SOME RESULTS OBTAINED IN INBREEDING ANGUILLULA ACETI (EHRB)

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Submitted to the Department of ZOOLOGY and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

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RESULTS OBTAINED IN INBREEDING ANGUILLULA ACETI

Since the rediscovery of Mendel's work in 1900, many experiments have been made, to determine whether characters appearing among plants and animals are inherited and, if so, whether they behave as Mendelian dominants or recessives? Whether these characters, if inbred are harmful or beneficial.

The problem of this investigation is first, to determine whether the vinegar eel, Anguillula aceti, a nematode, possesses characteristics which may be inherited according to the Mendelian law. Second, to determine whether inbreeding affects fertility.

This problem has been done at the University of Kansas under the direction of Dr. H. H. Lane. I wish to express my thanks to him, who suggested and directed my work; to Dr. W. R. B. Robertson, (University of Missouri) who so kindly started the work and to Dr. A. A. Schaeffer, Miss Larson and others for suggestions given.

I, also, wish to thank Dr. N. A. Cobb, of the U.S. Department of Agriculture for the determination of the species as Anguillulla aceti.

I, also, wish to thank Hannah Lee Gaw and Mr. I. H. Spencer, of the Lawrence Junior High School for making the charts.

LITERATURE

Since 1900, a great amount of literature has appeared which proves that many characters in plants and animals, are inherited according to the Mendelian law.

But there is no conclusive evidence presented in the literature by which one could forecast.whether inbreeding is a harmful or beneficial factor of this nematode. However, the evidence seems to show that inbreeding may be one way of eliminating undesireable qualities, especially in domesticated animals.

No attempt has been made to review all the literature but only those papers which parallel this problem. No papers have been found that deal with inbreeding in nematodes.

Bos ('94) and Guaita ('98) working with house mice have presented almost conclusive evidence that inbreeding is harmful in the development of animals, especially on the fertility and vitality of the race.

On the other hand, some authors have given evidence that inbreeding is not harmful but tends toward better and stronger breeds of plants and animals.

Gentry ('05) as the result of inbreeding Berkshires has shown that this practice is not necessarily harmful but may prove beneficial in that it conserves

and intensifies the good points in his breed.

Castle ('06) inbred Drosophila ampelophila for fifty-nine generations. He concluded from his experiments that inbreeding does not necessarily result in the reduction of productiveness and vigor.

Moenkhaus ('11) mated brothers and sisters among Drosophila ampelophila for seventy-five generations. In some of his experiments he found a decrease in the number of young, but in the majority of cases his inbred stock paralleled the control cultures. This led Moenkhaus to decide that inbreeding in itself has no harmful effects upon the fertility or vigor of the species.

Wright ('22) working with guinea pigs, found that at first inbreeding tends to lessen the vigor of the race. By crossing with other inbred races, the combination of desired characters produces a race superior to the original stock. In ('23) after working with 35000 guinea pigs, he came to the conclusion that inbreeding has resulted in a decline in average vigor in every respect studied. This includes frequency and size of litter, growth from birth to maturity, mortality at birth and later, and resistance to tuberculosis. Some families, however, have not shown this decline and were as vigorous after more than twenty generations of brother and sister matings as the normal matings.

King ('23), working with albino rats for forty generations, found that inbreeding in the early generations brought to light abnormalities and weaknesses. Following this, she found that the inbred animals were equal to or superior to the original stock in the folowing respects, (1) Body weight: heavier than the stock rats; females inbred 36 to 38 generations, 20 per cent heavier. (2) Fertility: females inbred 1--25 generations bore average litter of 7.39 young compared with average litter of 6.75 young in the stock culture.

Connor and Harper ('24) after inbreeding Blackhull kafir for seven generations, concluded that seeds from plants, selected for many seed branches continued very vigorous and thrifty. Seeds selected from plants for short rachis yielded exceedingly large heads, but seeds selected from plants for a few seeds in the head resulted disastrously.

MATERIALS AND METHODS

HISTORY AND OBSERVATIONS

Anguilla aceti, otherwise known as the vinegar eel, is a round worm or nematode found in vinegar, wines, decaying vegetables and fruits, glues, and pastes.

These nematodes are very small, the females averaging 1.4 mm. in length and the males about 1.2 mm.; both are transparent and no color is visible. The life cycle is rapidly completed, making it easy to obtain a large number of generations in a comparatively short period. These nematodes may be kept breeding the year round at ordinary room temperature.

These nematodes are bisexual and viviparous. They began to copulate two days after birth and often give birth to young five days later. Usually the life of the mating nematode is seven days. The female dies after giving birth to young and the males, a few days later.

Males and females, not mated, frequently live from two to three weeks.

The number of young varies from 3 to 20 and in many cases from 30 to 50 or more. (1) (See tables I, II, III, IV.

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(1) Lindner ('90) sometimes found the number of young to vary from 30 to 40, but usually from 6 to 12.

The material for this experiment was taken from mother of vinegar kept in a tightly covered earthen jar, during the winter, in a furnace heated basement.

The mother of vinegar was removed to a glass fruit jar. Sterilized vinegar and distilled water were added. A day later, the vinegar was alive with nematodes.

Every morning for a period of a week, the nematodes were observed migrating to the top of the jar. They appeared to be feeding on some fungus growth. Later banana, or sugar and yeast, was added to the vinegar and the migration ceased.

If mother of vinegar is present, the nematodes may be seen feeding on some growth. With a sucking motion food is taken into the mouth. Occasionally yeast may be observed within the body cavity. If the food supply is adequate, these nematodes remain in the mother of vinegar, if not, they may be seen swimming freely in the vinegar.

This experiment was worked at room temperature. During the summer, the temperature range was 19° to 32° C, during the winter, 15° to 29°C.

These nematodes were, also exposed to the out-of-door temperature for a period of three weeks. Whenever the temperature dropped to 0°C they died, if however, the temperature remained about 14°C, they continued to give birth to young as long as the food supply was sufficient for their needs. If the food supply decreased the nematodes lived their length of life but no young were produced, nor were there any eggs found in the culture medium(2)

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(2) Linder ('90) Zu ihrem Gedeihen bedürfen sie nämlich hauptsachlich einer Wärme von +16 bis 30°C und darüber Bei dieser Temperature lassen sie sich sowohl im Sommer im Freien wie im Winter in der Stubenwärme leicht züchten und sie vermehren sich hierbei in jeder Jahreszert mehr oder weniger rasch, als viviparae, bald als oviparae, je nach der Qualität ihres Nährboden. Nach der Geburt gehen die Weibchen gewöhmlich bald zu Grunde und auch die Männchen haben anscheinend keine veil langere Lebendauer.

METHODS OF STUDY

In order to study the nematodes, a binocular, with a 10X ocular and a 55mm objective; a microscope with 10X ocular and 16mm. 25NA; 3mm. 85NA were used. The high power objective of the microscope was rarely used because only a portion of the bodies of the nematodes are visible in the field. The low power objective of the microscope gave a view of the entire body at a glance.

The nematodes were placed on a glass slide in distilled water. Two or three drops of 2 per cent chloretone were added for the purpose of narcotizing them so that they could be studied.

Two kinds of culture glasses were used in rearing the young. The first type was made by fastening glass rings (5mm X 1cm) to glass slides (.15mm concavity) with vaseline. These slides could be placed beneathe the binocular and microscope without disturbing the cultures.

The second type consisted of small test tubes (7cm X 1cm). As the nematodes could not be observed easily in these tubes, the culture was poured into a Syracuse watch glass. This was then placed beneath the binocular or compound microscope for study.

METHODS FOR PREPARING CULTURES

Various methods were used in rearing nematodes. The first culture glasses were made by fastening glass rings to the glass slides with vaseline to prevent the escape of the liquid. Equal amounts of sterlized vinegar and distilled water were used. The nematodes did not thrive because of the lack of the proper food supply and died, subsequently:

(1) A few grains of sugar and Fleischmann's yeast, the size of a pin head were placed in several cultures. The next day a thin transparent mother of vinegar had formed and the nematodes were feeding.

