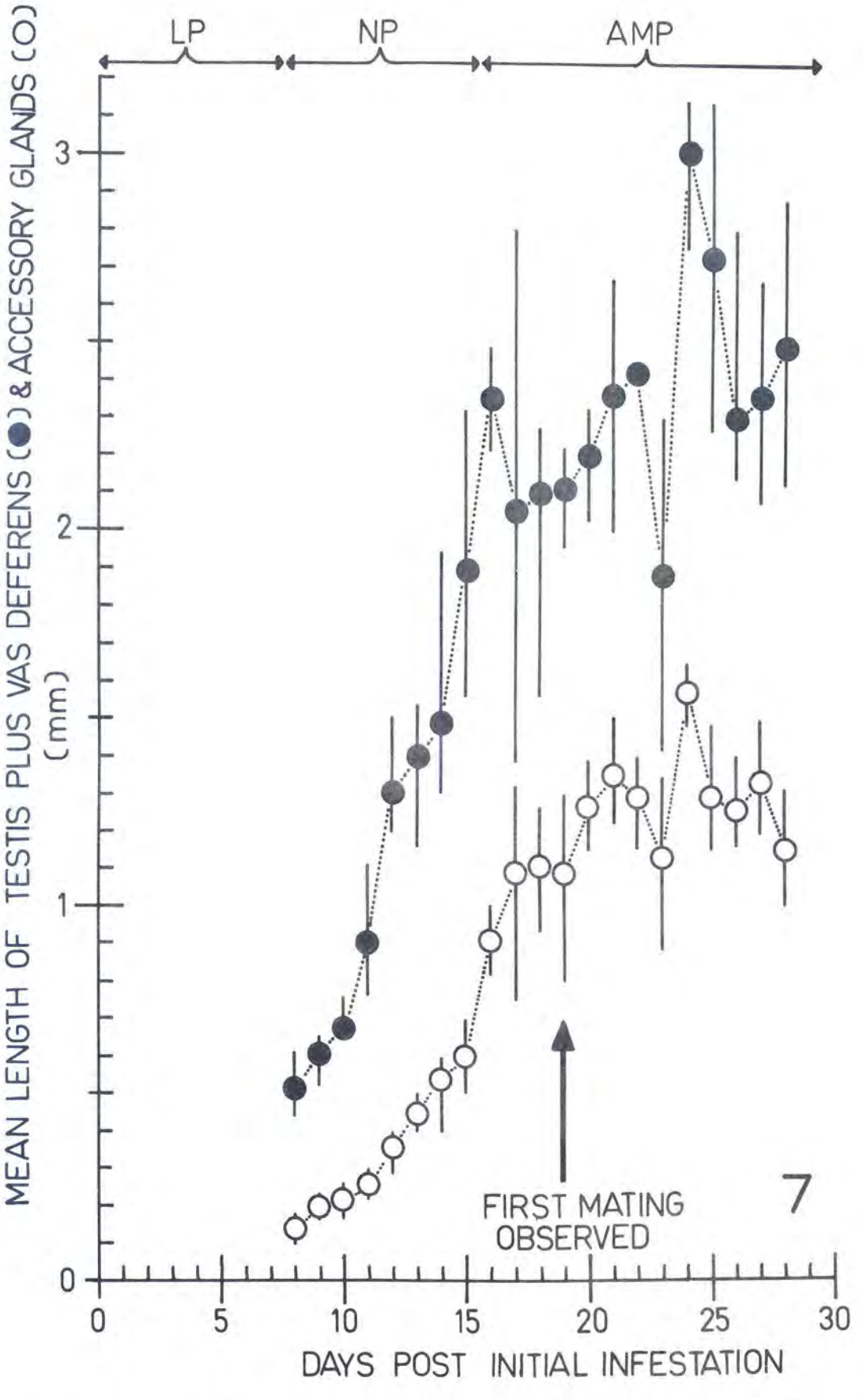


FIG. 7 Changes in the mean length of the testis plus vas deferens (●) and the accessory gland complex (○) of *Boophilus decoloratus* during the parasitic phase of the male ticks. Vertical lines indicate range. Abbreviations as in Fig. 1

Gross changes in the reproductive systems

Male

The changes in length of the testis plus vas deferens and the accessory gland complex of male ticks are shown in Fig. 7, while illustrations of the actual reproductive systems as seen on selected days during development are shown in Fig. 8–12. During the nymphal phase (i.e. Days 8–16 in Fig. 7) very rapid growth of the testes plus vasa deferentia occurred. Once the adult males had emerged, however, this growth ceased. There was considerable variation in the length of testis plus vas deferens and this is directly related to the variations in overall length recorded for males (Fig. 1). Arthur & Londt (1973) reported 2 distinct size groups among males of *B. decoloratus,* and, when dealing with small samples, it was inevitable, therefore, that mean lengths would show considerable variation. The fluctuations in mean length shown in Fig. 7 do not, however, mask the fact that the main growth of the testes takes place during the nymphal (and possibly larval) phase; these organs show little size increase once the ticks have moulted into adults. The accessory gland complex also shows considerable growth during the nymphal phase, but this growth continues into the adult stage and maximum size is only attained at the time of mating. Mating is generally accepted as taking place on Day 20 after initial larval infestation of the host, although a few males were seen to be paired with females on Day 19. After Day 20 no further change in the accessory gland size was observed.

Female

The mean overall lengths of ovaries plus oviducts were recorded from Day 8 after larval infestation until the end of the oviposition period of females that fell from the host on Day 21 (Fig. 13). The changes observed are illustrated in Fig. 14–21. Very little growth of the reproductive organs was recorded until the adults emerged on Day 16. From the 17th day onwards, when slightly more rapid growth of the ovaries took place, separate measurements of these 2 organs were possible. Although both increased in length during the adult phase the main growth phase of these organs was during the preoviposition period, i.e. on Days 21–24. This rapid increase in length continued during the first 2 days of the oviposition period (Fig. 13), but during this period the oviducts, and to a lesser extent the ovaries, became distended with eggs, and the apparent growth shown on the graph was probably the result of hypertrophy rather than hyperplasia. As oviposition proceeded, the overall length of the ovaries and oviducts decreased. Females dissected on Day 39 (i.e. the 15th and last day of oviposition) contained only 8–10 eggs in the ovaries and oviducts.

Gametogenesis

No chromosomes could be extracted from engorged nymphs, the gonadal nuclei showing no signs of divisional activity.

