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THE FEMALE GONAD OF *LONGIDORUS AFRICANUS* MERNY AND THE INFLUENCE OF THE HOST ON ITS DEVELOPMENT

by
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The effect of the host on the population levels of *Longidorus africanus* Merny has been studied by Lamberti (1968). He found that the nematode reproduced very well on sugar beet (*Beta vulgaris* L.) increasing the initial population levels by 362% in an experiment conducted from May to October 1967 and by 1100% in a second experiment carried out from November 1967 to April 1968. Plants such as Bermuda grass (*Cynodon dactylon* L.), Eggplant (*Solanum melongena* L.), spinach (*Spinacia oleracea* L.), and lettuce (*Lactuca sativa* L.), appeared to be indifferent hosts for *L. africanus* in the first experiment, but in the second one population increases of 180 to 280% were obtained with respect to the initial levels.

The results of these experiments suggest that the quality of the host, perhaps associated with different growth rates at different times of year, influence the reproductive behavior of the nematode. Possibly the plant supplies nutrients essential for nematode development, particularly for reproduction. In the case of an unfavourable host on the other hand, reproduction may not take place because the nematode is unable to feed on it or because the host does supply certain substances which negatively affect the sexual development of the nematode.

Therefore, having available the material of Lamberti's (1968) experiments and other populations of *L. africanus* grown for diffe-

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rent periods of time in soil planted with various hosts, it was thought that useful information could be obtained from a detailed study of the sexual development of the nematode in relation to its nutritional status.

Material and Methods

All the observations were made on groups of 20-25 females of *L. africanus* extracted from the soil by the Cobb wet sieving technique. After extraction the nematodes were killed in hot 5% formalin and mounted in glycerin by the slow infiltration method (Lamberti and Sher, 1969). Specimens were also dissected and the extracted gonads studied in detail.

The nutritional status of the nematodes was determined by observing the intestinal content as suggested by Van Gundy *et al.* (1967), each specimen being classed as starved when it appeared transparent and without granules within the intestine, well fed when the whole body content was retained and partially fed when about half of the body contents had been lost.

Initially evaluation of the gonad maturity was attempted on the basis of its total length but after a careful study of this organ (described in detail in this paper) on over 600 specimens, we have come to the conclusion that the degree of sexual maturity is better expressed by certain ratios, which are discussed later, and that the status of maturity of the oocytes must be also considered in this evaluation.

Description of the female gonad of Longidorus africanus

The anterior or posterior gonad of *L. africanus* (Fig. 1) has a scarsely differentiated uterus which is rather short when relaxed. A well developed sphincter having, as usually for nematodes, an inner layer of longitudinal fibres and an outer one of circular fibres, separates the uterus from the oviduct which appears clearly differentiated in two parts: one proximal to the uterus, probably glandular, which forms the sac of the oviduct and a second tubular part consisting of a single row of ring cells. The oviduct is inserted ventrally on the ovary and this point indicates the boundary be-

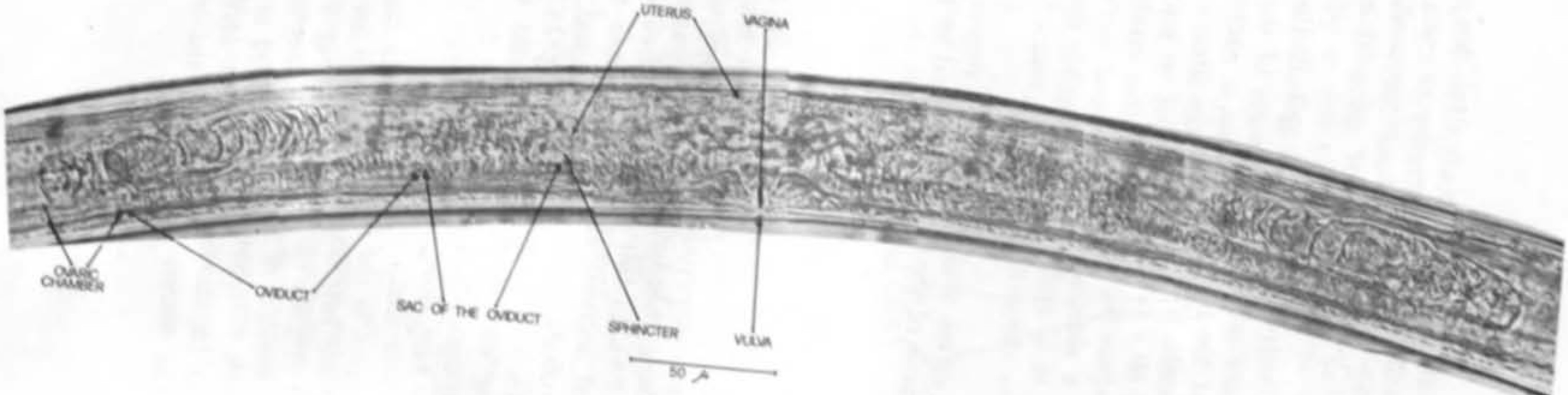

Fig. 1 - Female gonads of *Longidorus africanus*.

tween the ovaric chamber and the true ovary. The length of the gonad varies with the age and degree of maturity of the individual. In young females the averages length is about 300 μ and this increases to 900 μ or more as the process of maturation proceeds (Figs. 2 and 3).

Of the different parts that constitute the gonad, the uterus is the least variable. It appears more or less always of the same size independently of the age of the nematode, unless it contains oocytes which extend its elastic walls. The length of the uterus for all the stages is between 50 and 60 μ (Fig. 4), and only in gravid, mature individuals is it sometimes possible to observe values around 150-200 μ.

The oviduct sac has a glandular function and secretes the egg shell around the oocyte. It is remarkably extensible and is formed by a few cells of irregular shape and size when relaxed, but polygonal when stretched.

