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"Chemotaxis among different strains of the free-living nematode species complex *Panagrellus redivivus* - *silusiae*"

by

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Abstract

The behavioral response of three genetically different stocks of the dioecious free-living nematode species complex Panagrellus redivivus - Panagrellus silusiae to naturally occurring attractants was examined. The male response to mating attractant showed strain differences. Males are attracted to females of their own strain and to females of the other strains. P. redivivus (strains B and N) showed the greatest attraction to females of P. silusiae (strain C). The response of N and C males is dose dependant and showed optimal responses at specific concentrations of females. These differences are due to specificity of both orientation to and retention at the source of attractant and are under genetic control. In xenic cultures of Panagrellus several distinct attractants were isolated. At least one of which was protein, and none were ether soluble. Most attractants were of low molecular weight, with only one high molecular weight component.

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Introduction

Behavioral activities in simple animals primarily involve food and mate finding. These activities generally involve some modification of the basic behavior pattern of the organism. Mate finding behavior is limited to specific portions of the life cycle and as such provides a means for studying behavior in terms of gene activity during development (Cheng and Samoiloff, 1972). Another approach to studying the genetic basis of differences in behavioral activities is to compare the behavioral response of members of genetically dissimilar populations reared in the same environment and with the potential for genetic interchange. Genetic behavioral variance was found in populationsof Drosophila pseudo-obscura (Dobzhansky and Spassky, 1969).

Such conditions occur in the free-living nematode species - complex Panagrellus silusiae - P. redivivus. On morphological grounds Hechler (1971) finds one species while Anderson (personal communication) argues for separate species. Breeding experimentshave shown that P. redivivus and P. silusiae mate and produce fertile progeny (Behme and Pasternak, 1970). However, other breeding tests (Boroditsky and Samoiloff, unpublished) reveal that P. silusiae strain C breeds with the N strain of P. redivivus, but not with the B strain of P. redivivus. On these grounds it is more reasonable to consider the strains part of a species complex with C and B at the extremes and N intermediate. The advantages of nematodes for experimental studies of gene activity

have been presented by Samoiloff (1969) and Gershon (1970).

The complex nature of nematode attraction has been reviewed by Croll (1970). Both mating and food attraction have been reported in nematodes. Ward (1973) demonstrated attraction of the free-living Caenorhabditi elegans to cyclic AMP and a variety of ions. Attraction to ammonium ions was shown by Katznelson and Henderson (1963). Balan and Gerber (1972) found that the nematophygous fungus Arthrobotrys dactyloides produces compounds that attract the free-living nematode Panagrellus redivivus. Studies by Roche (1966) on Ancylostoma caninum, Jones (1966) on Pelodera teres, Green (1966) on Heterodera rotochiensis and H. schachtii, and Chin and Taylor (1969) on Cylindrocorpus longistoma and C. curzii show that males are attracted to females in these species. Mutual attraction between males and females has been shown by Greet (1964) in Panagrolaimus rigidus and in Cammalanus by Raymond and Freed (1973). In studying sex attraction in adult Trichinella spiralis, Bonner and Etges (1967), demonstrated that males are more strongly attracted to females although females are attracted to males. Tests on the interrelationships of 10 species of Heterodera by Green and Plumb (1970) led to the division of the genus into groups of similar mating attraction response. In the C-15 strain of P. silusiae males are attracted to females and the attractant, produced by adult females, is a diffusable chemical (Cheng and Samoiloff, 1971). The experiments of Cheng and Samoiloff (1971) were carried out using the attractant produced by 50 females diffusing through a distance of 2 centimeters.

No attraction was found between P. silusiae and a strain identified as Cephalobus persegnis. The latter strain has since been reidentified as P. redivivus strain B (Anderson, Personal communication).

The three strains of Panagrellus represent genetically different populations of the species complex, presumably with differences in their genetically programmed behavioral responses. This investigation was initiated to describe the differences in their responses to attractnats and to determine if such differences do, in fact, have a genetic basis.

Materials and Methods

Cultures and Sources of Attractant

All experiments were carried out with Panagrellus silusiae (strain C), P. redivivus (strain N) and P. redivivus (strain B) grown in standard culture as previously described (Samoiloff and Pasternak, 1968; Cheng and Samoiloff, 1971). The source and history of these stocks is presented in Table I. Mating studies (Boroditsky and Samoiloff, unpublished) show that the crosses C x N, and N x B produce fertile offspring, while C x B does not. Animals used for attraction test were removed directly from stock cultures for testing. Attractant was obtained in 2 ways: (1) females were placed over 2 ml of 1.5% agar in 35 mm plastic Petri plates for 24 hours and blocks of this agar (approximately 1x3x3 mm.) were removed and used as sources of attractant, (2) the fluid from dense Czapex dox cultures was filtered through tightly packed cotton in a funnel and refiltered through a fine porosity (0.2 micron) sintered glass filter.

