VOL. 7 APRIL, 1933 NO. 10

HILGARDIA

A Journal of Agricultural Science

PUBLISHED BY THE

California Agricultural Experiment Station

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UNIVERSITY OF CALIFORNIA PRINTING OFFICE
BERKELEY, CALIFORNIA

HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

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REPRODUCTION WITHOUT MALES IN ASEPTIC ROOT CULTURES OF THE ROOT-KNOT NEMATODE[1]

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Parthenogenesis and syngonism[3] are recognized phenomena among the Nematoda, yet in the root-knot nematode, *Heterodera marioni* (Cornu),[4] where males do occur, their function in reproduction has been taken for granted. Byars (1914), however, did suggest the possibility of parthenogenesis in this nematode. Gabriel (1926) assumed its probability because he found several generations in one gall.

1 Received for publication March 25, 1932.

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3 Maupas (1900), Cobb (1918), and others, have found that in certain free-living nematodes the gonad of the female produces spermatozoa, which are stored in a seminal receptacle and fertilize the eggs which are produced subsequently.

4 This nematode has long been known as *Heterodera radicicola;* but, as Goodey (1932) points out, the original description of *Anguillula radicicola* by Greeff (1872) obviously does not refer to the root-knot nematode. Cornu (1879), then, was the first to publish a name for the latter, which he called *Anguillula marioni.* His descriptions and figures, though inaccurate and incomplete, do apply unmistakably to the root-knot nematode.

The genus (or subgenus) *Caconema,* proposed by Cobb (1924), is founded on three points: the type of parasitism, the structure of the amphids, and the number of testes. None of these is a distinguishing generic character in the classification of nematodes. Plant-parasitic and free-living species are commonly contained within the same genus (Imperial Bureau of Agricultural Parasitology, 1932), e.g., *Anguillulina* and *Pathoaphelenchus* [now *Aphelenchoides* (Steiner, 1932)]. The amphids vary in position, size, and structure from species to species (Steiner, 1923). The reproductive organs also are highly variable, as was observed by Bütschli in 1874. According to Baylis and Daubney (1926), each of the following genera contains species with two testes and species with only one: *Diplogaster, Tylencholaimus, Monhystera, Oncholaimus, Linhomoeus, Cyatholaimus, Chromadora,* and *Laxus,* a genus "of doubtful status," in which Cobb (1894; 1914) himself combined the two types of males. That Baylis and Daubney do not consider the number of testes a significant taxonomic character is indicated by the fact that they make no mention of the point in their synopsis of many of the genera of Ascaroidea, including *Spilophora,* which may be added to the list on the authority of Bütschli (1874). In the descriptions for the other four orders, testes are mentioned for only one genus.

Reproduction without males is obviously a great advantage to a parasite which must develop and lay eggs in one isolated location. There is, however, no true evidence for the phenomenon without actual pedigrees of isolated females, for which controlled laboratory cultivation is necessary.

Berliner and Busch (1914) made preliminary attempts to raise the sugar-beet nematode, *Heterodera schachtii*, in seedlings in agar. Byars (1914) raised the root-knot nematode aseptically through one generation in seedling cultures.

METHOD

The method of cultivation described in the present paper permits the easy observation of individual nematodes in a fairly healthy environment. Complicating factors are eliminated by keeping the cultures bacteriologically sterile. Exact observations can thus be made on many phases of the life history.

Earliana tomato is used as the host plant. Seeds are disinfected with calcium hypochlorite (Wilson, 1915), and allowed to germinate in agar plates. The seedlings are transferred to fresh plates of a plant-nutrient medium.[5] One seedling is planted in each plate just before the agar sets, and for most of the cultures one nematode in the larval stage is placed beside the root tip. For the multiple infestations an egg mass was placed near the seedling, which was later found to harbor from 1 to 10 or more developing nematodes. Some larvae have difficulty in penetrating the root, but with good material galls appear in from one to five days, according to the temperature.