(2) In a few culture glasses, bits of crushed banana were added to the vinegar. This method was not successful because care had not been exercised in selecting the banana, mold covered the mother of vinegar, making study impossible.

(3) Sterilized banana with a very small amount of Fleischmann's yeast was substituted. The same results were obtained as in No. 1.

(4) Later ripe crushed bananas were, again, tried with satisfactory results.

This time care was exercised in selecting the bananas. Only those with unbroken skins

were used and the pulp taken from the inner portion, thus avoiding the mold contamination.

(5) Another medium used was made by mixing flour with distilled water and adding sugar and yeast. This was discarded since the liquid was not transparent enough.

To prevent the contamination of the culture media, the slides were placed in glass containers, (Dia. 21cm. X Height 7cm.) and protected by tightly-fitting glass covers. Each dish held 10 culture slides. Evaporation, however, went on quite rapidly and many of these nematodes were lost from drying or too great an acid concentration.

Sterilized cotton, moistened with distilled water, was then put in the bottom of the containers. This lessened the amount of evaporation but increased the mold contamination thus increasing the death rate.

Finally, the cotton was discarded but water was added in small quantities.

This method of keeping the culture slides was abandoned December 1923 for the test tubes. (Dia. 1cm X Height 7cm).

The test tube for holding the culture medium, proved very successful. It eliminated the mold contamination and also lessened the evaporation of the

vinegar. The test tubes took less space, therefore, more individuals could be reared. Less time was taken to fix the test tube cultures.

METHODS FOR ISOLATING MATERIALS

All tools and glassware were sterilized before using. The glassware was placed in boiling water and left over the fire for five to twenty minutes. The needles and metal tools were sterilized in a gas flame.

To commence the experiment, a number of fertilized females were isolated. A Barber's pipette for isolating bacteria, can be used successfully as long as the nematodes are swimming freely in the liquid. If the nematodes were lying in the mother of vinegar, the latter was teased apart with dissecting needles.

The dissecting needles are made by using a holder fitted with ordinary steel sewing needles, Nos 8 to 12.

To isolate the nematodes, the mother of vinegar was teased apart until small bits holding one or two specimens were available. These were transferred to a glass slide and covered with distilled water. After all the nematodes had been studied beneath the binocular and the low power objective of the microscope, they were transferred to the culture glasses, individually or in pairs.

In teasing apart the mother of vinegar many nematodes escaped freely into the vinegar. These were picked up with a glass pipette which had been drawn to a fine point.

METHOD USED TO LABEL AND MARK CULTURES

Small labels were used, with the date, generation and parentage written upon each. These were pasted on the sides of the test tubes or on the slides. This method did not prove very successful when used with the slides as the vaseline often melted, making the labels translucent; or, when the moisture content of the containers became too great, the labels were loosened.

If mold attacked the cultures, the labels were always destroyed.

When the test tubes were substituted for the cultures slides, this method of labeling proved entirely satisfactory.

PROCEDURE AND RESULTS

Nine fertilized females were placed in cultures and marked with the letters A, B, C, etc. Two days later, each female gave birth to a litter of young varying in number from nine to twenty-eight individuals each.

These young from female A and female B were isolated and marked A_1, A_2 etc and B_1, B_2 etc. The offspring from the other females were placed into the Syracuse watch glasses, food was added and they were permitted to mate at random. The brothers and sisters in the A_1 generation and B_1 generation were mated. In the A_2 generation there appeared a female differing from the rest in at least one character. She had a constriction located at a point about 1/3 her length from the anterior end of her body. She was mated with one of her brothers and gave birth to three abnormal offspring (one female and two males)(1). Her body cavity contained a number of embryos not developed when she died. These abnormal individuals did not mate. A normal male and female from generation A_2 gave normal offspring.

The B_2 generation left to mate at random produced no abnormal offspring, according to the data obtained by examining the culture from time to time.

(1) See chart I

The results of mating the A line for eight generations are given in the following table along with other matings made and observed at the same time.

DATA FROM JUNE 20 TO AUGUST 13, 1923

A. Total no. of abnormals studied----------48

Abnormal males---------- 6

Abnormal females----------42

Abnormal ♀ mated with normal ♂----------21

Abnormal ♀ mated with abnormal ♂---------- 2

Abnormal ♀ , dried out---------- 6

Abnormals undetermined---------- 5

Abnormal ♀ mated with normal ♂ producing young--- 6

Abnormal ♂ mated with normal ♀ producing young--- 0

B. Total normal matings studied----------12

Normal matings producing all normals---------- 4

Normal matings producing 36 A and 153 N---------- 8

C. Abnormal ♀ mated with normal ♂ ----------21

Matings giving all normals---------- 1

Matings producing 15 A and 30 N*---------- 2

Matings producing all abnormal (3)---------- 1

Matings producing no young----------17

D. Abnormal ♂ and abnormal ♀---------- 0

Abnormal females produced from 3 to 22 offspring each.

Normal females produced from 4 to 45 pffspring each.

Note* One mating is not in table I as it was not in A line.

Owing to pressure of other work during a period of three weeks, very little attention could be given to this investigation except to make occasional observations. Two weeks later some nematodes were seen in the sterilized stock vinegar. Therefore, the data secured at this time are not used in this paper.

Again, fertilized females were isolated from the original stock and marked WA, WB, WC and WD. WB and WC offspring were left to mate at random.

The WA line produced normal offspring until the WA_3 generations in which there was one abnormal male. Mating two normal WA_3 offspring, gave a normal WA_4 generation.

The WD line remained normal. There were only a few offspring to each mating probably due to the fact that the food supply was inadequate.

A normal male from the WA generation and a normal female from WA generation mated and the number of offspring produced was greater than in the control. Five of the males were abnormal.(1) This part of the experiment was carried until December 22, 1923 when all cultures were lost by accident.

The summary of results, from October 27, 1923 to December 22, 1923, is given in the following table.

(1) See chart II.

A. Total Abnormals studied----------22

Abnormal males, not mated---------- 2

Abnormal males mated---------- 6

Abnormal females not mated---------- 4

Abnormal females mated---------- 3

Abnormal undetermined---------- 7

Total No. of abnormal matings---------- 8

Total No. of normal matings----------10

No. normal matings producing normal offspring----- 8

No. normal matings producing 11 A and 33 N-------- 2

Results of Abnormal matings

Abnormal ♂ and abnormal ♀ ---------- 1

Abnormal ♂ and normal ♀ ---------- 5

Matings producing no results---------- 4

Matings producing 8 A and 19 N---------- 1

Abnormal ♀ and normal ♂---------- 2

Mating producing no results---------- 1

Mating producing 2 A and 17 N---------- 1

December 31, 1923

After the accident December 22, it was, again, necessary to make a new start. This time two unfertilized females and two males were taken from the original culture. These were marked WA and WB as before. WA produced all normal offspring and WB gave two normal males and one abnormal female.

The offspring from female WA were mated and those from WB_2 were left to mate at random. Examination from time to time showed that culture WB continued to give abnormal offspring although not in great numbers. The results of the WA line may be seen on Chart III and table III.

The small test tubes were used for the first time. The food consisted of vinegar and bananas or vinegar with sugar and yeast.

The results obtained from inbreeding WA young for nineteen generations are given in the following table.

