The copulatory behaviour of *Cruznema lambdiensis* (Nematoda : Rhabditidae)

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SUMMARY

Studies on the copulatory behaviour of *Cruznema lambdiense* revealed that copulation involved three distinct steps : i) attachment of male to female and localization of the vulva, ii) penetration by the spicules and iii) insemination. The bursa aided in gripping the female while the spicules located the vulval opening, but did not take part in channelizing the sperms from the male to the female reproductive tracts. Females continued feeding during copulation. The mean number of copulations per day varied from 3 to 7.2 and the sperms transferred per day from 61 to 176. On an average, 20-33 sperms were transferred per copulation per day. In its life span, a male copulated 15-32 times and transferred a total of 517-754 sperms. When males were isolated for more than two days, both the number of copulations and the number of sperms transferred decreased. As the isolation period of the males increased, the time required for the first copulation also increased.

RÉSUMÉ

Comportement de Cruznema lambdiense *(Nematoda : Rhabditidae) au moment de la copulation*

Les études sur le comportement de *Cruznema lambdiense* ont montré que la copulation comprenait trois phases distinctes : i) attachement du mâle à la femelle et localisation de la vulve, ii) pénétration par les spicules et iii) insémination. Les bursae aident à maintenir la femelle pendant que les spicules cherchent l'ouverture vulvaire mais ne jouent aucun rôle dans l'acheminement des spermatozoïdes dans l'appareil génital de celle-ci. Les femelles continuent à se nourrir pendant la copulation. Le nombre moyen de copulations varie de 3 à 7,2 par jour et le nombre de spermatozoïdes transférés de 61 à 176 pendant le même temps. En moyenne, 20-33 spermatozoïdes sont transférés par copulation et par jour. Au cours de sa vie, un mâle copule 15 à 32 fois et transmet au total 517 à 754 spermatozoïdes. Quand les mâles sont isolés pendant plus de deux jours, les nombres de copulations et de spermatozoïdes transférés diminuent. Quand la période d'isolement des mâles augmente, le temps nécessaire à la première copulation augmente également.

Little work has been done on the copulatory behaviour of plant and soil nematodes, and when studied, only the mechanism of copulation has been described (Greet, 1964 ; Jones, 1966 ; Chin & Taylor, 1969). Recently, a rather detailed account of the copulatory behaviour of the free-living nematode, *Panagrellus redivivus* has been given (Duggal, 1978) and it revealed that the rate of copulation was related to the rate of sperm transferred (Duggal, 1978). In *Rhabditis pellio* it was found that the number of matings decreased with age (Somers, Shorey & Gaston, 1977), while in *Aphelenchus avenae* the intervals between copulations increased with age (Fisher, 1972). In the present work, the copulatory behaviour of young and ageing *Cruznema lambdiense* (Maupas, 1900) Thorne, 1961 has been studied in detail.

Materials and methods

The nematodes were raised in xenic conditions on peptone agar supplemented with wheat flour.

Copulation behaviour

The copulatory behaviour was studied in microchambers (Ahmad & Jairajpuri, 1980). Single newly moulted adult males were placed with four or five newly moulted adult virgin females, and observed under the microscope. Observations on matings were also recorded in cultures.

Rate of copulation and sperm transfer

To determine the rate of copulation and the number of sperms transferred, freshly moulted adult males were kept separately with ten freshly moulted adult virgin females in a Petri dish containing 1% water agar. After 12 hr the females were removed, and examined, and a fresh batch of virgin females was added to the Petri dish. The males were observed till they died.

Effect of isolation of male on copulation and sperm transfer

Ageing effects of males on copulation and sperm transfer were studied by placing one, two, three and four-day-old virgin males with ten one-day-old virgin females in a Petri dish containing 1% water agar. The females were examined after 12 hr, then a fresh batch of virgin females was added to the Petri dish. Males were transferred to fresh media every 12 hr and were observed till they died.

Length of time required for the first copulation

Virgin males were placed singly with eight to ten young virgin females in a Petri dish containing 1% water agar and observed continuously under a binocular microscope. Tests were carried out with males isolated for zero, one, two and three days. There were five replicates for each set of experiments.

Results

Copulatory behaviour

Localization of the vulva and attachment to the female

When an active male came in contact with a female, it pressed its tail at once against the female body and moved over her backwards. If the male touched the female with its anterior end, there were quick back and forth movements giving the impression of searching behaviour.