The plates can be kept for observation for a week or two, but better growth is obtained if the infested host plant, or part of its root, is transferred to a test tube containing Pfeffer's solution. A few grains of sand are added to each tube for a possible value in aeration.[6] The tubes can be kept sterile for weeks or months, and incubated as desired.

For obtaining uncontaminated larvae, the most practicable source of material is an infested potato. Here the egg masses are protected by a tough brownish case, which appears to be a shell of crushed plant tissue possibly impregnated with the oöthecal secretion discharged by the

[5] An agar medium is made with Pfeffer's solution (Robbins, 1922), modified by the substitution of ferric tartrate for ferric chloride (Hoagland, 1919) and by the addition of boron (Sommer and Lipman, 1926). The following formula was used: $Ca(NO_3)_2 \cdot 4H_2O$, 4.0 grams; KH_2PO_4, 1.0 gram; KNO_3, 1.0 gram; KCl, 0.5 gram; $MgSO_4 \cdot 7H_2O$, 1.0 gram; ferric tartrate, trace; borax, 1:1,000 solution, 5 cc; glucose, 120 grams; agar agar, 60 grams; distilled water, 6,000 cc.

[6] Method used by G. Thorne.

nematode before egg-laying. The cases can be dissected out unbroken from the tuber. If they are originally free from contamination, a rinse of 3 to 5 minutes in hydrogen peroxide (c.p., full strength, i.e., 3 per cent) will clean them of any external organisms acquired during the manipulation, which need not be too carefully aseptic. From the peroxide, the egg masses are placed directly on agar plates, where any chance contamination can be discovered. Egg masses from tomato galls have also been used. They are considerably less convenient because the protecting cases are more fragile. It is also difficult to clean contaminating soil from the small galls.

The first larvae to emerge in the plates are those which have hatched some time earlier and lain packed, inactive, within the egg case. Later individuals are evidently newly hatched from the eggs. They wander on or through the soft agar and can be handled conveniently with a pointed splinter of bamboo, as used in Thorne's laboratory. A supply of splinters can be sterilized in test tubes in the hot-air oven.

ABILITY OF LARVAE TO ENTER ROOTS

In view of the abundance of nematode galls in the field, it was most surprising to meet with considerable difficulty in obtaining galls in cultures, even on healthy growing seedlings. A great difference in the ability of nematodes to penetrate plant tissue was observed in different lots of larvae.

In an attempt to analyze this behavior, over 8,000 larvae have been tested. It is seen in table 1 that larvae raised in root cultures are able to enter fresh roots readily, while an initial infestation from field material was obtained less often. In the itemized daily records, infestation by larvae from cultures has been as high as 90 and 100 per cent of the individuals tested. When larvae hatched from a poor root were active enough to use for F_1 cultures, they were as successful in entering the new host as were larvae from a better environment. By contrast, some lots of larvae from potato gave no galls, and only a few of the others gave 50 per cent infestation. Larvae from tomato gave 0 to 30 per cent.

There is no indication that external conditions affect the situation. It appears to be in the nature of some lots of larvae to enter the root tip readily and of other lots to find difficulty in penetration. Several possible explanations have been tested and discarded.

The first suggestion was that there might be a condition of dormancy of the nematodes in a dormant potato, or else that the larvae there were somewhat older and weaker than the normal. Egg masses were then taken fresh from tomato roots growing in pots. There is no reason to assume that dormancy occurs under greenhouse conditions, yet the proportion of galls formed by these larvae was even lower than by those from potato.

This comparison also answers Steiner's (1925) suggestion that a race of nematodes accustomed to potato might resist a change of host. On the contrary, larvae from potato make more galls in tomato seedlings than do those from greenhouse tomato roots (table 1). The larvae from one lot of potatoes[7] in particular showed a high power of infestation. These nematodes had been in potato only two or three generations, however. Their previous host was tomato.