A. Abnormal matings----------22

(1) Abnormal ♂ and normal ♀ ---------- 4

1 gave no result

1 gave 42 normals

2 gave 37 normals and 5 abnormals

(2) Abnormal ♂ and abnormal ♀ ---------- 3

1 gave 7 normal offspring

2 gave no results

(3) Normal ♂ and abnormal ♀----------15

8 gave no results

7 gave young

3 gave 3 A and 23 N

4 undetermined

B. Normal matings----------77

21 gave no results

7 gave 191 normals

14 gave 212 normals and 39 abnormals

16 no data (due to time)

19 gave normals and abnormals undetermined

C Abnormals not mated----------31

26 Abnormal females

3 Abnormal males

2 undetermined

D Total Abnormals determined----------57

Total Normal 178♂ + 348 ♀ + 125 Undetermined-------750

A ratio of 13½:1

During the summer of 1924, this investigation was practically in abeyance except for daily inspection as to food supply. August 13, 1924 new material was obtained and the experiments which had been carried on during the summer of 1923, and the fall and the winter of 1923--'24 were repeated. The results obtained during this period parallel those of the other period with one exception. The exception is that productivity seemed greater among the normal matings than among the abnormal.

For the beginning of this experiment, five fertilized females were isolated and marked NA, NB, NC, ND etc. All females produced young except one. Only the ND line was used for this experiment during sixteen generations. The summary of the results obtained from August 13, 1924 to February 7, 1925 is given in the following table.

A. Abnormal matings----------23

(1) Abnormal ♂ and abnormal ♀---------- 2

1 mating gave no results

1 gave 11 normals and 2 abnormals

(2) Normal ♂ and abnormal females----------21

6 gave 95 normals and 19 abnormals

1 gave 9 normals

2 undetermined

8 gave no results

4 no data as to normals and abnormals

(3) Abnormal ♂ and normal ♀---------- 0

B. Normal matings----------72

9 gave no results

7 gave all normals

15 gave 227 normals and 51 abnormals

12 no data (lack of time)

9 undetermined

20 gave normals and abnormals not counted

C. Abnormals not used in matings

4 abnormal males

37 abnormal females

8 undetermined

D. Total number abnormals determined----------84

Total number normals determined----------527

A. Total normal matings----------171

Matings giving all normals---------- 26

Matings giving both N and A---------- 59

Matings giving no results---------- 30

Matings young undetermined---------- 28

Matings, no data---------- 28

B. Total Abnormal matings---------- 74

(1) Abnormal ♂ and abnormal ♀---------- 6

1 gave 7 normals

1 gave 11 normals and 2 abnormals

4 gave no results

(2) Abnormal ♂ and normal ♀---------- 9

1 gave 42 normals

3 gave 56 normals and 13 abnormals

5 gave no results

(3) Normal ♂ and abnormal ♀---------- 59

3 gave all normals

16 gave 160 normals and 35 abnormals

34 gave no results

1 gave 3 abnormals

5 not determined

C. Total Individuals worked with----------1536 N + 209=1735

Group I 213 N + 47 A=260

Group II 46 N + 21 A= 67

Group III 750 N + 57 A=807

Group IV 527 N + 84 A=611

DISCUSSION

Anguillula aceti has very few characteristics which may be used in testing for Mendelian inheritance. No color is visible, therefore, body length seemed about the only available character. The size of the spicule in the male, also, offers a possible problem.

After inbreeding a number of these nematodes a constriction, or pinching in of the body appeared and this forms the basis of a part of this experiment.

According to the Mendelian expectation, if the abnormal is dominant, there are three classesto be considered.

(1) If neither parents are abnormal, theoretically there are four possibilities:

(a) Where both parents are homozygous for normal, all the offspring should be normal.

(b) Where one parent is homozygous for normal and the other heterozygous, the offspring should all appear normal; however one-half would be homozygous for normal and one-half heterozygous.

(c) Where both parents are heterozygous the expectation is a 1:2:1 ratio, or one-fourth homozygous for normal, one-half heterozygous and one-fourth homozygous for the abnormal character.

(d) Where both parents are homozygous for the recessive all the offspring should be abnormal.

(2) If only one parent is abnormal, two possibilities are presented;

(a) either one-half the offspring are normal and one-half abnormal, or,

(b) all the offspring are normal, in which case they should be heterzygous.

(3) If both parents are abnormal, all the offspring should be abnormal.

According to the data shown in Tables I, II, III and IV the individual offspring from matings made do not fall within the Mendelian expectation because in one case of abnormal matings all the offspring were normal. Chart III, V WA_1 and V WA_2; another mating gave two abnormals to eleven normals as shown in Chart IV, VIII WD_9 and VIII WD_{10}.

Four matings of abnormal males and normal females gave the following results one, (Chart III, X WA_1 and X WA_2) gave all normal offspring, which is contrary to the expectation. VII WA_1 and VII WA_9, chart III gave both normals and abnormal offspring but not according to the Mendelian expectation.

The normal male and abnormal female matings gave varied results; five matings gave all normals; sixteen gave both normals and abnormals; one gave all abnormals, but this mother died before giving birth to all the embryos within the body cavity.

ABNORMAL CONDITION

The abnormal condition is a rather peculiar one. It is a constriction or a pinching in of a portion of the body. This constriction may vary. It may lie in the anterior part of the body, in the posterior part of the body or quite frequently, it is found near the middle of the body as shown in plates II.

Occasionally two or three constrictions may be seen.

The cause of these constrictions is not known but it is quite evident that there are several factors responsible for this condition.

Whenever the constriction occurs midway between the anterior and the posterior portions of the body, each portion of the body turns in the same direction so as to resemble a pair of horns. These horns move in unison. The muscles of the body do not coördinate.

In the normal matings, copulation takes place by the male wrapping the posterior portion of the body containing the glands about the body of the female.

If the abnormal condition appears in the posterior part of the body, the anterior part moves about as in the normal nematode but over a limited area. The posterior portion did not move in accord with the anterior portion, which shows that the muscles of the two portions of body do not coördinate. If this con-

dition appears in the female mating and parturition are normal. If it appears in the male, mating is impossible as only a slight movement of the tail can be observed.

If the abnormal condition appears in the anterior portion of the body, the nematodes feed over a very limited space. Mating and giving birth to the young appear normal although the females do not move very far. A male with this abnormality may copulate with females if they are lying within the area of the body movements.

Another type of this abnormal condition is the appearance of either two or three constrictions in the middle portions of the body. Once in a while such females will mate with normal males. Invariably these females die before giving birth to young, although the embryos develop within the body cavity.

If the mother dies and one or two of the embryos are fully developed before death, they force their way out of her body cavity, mutilating the body.

The constriction appears to be due to muscular tetanus, but some histological work is necessary to prove the statement. (Further study is planned along this line.)

There is such a varied range in the ratios of abnormals to normals, as shown in Tables I, II, III, and IV, that one is convinced that some factors such as temperature change, food supply, oxygen, acetic

concentration or the lack of CO_2 have had some influence in bringing about this abnormal condition. A few factors will be discussed.

TEMPERATURE CHANGE

The temperature range during the summer months read 20.5° to 32°C. The greatest temperature change, between any two days during the summer months, was 11.5°C between June 27 and 28, 1923. The greatest drop in temperature during the winter was 9°C between December 22 and 23, 1924. The rises and falls in temperature both for summer and winter averaged from two to four degrees.

The first abnormal nematode appeared two days after the drop in temperature of 11.5°C. The embryos were quite well developed by the time, the drop occured. Therefore, there is a question as to whether the temperature change caused the abnormality as there were no abnormals in the control cultures. In the IV A_1 generation, there were five abnormals. In the fifth generation, there was a temperature change of 2°C. The data of three matings, in the fifth generation, show that one had all normal offspring, one had four abnormal offspring and the other had six, as shown in Chart I. The temperature, frequently, dropped four degrees and occasionally six to seven degrees during the winter months, but there was no evidence that this temperature change brought about an increase in the number of abnormal nematodes.

FOOD FACTOR

The question arises as to the amount of food present in the cultures. During the inbreeding of the groups I and II the food supply was often inadequate for the needs of the nematodes as the problem of feeding had not been solved. The ratios of normals to abnormals are 4.5: for group I and 2.1:1 for group II. During the period of inbreeding from December 31, 1923 to May 1924, the food supply was adequate and the ratio of normals to abnormals is 13.1:1 ; for the period from August 1924 to February 1925, the ratio was 6.2:1.