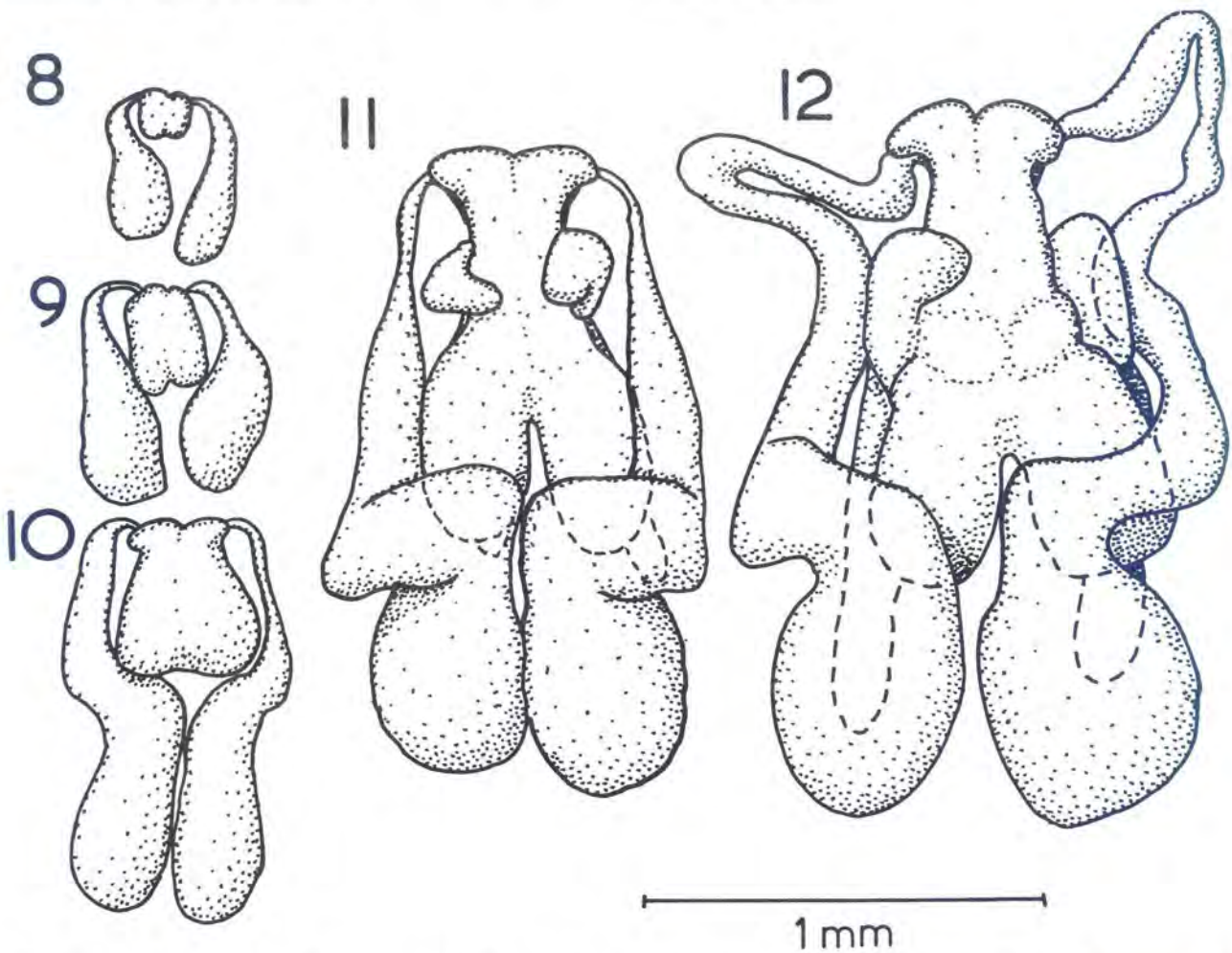


FIG. 8–12 Selected examples of the appearance of the male reproductive organs of *Boophilus decoloratus* during development. (8) Day 8 nymph. (9) Day 10 nymph. (10) Day 12 nymph. (11) Day 17 adult. (12) Day 24 adult

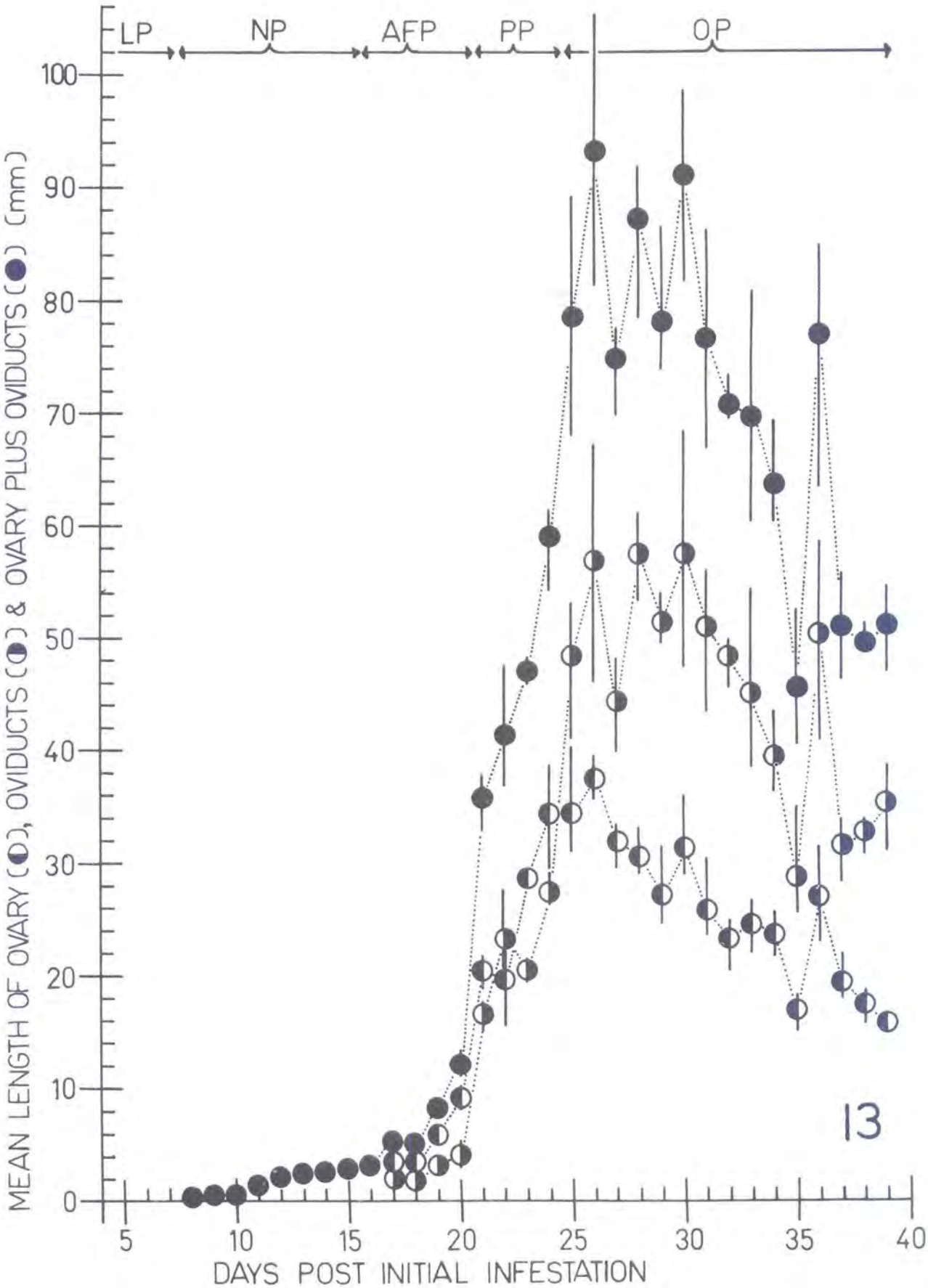


FIG. 13 Changes in the mean length of the ovary (◐), oviducts (◑) and ovary plus oviducts (●) of *Boophilus decoloratus* during the parasitic phase of nymphs and adults and the non-parasitic phase of engorged adult females. Vertical lines indicate range. Abbreviations as in Fig. 1