TABLE 1

Root Penetration by Larvae from Different Sources

Material	Larvae tested	Per cent infestation
Potato (3 lots, various ages)	1,810	6.0
Tomato (fresh from greenhouse)	1,599	4.0
Potato No. n464	91	27.5
Tube cultures (mostly from isolated females)	4,933	54.2

There is no reason to suspect the peroxide treatment of weakening the eggs or larvae. All the egg masses from No. n464 received this treatment, as did also a few of the galls from cultures. Material so treated gave infestations up to 90 per cent.

Cultured larvae appear exceptionally large and vigorous. The absence of other organisms cannot account for this because the egg masses within an otherwise healthy potato are equally aseptic.

Only two observations seem to have significance: (1) There is an apparent stimulation of larvae which have hatched in a liquid. This has been observed in sterilized Pfeffer's solution, and in tap and distilled water. (2) Larvae hatched and "dormant" within the egg mass in potato, and larvae kept in tubes of any liquid longer than a few weeks, seem to

[7] In December, 1928, potatoes were received from Goodyear's Bar, in the Sierra Nevada foothills. Though stunted by nematode attack, the tubers seemed to have resisted the invaders by walling off many of them with heavy brown tissue. Potatoes grown from this strain failed to carry on the resistance. Small tubers of the third generation were harvested from one can (No. n464) in November, 1929, after four months' growth outdoors in Berkeley. The potatoes were kept in the laboratory all winter, and dissected April 17, 1930. Nematodes were found in only 5 of the 28 tubers, but one of these was packed with nematodes in all stages of development. It was a potato only ¾ inch in diameter. Over a thousand females, large and small, were counted with the naked eye. Three-fourths of the tissue of the tuber showed a watery degeneration from the heavy infestation. The other four tubers had small watery spots packed with nematodes.

be less active in infesting a root. Even cultured larvae become weakened if kept too long in their sterile tubes. Their reserve energy[8] is used up in fruitless activity. In the field, larvae are believed to survive 15 months' starvation (Bessey, 1911). Dryness, temperature, and aeration may have much to do with their survival, and there may in reality be only a small proportion of a population which is able to start the new infestation.

NUTRITION AND DEVELOPMENT OF NEMATODES IN ROOT CULTURES

Among the seedlings used as host plants, there occurred wide differences in the vigor of root growth. In general, the development of the nematode corresponded to the condition of the host. This statement may seem obvious, yet it becomes significant as the progressive effects of malnutrition are seen, culminating apparently in an alteration of the sex ratio.

In primary cultures, when only one nematode was used for each plant, 50 per cent of the population completed the life cycle from larva to larva ("per cent hatch," table 2), but when one seedling had to support several parasites, only 10 per cent completed the cycle. Again, in single-nematode cultures, only 12 per cent failed to develop as far as sexual differentiation, while in multiple infestations, 61 per cent remained undifferentiated.

A more extreme case of undernourishment occurred in long-unopened cultures ("secondary galls," table 2). Some of the F_1 larvae in tubes attacked the root of the host plant from which they had hatched. The plants had been weakened by age and artificial conditions to the point where they no longer provided healthy growing tissue for their parasites. These secondary galls turned brown, and the effect of starvation on the nematodes was conspicuous: 79 per cent remained undifferentiated, and only 2 of the 20 females contained ova, which failed to develop.

Cultural conditions were not always unfavorable for the development of nematodes. In culture No. 2391, one female laid 1,998 eggs, counting 1,406 larvae and 592 unhatched eggs, the highest number of eggs reported for a single female.