However this movements did not occur consistently were exhibited only by those males which eventually copulated. Those that did not exhibit this behaviour did not copulate. In cultures, the males could be divided into two distinct groups : i) those apparently unaware of the presence of females that kept on feeding without attempting to copulate, and ii) those which actively attempted copulation. Once the male tail had pressed against the female body, both spicules working together, probed the female regularly.

For a successful mating it was essential that the male and the female body be properly oriented with respect to each other, i.e., with anterior ends in opposite directions (Fig. 1) and that their ventral surfaces be mutually opposed. No mating was observed when male and female pointed in the same direction. In cultures, the males apparently showed no preference for virgin females and copulated as readily with gravid females.

Penetration

Once the spicules had penetrated the vulval opening, the female was held firmly. The spicules made repeated thrusts into the vagina and penetrated deep into it. The male stopped feeding at this stage.

Insemination

The males produced anteriorly directed waves of the body soon after penetration resulting in a shortening of the body. Simultaneously, the body swept across in wide arcs with the anterior region oscillating vigourously (Fig. 1). Just prior to the release of sperms, all activities

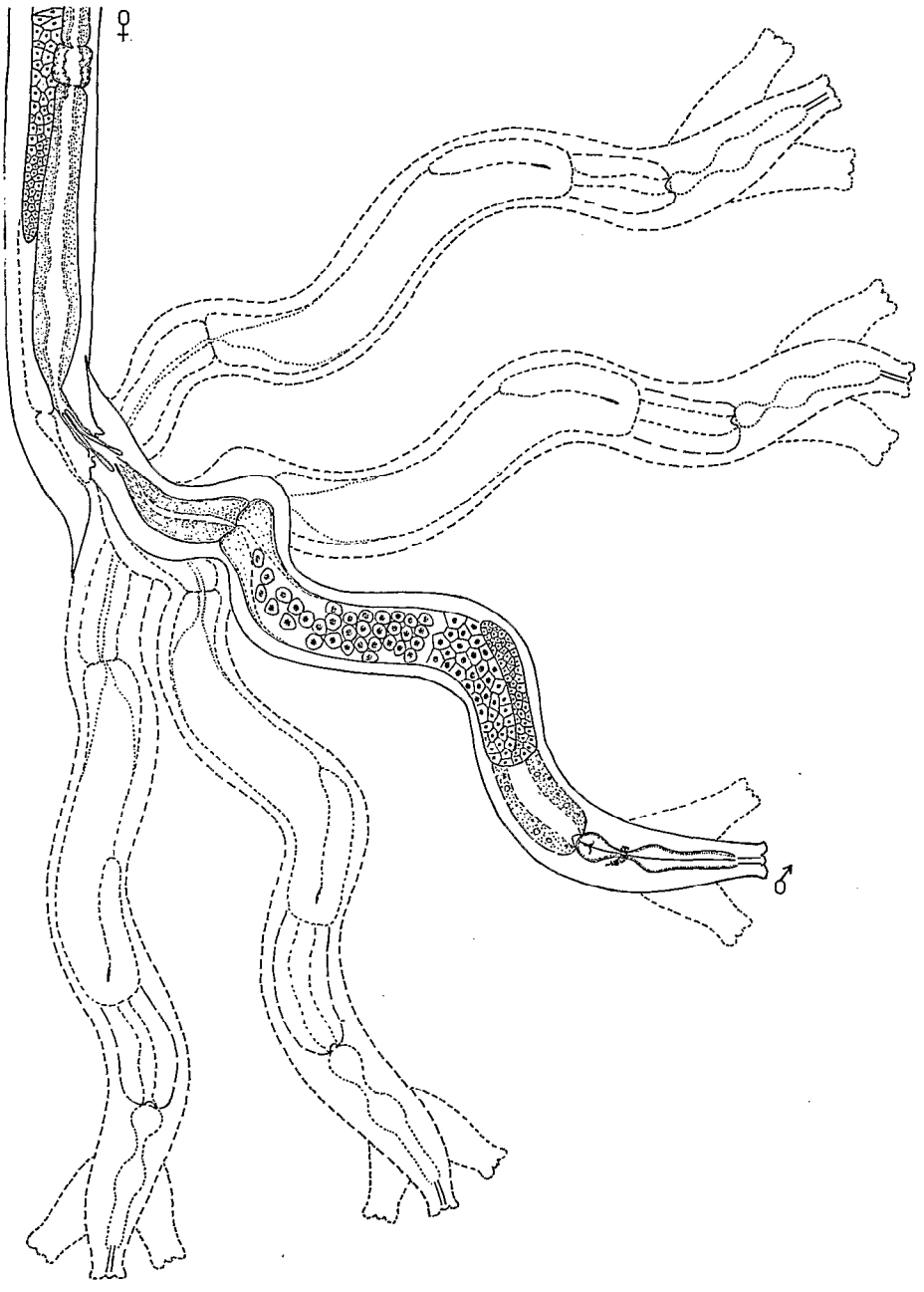

Fig. 1. Copulating postures of *Cruznema lambdiense* males.

ceased and the male lay motionless for a few seconds with its spicules inserted into the vagina. Soon afterwards, there was a simultaneous retraction of the spicules and shortening of the body which resulted in the flow of sperms into the uterus. On the average, 20-33 sperms were transferred per copulation per day (Fig. 3). However, six-day-old males transferred fewer sperms.