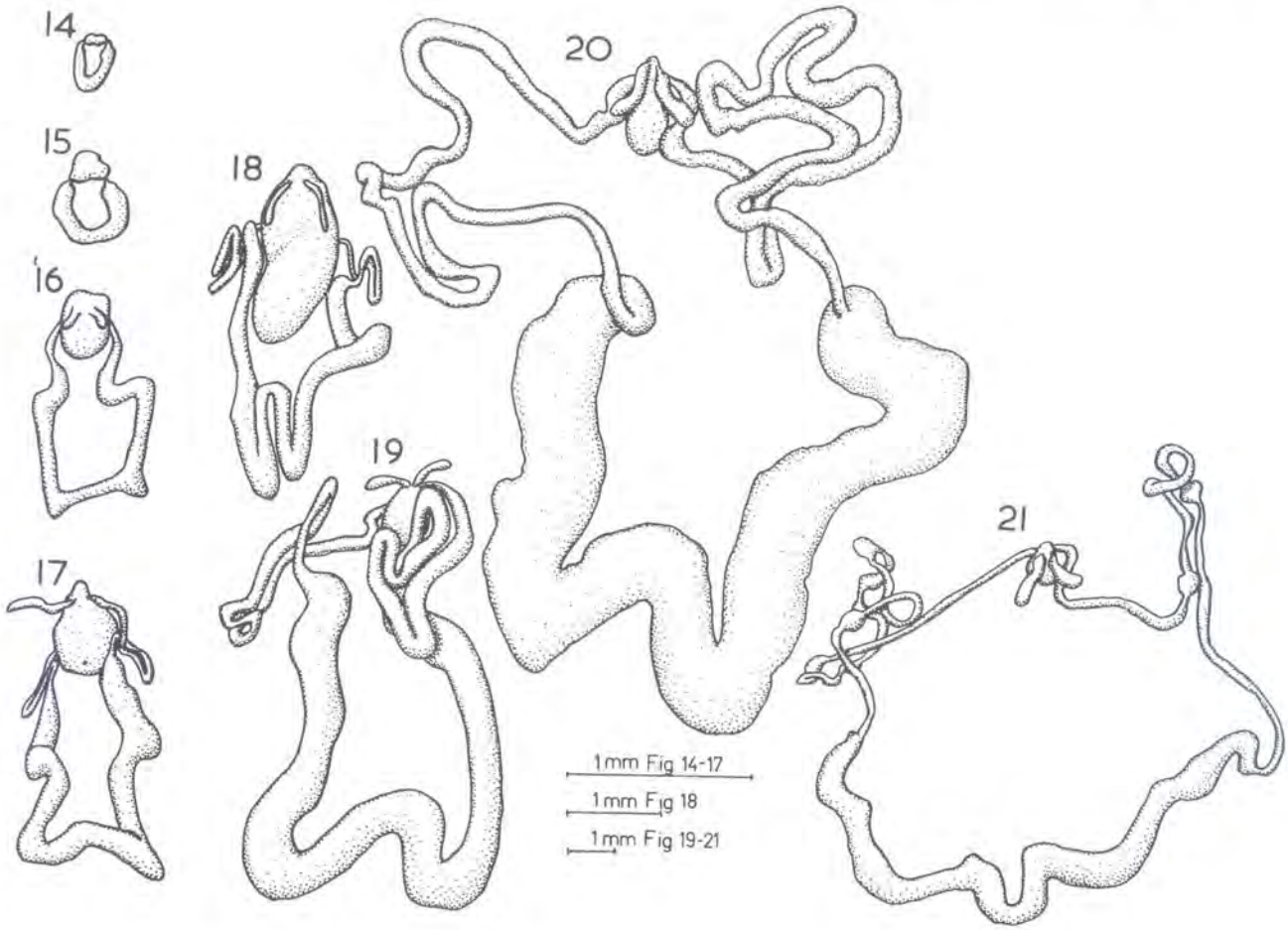


FIG. 14–21 Selected examples of the appearance of the female reproductive organs of *Boophilus decoloratus* during development and oviposition. (14) Day 8 nymph. (15) Day 10 nymph. (16) Day 12 nymph. (17) Day 17 adult. (18) Day 20 adult (just prior to falling from host). (19) Day 23 adult (2nd day of preoviposition period). (20) Day 29 adult (4th day of oviposition period). (21) Day 39 adult (15th and last day of oviposition)

Spermatogenesis

Males yielded typical mitotic-metaphase rings (Fig. 23) and telophase groups (Fig. 24) in the anterior portion of the testes immediately after moulting. The telophase groups showed typical chromosomal bridges formed by the lagging x-chromosome (Fig. 24). Mitosis was evident up to Day 9 after adult emergence (i.e. Day 28 of the parasitic phase) when observations on the males ceased.

Males that were forcibly removed while moulting showed no dividing cells, the testicular nuclei being uniformly inactive. Testes of males, removed while moulting and kept unfed for 3 days, also showed uniformly dense staining nuclei.

In feeding males, meiosis occurred in the posterior portion of the testis soon after moulting, diplotene-diakinesis being evident after 6 hours. Twenty-hour-old males yielded diplotene-diakinesis, metaphase I (Fig. 25, 26) and anaphase I (Fig. 27). In all these stages the x-chromosome was single-stranded (univalent), the only way in which it could be distinguished from the 10 autosomal bivalents. At anaphase I it lengthened to 2–3 times the length of the autosomes and lagged behind in its undivided migration to 1 pole (Fig. 28). The first meiotic division is thus reductional for the x-chromosome.

On Day 2 after moulting, adult testicular tissue yielded the above-mentioned stages as well as anaphase II and telophase II (Fig. 31, 32). Anaphase II is equational for the x-chromosome, which at this stage is again 2–3 times the length of the autosomes (Fig. 29, 30). All stages of meiosis remained evident until the last observations were made.

The males have a chromosomal complement of 20 autosomes and 1 long x-chromosome (Fig. 23).

Oogenesis

Engorging females yielded no dividing cells 1 day after moulting. Prophase cells and mitotic-metaphase groups (Fig. 22) were found in 2-day-old females. Mitotic-metaphase and anaphase-telophase, which showed typical chromosomal bridges caused by the lagging x-chromosomes, were most numerous in 3–4 day-old engorging females. In 5-day-old females early and late telophase were the most numerous. No dividing cells could be detected after females had fallen from the host.

The females had a chromosomal complement of 20 autosomes and 2 long x-chromosomes (Fig. 22). Depending on the divisional stage, the x-chromosomes were 3–6 times longer than the autosomes. All the chromosomes appeared to be cephalobranchial in centromere position.