Test for sexual attraction within and between strains

Fifty females placed over agar were used as sources of attractant. The tests were performed on "Mickey Mouse" mazes (Fig. 1). These mazes were made of 1 mm thick Perspex with a 2.3 cm hole and two 1.8 cm holes. The latter were situated 1 cm. from the larger hole, with a distance of 2 cm separating their peripheries. A 1 mm wide channel connected each small hole with the larger hole. The maze was placed in 9 ml of molten 1.5% agar in a 100 mm Petri plate

and the agar was allowed to solidify. A source of attractant was placed in one of the small openings, which was termed the test chamber. Twenty-four hours was allowed for the attractant to diffuse from the test chamber. Ten males were placed in the larger opening, the inoculation zone. A fresh agar block, without attractant, was placed in the other opening (the control chamber). The distribution of the inoculated worms was recorded at the end of three hours. Experiments were arranged so that in 50% the test chamber was the left hand zone and in 50% of the experiments that test chamber was to the right.

Nine experiments were carried out with P. redivivus (strain B), P. redivivus (strain N) and P. silusiae (strain C) males to attractant from females of B strain, C strain and N strain. Each experiment was repeated 30-40 times.

As the greatest differences were observed between C males and N males migrating to attractant from B females and C females subsequent experiments were limited to these combinations.

Test for preference

Two different assays for mating attraction preference were used. In one series of tests, using the "Mickey Mouse" maze, one test chamber contained two drops of extract from the N strain while the other test chamber contained 2 drops of extract from C strain. One male per plate was tested by placing the male in the inoculation zone. One hundred and thirty-six of the N strain males and 123 of the C strain males were tested in this manner.

Another series of tests for attractant was carried out on crosses cut from agar in 65 mm. Petri plates (Fig. 2). These were similar to the "Maltese cross" apparatus used by Green (1967). In these tests attractant from B females and C females were placed at the end of two arms 180° apart. After 12 hours to permit diffusion of attractant 10 or 20 males were placed in the center of the cross. The distribution of males was determined three hours after inoculation. This latter method was a more sensitive test. Fifteen experiments for each strain were repeated.

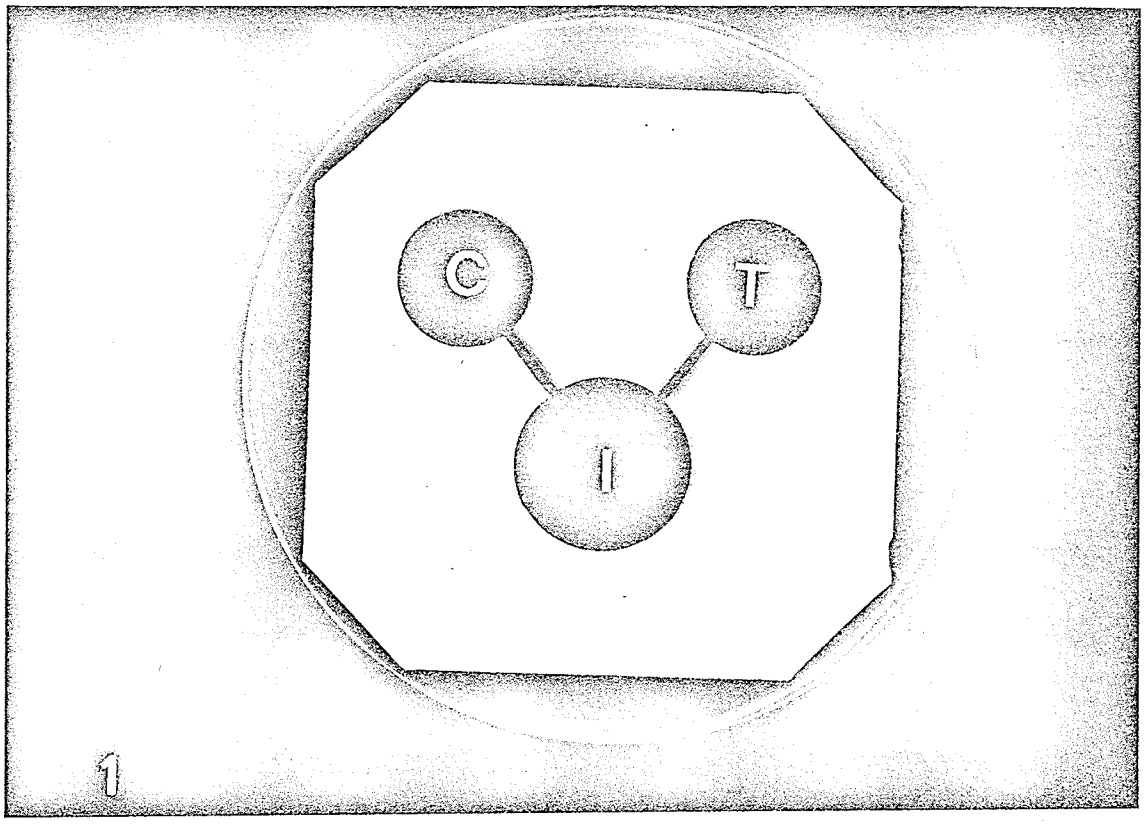
Test for changing of response to relative concentration of the attractant