The oviduct length varies slightly with the degree of maturity of the gonad mainly because of the traction exerted by the increasing size of the ovary. In fact, while in young gonads the length ranges from 100 to 150 μ (mode 135 μ), at a more advanced stage it reaches a size of 150-180 μ (mode 165 μ), and is 250-300 μ or even 400 μ during the passage of the oocytes (Fig. 5).

The ovary is the most variable structure of the gonad (Figs. 1 and 6). In young females it is rather thin and has the length of the oviduct. It contains numerous small oocytes which have not yet passed into the ovaric chamber and the process of vitellogenesis is not initiated (Fig. 2 A). In full developed individuals it occupies, as seen in transversal section, almost the whole body cavity, its length is about 50% of the total gonad length and its reflexed germinal apex usually reaches the uterus level (Fig. 2 C). This is mainly due to the increasing size of the oocytes whose volume increases gradually as the vitellogenesis takes place (Fig. 2 C). The number of the germinal cells, in fact, does not vary with the age of the animal; what is varying is their size.

Vitellogenesis begins in the ovary when the oocytes are at phase « I ». Usually there are few oocytes (oogonia are more numerous) in specimens in the process of maturation (Fig. 2 B).

Their germinal portion of the ovary is enveloped by a thin epithelium (2-3 μ) which becomes thicker (4-5 μ) at the junction with the oviduct and has its maximum thickness at the ovaric chamber (7-8 μ) (Fig. 2). The oocyte « I » during the vitellogenesis grows

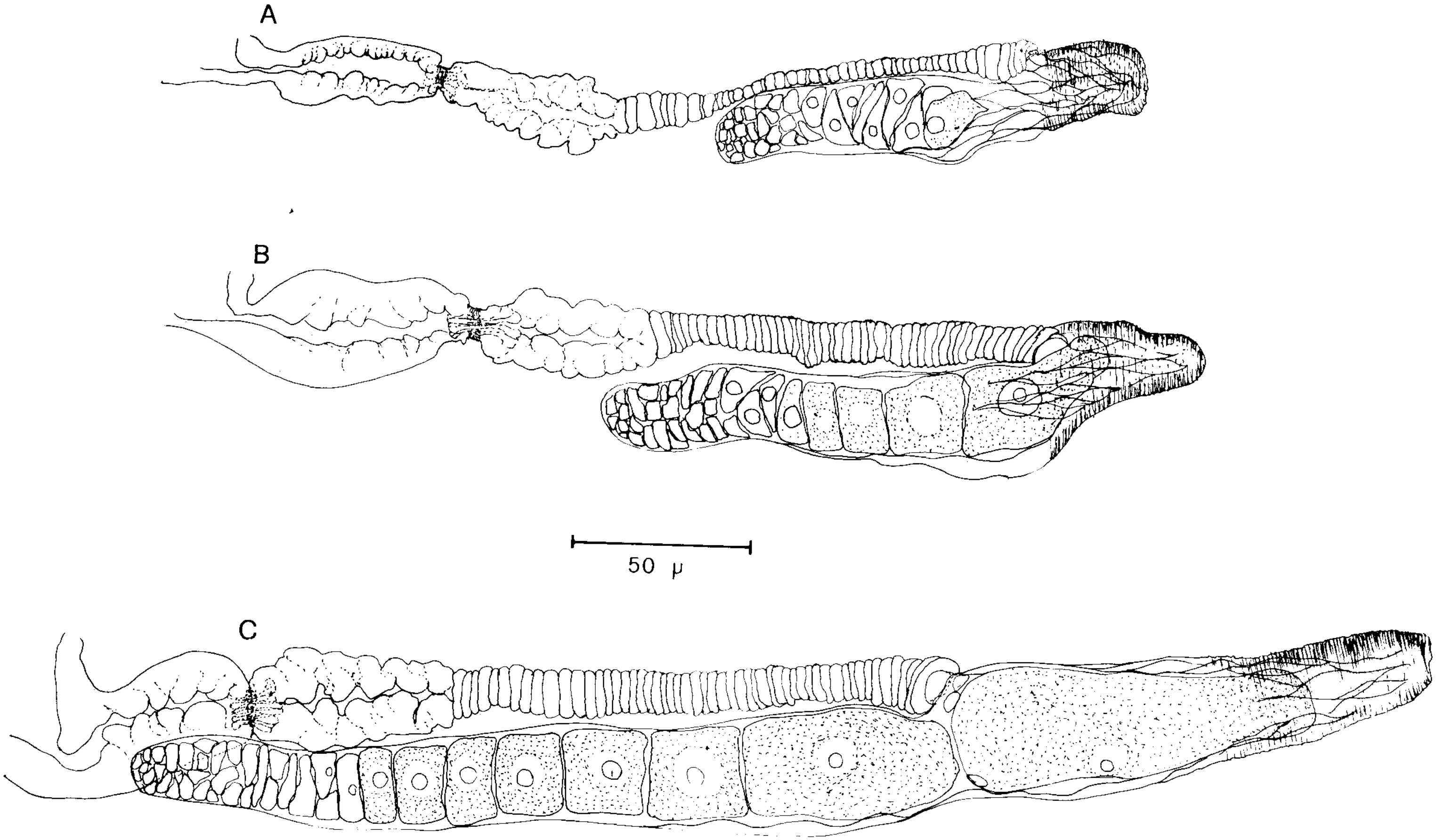

Fig. 2 - Different stages of maturation of the female gonads in *L. africanus*: A, young; B, in the process of maturation; C, mature.