Development from larva to larva is possible even in some very unfavorable roots, but the number of eggs laid, and the number, size, and

[8] Godfrey, Oliveira, and Gittel (1933) have demonstrated the food material stored by *Heterodera marioni* as layers of fat in the cells of the intestine.

vigor of larvae are subject to nutritional conditions. Galls can be formed in bits of root tips as short as 5 mm. These conditions are far from normal, but the life cycle has been completed in several such cultures.[9]

From time to time a gall has been found in which the nematode was apparently too weak to lay her eggs, or where external pressure left no room for an egg mass. Larvae inside the body of the female appeared healthy. A similar situation occurs in potatoes, where sometimes the last eggs are not laid by an exhausted female. Nagakura (1930) describes such a female as a *"Brutkapsel"*; it may be also the "cyst form" of Jones (1932).

TABLE 2

SEX AND AMOUNT OF DEVELOPMENT OF NEMATODES IN CULTURES

Type of gall	Infestation	Root growth	Sex undifferentiated*	Males	Females: No eggs or no hatch: Insufficient time	Females: No eggs or no hatch: Sufficient time	Females: Eggs hatched	Total number of nematodes	Per cent undifferentiated	Per cent males†	Per cent hatch‡	Per cent infestation by F_1 larvae¶
Primary	Single	Poor	63	6	49	94	95	307	20.5	2.5	37.7	58.5
		Fair	61	2	130	115	158	466	13.1	0.5	47.3	54.4
		Good	39	1	117	158	285	600	6.5	0.2	59.1	53.7
		All roots	163	9	296	367	538	1,373	11.9	0.7	50.4	54.2
	Multiple	Poor	41	4	....	18	7	70	58.6	13.8	10.6	....
		Fair	52	6	....	14	5	77	57.5	24.0	7.0	....
		Good	3	0	....	4	3	10	30.0	0.0	30.0	....
		All roots	96	10	....	36	15	157	61.1	16.4	10.2	....
Secondary	Multiple	Old	174	26	....	20	0	220	79.1	56.5	0.0	....

* These nematodes were allowed sufficient time to complete the life cycle.

† Per cent males is the ratio of males to the total adult population.

‡ Per cent hatch is the percentage of all the nematodes in any series (excluding males, and the females which had insufficient time for development) which completed the life cycle.

¶ Per cent infestation is based on the number of larvae tested. Only a few subcultures were made with F_1 larvae from multiple infestations.

PEDIGREES

The conditions of the method outlined above are such that, if each plate is planted with only one larva, the fact of reproduction without males can be clearly established. In most cases the unmated females in cultures laid viable eggs. When these eggs hatched, subcultures were started using the aseptic F_1 larvae, one to a plate.

9 Five out of 12 cultures gave the complete cycle. Of the other 7 individuals, 1 remained immature, 2 were males, and 4 were females.