Behaviour of the male when disturbed during copulation

When disturbed the males displayed consistent behavioural characteristics depending on the stage of copulation. In the early stages, before the penetration of the spicules, if the female moved out of its grip, the male showed sharp reversals alternating with forward movement, perhaps indicating searching behaviour. If it did not make contact again, it started its normal feeding behaviour. If separated after spicule penetration and the subsequent shortening of the body, they became motionless with their spicules protuded. Occasionally, a few sperms were discharged out of the body passively. The males lay motionless for about 50 min and afterwards resumed activity with slight movements first in the anterior end and then gradually spreading to the entire body. However the spicules were retracted only after the male had moved a short distance.

Female behaviour during copulation

Females continued feeding through the process of copulation. Occasionally, when the male probed their anterior end, there was a sharp withdrawal response. After insemination, when the male had moved apart, vulval twitchings were observed both in virgin and gravid females.

EFFECT OF AGEING OF MALES ON THE RATE OF COPULATION AND SPERM TRANSFER

Copulating males

The mean rate of copulation and the number of sperm transferred per day were closely related over the first five-day-period (Fig. 2). Only two of the five males copulated on day six and hence it is considered to be of abnormal occurrence and no inferences are drawn from it. During the first five days, the mean rate of copulations per day varied from 3-7.2 and the mean number of sperms transferred from 61-176. The mean number of sperms transferred per copulation per day ranged from 20-33 (Fig. 3). Difference between the maximum number of sperms transferred per day (on day two) and the minimum per day (on day five) was statistically significant ($P < 0.025$). Comparisons between the mean number of copulations per day and the mean number of sperms transferred per copulation per day showed an inverse relationship (Fig. 3). However, the general trend indicated a decrease in the rate of sperms transferred (Fig. 2).

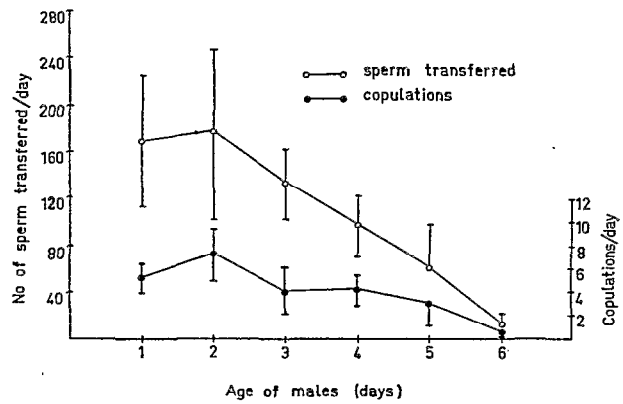

Fig. 2. Rate of copulation and sperm transfer during the life span of *Cruznema lambdiense*.

Normally copulating males which were allowed to copulate freely after the final moult copulated 15-32 times and transferred a total of 517-754 sperms in their entire life span.

Isolated males

As the isolation period of the males increased beyond two days, both the total number of copulations and the total number of sperms transferred decreased significantly (Fig. 4; $P < 0.001$). Males isolated from females for two days or more became sensitive and usually displayed a sharp withdrawal response when

they made their first contact with females. Further, in these males, the accumulated sperm in the seminal vesicle began to show degenerative changes. Males isolated for four days failed to inseminate females and only in one was a single copulation observed.

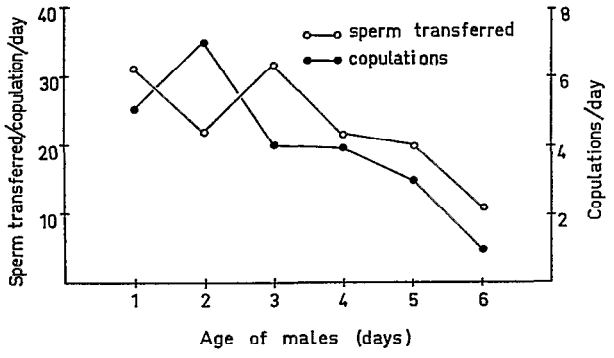

Fig. 3. The effect of age of male on the number of sperm transferred per copulation per day and the number of copulations per day.