The original culture control was examined from time to time, but no abnormal nematodes were seen at any time. Sometimes the food supply was limited and at other times abundant.

Cultures taken from those that were inbred were left, in Syracuse watch glasses on the table. From time to time these were examined. These nematodes always contained abnormal ones. Some of these control cultures had abundant food and the others not so much.

Whether the abundant food supply produced the decrease in the number of abnormals forms cannot be determined from the evidence in hand, though it would seem that the food supply is a negligible factor.

THE OXYGEN FACTOR

The air could always pass through the containers holding the culture plates because the liquid in the cultures evaporated quickly. During the period from June 1923 to December 1923, forty-one cultures were lost on account of their drying up. The cultures that did not dry had nematodes producing abnormals and others producing all normals under the same conditions. As long as evaporation goes on within a culture, air surely circulates thus supplying abundant oxygen.

ACETIC CONCENTRATION

That the acetic concentration apparently has little influence on the nematodes unless the vinegar becomes too acid (and then death) is evident from the fact that at various times the vinegar kept in ordinary round stender dishes had evaporated until the volume was one-half of the original volume. Upon examination no abnormal nematodes were found. In a number of the cultures the vinegar would evaporate until the acetic acid crystals had formed, yet usually no abnormal nematodes were found unless somewhere within the family the abnormal character had already appeared.

CO_2 FACTOR

Carbon dioxide is formed when fermentation takes place within the cultures. Since evaporation goes on freely, there is evidence that the air is circulating and thus carrying away some of the CO_2.

The Syracuse watch glasses, placed one on top of the other showed no difference as to the number of abnormals and normals. These nematodes are the ones spoken of previously as mating at random. These were sometimes liquid sealed and this prevented the free circulation of air. October 24, 1924 a line which appeared to be pure as no abnormal nematodes had appeared two generations was marked for a pure line. It was watched carefully for two generations. Then suddenly in the fifth generation there appeared two abnormal females. These cultures had been treated as those mentioned above. The CO_2 was no greater, the other factors equal. The question remains. Is it a mutation?

There is no doubt that CO_2 could mix freely with the air except in the containers which were liquid sealed and since the number of abnormal did not increase, the CO_2 factor surely can not be responsible for the abnormal condition.

EFFECT OF INBREEDING ON STERILITY

Out of 171 normal matings 30 or 17.5% gave no results whatsoever and 141 or 82.5 % produced young.

Out of 74 abnormal matings 43 or 58.1 per cent gave no results and 31 or 41.9 per cent produced young ranging from 3 to 42 in number, the average, however, was 16.6 each. The abnormal matings were made under the same conditions as the normal and kept in the same containers, no attempt being made to rear the abnormals separately.

The young apparently were strong physically for in cases where no matings were made the nematodes lived three to four weeks although the average length of life for both normals and abnormals, not mated, is two weeks.

The abnormal young lived the same length of time provided the food supply was sufficient, as they could not feed over a large area due to the impossibility of moving about.

EFFECT OF INBREEDING ON PRODUCTIVITY

The claim has been made by some investigators that inbreeding has a tendency to produce a harmful effect while others claim to have conclusive proof that it does not. Out of the ninety-one matings shown in the Tables I, II, III, and IV, thirty-one matings,

whether normal or abnormal, produced from twenty to fifty young and fifty-six matings produced from three to nineteen young.

Many of the first nematodes were probably injured when transferred from one medium to another, for many of the females died before giving birth to more than three or four offspring.

Another possible reason for so few young, is that when the food supply was inadequate, the females did not produce as many young as otherwise.

SUMMARY

1. Anguillula aceti does show characters which may be studied i, e. an abnormal condition.

2. The abnormal condition is apparently not inherited according to the Mendelian law of inheritance, though the data and results are not offered as a final analysis of the work.

3. The data and results indicate that several factors are apparently responsible for the abnormal condition.

4. The abnormal condition, as far as determined is due to muscular tetanus.

5. Inbreeding per se does not affect sterility to a great extent among the normal nematodes as 82.5 per cent produce young.

6. Sterility among the abnormal nematodes is largely due to the inability to copulate and the inability on the part of the female to give birth to young. Only 41.9 percent produce young.

7. Inbreeding does not affect productivity among these nematodes, if the line is vigorous to begin with.

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TABLE I

		Undetermined		Males		Females		Total	
	Parentage	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Offspring	Ratio
F_1	Normal Mother	3	0	2	0	5	0	10	
F_2	Normal ♂ + Normal ♀	5	0	8	0	10	1	24	23:1
F_3	Normal ♂ + Normal ♀	0	0	4	0	4	0	8	
F_3	Normal ♂ + Abnormal ♀	0	0	0	1	0	2	3	
F_4	Normal ♂ + Normal ♀	5	0	2	1	3	4	15	2:1
F_5	Normal ♂ + Normal ♀	1	0	5	0	15	0	21	
F_5	Normal ♂ + Abnormal ♀	6	1	3	0	7	5	22	2+:1
F_5	Normal ♂ + Normal ♀	1	1	10	1	13	2	28	6:1
F_6	Normal ♂ + Normal ♀	1	3	1	0	2	0	7	1:1
F_7	Normal ♂ + Normal ♀	0	0	2	0	2	0	4	
F_8	Normal ♂ + Normal ♀	10	0	7	2	15	7	41	3+:1
F_8	Normal ♂ + Normal ♀	13	1	11	0	14	6	45	5+:1
Aug 27 1923									
	Normal ♂ + Normal ♀								
	from VIII 2 + 4	3	0	5	0	4	1	13	12:1
	Normal ♂ + Normal ♀	1	0	1	0	1	2	5	1:1
	Normal ♂ + Normal ♀	6	1	0	0	1	2	10	2:1
	Normal ♂ + Abnormal ♀	2	0	8	2	4	4	20	3:1
	F_1, F_2, etc has reference to the offspring								

TABLE II

		Undetermined		Males		Females		Total	
	Parentage	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Offspring	Ratio
F_1	WA, Normal Female	0	0	1	0	1	0	2	
F_2	Normal ♂ + Normal ♀	1	0	1	0	2	0	4	
F_3	Normal ♂ + Normal ♀	0	0	0	0	3	1	4	3:1
F_3	Normal ♂ + Normal ♀	0	0	2	0	0	0	2	
F_4	Normal ♂ + Normal ♀	0	0	2	0	2	0	4	
F_1	WD Normal Female	3	0	3	0	4	0	10	
F_2	Normal ♂ + Normal ♀	0	0	4	0	3	0	7	
	Normal ♂ + Normal ♀	3	0	2	0	3	0	8	
	Normal ♂ + Normal ♀	0	0	4	0	3	0	7	
	IV WA, N ♀ + III WD, ♂	1	3	1	5	11	0	21	1+:1
	Abnormal ♂ + normal ♀	0	1	9	3	11	4	27	2+:1
	Normal ♂ + Abnormal ♀	13	1	3	0	1	1	19	8+:1
	Normal ♂ + Normal ♀	5	1	6	0	5	1	18	8:1