TABLE 3

REPRODUCTION BY ISOLATED FEMALES IN ONE FAMILY OF THE ROOT-KNOT NEMATODE

Generation											
First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth
408	[898; 1018,♂; 1026]	[1522; 1529; 1600; 1601; 1603; 1604; 1606; 1617; 1618; 1620; 1653; 1661; 1662; 1664; 1673; 1675; 1678; 1680] [1382; 1383; 1543; 1553; 1557; 1558; 1562; 1568; 1572; 1575; 1578; 1579; 1580; 1586; 1589; 1590; 1622; 1624; 1625; 1626; 1630; 1632,mult. 12 imm. 1♂,1♀; 1633; 1635; 1636; 1637; 1638; 1641; 1683; 1887; 1888]	[2054] [2215] [3456; 3465; 3466] [3391; 3392] [1♂*] [1854; 1857; 1858; 1861; 1864; 2022; 2024] [2104; 2108; 2118] [2658; 2662] [3378; 3380; 3383; 3385; 1♂*] [2671] [2629; 2630; 2631; 2644; 2646] [1♂,1♀*] [2307; 2309; 2311; 2321; 2322; 2324; 2328; 2331; 2339; 2347; 2348; 2353; 2354; 2355; 2358; 2359; 2360; 2370; 2382; 2384; 2391; 2392] [2532; 2543; 2548; 2550; 2556; 2564; 2568; 2570; 2572; 2574; 2577; 2591; 2774; 2778; 2785; 2786] [3422; 3426]	[4♂,2♀*] [2272; 2273] [3439; 3445; 3451] [3567; 3572; 3575; 3579; 3581; 2♂,3♀*] [3538; 3554; 3555] [3772; 3773] [3751; 3768] [3732; 3734; 3747; 3788; 3792; 3793; 3802; 3803; 3815; 3817] [1♂*] [3588; 3591; 3593; 3596] [3601; 3603; 3605; 3606; 3607; 3608; 3610] [4677; 4694; 4695; 4700; 4701; 4705; 4708; 4710] [4154; 4162; 4163; 4166; 4167] [3638; 3647; 3649] [3677; 3689] [3618; 3620; 3623; 3633; 3637] [3849] [12♂,3♀*]	[3703; 3705; 3707; 3720; 3727; 3800] [2♂*] [3♀**; 2♂,1♀*] [5132; 5141; 5146,♂] [5498; 5499; 5505] [4994; 4999; 5000; 5002; 5005] [5019; 5026]	[4784; 4788; 4792; 4793; 4798] [4856; 4868; 4869; 4873; 4874; 4888; 4891; 4894; 4905; 4910; 4940; 4947; 4953; 4961] [6193; 6206; 6220; 6231; 6235] [6274; 6279; 6381; 6387; 6388; 6482; 6484] [5726; 5764; 5766; 5829] [5548; 5556]	[5474] [5699] [5350] [5333] [6759; 6771; 6775; 6959] [7068; 7075] [6802; 7034; 7040; 7352; 7509; 7525; 7533; 7540; 7549; 7578; 7584; 7587; 7591; 7594; 7607; 7610; 7804; 7815; 7849; 7856; 7860] [7179] [7466; 7481; 7488; 7556] [6303; 6327; 6340; 6432; 6439; 6447; 6456; 6463; 6493; 6499; 6517] [6261]	[6002; 6005; 6009; 6117; 6119; 6121; 6124; 6125; 6131; 6288] [6012; 6015; 6017; 6023; 6038; 6046; 6054; 6060; 6072; 6073; 6074; 6076; 6141; 6144; 6161; 6173] [7763; 7764; 7765; 7770] [7669; 7671; 7676; 7867; 7871; 7872; 7879; 7881; 7886; 7895; 7897; 7898; 7901; 7909; 8012; 8016; 8029] [7678] [7637; 7639; 7658] [8046; 8054; 8066; 8067; 8069; 8080; 8083; 8084; 8088; 8089; 8098; 8110; 8111; 8128; 8129; 8132; 8134; 8135; 8137; 8141] [6881] [7144; 7274; 7305; 7315; 7409; 7432; 7443; 7454; 7560; 7564; 7566] [7118; 7120] [7382]	[6832,♂; 9♀**] [6536; 6560] [6731] [6686] [6715] [7723; 7742; 7745; 7753; 7756; 7757; 7987; 7990; 7992] [7695; 7705,♂; 7711; 8210] [8204] [7930; 8143] [8217; 8226; 8227; 8231; 8234; 8247; 8250; 8251; 8252; 8253; 8254; 8256; 8262; 8268]	[7268] [7197; 7374]	[7786; 7798; 7799; 8174] [7939,♂; 7945; 7956; 7961; 7962; 7963; 7970; 7981]

* In secondary galls.
** In primary galls; life histories not completed.