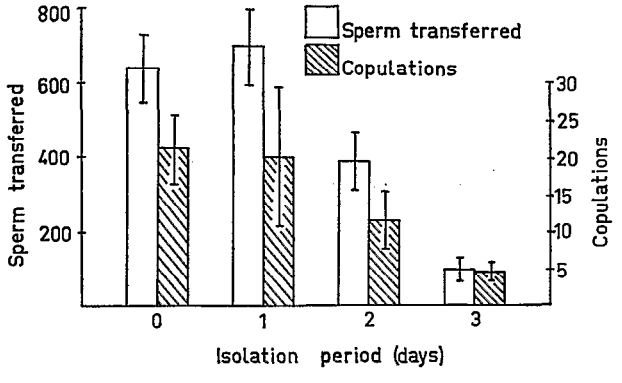

Fig. 4. The effect of isolation of males on the total number of sperms transferred and the total number of copulations.

Time required for the first copulation

Isolation of virgin males resulted in a gradual increase in the time required for the first copulation (Fig. 5). Differences between one and three, and zero and three day isolated males were significant ($P < 0.001$).

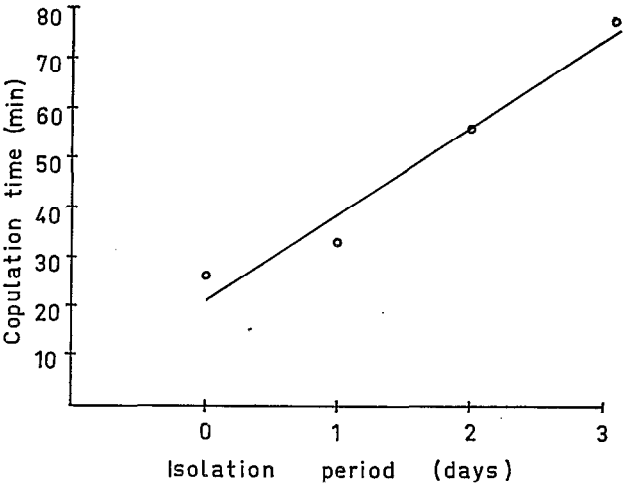

Fig. 5. The effect of isolation of males on the time required for the first copulation. Fitted regression line $y = 21.6 + 17.6\ x$.

Discussion

Studies on the copulatory mechanism of nematodes have shown that the spicules were inserted into the vagina during copulation, and aided in the transfer of sperms from the male to the female reproductive tracts (Greet, 1964 ; Jones, 1966 ; Chin & Taylor, 1969 ; Duggal, 1978). Further, ultrastructural studies on the male copulatory apparatus of *Panagrellus redivivus* (Duggal, 1978) and *Hoplolaimus galeatus* (Hogger & Bird, 1974) have revealed that the vela membranes of the spicules form a tube through which sperms pass during insemination. In species of *Heterodera* such a tube is formed by the dorsal and ventral wings (Clark, Shepherd & Kempton, 1973) and in *Pratylenchus* by the ventral wings (Wen & Chen, 1976) while in *Aphelenchoides blastophthorus* the dorsal and ventral limbs of the spicules form a kind of a channel (Clark & Shepherd, 1977). The formation of a tube by the flanged spicules of *Ancylostoma* was considered to help in the flow of sperms as early as 1905 (Loos, 1905). But in species where the spicules were not flanged and a tube was not formed, it was suggested that they were withdrawn during the movement of sperms (Mueller, 1930). The mechanism of sperm transfer in *C. lambdiense* seems to add weight to the suggestion of Mueller (1930). However, a

more detailed study of the spicules and associated structures is required to understand the actual mechanism of insemination. In *C. lambdiense* males, the well developed bursa and cloacal lips maintain the continuity between the male and female reproductive tracts, even when the spicules are withdrawn.

The frequency of copulation and the number of sperms transferred by normally copulating males began decreasing after two days, and similar results were obtained when males were isolated for varying periods of time. Hence, both these activities may be dependant on the age and physiological state of the males. A significant decline in the frequency of copulation was also observed in the males of *P. redivivus* isolated for long period (Duggal, 1978). The inverse relationship between the mean number of sperms transferred per copulation per day and the mean number of copulations per day suggests that when males copulated often, they transferred fewer sperms per copulation.

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