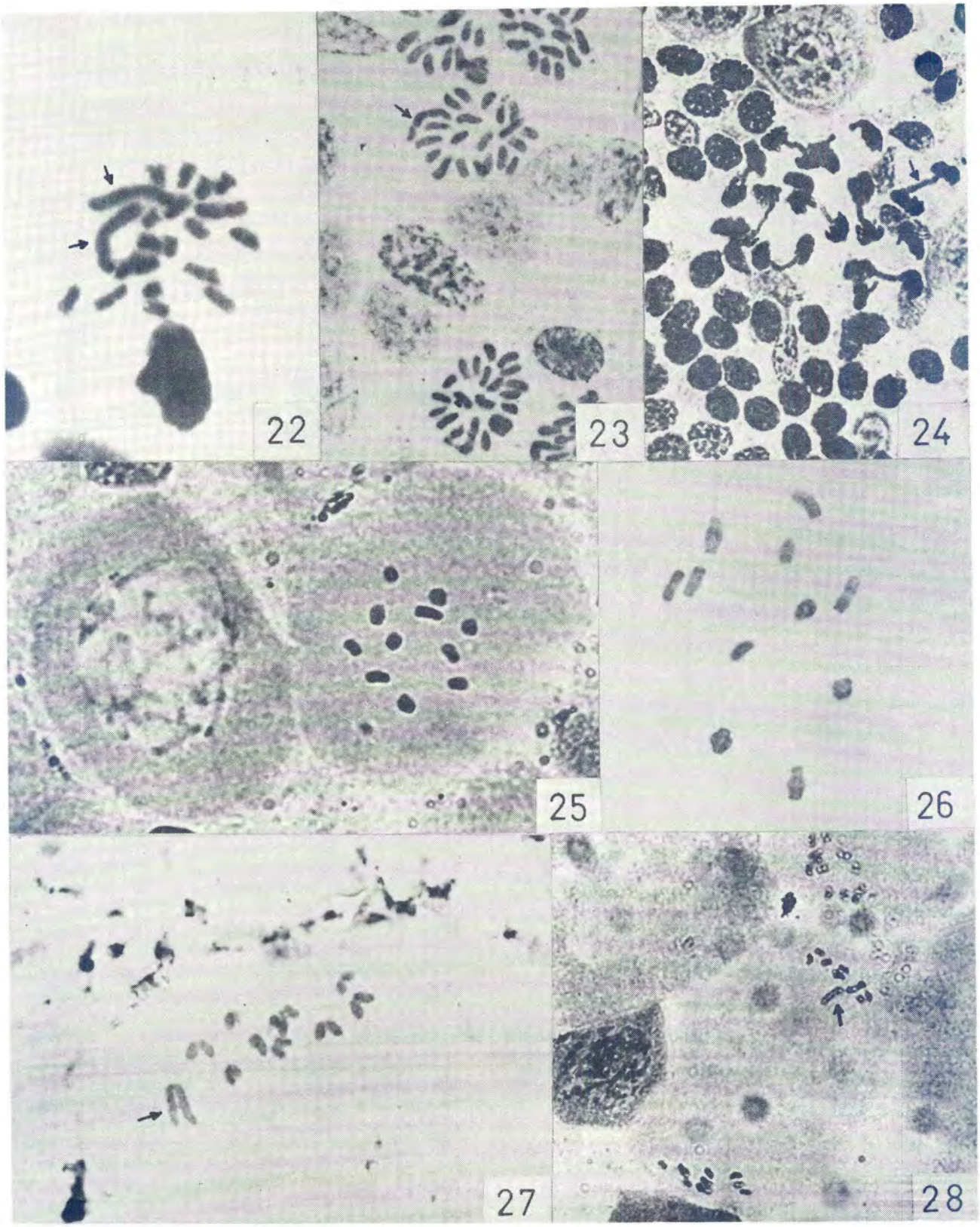


FIG. 22–28 Gametogenesis in *Boophilus decoloratus*. (22) Mitotic-metaphase in a 4-day-old adult female showing 20 autosomes and 2 long x-chromosomes (arrows). (23) Mitotic-metaphase rings in a newly-moulted male showing 20 autosomes and 1 long x-chromosome (arrow). (24) Mitotic-telophase in a newly-moulted male showing typical chromosomal bridge formed by lagging x-chromosomes (arrow). (25) Prophase (left) and metaphase I (right) in a male fed for 20 hours. (26) Metaphase I in a male showing 10 autosomal bivalents and a univalent x-chromosome. (27) One pole of anaphase I in a 1-day-old male adult; note length of x-chromosome (arrow) in relation to migrating autosomes. (28) Anaphase I in a 1-day-old male showing reductional division of x-chromosome (arrow)

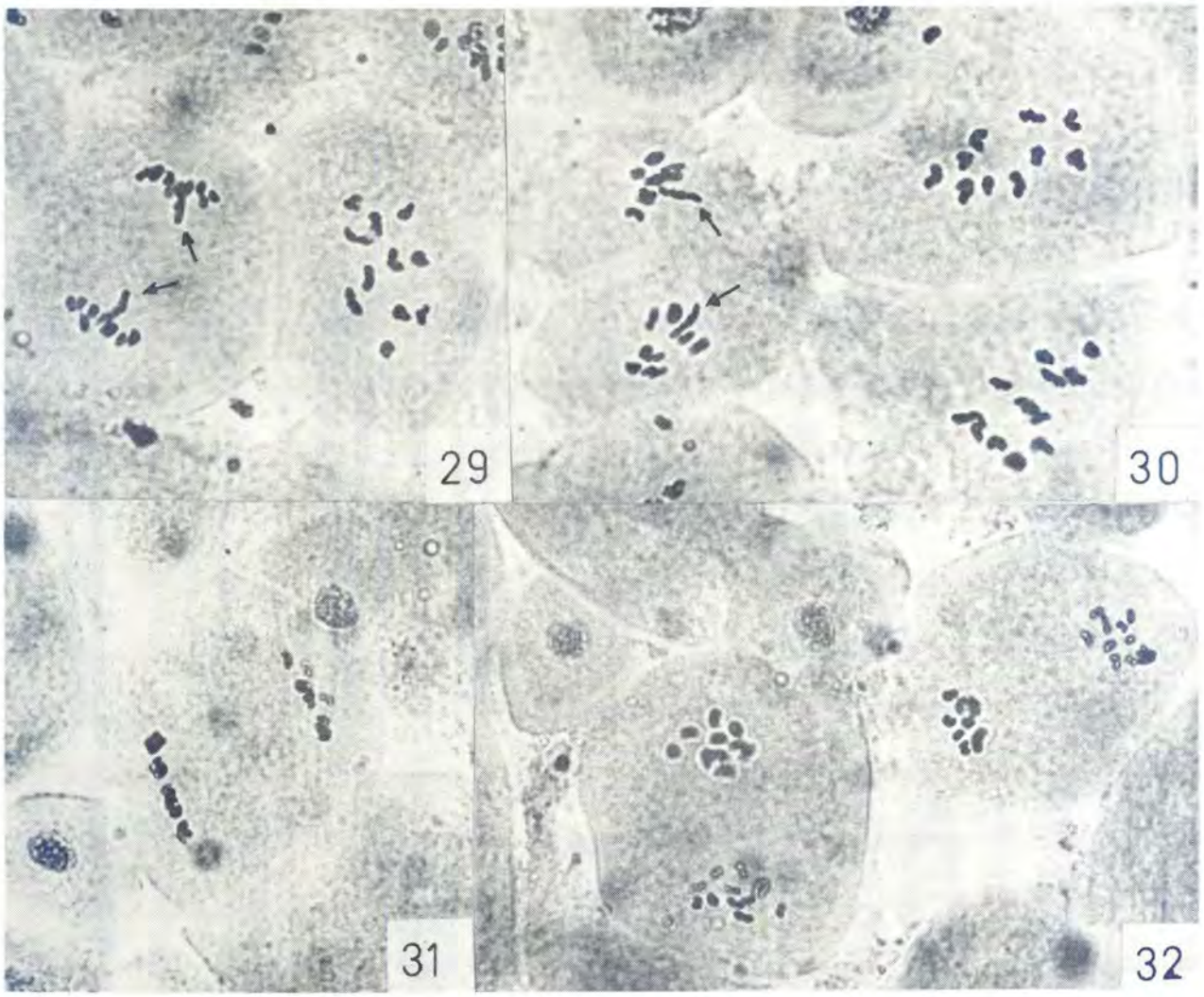


FIG. 29–32 Meiosis II in *Boophilus decoloratus* males. (29–30) Anaphase II showing equational division of x-chromosome (arrows). (31–32) Anaphase II—Telophase II in cells containing only autosomes; products of the reductional Anaphase I divisions