TABLE III

	Parentage	Undetermined		Males		Females		Total	
		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Offspring	Ratio
F_1	Normal ♂ × Normal ♀	0	0	3	0	8	0	11	
F_2	IWA 1 N ♂ × IWA 2 , N ♀	3	0	4	0	3	2	12	5:1
F_2	IWA 7 N ♂ × IWA 8 , N ♀	0	0	9	0	11	2	22	10:1
F_3	II WA 3, N ♂ × II WA 15 A ♀	0	0	4	0	3	1	8	7:1
F_3	II WA 11, N ♂ × II WA 12, N ♀	1	0	3	0	3	0	7	
F_3	II WA 5 N ♂ × II WA 4 N ♀	0	0	6	0	5	2	13	5+:1
F_4	III WA 1 N ♂ × III WA 5 A ♀	1	0	4	0	3	1	9	8:1
F_4	III WA 2 N ♂ × III WA 3, N ♀	0	0	9	1	15	4	29	5:1
F_4	III WA 4 N ♂ × III WA 5 N ♀	0	0	1	0	3	0	4	
F_4	III WA 11 N ♂ × III WA 12 N ♀	3	0	6	0	5	0	14	
F_4	III WA 7 N ♂ × III WA 8 N ♀	2	0	3	0	3	0	8	
F_5	IV WA 7 N ♂ × IV WA 5 N ♀	0	0	1	1	0	4	6	
F_5	IV WA 7 N ♂ × IV WA 4 N ♀	7	0	2	0	3	0	12	
F_6	V WA 1 N ♂ × V WA 2 N ♀	1	0	1	0	3	0	5	
F_6	V WA 4 N ♂ × V WA 5 N ♀	4	0	3	0	10	2	19	8+:1
F_6	V WA 1 A ♂ × V WA 2 A ♀	3	0	1	0	3	0	7	
F_7	VI WA 2 N ♂ × VI WA 7 N ♀	4	0	2	0	2	0	8	
F_7	VI WA 10 N ♂ × VI WA 5 N ♀	0	0	2	0	5	0	7	
F_7	VI WA 14 N ♂ × VI WA 2 N ♀	1	0	1	2	1	6	11	1:2+
F_7	VI WA 1 N ♂ × VI WA 2 N ♀	1	0	0	1	1	0	3	2:1

TABLE III con't.

		Undetermined		Males		Females		Total	
	Parentage	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Offspring	Ratio
F_8	VII WA 3 N ♂ × VII WA 4 N ♀	0	0	1	0	2	0	3	
F_8	VII WA 1 N ♂ × VII WA 4 N ♀	9	0	1	0	4	0	14	
F_8	VII WA 9 A ♂ × VII WA 1 N ♀	0	1	5	0	8	2	16	4+:1
F_9	VIII WA 2 N ♂ × VIII WA 3 N ♀	12	0	3	0	5	0	20	
F_9	VIII WA 1 N ♂ × VIII WA 2 N ♀	2	0	2	0	5	0	9	
F_{10}	IX WA 2 N ♂ × IX WA 19 N ♀	0	4	1	0	5	0	10	1+:1
F_{10}	IX WA 20 N ♂ × IX WA 19 N ♀	4	0	12	0	8	1	25	24:1
F_{10}	IX WA 2 N ♂ × IX WA 3 N ♀	6	0	7	0	9	2	24	7+:1
F_{10}	IX WA 4 N ♂ × IX WA 5 N ♀	4	0	2	0	2	0	8	
F_{11}	X WA 2 A ♂ × IX WA 3 N ♀	10	0	11	0	21	0	42	
F_{12}	XI WA 20 N ♂ × XI WA 34 N ♀	1	0	7	0	14	0	22	
F_{12}	XI WA 1 N ♂ × XI WA 2 N ♀	1	0	3	0	1	2	7	2+:1
F_{13}	XII WA 18 N ♂ × XII WA 19 N ♀	3	0	3	0	4	0	10	
F_{13}	XII WA 20 N ♂ × XII WA 15 N ♀	5	0	8	1	8	3	25	5+:1
F_{14}	XIII WA 7 N ♂ × XIII WA 8 N ♀	10	0	8	0	16	0	34	
F_{15}	XIV WA 17 N ♂ × XIII 24 N ♀	4	0	1	0	5	3	13	3+:1
F_{15}	XIV WA 5 N ♂ × XIV WA 18 N ♀	4	0	14	0	12	2	32	15:1
F_{16}	XV WA 1 N ♂ × XV WA 2 N ♀	2	0	4	1	8	2	17	4+:1
F_{17}	XVI WA 13 A ♂ × XVI WA 14 N ♀	11	0	6	1	7	1	26	12:1
F_{18}	XVII WA 7 N ♂ × XVII WA 8 N ♀	6	0	4	0	13	1	24	23:1

TABLE IV

	Parentage	Undetermined		Males		Females		Total	
		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Offspring	Ratio
F1	ND1 N♂ + ND2 N ♀ parents	1	0	6	0	13	2	22	9+:1
F2	I ND10 N♂ + I ND9 ♀	0	0	6	1	9	1	17	7+:1
F3	II ND7 N♂ + II ND8 N ♀	5	2	10	0	7	2	26	5+:1
F4	III ND2 N♂ + III ND1 A ♀	0	0	5	0	4	0	9	
F5	IV ND6 N♂ + IV ND1 N ♀	10	0	11	0	17	2	40	19:1
F6	V ND1 N♂ + V ND40 N ♀	3	0	13	1	15	6	38	4+:1
F7	VI ND21 N♂ + VI ND20 N ♀	4	2	3	0	6	1	16	4+:1
F7	VI ND5 N♂ + VI ND6 N ♀	3	0	6	0	13	11	33	2:1
F8	VII ND13 N♂ + VII ND14 N ♀	1	0	6	1	4	6	18	1+:1
F8	VII ND25 N♂ + VII ND26 N ♀	0	0	2	0	4	1	7	6:1
F8	VII ND17 N♂ + VII ND18 A ♀	0	0	18	1	16	7	42	4:1
F9	VIII ND10 A♂ + VIII ND9 A ♀	5	0	3	2	3	0	13	5:1
F9	VIII ND11 N♂ + VIII ND12 N ♀	0	0	9	0	10	6	25	3+:1

TABLE IV cont.

		Undetermined		Males		Females		Total	
	Parentage	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		Ratio
F_9	VIII ND34 N♂ × VIII ND32 A♀	3	0	3	0	1	1	8	7:1
F_{10}	IX ND8 N♂ × IX ND9 A♀	1	0	4	1	2	3	11	1+:1
F_{10}	IX ND11 N♂ × IX ND12 N♀	0	0	8	0	6	3	17	4+:1
F_{10}	IX ND6 N♂ × IX ND7 A♀	4	1	12	0	11	1	29	13+:1
F_{10}	IX ND9 N♂ × IX ND10 N♀	3	0	14	0	25	0	42	
F_{11}	X ND13 N♂ × X ND42 N♀	10	0	8	0	10	1	29	28:1
F_{12}	XI ND6 N♂ × XI ND7 A♀	2	1	13	0	12	0	28	27:1
F_{12}	XI ND1 N♂ × XI ND2 N♀	4	0	2	0	4	0	10	
F_{13}	XII ND3 N♂ × XII ND4 N♀	0	0	5	0	2	1	8	7:1
F_{13}	XII ND5 N♂ × XII ND9 N♀	0	0	1	0	3	0	4	
F_{14}	XIII ND2 N♂ × XIII ND1 N♀	0	0	8	0	10	2	20	9:1
F_{10}	XIV ND34 N♂ × XIV 7 N♀	2	0	16	0	23	8	49	5:1
F_{16}	XV ND33 N♂ × XV ND27 N♀	8	2	5	0	2	5	22	2:1

EXPLANATION FOR CHART I

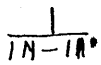
IA , IIA etc. represent the generation, the individual. The Roman numerals designate the generation, the letter the line worked with and the Arabic numeral the individual.