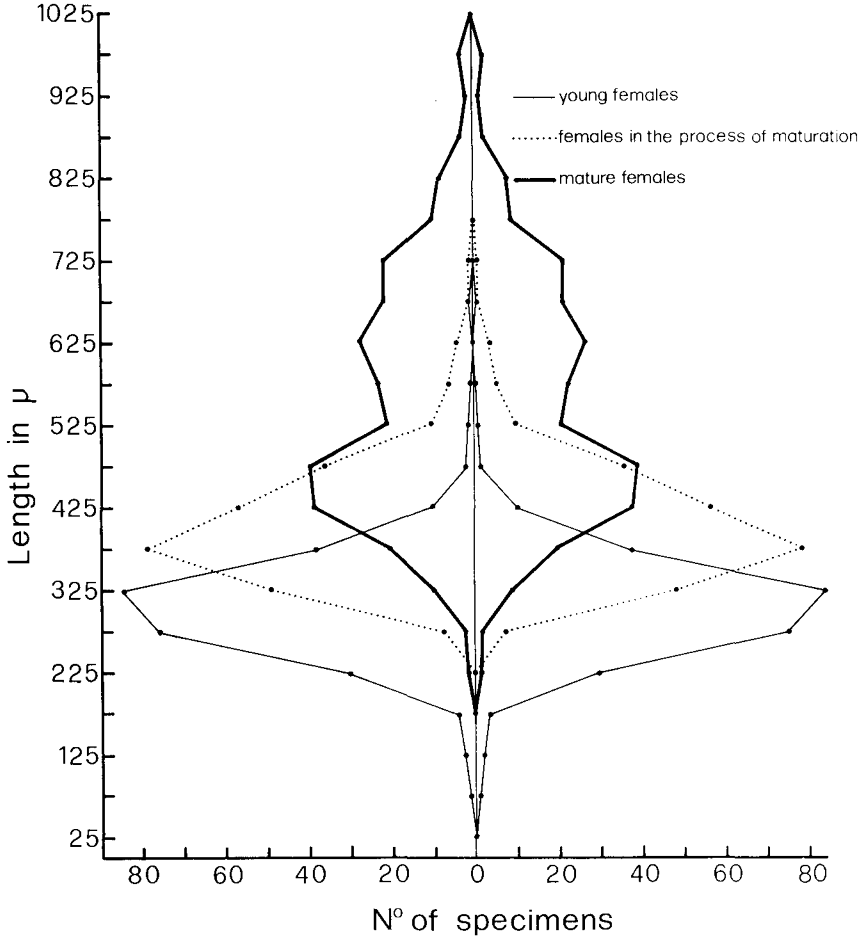

Fig. 3 - Variability of the total length of the female gonads in L. *africanus* (n = 500).

towards the ovaric chamber where this process is completed. In fact, we were able to observe clearly some cytoplasmic extensions joining the egg to the wall at the ovaric chamber in gonads just starting the process of maturation (Fig. 7).

The ovaric chamber has, therefore, two functions: trophic because it completes the vitellogenesis and mechanical because it pushes the oocyte into the oviduct. This structure, together with the

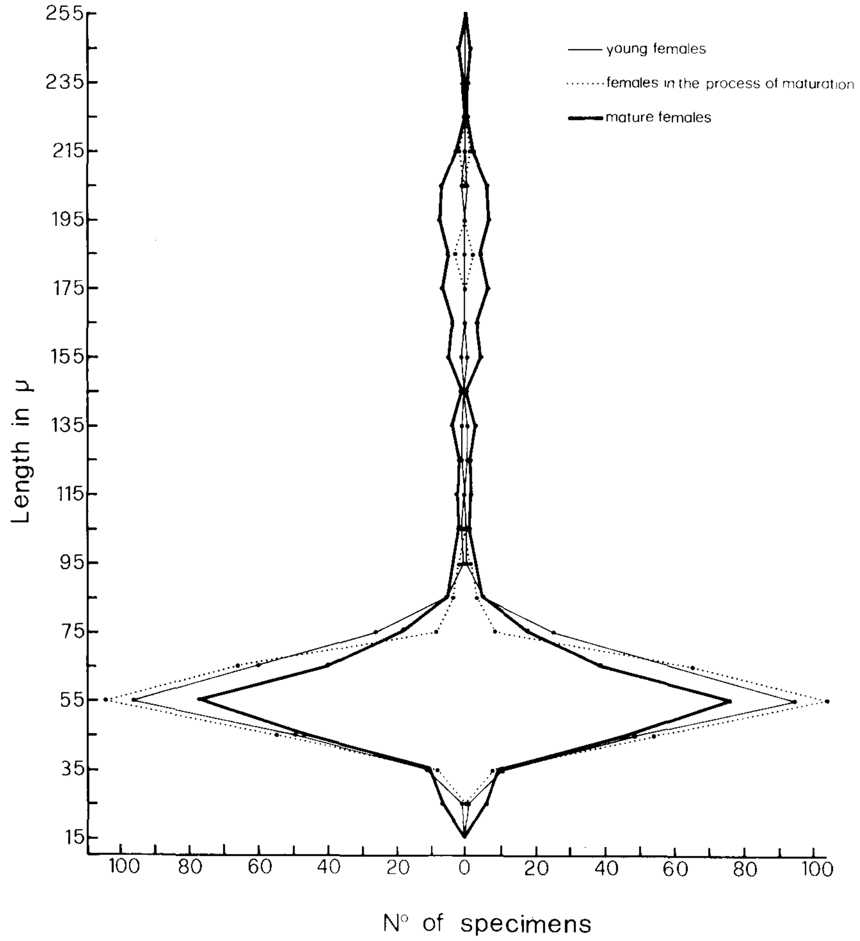

Fig. 4 - Variability of the uterus length in *L. africanus* (n = 500).

ovary, is the most variable portion of the gonad in relation to the age of the individual. It appears strongly contracted with a reduced lumen and robust walls, constituted by well developed spindle shaped cells longitudinally arranged, in very young gonads (Fig. 2 A). These cells are very extensible and enveloped by a thin epithelium folded many times when the chamber is relaxed (Fig. 2 A). As the oocytes increase in size and enter the ovaric chamber where they reach their largest dimensions, the ovaric chamber becomes stretched and its walls thinner (Fig. 2 C). Usually a single oocyte is present in the ovaric chamber, but in some cases it was possible to observe two or three of them. The alterations of the ovary affect the oviduct dimension, expecially the length but not the uterus whose size is more or less constant during all the stages unless eggs are inside.