Males of N and C strains were tested at different dilutions of attractant. Two methods were used to obtain the different concentrations. The first method involved dilution of the extracts of C and B cultures with distilled water. Males of the C and N strain were tested to full attractant, 1/2, 1/3, 1/4, 1/5 and 1/9 strength extracts of B and C strain fluid. Two drops of attractant from the same source were placed at the end of two opposite arms of a "Maltese cross" maze and ten males were inoculated at the center. Ten replicate tests were performed at each dilution.

The second method used ten spot depression slides containing 4 drops of 1.5% agar in each 1.5 cm diameter chamber. These circular sheets of agar were covered with a 1 cm circle of Metricel GA-8 filter (pore size 0.10) which supported a polythylene ring 0.5 cm high (cut from "Beem" capsules) forming a chamber from which animals could not

Figure 1. Diagram of the " Mickey Mouse " maze. Attractant is placed in test chamber (T), water in control chamber (C). After gradient is established males are placed in innculation zone (I).

Figure 2. Maltese cross apparatus. Attractant is placed at end of one or two arms (1,2,3,4). After gradient is formed males are placed in inoculation zone (I).



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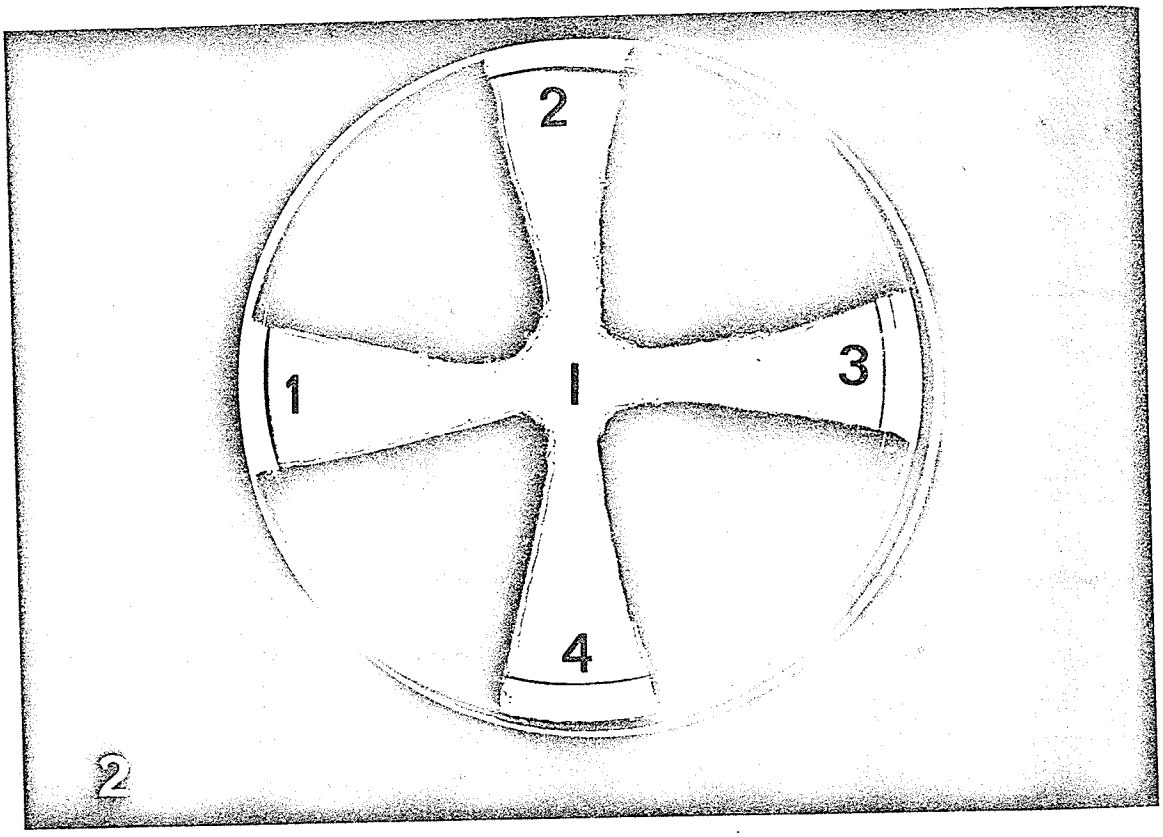