Spermiophore capacitation

The newly-formed spherical spermatid is immediately involved in morphological transformations (Fig. 33–64), culminating in the production of the mature spermiophore. The transformations seen in *B. decoloratus* males, which are basically similar to those described by Khalil (1969, 1970) for the argasid *A. arboreus* and *H. anatolicum excavatum,* respectively, are illustrated diagramatically in Fig. 33–48, and photographically in Fig. 49–64. The main changes observed involve relocations of the folded ectoplasm, endoplasm and nucleus. Transformation commences when the nucleus migrates to the side of the spherical spermatid. The folded ectoplasm retreats from this side of the cell (Fig. 33, 49), at the same time thinning in the region opposite the pole containing the nucleus. The ectoplasm then evaginates (Fig. 35), forming a sac surrounded by the nucleus containing endoplasm (Fig. 36, 50). The spermatid begins to elongate (Fig. 37, 51) and, as elongation proceeds, the nucleus becomes confined to a region of limited endoplasm at one end of the cell. This endoplasm forms a small process (Fig. 37, 38, 51, 52), of unknown function into which a similar process of the nucleus migrates temporarily (Fig. 39, 53). The nucleus then withdraws from this endoplasmic process, begins to elongate and moves into the very thin layer of endoplasm surrounding the by now completely elongated ectoplasmic sac (Fig. 40, 41, 54, 55). At the same time the endoplasmic process previously formed in the region of the nucleus (Fig. 37, 51) begins to decrease in size and eventually disappears. At about the time of nuclear elongation, the ectoplasmic sac begins to invaginate in the region of the nucleus (Fig. 38, 39, 54), and this invagination continues apparently uninterruptedly until the spermatids become spermiophores. During the first half of this invagination process, the nucleus migrates in ribbon-like form down to the opposite pole of the spermatid (Fig. 41–44, 55–60). Once the spermatids reach the stage illustrated in Fig. 44, 60, they leave the testis and migrate down the vas deferens into the seminal vesicle (surrounded by the accessory gland complex). Here they continue invagination and squashes of accessory gland complex contain spermatids in the stage shown in Fig. 45, 46, 61. These spermatids are then enclosed in a spermatophore and transferred to the female during copulation. Spermiophore capsules removed from the female seminal receptacle contain spermatids in the stage illustrated in Fig. 47, 62, 63, and mature spermiophores (Fig. 48, 64). During the final stages of spermatid invagination the nucleus wraps itself around the spermatid in a spiral before entering the posterior end of the spermatid. In the mature spermiophore the nucleus can be seen as a long, thin and often undulating strand in the posterior region of the endoplasm.

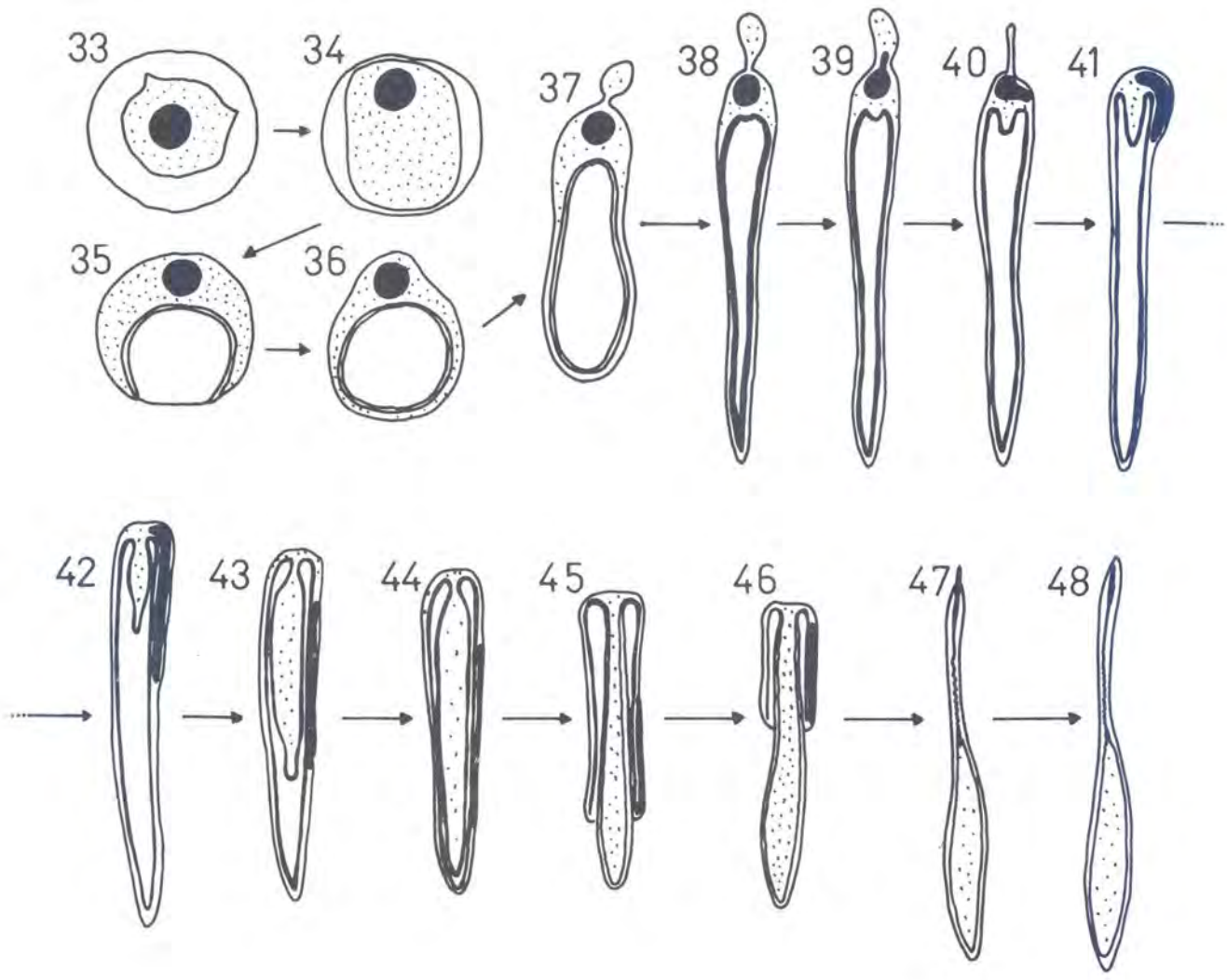


FIG. 33–48 Diagrammatic representations of the changes which take place during spermatid capacitation in *Boophilus decoloratus*