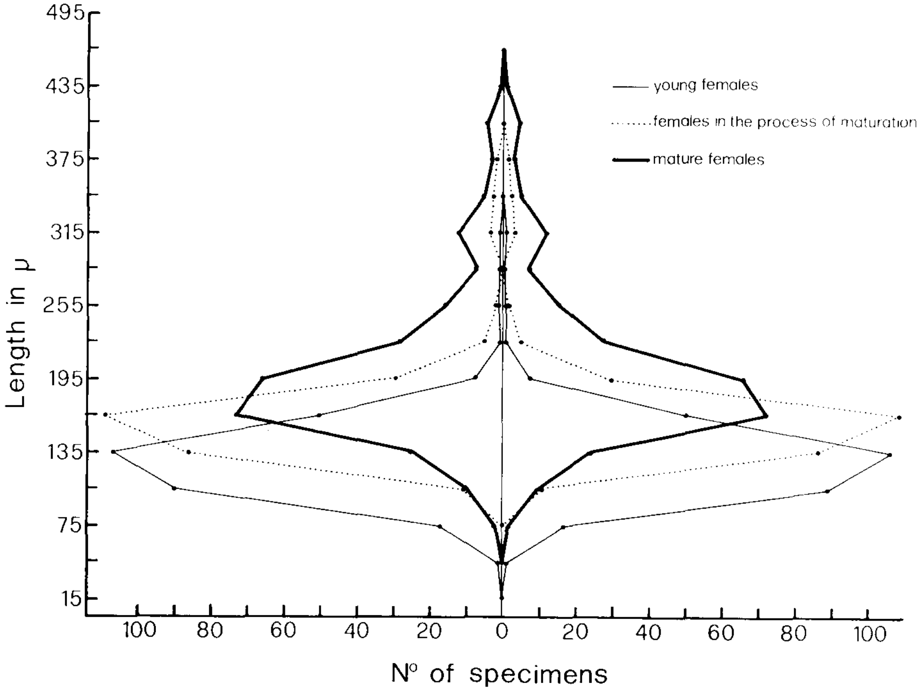

Fig. 5 - Variability of the oviduct length in *L. africanus* (n = 500).

The average length of a young ovary is about 105 µ (Fig. 6) reaching 300-350 µ at full maturity which is, as already stated, a half of the total gonad length. Therefore, considering the ratio: length of the ovary/length of the oviduct, we have a value of less than 1 for young gonades, 1 for those in the process of maturation, and greater than 1 for the fully developed ones (Fig. 8). Moreover, the ratio: length of the ovary/number of oocytes varies from values of 4-5 for young females, 9-24 for mature individuals, and 6-8 for intermediate stages (Fig. 9). The variability of the value for mature individuals (9-24) is due to the decrease in the number of oocytes as the eggs are laid. Egg laying, in fact, is not followed by production of new oogonia; we have never observed mitotic phases in the germinal apex of dissected gonads. This means that the number of oocytes of each gonad is probably determined when the organ is differentiated, being unaffected by successive alteration of the host-parasite relationship, and might be, within certain limits, a specific character (Tab. I).

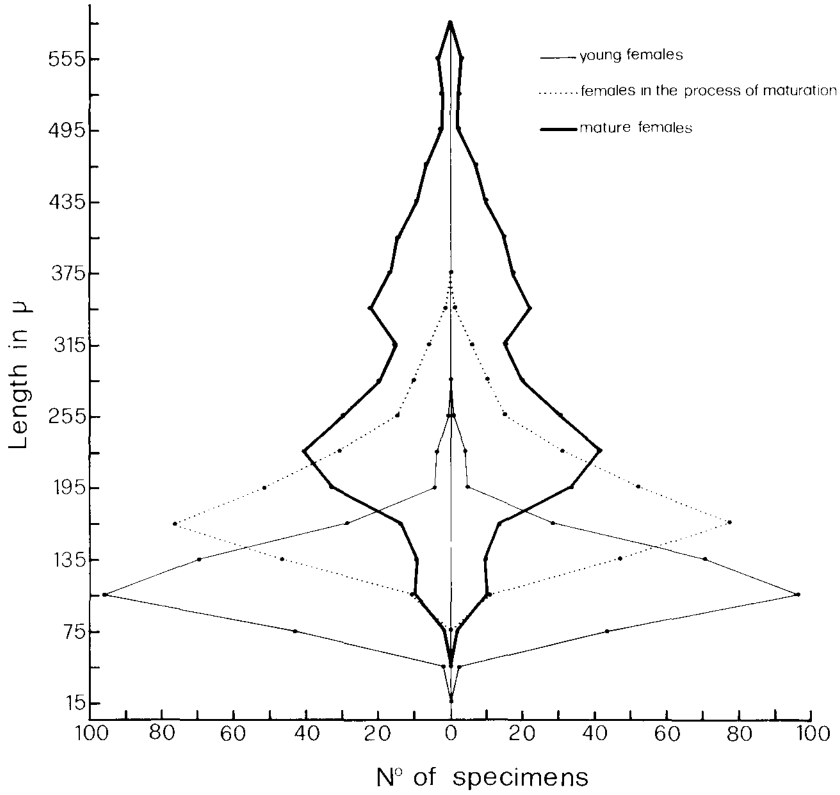

Fig. 6 - Variability of the ovary length in *L. africanus* (n = 500).