TABLE 4

REPRODUCTION BY ISOLATED FEMALES IN FAMILIES FROM VARIOUS SOURCES

Source	Generation: First	Second	Third	Fourth	Fifth
From commercial potatoes	1261	1688			
	1282	1770, 1775, 1779, 1790, 1791, 1792, 1808			
	1294	1849			
From greenhouse tomato roots	1410	2133, 2256, 2264			
	1498	2171, 2177			
From potato No. n464	2280	4094, 4095, 4102			
		4094	4808, 4810		
		4095	4748, multiple. 29 immature 6 ♂, 4 ♀; 4752, 4754, 4755, 4758, 4763, 4765, 4768, 4769, 4770, 4772, 4774, 4778		
			4752	5798	
			4755	5528	
			4758	5286	
			4774	5801, 5805, 5816, 5844,♂, 5890, 5895, 5917, 5927, 5928, 5930	
				5917	6412

TABLE 5

REPRODUCTION AND INCIDENCE OF MALES IN FIRST-GENERATION CULTURES OF NEMATODES FROM VARIOUS SOIL POPULATIONS

Source of larvae		Infestation	Males	Females: sufficient time		Per cent males
				No eggs or no hatch	Eggs hatched	
Commercial potatoes	California, 1929	Single	3	9	17	10.3
		Multiple	2	6	2	20.0
	Nevada, 1929	Single	0	4	9	0.0
		Multiple	0	11	4	0.0
	California, 1930	Single	0	0	5	0.0
Potato No. n464		Single	0	1	11	0.0
Greenhouse tomato roots		Single	0	3	25	0.0
		Multiple	1	13	3	5.9

One family of the root-knot nematode has been carried through 12 generations by repeated isolations. The pedigree is given in table 3, in which individual nematodes in primary galls are represented by culture numbers. The original parent of the family, No. 408, was a nematode from a commercially grown potato ("California, 1929," in table 5). Each culture number, including No. 408 but excepting those designated as males and one culture designated as multiple, stands for an isolated female with life history complete from larva to larva. Females with incomplete life histories have been omitted from the pedigree, except that in cases where males would otherwise appear to predominate, the number of females with incomplete life histories in primary or in secondary galls is also given, marked with a double or a single asterisk. The occurrence of males in secondary galls is indicated by the number of males, marked with an asterisk.

Additional pedigrees are shown in table 4, one family with 5 complete generations and five with 2 complete generations of isolated females. The 6 nematodes which form the first generation of these 6 families respectively were taken in the egg stage from commercial potatoes ("California, 1929," in table 5), from the home-grown potato, No. n464, and from tomato galls grown in the greenhouse.

Table 5 shows the first generation of nematodes in test-tube cultures started from field or greenhouse populations. It includes the 7 females of the first generation which appear also in tables 3 and 4, making a total of 67 unmated females whose eggs hatched normally, sufficient to show that reproduction without males occurred readily in the first generation of the 5 populations tested. There was a high percentage of males from the first potato population, but because the 5 males were all in poor roots, their occurrence is not inconsistent with the behavior of other material.

OCCURRENCE OF MALES IN CULTURES

In single-nematode cultures (table 2), there were only 9 males to 1,201 females. There were 10 males to 51 females in the multiple primary infestations, and a still higher ratio of males in the secondary galls, which contained 26 males to only 20 females. Some of the males were less than half the normal length.

The difficulty of development in the more heavily infested roots can be seen by comparing the three types of infestation in table 2. Conditions of nutrition were most favorable in single primary infestations, less so in multiple primary infestations, and conspicuously unfavorable in the secondary infestations, which were also multiple. Accordingly, the percentage of larvae which were unable to reach the stage of sexual differentiation increased through the series, while the percentage of females whose eggs were able to hatch decreased from single to multiple primary infestations, and in the secondary infestations there were no eggs laid. The increase in percentage of males developed in the cultures is correlated with the increasingly adverse conditions of nutrition.

There is no indication that this is merely a female-producing strain of the root-knot nematode. The occurrence of males appears to follow the same pattern in the different populations used. Table 2 shows 19 males in primary and 26 in secondary infestations. This is the total number of males developed in all cultures. Tables 3, 4, and 5 give their positions in the families, where no genetic relation can be found. The 6 males in original infestations from potato or tomato material (table 5) occurred in poor roots, and unfavorable cultural conditions also prevailed in the secondary galls, which provided by far the highest percentage of males.