○ , the normal female

● , the abnormal female

□ , the normal male

■ , the abnormal male

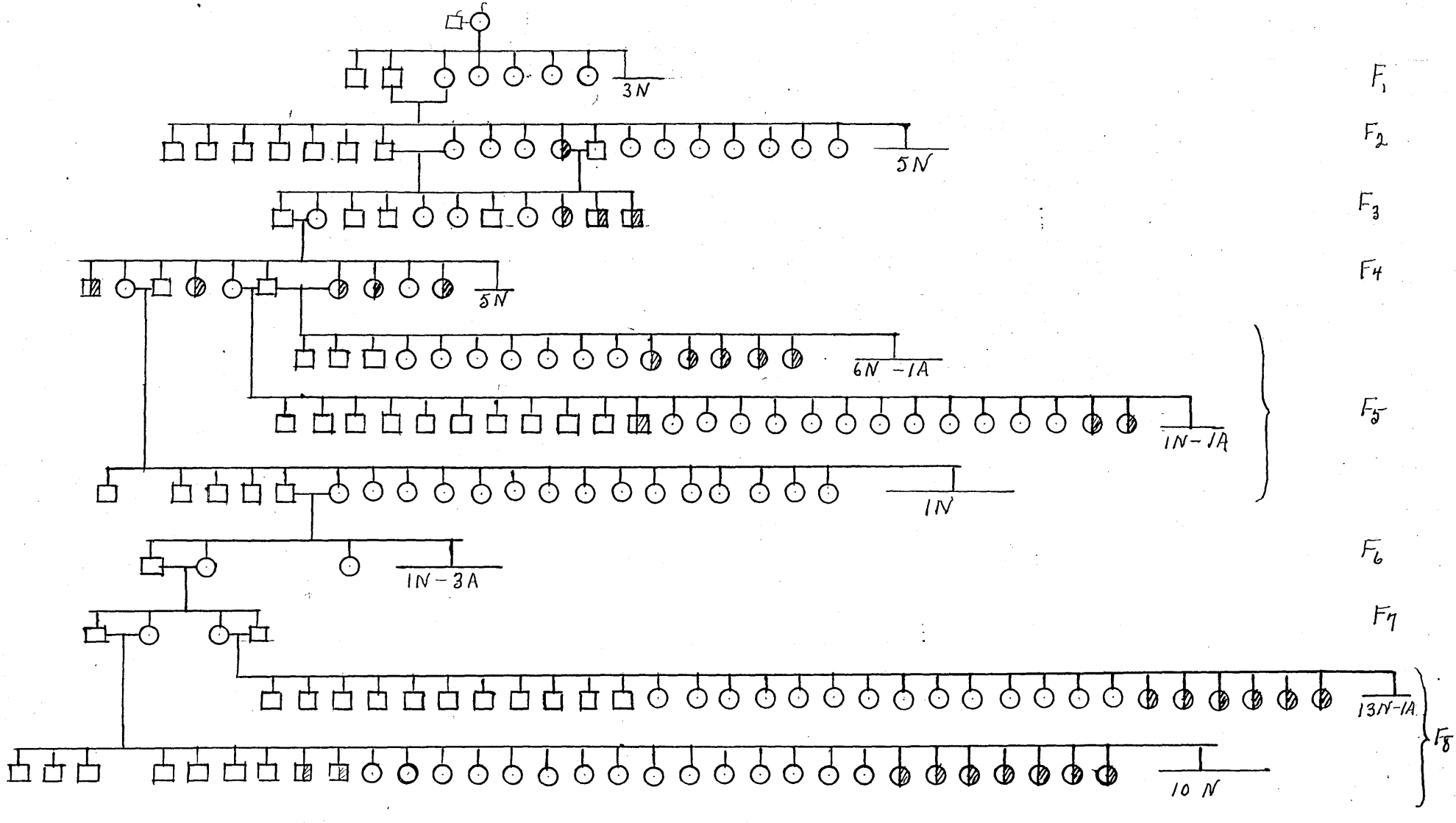


1N represents, one normal nematode, sex not not determined and 1A, the abnormal nematode not determined as to sex.

N, Normal

A, Abnormal

CHART I



EXPLANATION FOR CHART II

WA, the one line of nematodes used.

WD, the other line of nematodes

After mating the WD and WA line, the offspring were designated as the WA line.

 , the normal female

 , the abnormal female

 , the normal male

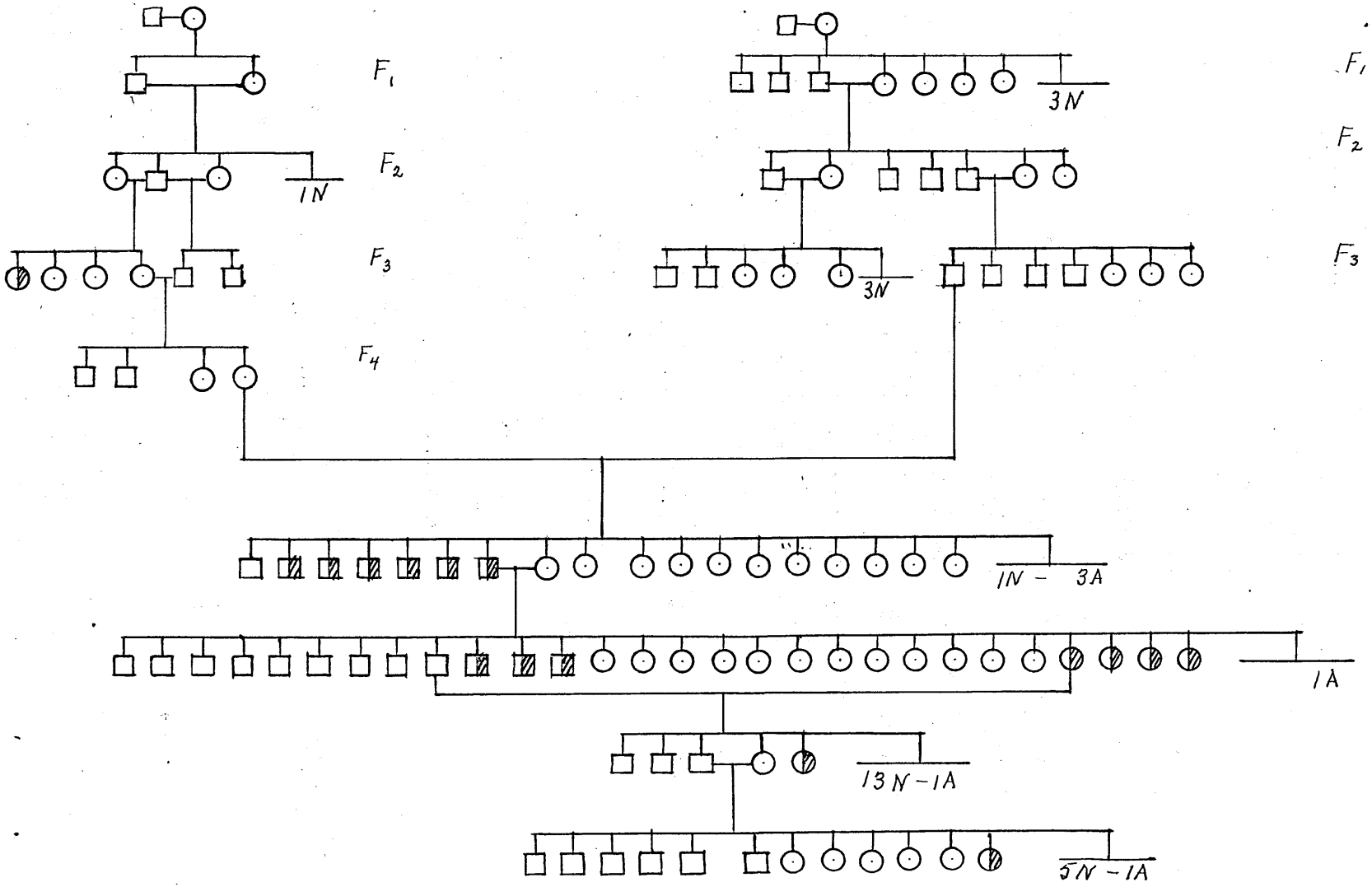
 , the abnormal male

N, Normal

A, Abnormal

$\frac{1}{N-A}$, Normal and abnormal undetermined

CHART II



EXPLANATION FOR CHART III

The black symbols used represent the abnormal nematodes.

The notation above the line refer to the nematodes mated. As IWA_{1} represents the male and IWA_{10} the female.