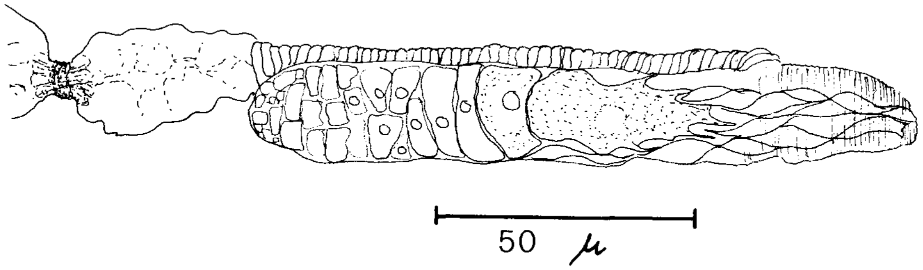

Fig. 7 - Cytoplasmic extensions of an oocyte I in the ovaric chamber of *L. africanus.*

Table I - *Number of oocytes observed in gonads of different populations of* L. africanus *reared on various hosts, for different periods of time and at different temperatures.*

	Period of time in months	Number of oocytes		Mode (class of highest frequency)	Mean	S E
		Min.	Max.			
Spinach	5	17	32	23	24	± 3.6
Okra	5	14	31	22	22	± 3.6
Eggplant	5	13	35	23	25	± 4.8
»	7	16	37	27	24	± 4.9
Sunflower	5	14	36	23	24	± 4.4
Cantaloup	5	13	27	19	20	± 3.7
Cucumber	5	13	30	30	21	± 4.1
Bermuda grass	5	13	27	23	22	± 2.9
» »	12	15	43	25	25	± 4.6
Snap bean	5	12	30	21	22	± 4.2
Squash	5	15	31	20	22	± 4.8
Mint	5	15	33	25	23	± 3.8
»	12	4	38	22	25	± 5.9
Grapevine	5	18	32	27	26	± 4.3
»	12	17	32	25	24	± 3.6
Tomato	5	12	38	24	25	± 5.8
»	12	16	38	20	25	± 7.0
» at 30°C	3	15	48	32	29	± 7.4
» at 35°C	3	20	49	30	29	± 9.2
Fig	5	14	36	26	25	± 5.0
»	12	11	31	20	22	± 5.0
Sugarbeet	5	5	34	26	25	± 5.0
»	7	4	37	22	24	± 6.7
»	12	18	31	24	24	± 3.7
Sorghum	7	15	32	24	24	± 3.8

The ratios referred to above can be used graphically to express patterns of the status of maturity of a population (Figs. 8 and 9). Moreover, since the uterus is a structure of constant dimension independent of the status of maturity of the animal, whereas the ovary length varies with the degree of maturity of the gonad, plotting the ratios $\frac{\text{ovary length}}{\text{oviduct length}}$ versus $\frac{\text{ovary length}}{\text{oocytes numbers}}$, provides another pattern of the degree of maturity of the population (Fig. 10).

Results

On the basis of the above observations, we have evaluated the sexual maturity of about 600 females (1147 gonads observed) of *L. africanus* reared for periods of five, seven and twelve months in metal pots croped with different plants species and kept in a lathhouse under the climatic conditions of Southern California (Lamberti, 1968).

Differences in the sexual development of the nematodes can be observed after five months association with the different hosts (Fig. 11). The gonads of most females extracted from the rhizosphere of plants on which Lamberti had observed severe decreases of the initial population [Okra (*Hibiscus esculentus* L.), Eggplant, Cantaloup (*Cucumis melo* L.), and Cucumber (*C. sativus* L.)] were still in a young stage (Fig. 11). On the other hand, with plants on which less drastic reduction of the initial nematode populations occurred, about only 50% of the females were still young [Bermuda grass, Sunflower (*Helianthus annuus* L.), Snap beans (*Phaseolus vulgaris* L.), Cucumber and Squash (*Cucurbita pepo* L.)]. At the same time, the populations extracted from the soil cropped with plants that had led to increases in the initial level [Spinach, Eggplant second experiment, Mint (*Mentha spicata* L.), Grapevine (*Vitis vinifera* L.), Tomato (*Lycopersicon esculentum* Mill.), Fig (*Ficus carica* L.) and Sugarbeet] contained always less than 50% of young females whereas the percentage of the mature ones was clearly higher. The only exception is represented by Sorghum (*Sorghum vulgare* Pers.), but is probably because in our experiment a summer plant was cultivated out of season (from November to May).