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excape. This is a modification of the apparatus used by Cheng and Samoiloff (1971). Five, 10, 20, 50, 100, 200 and 300 females were placed in each chamber. After 24 hours females were removed and 2 x 2 mm blocks of agar were cut and used as the source of attractants. A block of attractant was placed at the end of one arm of the maltese cross and a block of pure agar was placed at the opposite arm.

Test for movement patterns

In order to observe the pattern of movement of males to attractant it was necessary to observe the tracks of the nematodes over an agar surface. Tracks were demonstrated in 2 different ways. The first was accomplished by placing a source of attractant onto a point on a 100 mm Petri plate containing 2 mm depth of Nutrient agar. After 12 hours to permit diffusion of attractant a single male grown in E. coli was placed at the center of the plate, 1.5 cm from the source of attractant. As the male moves toward the attractant it leaves a track of E. coli. When the males reached the source of the attractant the nematode was removed from the plate and the plate was incubated for 24 hours at 37° C to permit growth of the bacteria along the track. The path of the nematode, outlined by bacterial grown, was traced or photographed. In some experiments two different attractants were placed 3 cm apart on the agar and the male was inoculated midway between them.

The second method (Croll, personal communication) was done by pouring warm (80° C) 6% Czepex dox agar over a Petri dish and quickly removing the excess agar. The agar formed a very thin layer on the

plate. Worms were placed on this layer. Tracks were photographed by placing photographic film (Kodak Kodalith Type 3) below the Petri dish and exposing the film to light from a photographic enlarger for 2 seconds, then developing the film with fine line developer.

Test for inheritance of behavioral response

To obtain virgin animals for genetic studies second stage juveniles were individually cultured on Nigon's agar (Nigon, 1949). When these animals reached the adult stage they were mated. Reciprocal crosses were made with C males x N females and N males with C females. Crosses between N and B were not made because major differences in mating response was not observed. Crosses between C and B were not made due to infertility.

The male progeny of the crosses were tested by using concentrations of attractants from B and C strains such that C males showed preference for B females and N males showed a preference for C females.

Assay for differential solubility of attractant

One hundred ml of extract was mixed with 100 ml of ethyl ether in a separatory funnel. Material from the ether layer, the water layer and the interface layer were tested by placing 2 drops of extract in the test chamber of "Mickey Mouse" mazes and 2 drops of distilled water in the control arm. After 24 hours to form a gradient 10 males were placed in the inoculation zone. Ten experiments were performed with each extract. The water extract was further tested by treatment for 1 hour at 37° C with the proteolytic enzyme Pronase (Calbiochem) at a concentration of 0.4 mg/ml. The pronase treated extract was tested

against untreated water extract and pronase solution. The response of both C males and C females was noted.

Assay for molecular weight distribution of attractants

To further examine for strain differences in the response to attractants contained in the medium culture, extract was passed through a column of Sephadex G-25 (1 cm x 17.5 cm) with a 60 cm pressure head at a flow rate of 3 ml/minute. Eighty fractions were obtained. Two drops from every other fraction were placed at the edge of a 100 mm Petri plate containing 10 ml of 1.5% agar. After 12 hours to form a gradient twenty-five C males, N males or C females were placed in the center of the plate and the number of animals in 1 cm^2 containing the extract was recorded at 1, 2, 3 and 14 hours after inoculation.