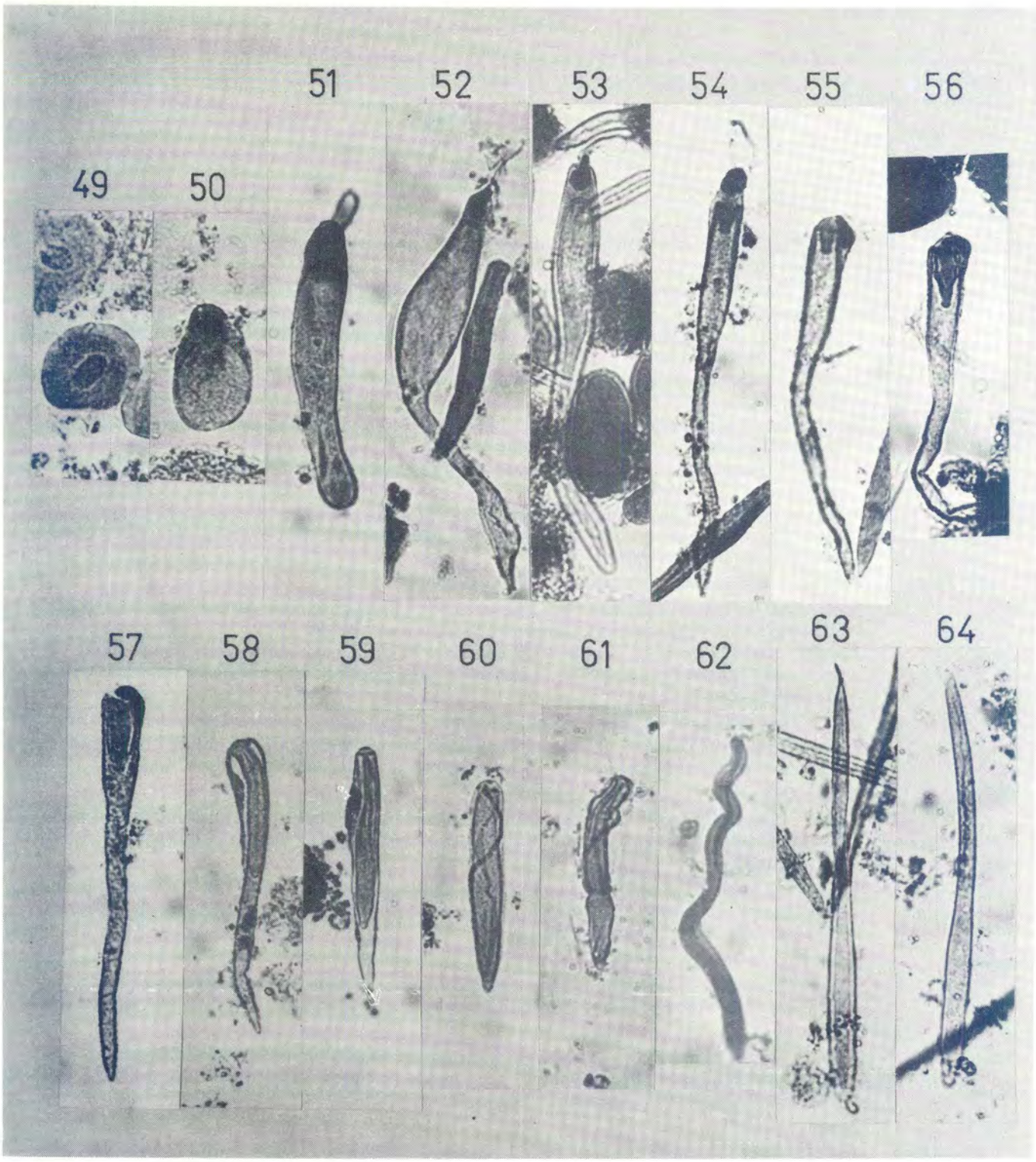
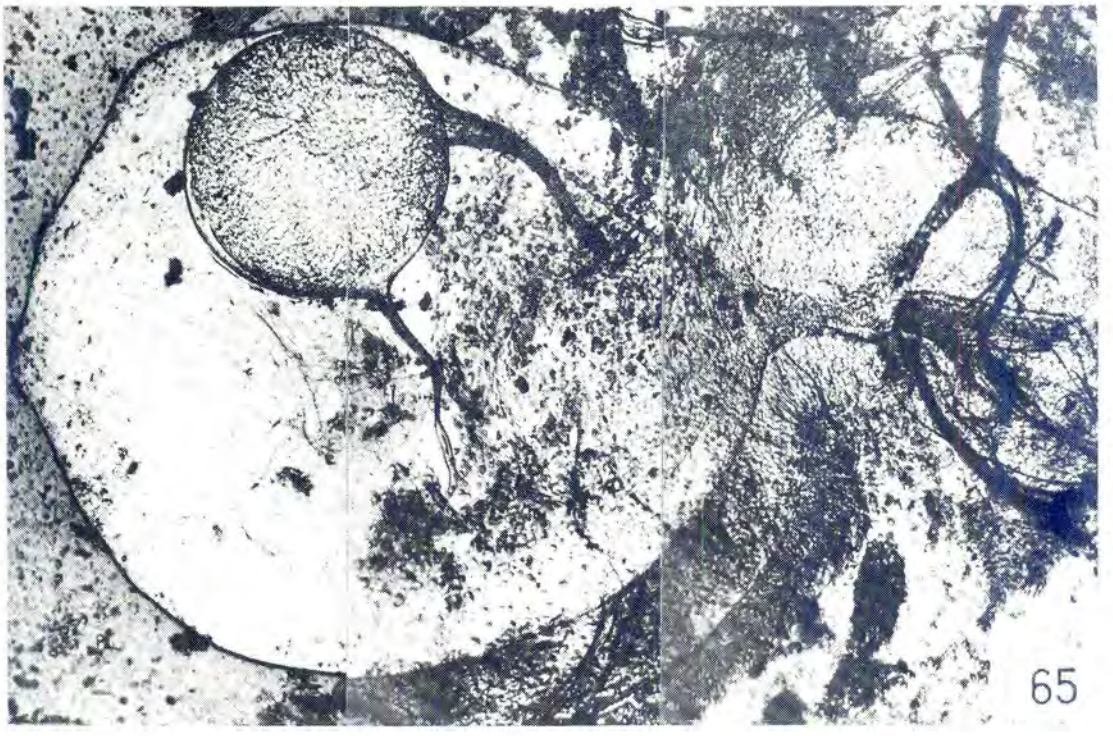


FIG. 49–64 Photographic representations of the changes which take place during spermatid capacitation in *Boophilus decoloratus*

Timing of Gametogenesis, Meeting of Sexes, Mating, Spermatophore Transfer, Spermiophore Relocation and Syngamy

The following notes describe the timing of the essential stages in the reproductive behaviour of *B. decoloratus* in relation to the parasitic phase of the nymphs and adults, and the preoviposition and oviposition periods of the engorged female ticks.