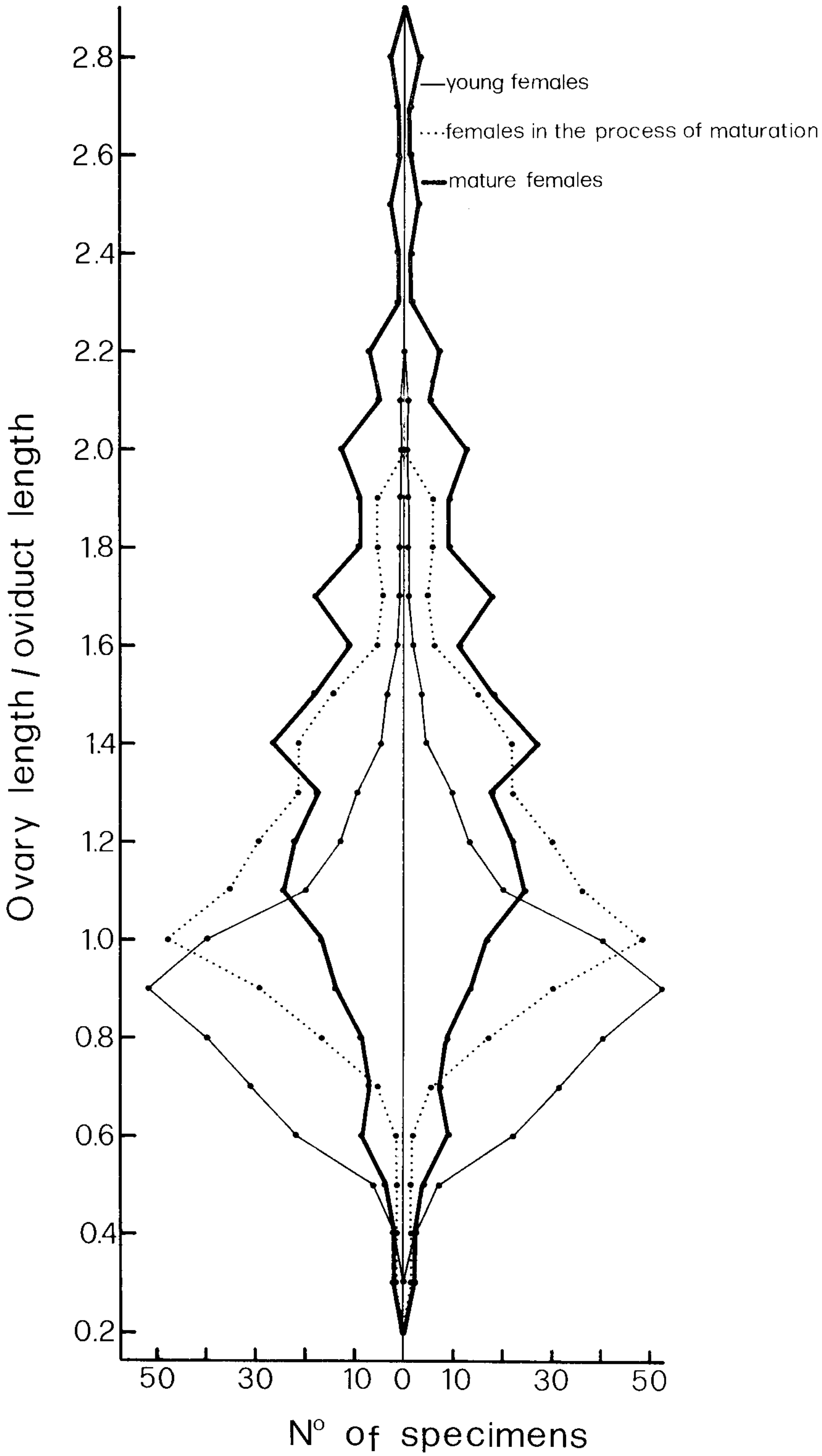

Fig. 8 - Variability of the ratio ovary length/oviduct length in *L. africanus* (n = 500).

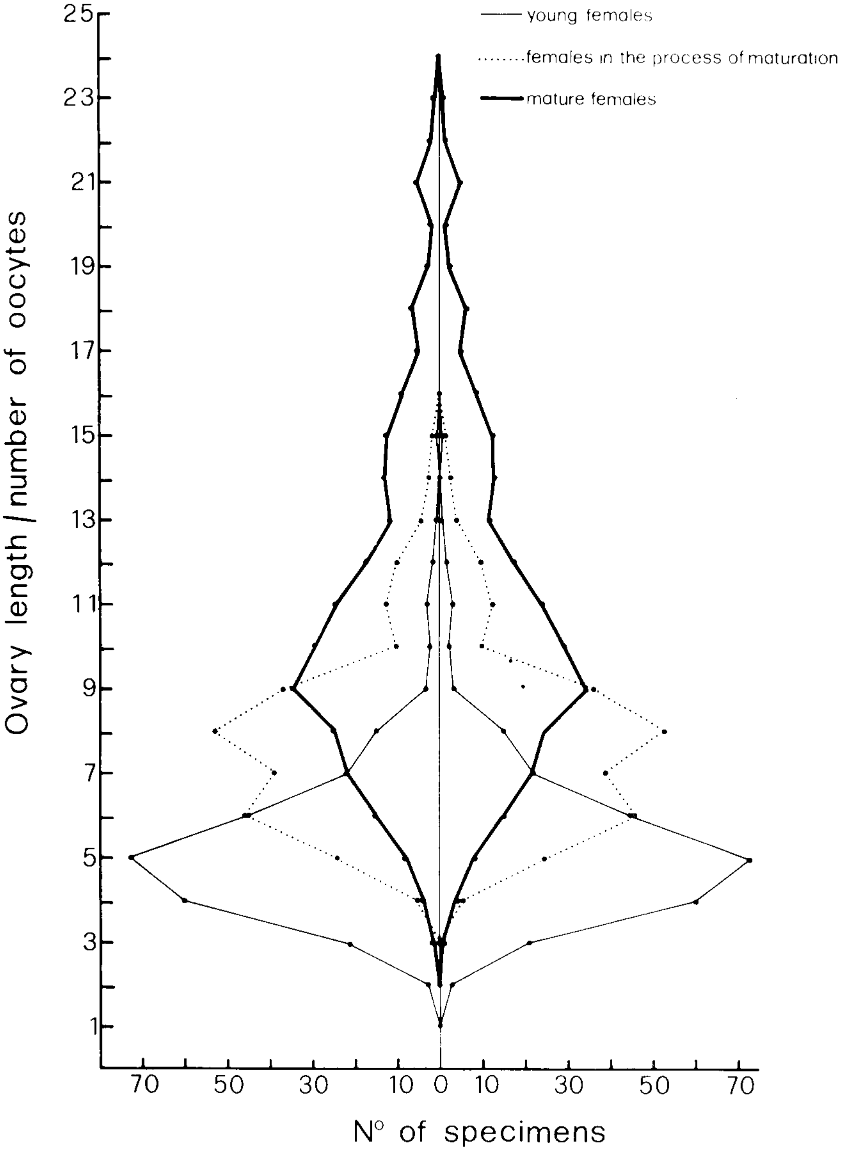

Fig. 9 - Variability of the ratio ovary length/number of oocytes in *L. africanus* (n = 500).