Results

Using attractant obtained by placing females over agar and testing on "Mickey Mouse" mazes it was determined that males were attracted both to females of their own strain and to females of the other strains (Table II). Both B and N strain males were more strongly attracted to the C strain attractant than to their own strains. Fifty-six percent of B males tested against C attractant migrated to the test chamber, while only 40% of the B males tested against B attractant migrated to the test chamber. Fifty-three percent of the N males tested against C attractant migrated to the test chamber, while only 37% of those tested against N attractant migrated to the test chamber. This was not due to higher production of attractant by C females since C males showed a preference for B attractant over that of their own strain. Only 38% of the C males tested against C attractant migrated to the test chamber as compared to 45% migrating to the test chamber containing B attractant.

Chi-square comparison of the total distribution of males between pairs of experimental combination from Table II provides a means of ranking the differences between the distribution of males of the three stocks to attractant from the three stocks (Table III). The greatest differences were observed between N males migrating to C or B attractant (X^2 = 52.39) and C and N males migrating to B attractant (X^2 = 40.57). B males migrating to C or N attractant showed no significant difference (X^2 = 1.883), and no difference was detected between N and C males migrating to N female (X^2 = 3.137), B and C males migrating to B females (X^2 = 3.158) or N and B males migrating to C

females ($X^2 = 3.431$).

Tests using filtrates of culture fluid on "Maltese crosses" again demonstrated that N males show greater attraction to C and that C males show greater attraction to B attractant (Table IVa). The preference is due to differences in sensitivity to attractant as standard dilutions of B and N culture filtrates placed at opposite poles of the cross showed that 62% of C males went to C extract, while 62% of the N males tested went to C extract (Table IVb). However, when the C attractant was diluted 1:1 with distilled water, with the B extract kept at standard dilution, 75% of the C males moved to B extract and 49% of the N males moved to C extract. Under these condition only 18% of the C males moved to C and only 26% of the N males moved to B attraction (Table IVc). This result led to examination of concentration effects.

Test using different concentration of attractant revealed that the response of males of both the N and C strain is strongly dose dependant (Table V, VI; Fig. 3, 4). Dilution experiments (Table V) indicate that N males show increased attraction to increasing concentration (Fig. 3), while C males show distinct optimal concentration (Fig. 3) to both C and B extract. Experiments with attractant from varying numbers of females (Table VI) demonstrate that both N males and C males show optimal response at specific concentrations of females. The optimal response for N males occurs with 200 C females or 100 N females (Fig. 4). The optimal response for C males occurs at 100 C females or 100 N females (Fig. 4).

The male progeny of the crosses N female x C male and C female x N male showed patterns of mating attraction different from either parental strain (Table VII). The distribution of the parental strain males was the same 1 hour after inoculation as it was 12 hours after inoculation. The progeny of the crosses between the strains showed an equal distribution to C and B attractant after 1 hour, but after two hours more of the animals were found near the C attractant. Male progeny of the cross N female x C male showed a greater attraction to C with 86% of the worms at C attractant after 5-12 hours, as compared to 66% of the male progeny of the C female x N male cross.

Examination of the tracks of the males responding to the attractant of the different strains again demonstrated strain specific response to the different attractants (Fig. 5-8). C males migrating to C attractant (Fig. 5) follow a wide arcing path in which the nematodes initially move at right angles to the gradient of the attractant. N males migrating to B attractant (Fig. 8) usually follow a similar path. C males migrating to B attractant (Fig. 6) move in a more linear manner to the source of attractant. This linear movement is also seen in N males migrating to C attractant (Fig. 7). The presence of C attractant had little effect on the movement of C males to B attractant and B attractant had little effect on the movement of N males to C attractant. Previous studies have established that the behavioral patterns of males responding to female attractant consists of two phases. During the initial phase the animal moves in a series of tight circles with many changes in orientation. This is "activated" behavior in which the animal is perceiving the attractant and is

attempting to orient itself to the source of attractant (Samoiloff, McNicholl, Cheng and Balakanich, 1973). Once oriented the nematode moves toward the source of attractant. C males moving to C attractant (Fig. 5) usually show an extensive period of activated behavior, while C males responding to B attractant generally show only limited activated behavior (Fig. 6). During the migration to attractant C males undergo a great deal of looping and reversal. N males responding to C attractant usually undergo only limited activated behavior (Fig. 7), while extensive activated behavior is common in N males responding to B attractant (Fig. 8). N males migrating to C attractant commonly move in a linear manner, while N males moving to B attractant show many loops and reversals. Males moving without attractant show a more or less linear pattern broken by widely spaced changes in direction and decreases in the number of loops, reversals and turns as compared to movement in response to attractant (Fig. 9, 10).