Days after the initial infestation of the host	*Notes*
Days 8–15.....	*Male:* Feeding nymphs show marked growth of testes and accessory glands. No mitosis or meiosis seen
	Female: Feeding nymphs show little growth of ovaries and oviducts. No mitosis or meiosis seen
Day 16.....	*Male:* Newly moulted adults show mitotic cell divisions in anterior region of testes and vasa deferentia; meiotic divisions in posterior region of testes. Accessory gland complex shows marked increase in size
	Female: Newly moulted adults show slight growth of ovaries and oviducts. No mitosis or meiosis seen
Day 17.....	*Male:* Mitosis in anterior region of testes; meiosis in posterior region; early stages of spermatid capacitation. Accessory glands continue to grow
	Female: Slight growth of ovaries and oviducts. Mitosis seen primarily at junction of ovaries and oviducts
Day 18.....	*Male:* Mitosis as before; meiosis in most regions of testes; all stages of spermatid capacitation up to the stage illustrated in Fig. 44, 60. Vasa deferentia full of spermatids. Accessory gland complex completes growth
	Female: Little growth of ovaries and oviducts. Mitosis only
Day 19.....	*Male:* First pairing of sexes observed. Spermatophore transfer in these pairs already completed. All stages of spermatogenesis seen in all males as on Day 18
	Female: Slight growth of ovaries and oviducts. Mitosis most noticeable. Final stages of spermatid capacitation and mature spermiophores found in spermiophore capsules within seminal receptacle
Day 20.....	*Male:* Most males paired with females. All stages of spermatogenesis and spermiogenesis seen
	Female: Rapid engorgement of blood commenced. Mature spermiophores found in capsules in seminal receptacle. Slight growth of ovaries and oviducts. Mitosis only
Day 21.....	*Male:* All stages of spermatogenesis seen
	Female: First fully fed females detached from host. Spermiophore capsules intact and containing mature spermiophores. Rapid growth of ovaries and oviducts. Mitosis only
Day 22.....	*Male:* As on Day 21
	Female: 1st day of preoviposition period. Spermiophore capsules intact; few spermiophores in capsule and ovary lumen; many spermiophores in lumen of oviducts. Rapid growth of ovaries and oviducts. No cell divisions seen
Day 24.....	*Male:* As on Day 21
	Female: Third day of preoviposition period. Few spermiophores still in intact capsule; many in oviducts; few in ovary. Rapid growth of ovaries and oviducts. No cell divisions seen
Day 25.....	*Male:* As on Day 21
	Female: Fourth and final day of preoviposition period. Empty but intact spermiophore capsules in seminal receptacle. Spermiophores seen in low numbers in oviducts and ovary lumen. Growth of ovaries and oviducts continues to be rapid. No cell divisions seen
Day 26.....	*Male:* As on Day 21
	Female: First day of oviposition period. Oviducts packed with eggs. Spermiophore capsules empty and intact. Few spermiophores seen only in ovary lumen; none in oviducts. Growth of ovaries and oviducts ceased. No cell divisions seen
Day 27.....	*Male:* As on Day 21
	Female: Second day of oviposition period. Oviducts packed with eggs. No spermiophores seen anywhere in female system. No cell divisions seen
Days 28–38....	*Male:* No males examined after Day 28 when they were as before
	Female: Continued oviposition. Ovaries and oviducts with progressively fewer eggs and reducing slowly in length. Spermiophore capsules still intact, and empty, in seminal receptacle. No cell divisions seen
Day 39.....	*Male:* None examined
	Female: Last (i.e. 15th) day of oviposition period. Few eggs remaining in oviducts and ovaries. Spermiophore capsules intact and empty. No cell divisions



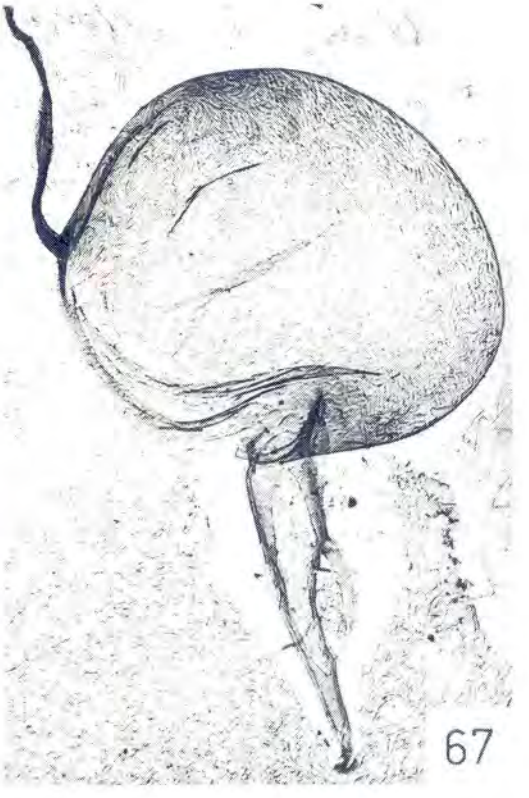


FIG. 65–67 Spermiophore capsules of *Boophilus decoloratus*. (65) Single spermiophore capsule in the seminal receptacle of a female. (66) Two spermiophore capsules in the seminal receptacle of a female. (67) A single spermiophore capsule extracted from a female seminal receptacle (Note that this capsule broke during extraction thus liberating mature spermiophores)

From the above notes a number of interesting facts emerge. Firstly, with respect to male ticks, nymphs, although showing considerable gonadal development, did not demonstrate either mitotic or meiotic divisions. When mitosis was seen in newly moulted adult males, these divisions were few in number and limited to the small cells at the junction of the vasa deferentia and testes. The testicular cells enlarged considerably during the nymphal phase and appeared to undergo meiotic divisions soon after the emergence of the adults. It is therefore believed that these large cells are primary spermatocytes and that multiplication of spermatogonia by mitosis was for some reason never observed. Another interesting observation is that the spermatophore capsules of *B. decoloratus* did not dissolve but remained intact for the duration of the female's oviposition period. The capsules did not break open to liberate the spermiophores and it may therefore be concluded that they exit via 1 or both of the 2 small tubes possessed by each spermiophore capsule (Fig. 65, 67).

With regard to the female, it is interesting to note that no cell divisions were observed during the nymphal stage. Adult female ticks contained only a few small cells undergoing mitosis. As in the males, the mitotic multiplication of gametogonia was apparently not observed, nor were reduction divisions or primary oocytes seen. It is possible that these divisions take place only after spermiophore penetration of oocytes and egg shell formation.

TABLE 1 The number of male spermiophore capsules found in the seminal receptacles of female *Boophilus decoloratus* ticks

No. spermiophore capsules	Female ticks examined	
	Number	%
1	12	14,5
2	32	38,6
3	13	15,7
4	17	20,5
5	2	2,4
6	4	4,8
7	0	0
8	2	2,4
9	1	1,2
Total	83	100,1

Multiple mating

Although Londt (1976) has shown that male *B. decoloratus* are able to mate more than once, it was not known how many times a female could be impregnated. Actual copulation and spermatophore transfer have only once been witnessed and it was impossible to determine the number of times a female was mated by observing copulatory behaviour. It has been possible, however, to draw a number of conclusions from counts made of the number of spermiophore capsules found in the female seminal receptacle. Eighty-three fully engorged female ticks that had dropped from the host were dissected. The data obtained are shown in Table 1. Female seminal receptacles were found to contain from 1–9 capsules. As spermiophore capsules were more often found in even numbers, 2 being the commonest number (i.e. in 38,6% of the females), it is assumed that with each mating 2 capsules are usually deposited in the receptacle (Fig. 66). A single capsule only can be produced by males (Fig. 65). In cases where uneven numbers of capsules were found, at least 1 was often somewhat larger than the others, suggesting that when 2 capsules are not produced, the single one which results is often larger than normal. On the assumption that each mating results in either 1 or 2 capsules it is suggested that just over half the dissected females (53,1%) had mated only once, while the remaining females had mated more than once. The female containing 9 capsules must have mated at least 5 times, and possibly as many as 9 times. Multiple mating is therefore quite common.