The cultures of the sugar-beet nematode made by Berliner and Busch (1914) also produced males in host roots which apparently made little growth. A male developed in a decaying bit of root, in which a second larva failed to grow at all. The male was perfectly formed but abnormally short, because of undernourishment. Another male is described, but only one female, "almost mature."

Although it was not the purpose of this investigation to deal with genetic problems, it does not seem unreasonable to suggest that conditions of nutrition may be important enough in the early stages of development to change the physiological balance of certain individuals. This point of view has been recognized by Babcock and Clausen (1927), who conclude their chapter on sex determination as follows:

> The thesis that sex under normal conditions is determined at the time of fertilization is supported by abundant cytological and genetic investigations. This theory is not inconsistent with the view that the differentiation of sex during development depends upon a complex series of interactions between factors located in the sex chromosomes and autosomes; nor with the observation that internal secretions of the gonads and other glands may play an important part in the process. Nor is it inconsistent with the view that the sexes may be characterized by differences in metabolic rate, nor that changes in the metabolic rate or alterations in the type of internal secretions circulating in the blood during development may go so far as to reverse completely the sex determined by the original zygotic constitution.

It may be interesting to note that Allen (1932) compares hermaphroditism in animals with the situation in monoecious plants, where "the potentialities for the production of the characters of both sexes must reside in each individual," and the expression of either set of characters may be favored or inhibited by additional factors, either genetic or environmental.

There is a temptation to ask whether fertilization may not be necessary or helpful at the very time the males appear, i.e., when a population has been weakened by malnutrition; but that would be to plead the direct influence of nutrition on sex. Furthermore, it is not known whether these or any of the males are functional, nor whether fertilization may be haphazard. Maupas (1900) considers that the few males which occur in parthenogenetic species are only atavistic, and lack the mating instinct.

Any conclusions must depend, of course, on a study of the germ cells. In *Ascaris* (Edwards, 1910) and in some other nematodes (Gulick, 1911) sex determination is of the XY type. There is no information on the chromosome behavior of *Heterodera,* nor is there cytological evidence on the question of parthenogenesis vs. syngonism.[10]

OCCURRENCE OF MALES IN ROOTS GROWN IN SOIL

Occasional lots of field material have been found with a preponderance of males. This condition was conspicuous in strawberry roots from San Luis Obispo County. Nearly every gall contained 1 or 2 males, and some showed 5 and 6 males to 1 female. These males seemed unusually short and broad. Strawberry roots obtained from the same field a month later yielded fewer males.

The value of a census taken by dissection of galls and egg masses is limited because of the free traveling of the males. However, when the question was raised, counts were made by dissection of various lots of material. They are given in table 6 as a matter of general interest. There is no correlation between the different percentages, and more cases would be needed before conclusions could be drawn.

The lack of males was not observed in seedlings grown in jars of soil for temperature experiments in 1925 and 1927. The roots were dissected before the males had time to wander, and data are thus more complete than for random field collections. But because the small seedlings were heavily attacked from the beginning, neither the plants nor the

[10] Atkinson (1889) found spermatozoa in the oviducts of the root-knot nematode. Nagakura (1930) describes and figures the seminal receptacles, in which he saw many spermatozoa. He states that dead males were frequently found among the eggs in the oötheca. These reports raise more questions for the cytologist to answer. Von Sengbusch (1927) observed copulation twice in experiments with the sugar-beet nematode, and found spermatozoa in most of the females examined. Considering the abundance of males in the sugar-beet nematode (Molz, 1920), it is very possible that normal mating may occur in that species.

nematodes had a fair chance to develop normally. For completeness of count, the tube cultures are of course the most accurate of all, and it is there that the fewest males are found.