Attractant is not found in the ether soluble fraction of culture fluid extract but is found in the water soluble fraction and the ether-water interface (Table 8). Greatest attraction is to the water soluble fraction. Treatment of the water soluble fraction with pronase decreases the attraction but does not eliminate it (Table 9).

Extracts from cultures of C animals contain at least two main attractants (Fig. 11) a high molecular weight component (or components) which attracts and retains males of both the N and C strain and retains C females, and lower molecular weight material which attracts and retains N males well, attracts C males with only limited retention and has little attraction but good retention of C females.

Discussion

Responses to various attractants and specificity of attraction in different species of nematode have been observed (Chin and Taylor, 1969, Green and Plumb, 1970). Attraction in the three strains of Panagrellus studied do show strain specificity as well as sex specificity. Differences in the response to mating attractant in these three strains are obvious. The B and N strains show a distinct preference for the attractant of the C strain, while the C strain shows a preference for the B strain attractant. The response of the C strain to N and C attractant is similar. Both the B and N strains have been identified as P. redivivus (Anderson, personal communication) while the C strain was considered a separate species. N and B, and N and C will readily mate, while C and B rarely, if ever, mate. This evidence indicates that the C, N, and B strains form a species complex with N the intermediate between C and B. The mating attraction patterns indicate that the three strains are outbreeders by mating behavior with B and N forming one group and the C strain a more distant group. This is consistent with the identification provided by Anderson. The intermediate strain, N, is more closely related to the B strain than to the C strain. It is of interest to note that the C and B strains are strongly attracted via mating attraction although matings are rare, indicating that the divergence of the mechanisms for copulation behavior was more rapid than that for mating attraction.

Test using different concentration of attractant also show some differences in behavioral response of N and C strain. In the dilution experiments, although N males show increased attraction to increasing concentration of the extracts of both B females and C females, they still show preference to C females at each point except at the full concentration and the lowest concentration. This probably indicates that at very high or very low concentrations, the receptors of N males can not function normally. The response of C males is different from N males in that C males show distinct optimal concentrations to both C and B extracts and at the full concentration C males are more strongly attracted to B females than to their own females. Both N males and C males show optimal responses at specific concentrations of females, but their responses decrease similarly when concentrations increase from the optimal point. This may be due to the differences in the attractants of the two strains.

The optimum attraction of males to both C and N females occurs when attractants are obtained from 100 females. At this high concentration N malesshow preference to C females while C males show preference to B females. At 1/9 concentration and at 50 female extracts, C males migrate to C females but not to B females. This indicates that mating attraction promotes outbreeding especially at high population levels, resulting in maximum genetic variability. Perhaps species, when overly abundant, may inhibit further increase in its numbers or increase its genetic heterogeneity in response to increased selective pressures. At low concentration of attractant C males are attracted

to C females, leading to inbreeding under these conditions.

The genetic crosses show that the N pattern of attraction is dominant, and probably polygenic. Differences in the behavior of the hybrid males can be observed since approximately 50% of males that initially responded to attractant went to the B attractant, but were not retained, later moving to the source of C attractant. This indicates that the mechanisms for retention of males at the source of attractant (Cheng and Samoiloff, 1971) are different from those resulting in movement or alignment to the sources of attractant. Fractionation of culture extracts show that different molecules can produce migration or retention, so that different reception mechanisms may be involved.

The observation that C males respond to B attractant and that B males respond to C attractant are not in agreement with those of Cheng and Samoiloff (1971), who reported no attraction between these stocks. However the source of attractant used in the present study was stronger than previously used and the test using the "Mickey Mouse" maze more sensitive than the V-shaped maze used in the earlier study. In the "Mickey Mouse" maze the most probable behavior for any nematode is to remain in the inoculation zone, with migration to the test chamber primarily in response to some attractant. In the V-shaped maze the test for attraction involved examination for deviation from a 1:1 ratio of migration up to test and control arm. The increased sensitivity of the "Mickey Mouse" apparatus can be seen by comparing the time course of migration. In the V-shaped maze differences in

response to attraction could only be detected after 4 hours, but in the "Mickey Mouse" apparatus attraction can be demonstrated after 15 minutes. In this study, there are certain concentrations in which attraction cannot be detected between strains but only in the same strain. The previous study might be working at this concentration.

The differences in behavioral pattern as demonstrated by the tracks may be due to several factors. The males may be utilizing different sensory-motor pathways in response to the different attractants, or they could be responding to different optimal concentration of the attractants or, in light of the high concentration of attractant used in this study the males could be showing saturation of receptors for their own attractant but normal, non-specific responses to extremely high concentrations of the attractant of the more distantly related strain.