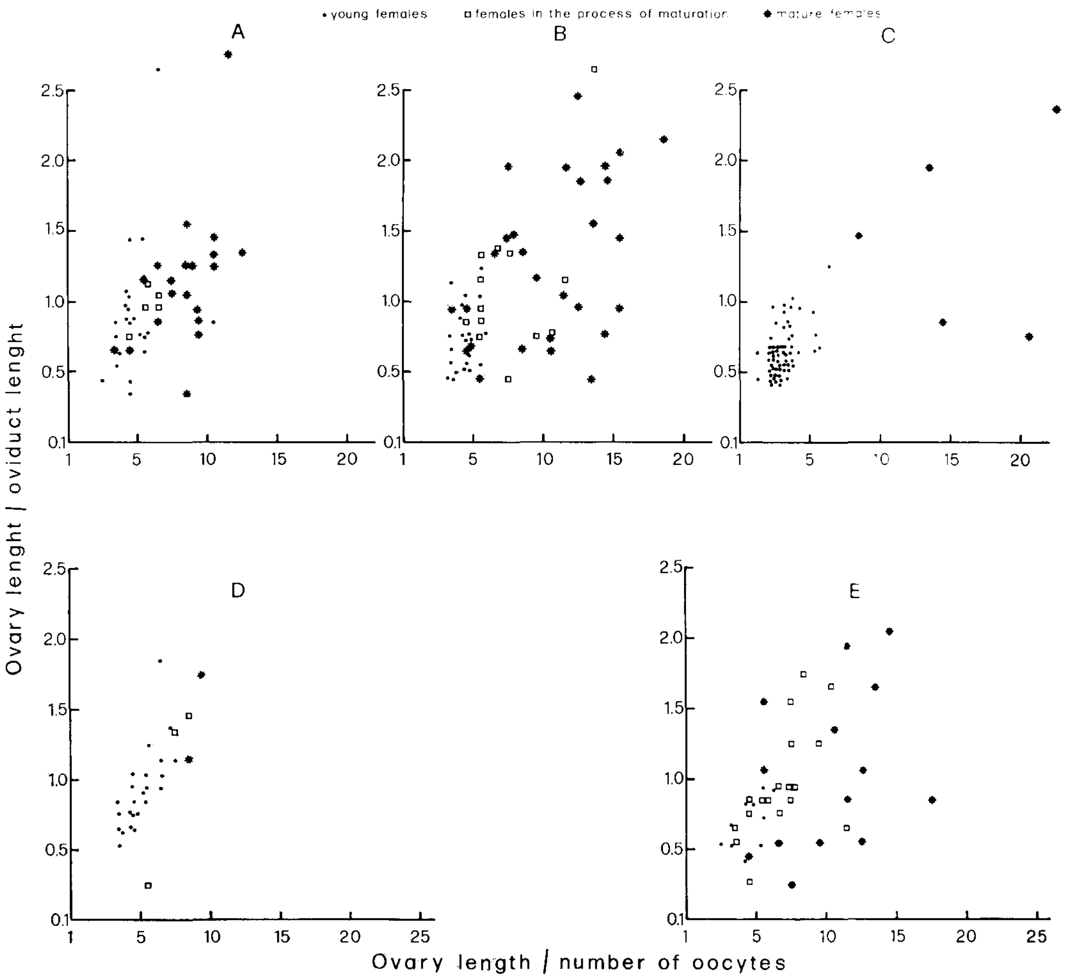

Fig. 10 - Status of maturity of five populations of *L. africanus*: A, sugarbeet after 5 months; B, sugarbeet after 7 months; C, sugarbeet after 12 months; D, eggplant after 5 months; E, eggplant after 7 months.

It is evident in Fig. 11 that the sexual maturity of the populations is directly correlated with the host-nematode relationship. On the roots of some plants, not illustrated in the graph, the populations decreased below the level of detection. On the other plants nematode numbers either increased, under conditions suitable for reproduction, or decreased more or less drastically and although maintaining their reproductive capability the life cycle was retarded probably due to the status of the host. This is confirmed by further observations when in pots cropped seven months with eggplant we have found that the percentage of females with mature gonads had increased considerably. A remarkable augmentation of matures, with respect to the situation at five months, had occurred also in the rhizosphere of Bermuda grass in one year (Fig. 11).

The process of sexual maturation continued regularly on the hosts on which had already been observed at five months a large number of specimens with well mature gonads. After seven months on sugarbeet the number of young females was much less than at five months; but after a year the adult population was almost totally composed of young females (Fig. 11) indicating that the nematode had completed its first cycle and the individuals of the successive generation were approaching the reproduction phase. Analogous patterns, with minor variations on the length of the life cycles occurred on mint, grapevine, tomato and fig.

Also of interest are observations made on two populations of *L. africanus* having the same origin as those referred to above and reared for three months on tomato at a constant temperature of either 30 or 35°C (Lamberti, 1969). The higher temperature, or the status of the host at higher temperatures, seems to retard the life cycle of the nematode (Fig. 11). This explains the decrease of the reproduction rate observed by Lamberti (1969) at 35°C.

On the basis of these informations we have tried to correlate the sexual maturity of the specimens with their nutritional status. Our observations indicate that most individuals reared for five months on plants on which they had reproduced actively, such as sugarbeet, grapevine, fig, etc., had fully mature gonads independently of their nutritional status, although a high percentage of them appeared to be well fed. On the other hand, nematodes reared for the same period of time on cantaloup, cucumber, okra, etc., even when apparently well fed had very poorly developed reproductive systems or, as in the case of sunflower, one of the gonads was completely atrophic.