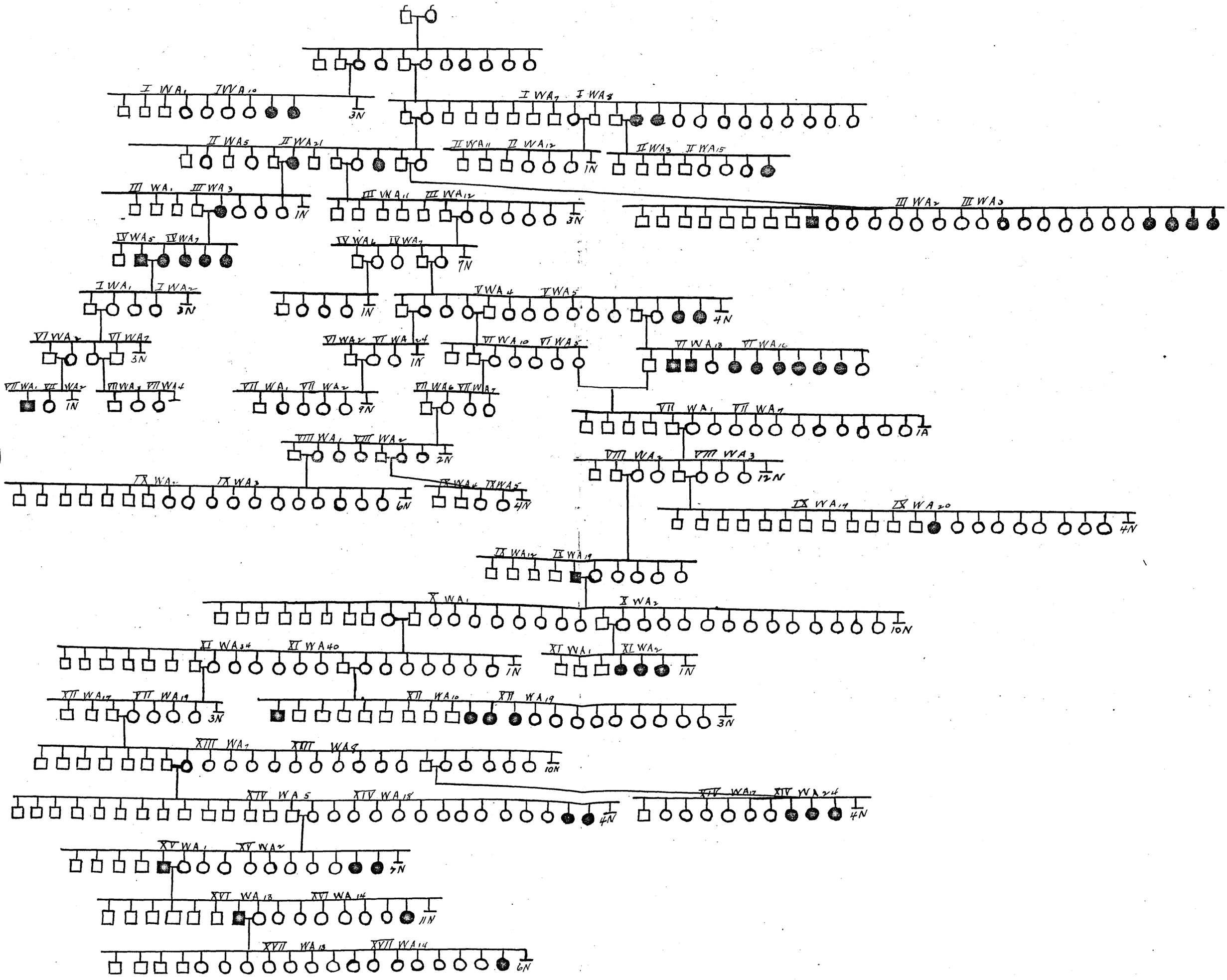
The Roman numeral I, II, etc represents the generation.

WA, WA etc represents the line of nematodes. And the small numerals 1, 2, 3 etc the individual nematode used in the matings.

$\frac{1}{1N}$ or $\frac{1}{1N}$, represents the normal nematodes whose sex were not determined.

$\frac{1}{1A}$, shows the undetermined abnormal nematode.

CHART III


I WA 1
I WA 10
3N
I WA 7
I WA 8
II WA 5
II WA 21
II WA 11
II WA 12
1N
II WA 3
II WA 15
III WA 1
III WA 3
1N
III WA 11
III WA 12
3N
III WA 2
III WA 3
IV WA 5
IV WA 7
IV WA 1
IV WA 7
7N
I WA 1
I WA 2
3N
1N
V WA 4
V WA 5
4N
VI WA 2
VI WA 7
3N
VI WA 2
VI WA 4
1N
VI WA 10
VI WA 5
VI WA 13
VI WA 16
VII WA 1
VII WA 2
1N
VII WA 3
VII WA 4
VII WA 1
VII WA 2
9N
VII WA 6
VII WA 7
VII WA 1
VII WA 7
1A
VIII WA 1
VIII WA 2
2N
VIII WA 2
VIII WA 3
12N
IX WA 2
IX WA 3
6N
IX WA 4
IX WA 5
4N
IX WA 19
IX WA 20
4N
IX WA 12
IX WA 19
X WA 1
X WA 2
10N
XI WA 34
XI WA 40
1N
XI WA 1
XI WA 2
1N
XII WA 17
XII WA 19
3N
XII WA 10
XII WA 19
3N
XIII WA 7
XIII WA 9
10N
XIV WA 5
XIV WA 18
4N
XIV WA 17
XIV WA 24
4N
XV WA 1
XV WA 2
7N
XVI WA 13
XVI WA 14
11N
XVII WA 13
XVII WA 14
6N

EXPLANATION FOR CHART IV

The black symbols used represent the abnormal nematodes.

■ , represent the abnormal male

● , represent the abnormal female

□ , represent the normal male

○ , represent the normal female

$1ND_9$, etc. is used to designate the generation, line of nematodes used, and the individual.

$\frac{1}{2N-1A}$, is used to show that two normal nematodes and one abnormal nematode were not determined as the sex.