A model for the swimming and orientation of nematodes based on transitions between two behavioral states has been proposed (Samoiloff, McNichol, Cheng and Balakanich, 1973). The basis of this model is that under normal, unstimulated conditions the nematode follows a more or less linear path with the anterior and posterior ends of the animal sweeping through a minimal area. This is termed the normal behavior state. When stimulated by chemical or physical means the animal enters the activated state, making rapid changes in orientation with the ends of the animal sweeping through a maximal area. In the case of a gradient of an attractant, the animal remains in the activated state until it orients to the gradient by sensing the maximum difference in

intensity of the stimulus between the posterior and anterior sensory structures, at which point the animal returns to the normal behavioral state and migrates to the source of attractant. Laser microbeam studies indicated that the copulatory spicules of the free-living Panagrellus silusiae function as the posterior receptors for oreintation to mating attraction (Samoiloff, McNichol, Cheng and Balakanich, 1973), and recently a sensory function has been found for the spicules in other species (Lee, 1973). It has also been proposed that nematode chemotaxis is mediated by comparisons of stimuli by the anterior sensory receptors at successive lateral sweeps (klinotaxis) (Ward, 1973). This mechanism requires some type of memory system in nematodes, which, in fact, has been demonstrated in P. silusiae by Samoiloff, McNichol, Cheng and Balakanich (1973).

Analysis of the orientation patterns indicate that males of the two strains used different mechanisms in response to the same attractant. The pattern of the tracks of N males responding to C attractant (Figure 7) agrees with the two state model. They initially made rapid, constant changes in their orientation. This was only partially activated behavior. After aligning themselves, they oreinted well. Then normal movement in a linear manner occurred as they neared the attractant of females where the gradients was steeper or greater concentrations occurred. The C attractant was good in retention and less attraction to C males. This might be due to C attractant being at too high a concentation for its own strain, resulting in looping, turning, and wide arc tracks. C males migrating to C attractant

showed an extensive period of activated behavior. In steeper gradients a klinotaxic response should lead more directly to the female attractant, but it seems that the receptors of C males became fatigued and a less well oriented response resulted. Dilution of the extract of C females caused more direct paths of C males. The responses of N males to 200 C females was similar to the responses of C males to 100 C females, which also indicates that C female attractant acts more efficiently on the receptors of C males than N males. The preference of C males to B extract at higher concentrations of extract seems most probably due to saturation of the specific sex attraction receptors and a response to a non-specific attractant in the extract. Supporting this interpretation are the observations from the fractionation experiment which show that lower molecular weight material from C cultures attracted and retained N males well but affected C males with only limited retention and little attraction. As sex attractants may act to inhibit normal non-specific klinotactic response, I believe that the low molecular weight components of the extracts play a major role in these sexual attraction preferences.

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Table 1

The Designation of the Stocks

B strain	C strain	N strain
- in culture at U. of Manitoba prior to September, 1969. Source unknown. Initially labelled Cephalobus persegnis. Identified as Panagrellus redivivus by R. V. Anderson in January, 1972.	- derived from culture of Panagrellus silusiae obtained from Dr. A. C. Coomans in 1966. The C strain has been generated by over 20 generations of sib - sib mating.	- laboratory stock of Panagrellus redivivus. Obtained from Dr. E. Hansen, Berkeley in 1971. This stock has been in axenic culture for over 10 years.

Table II

Test of Response of B, C or N Strain Males in Inoculation Zone to Secretions from B, C or N Strain Females in Test Chamber

Migrating	Source of Attachment	Number Observed Control	Inoculation	Test	Total
B males	B females	71	112	121	304
	C females	49	93	182	324
	N females	64	92	178	334
C males	B females	78	93	138	309
	C females	47	153	124	324
	N females	66	123	115	304
N males	B females	35	167	110	312
	C females	71	90	184	345
	N females	56	153	122	331

Table III

Chi-square Comparison of the Attraction of Males to Females of the Same Strain and Between Strains