Discussion

Oliver (1974) briefly reviewed the present state of our knowledge of reproduction in ticks. In addition to the publications he has listed, the following papers contain useful information: Rothschild (1961), Chinery (1965), Rechav & Oppenheim (1969), Gregson (1969), Feldman-Muhsam (1971), Gladney & Drummond (1971), Aeschlimann & Grandjean (1973), Oliver, Al-Ahmadi & Osburn (1974), Norval (1974), Norval & Capitini (1974), Khalil & Shanbaky (1975).

For descriptive and comparative purposes Oliver (1974) has divided spermatogenesis into 4 main phases:

1. Multiplication of spermatogonia by mitosis.
2. Enlargement and differentiation of primary spermatocytes.
3. Two meiotic divisions and partial spermatid development.
4. Capacitation (final growth and differentiation) of spermatids to mature spermiophores after transfer to the female.

Testes development (growth) takes place primarily during the nymphal feeding phase in *B. decoloratus*, not during the first few days of feeding by the adults as in the case of the 3-host ticks *D. occidentalis*, as described by Oliver & Brinton (1972), and *A. hebraeum*, as described by Norval & Capitini (1974). No mitotic divisions were observed during the nymphal feeding phase of *B. decoloratus*, but the scarcity of these divisions and the onset of meiosis very soon after moulting (6 hours) suggest that spermatogonial mitosis and the formation of primary spermatocytes do in fact take place during nymphal engorgement and that, for some reason, this was never seen even though many squashes were made. As in other ixodids (Oliver, 1974) maturation of primary spermatocytes in *B. decoloratus* begins in the posterior region of the testis and proceeds in a wave-like manner anteriorly.

Meiosis proceeds normally, the first meiotic division being reductional for the x-chromosome and the second equational. The only reported exception to this seems to be *Argas reflexus*, in which the situation is reversed (Oliver, 1964). Chiasma encountered during diplotene-diakinesis suggests that genetic crossing over takes place in *B. decoloratus*. The timing of spermatogenesis in *B. decoloratus* is similar to that found in most other Metastriata in that meiosis does not take place in unfed adults. It does, however, take place sooner and proceeds faster after moulting (attachment) than is the case in the 3-host tick *D. variabilis*, as reported by Homsher & Sonenshine (1972). Apart from the fact that males that had moulted in the laboratory and were thus prevented from attaching to a host did not show meiotic divisions, there is as yet little evidence to suggest that

males feed during the pre-mating period. Spermatid capacitation takes place in the usual manner and the elongating spermatids are transported through a central canal into the vasa deferentia and seminal vesicle, where they are enclosed in spermatophores for transfer to the female. There is no evidence to suggest that spermatids are stored in the spermatophores for any length of time and it is thought that capacitation continues uninterruptedly within the spermatophore and that the spermatophores are transferred to a female as soon as they are ready. Three to 4 days of attachment to the host are required by male *B. decoloratus* before mating behaviour and insemination of the female takes place.

The various stages of oogenesis in *B. decoloratus* were not observed. It appears that oogonia do not undergo reduction divisions until they have been penetrated by spermiophores. Certainly no meiotic divisions were seen before the deposition of the egg shells; after this it was impossible to study the egg contents by the method used. Female *B. decoloratus* require a blood meal before final gonad development and oogenesis take place. During the last 2 days of engorgement and the 4 days prior to oviposition, the total length of the ovary plus oviducts increased from about 5 mm to over 90 mm. The cytogenetic findings for *B. decoloratus* agree closely with those of Oliver & Bremner (1968) for *B. microplus* in Australia and with those of Newton, Price, Graham & Trevino (1972) for *Boophilus annulatus* (Say) and *B. microplus* in Mexico on the following points: 1. The chromosome complement of 2n=22 for females and 2n=21 for males; ii. The cephalobranchial position of the centromere on all the chromosomes; iii. The relative lengths of the autosomes and x-chromosomes; iv. The xx:xo sex mechanism, the male being heterogametic; and v. The 1st male meiotic division being reductional and the 2nd equational for the x-chromosome.

Oliver (1974) and Feldman-Muhsam & Havivi (1967) have shown that the first spermatophore to be produced by males commonly contains no spermiophores. Many spermiophore capsules were examined in female *B. decoloratus* seminal receptacles and all were found to contain mature spermiophores. The act of copulation in *B. decoloratus* was observed only once, although many ticks were found to be paired. The males of these pairs were, however, invariably attached to the host with their ventral sides uppermost and in close contact with the female, spermatophore transfer having already taken place. In the single copulation observed, the male was found with its mouthparts inserted into the female's vagina. The mating ticks were removed from the host and studied under a microscope, where it was seen that a newly formed ectospermatophore protruded from the genital opening of the female. The contents of this ectospermatophore were seen to be discharged into the female and the empty ectospermatophore then fell away from the female opening. On subsequent dissection of the female, 2 spermiophore capsules were found in the seminal receptacle. It thus appears that mating in *B. decoloratus* is similar to that of other ixodids. According to Galun & Warburg (1967), the male accessory glands contain several pharmacologically active compounds which probably initiate peristalsis in the female oviducts, resulting in spermiophore relocation. Many female *B. decoloratus* were found to possess a whitish, sometimes very large, mass of waxlike consistency in the seminal receptacle, together with the spermiophore capsules. This substance, absent in unmated females, is almost certainly a product of the male accessory glands and may be worthy of further investigation with a view to discovering whether it is indeed involved in the initiation of spermiophore relocation.

Syngamy in *B. decoloratus* probably occurs in the ovary as spermiophores were commonly observed in the ovary lumen as well as in the oviducts. By the time oviposition was under way, however, no further spermiophores were seen in the oviducts. Observations on the timing of sperm relocation under laboratory conditions suggest that the male gametes reach the oocytes between Day 2 and Day 4 of the female's preoviposition period.

Multiple impregnation of female ticks is well known, and this now includes *B. decoloratus*. Oliver *et al.* (1974) found that *D. occidentalis* and *Haemaphysalis leporispalustris* (Packard) produce a single spermiophore capsule per mating (i.e. 1 endospermatophore per ectospermatophore) and Feldman-Muhsam & Borut (1971) confirmed that this was true also of *H. anatolicum excavatum*. This feature has been thought to be a consistent family character, Argasidae producing 2 spermiophore capsules per ectospermatophore (Feldman-Muhsam, 1967; 1971). *B. decoloratus* can, however, produce either 1 or more usually 2 capsules per ectospermatophore. Female *B. decoloratus* ticks are sometimes impregnated as many as 5 times. As females complete engorgement and leave the host within a short time of being mated for the first time, it is likely that females containing more than 2 capsules receive all the spermiophores from the same male. Females mated only once produce as high a number of fertile eggs as females which have been mated more than once. This suggests that multiple mating in *B. decoloratus* is probably wasteful of male gametes. If this is true, then it may mean that multiple mating was a result of the experimental design. A single large infestation of larvae was made, which means that all the ticks were in more or less the same stage of development at any one time. This in turn means that meeting of the sexes and first copulation takes place for all the ticks on the host at more or less the same time. It is possible that each male, on completion of its first mating, cannot be attracted to other unfertilized females as there are none available. This may therefore lead to the re-impregnation of females which have already been successfully fertilized.

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