In the 1931 temperature experiments, in soil between 12° and 26° C, only 8 males were found, mostly in decaying galls, while there were 992 females, in roots of every degree of vigor. However, in 3 cages at 28° C, there were 74 males to 354 females. The air bath used in this experiment did not favor a healthy growth of the host plants. The soil dried rapidly.

TABLE 6

MALES FOUND IN ROOTS GROWN IN SOIL

Host plant	Source	Males	Females	Per cent males
Strawberry	San Luis Obispo County	48	29	62.3
	San Luis Obispo County, second shipment	27	72	27.3
	Carlsbad, California	1	36	2.7
	Old roots in formalin	1	25	3.8
Potato	From Nevada, 1929	0	41	0.0
	From California, 1930	0	24	0.0
Tomato	Fresh from greenhouse	19*	178	9.6*
	Later generations of strawberry strain from Carlsbad	6	23	20.7
	Later generations of strawberry strain, second shipment from San Luis Obispo County	22	329	6.3
	Temperature experiments (not at extreme temperatures) 1925	15	89	14.4
	1927	70	1,014	6.5
	1931 28° C	74	354	17.3
	1931 Other cages	8	992	0.8
Totals		291	3,206	8.3

* Of the 19 males, adult or developing, noted above from tomato, 10 were found in one large gall. he percentage of males is raised by this one gall.

The roots were too heavily infested with nematodes: 46 of the 74 males were developed in crowded galls. Temperature does not directly influence the sex ratio, for in experiments with 835 single-nematode cultures at all temperatures, including the biological extremes, there were only 5 males—all in poor roots. There were no males at all in the culture-solution experiments between 25° and 35°.

On two other occasions a "nest" of males has been found in one gall, an obvious case of overcrowding. One was a tomato root fresh from the greenhouse. Ten males were found in one large gall. In the 1925 temperature experiments, one gall contained 6 males and 2 young females.

There was crowding in tube cultures also. The 14 secondary galls in culture No. 2785 gave a count of 12 males, 3 immature females, and 9 individuals in earlier stages of development. In culture No. 4748 the primary infestation was too heavy for good growth, and dissection of 6 galls showed 29 individuals in early stages, 6 males, and 4 females without eggs. These two cultures occurred in different families, as can be seen in tables 3 and 4.

Molz (1920) found that in the sugar-beet nematode the development of females was favored by a heavy nitrogen fertilization and by other conditions which promoted a vigorous growth of the host plants, and that a larger proportion of males appeared when the host plants were weakened for any reason. According to his figures, males of that species are usually more numerous than females.

Hornburg (1929) was able to raise the ratio of males of the sugar-beet nematode from 90 to 500 per 100 females, or to reduce it from 300 to 90 per 100 females. His treatment, watering the host plant with infusions of other plants or of seeds, did not affect the amount of infestation.

SUMMARY

A method of obtaining uncontaminated larvae and of raising them in sterile seedlings is described. This method of cultivation lends itself to the detailed study of a variety of problems.

Larvae raised in root cultures were much more active than larvae from the field in entering growing seedlings in vitro. There was also a great variation in this respect among different lots of nematodes grown in soil, related in part but not wholly to the freshness of the larvae.

A healthy condition of the host plant is important for the development of its parasites.

Reproduction without males appears to be regular and normal for the root-knot nematode. One family has been carried through 12 complete generations by repeated isolations, and the same type of reproduction has been demonstrated in other families from various sources.

The occurrence of males is rare: only 0.7 per cent have been found in single-nematode cultures. Males appeared more frequently in old, unhealthy, or heavily parasitized roots. There were 16.4 per cent in multiple primary infestations, while in secondary infestations, also multiple, 56.5 per cent of the nematodes which were able to develop were males, a ratio of 130 males to 100 females. Observations are presented which suggest that in the field also males occur under adverse conditions.

ACKNOWLEDGMENT

The writer appreciates the assistance of Professor W. B. Herms, who has been most helpful in the preparation of the manuscript and in discussion of the fundamental problems involved.

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