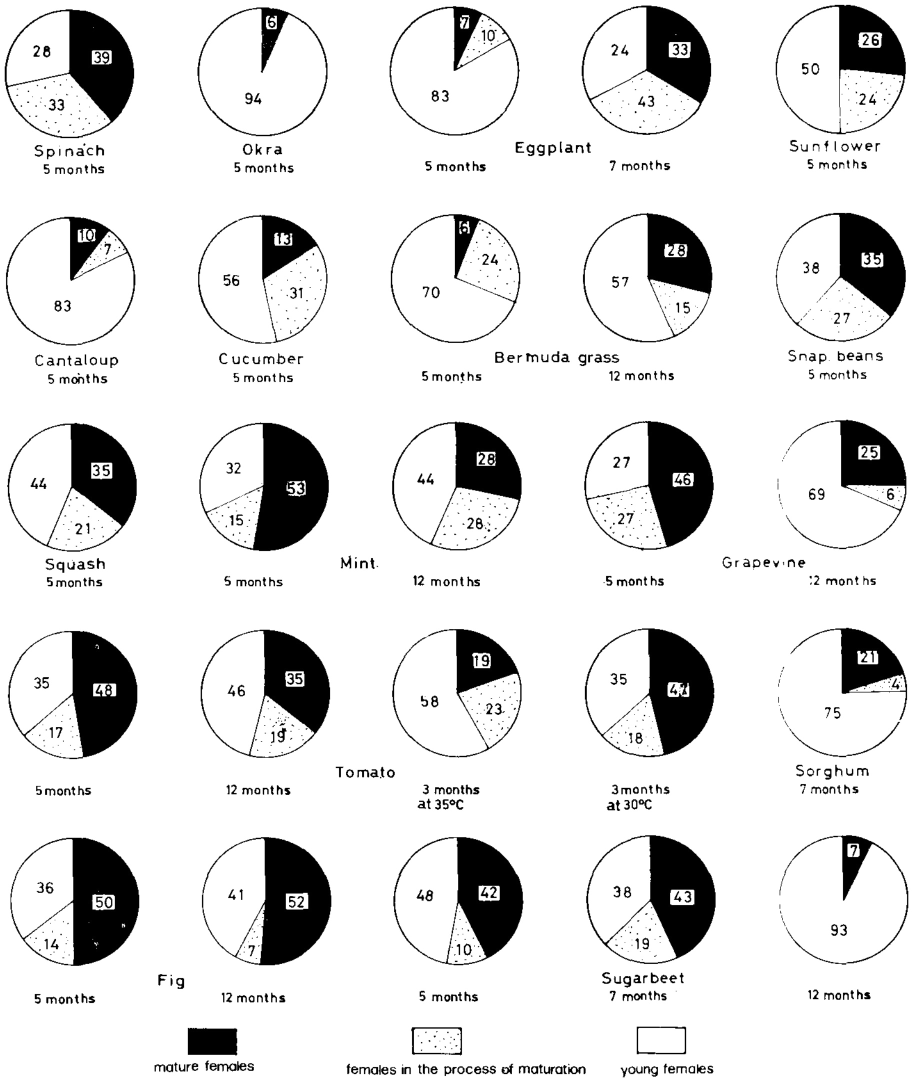

Fig. 11 - Per cent composition as referred to the sexual maturity of different populations of *L. africanus* reared on various hosts for different periods of time and at different temperatures.

Conclusion and Discussion

From our results we conclude that the sexual development in nematodes is affected more by the quality of the food intake than the quantity imbibed, and indeed, that there does not seem to be a close relationship between nutritional status and sexual maturity. In fact, many well fed specimens have often been found in populations reared on hosts which, more or less inhibited the sexual development to the extent that the gonads were atrophic or rudimentary; conversely, nematodes extracted from the rhizosphere of sugarbeet or grapevine, etc. had fully matured gonads although they appeared to be in a very poorly nutritional status. A greater importance must be attributed to the status of the host. Bermuda grass, a winter weed, which in an experiment done from May to October induced a decrease of the initial population level, in a second experiment carried out from October to May stimulated fairly active reproduction of the nematode (Lamberti, 1968). Moreover, tomato plants grown at two different temperatures (Lamberti, 1969) differently affected sexual development and rate of reproduction of *L. africanus*, shortening the life cycle of the nematode under conditions which were more favourable for the host. On certain plants, such as snap beans and squash, the maturation of the gonads and consequently the life cycle of the nematode seemed to need a longer period of time to be completed. Possibly these plants contain a substance, which stimulates sexual development, but is more diluted than in more favoured hosts and therefore the nematodes need to feed on them for a longer time to accumulate the effective concentration.

It is impossible to say, on the basis of our knowledge, which of these factors are involved in the sexual metabolism of the nematode. The length of the life cycle of nematodes seems to be affected by mineral concentration within the plants: nitrogen, according to Bird (1960 and 1970), Evans and Fisher (1970) and Baker *et al.* (1971); potassium, according to Marks and Sayre (1964) and Dropkin and Boone (1966); and calcium, according to Dolliver *et al.* (1962). Krusberg and Blickenstaff (1964) demonstrated that cinetine increases the rate of reproduction in *Ditylenchus dipsaci* but decreases that of *Pratylenchus penetrans* and *P. zeae*. However, our observations might suggest that the host produces substances which are toxic or inhibitory to the sexual development of the nematode. The concentrations of these substances could vary with the plants species and the density of the parasite population feeding upon them. This would explain, in some cases, the presence of high percentages of females with immature or rudimentary gonads in the rhizosphere of plants considered as good hosts. Evans and Trudgill (1971) have, in fact, experimentally proved that aminoacids such as ethionine, methionine, valine, and others, have inhibitory effects on nematode development, whereas Webster (1967) has demonstrated that the reproduction of the plant parasitic nematodes is more active and faster on growing hosts due to the presence within the tissues of high concentrations of giberellin, triptofan and indolacetic acid. Several workers have detected an increase of free aminoacids in nematode-affected roots and it is claimed that this is due to the action of proteolytic enzymes produced by the parasite (Viglierchio, 1971). The balance between incitants or inhibitors at different sites of the host plant could then account for the differing degrees of sexual development of individuals seen in nematode population.

SUMMARY

The female gonad of *Longidorus africanus* Merny is described in detail with observations on its status of maturity. Investigations on the effect of the host on the sexual development of the nematode lead to the conclusion that the quantity of food uptake is not as important as the quality in affecting nematode reproduction.

RIASSUNTO

La gonade femminile di Longidorus africanus *Merny e l'influenza dell'ospite sul suo sviluppo.*

È descritta la gonade femminile di *Longidorus africanus* Merny anche in relazione al suo stato di maturità. Indagini sull'effetto dell'ospite sullo sviluppo sessuale di questa specie, portano alla conclusione che la quantità di cibo assunta non è altrettanto importante, quanto potrebbe essere la qualità, nella riproduzione dei nematodi.

RESUMÉ

La gonade des femelles de Longidorus africanus *Merny et l'influence de l'hôte sur son développement.*

La gonade des femelles de *Longidorus africanus* Merny est décrite en detail avec des observations sur son état de maturité. Des recherches sur l'effet de l'hôte sur le développement sexuel de cette espèce prouvent, en conclusion, que la qualité est plus importante que la quantité de nourriture dans la reproduction des nématodes.

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