CHART IV

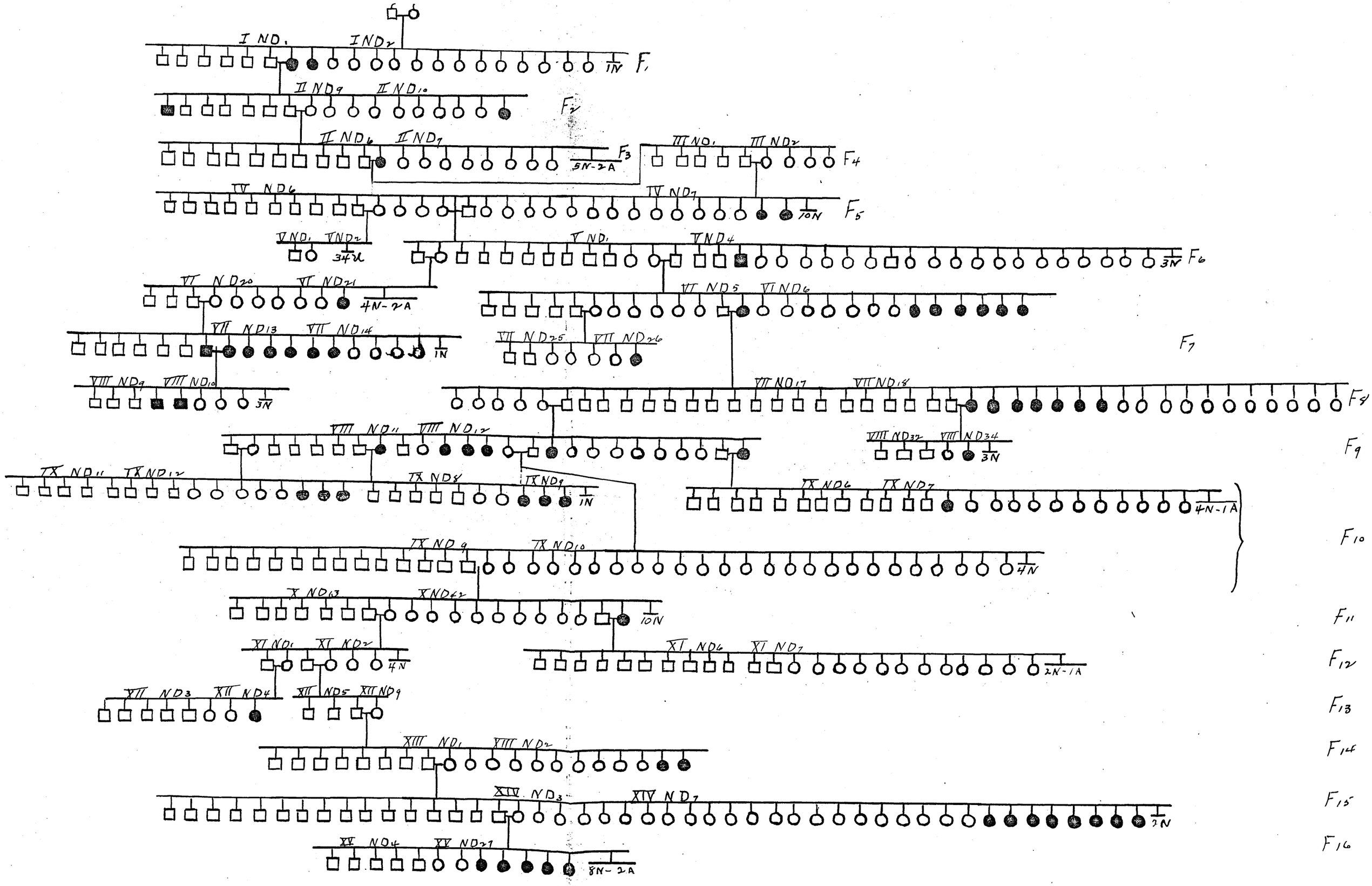

I ND1
IND2
1N
F1
II ND9
II ND10
F2
II ND6
II ND7
5N-2A
F3
III ND1
III ND2
F4
IV ND6
IV ND7
10N
F5
V ND1
V ND2
34N
V ND1
V ND4
3N
F6
VI ND20
VI ND21
4N-2A
VI ND5
VI ND6
VII ND25
VII ND26
F7
VII ND13
VII ND14
1N
VIII ND9
VIII ND10
3N
VII ND17
VII ND18
F8
VIII ND11
VIII ND12
VIII ND32
VIII ND34
3N
F9
IX ND11
IX ND12
IX ND8
IX ND9
1N
IX ND6
IX ND7
4N-1A
IX ND9
IX ND10
4N
F10
X ND43
X ND42
10N
F11
XI ND1
XI ND2
4N
XI ND6
XI ND7
2N-1A
F12
XII ND3
XII ND4
XII ND5
XII ND9
F13
XIII ND1
XIII ND2
F14
XIV ND3
XIV ND7
2N
F15
XV ND4
XV ND21
8N-2A
F16

PLATE I

Drawings showing the normal female, Fig. 1 and the normal male, Fig. 2.

These drawings were made free hand from outlines made by a camera lucida. A Bausch and Lamb 4X ocular and 16mm. objective were used.

PLATE I

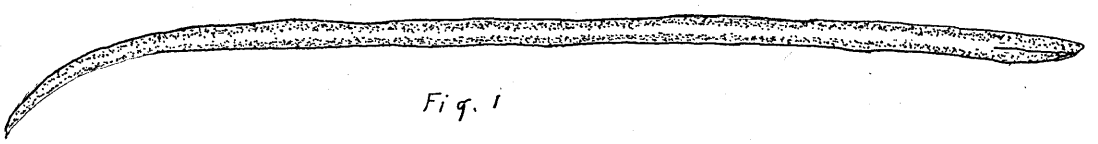


Fig. 1

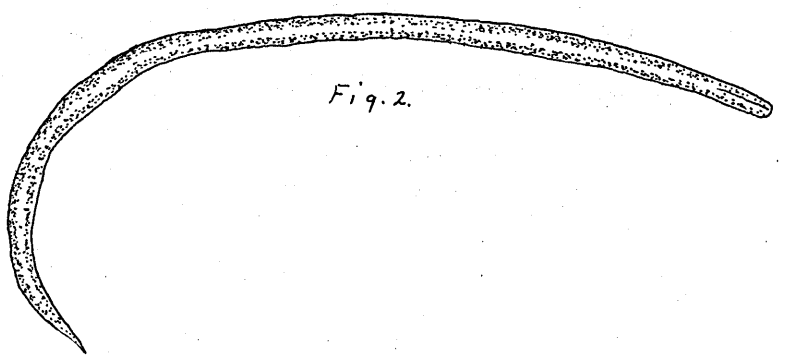


Fig. 2.

PLATE II

Drawings showing some of the abnormal conditions in Anguillula aceti.

All drawings were made free hand from outlines made by a camera lucida. A Bench and Lamb 4X ocular and 16mm. objective were used.

PLATE II

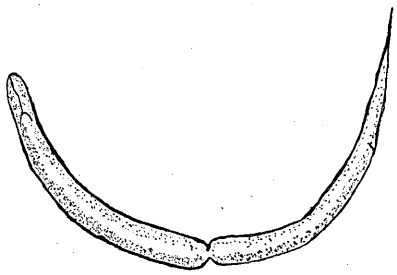


Fig. 1.

Fig. 2

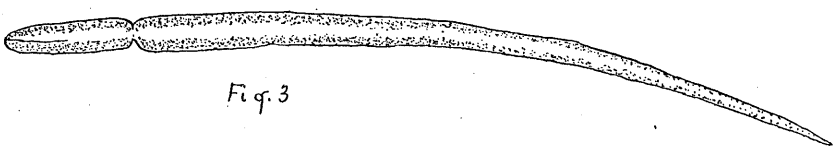


Fig. 3

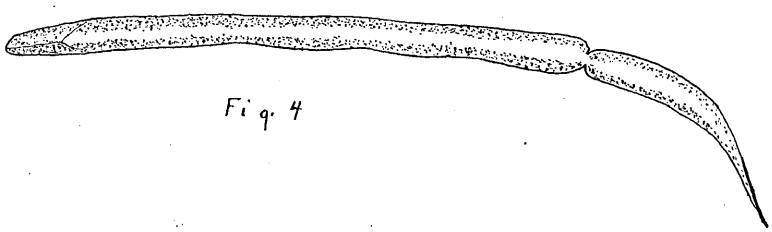


Fig. 4

PLATE II con't.

Fig 5

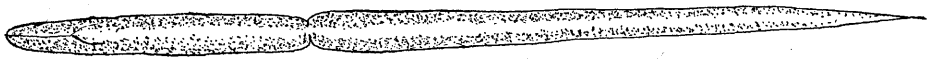


Fig 6

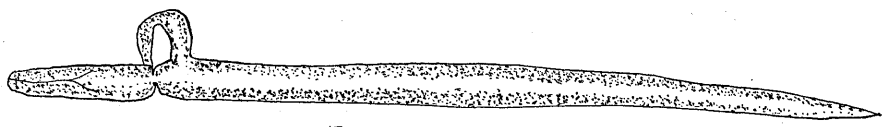


Fig 7

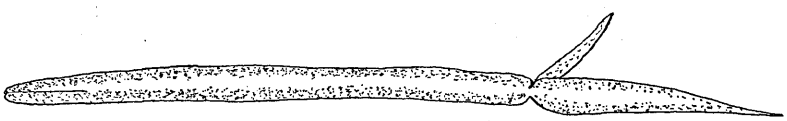


Fig 8

Fig 9.