Constant	Comparison	X^2	Remarks
B males	C females, B females	17.452	B males prefer C females
	C females, N females	1.883	
	B females, N females	11.799	B males prefer N females
C males	C females, B females	22.724	C males prefer B females
	C females, N females	6.156	
	B females, N females	7.211	
N males	C females, B females	52.39	N males prefer C females
	C females, N females	30.382	N males prefer C females
	B females, N females	5.519	
B females	N males, B males	23.483	B males more attracted to B females
	B males, C males	3.158	
	N males, C males	40.566	C males more attracted to B females
C females	N males, B males	3.431	
	N males, C males	32.271	N males more attracted
	B males, C males	25.664	B males more attracted
N females	N males, B males	26.154	B males more attracted
	N males, C males	3.137	
	B males, C males	16.665	B males more attracted

Table IV

Testing of Preference of C Males and N Males to Culture Fluids by Using the "Maltese Cross" maze

a)	Migrating	B Fluid #1	C Fluid #1	Control	Total
	C males	98	48	34	180
	N males	57	94	49	201
b)	Migrating	B Fluid #1	C Fluid #2	Control	Total
	C males	15	65	25	105
	N males	29	87	25	141
c)	Migrating	B Fluid #2	½ C Fluid #2	Control	Total
	C males	53	13	5	76
	N males	24	45	23	92

Table V

Response of Males to Different Dilution of Culture Fluids

Migrating	Attractants	No. of Worms Found in: Test Chambers	Control	Total
N males	Full B extract	94	10	104
	1 extract : 1 water	83	26	109
	1 " : 2 "	70	33	103
	1 " : 3 "	69	25	94
	1 " : 4 "	54	62	116
	1 " : 8 "	56	31	87
N males	Full C extract	101	12	113
	1 extract : 1 water	83	22	105
	1 " : 2 "	81	19	100
	1 " : 3 "	66	42	108
	1 " : 4 "	64	38	102
	1 " : 8 "	55	36	91
C males	Full B extract	91	11	107
	1 extract : 1 water	89	22	111
	1 " : 2 "	94	13	107
	1 " : 3 "	68	50	118
	1 " : 4 "	73	18	91
	1 " : 8 "	54	48	102
C males	Full C extract	70	20	90
	1 extract : 1 water	78	25	91
	1 " : 2 "	75	25	100
	1 " : 3 "	69	36	105
	1 " : 4 "	73	20	113
	1 " : 8 "	62	42	104

Table VI

Attraction of Males to Varying Numbers of Females

Used as Source of Attraction

Migrating	No. of Females	No. of Worms Found in: Test	Control	Blank	Blank	Inoculation Zone	Total
N males	0 B females	24	16	21	16	28	105
	5 " "	15	16	21	15	9	76
	10 " "	35	51	29	26	28	169
	20 " "	29	35	32	42	22	160
	50 " "	55	50	43	54	21	223
	100 " "	68	37	43	34	25	207
	200 " "	45	47	38	44	27	201
	300 " "	34	28	28	26	21	137
N males	0 C females	24	16	21	16	28	105
	5 " "	31	15	26	25	26	123
	10 " "	30	23	13	31	32	129
	20 " "	46	27	29	23	41	166
	50 " "	58	19	28	21	25	151
	100 " "	71	30	26	32	24	183
	200 " "	83	12	31	41	33	200
	300 " "	45	15	25	25	34	144
C males	0 B females	27	24	19	26	22	118
	5 " "	22	19	14	16	6	77
	10 " "	48	30	25	38	19	160
	20 " "	33	25	28	29	17	132
	50 " "	36	45	45	45	19	190
	100 " "	109	39	43	34	19	244
	200 " "	49	32	27	41	34	183
	300 " "	24	18	18	25	16	101
C males	0 C females	27	24	19	26	22	118
	5 " "	35	35	20	30	29	149
	10 " "	39	33	32	44	30	178
	20 " "	41	19	25	32	32	149
	50 " "	48	29	25	15	38	155
	100 " "	84	30	18	19	39	190
	200 " "	70	24	23	27	37	181
	300 " "	58	15	18	15	49	155

Table VII

Test of Resonse of the Progeny of Crosses Between Strains C and N to Culture Extracts Tested on "Maltese Crosses"

Migrating	Time	B Extract	C Extract	Inoculation Zone	Control Arms	Total
C males	1 hr.	116	54	20	17	207
N males		29	87	11	14	141
F_1(C females x N males)		149	150	31	41	370
F_1(N females x C males)		81	73	23	46	223
F_1(C females x N males)	2 hrs.	80	88	5	12	185
F_1(N females x C males)		91	154	22	23	290
F_1(C females x N males)	5-12 hrs.	70	114	4	1	189
F_1(N females x C males)		15	186	20	0	221

Table VIII

Test of Response of C Males to

Fraction of Culture Fluid Extract

	Test	Inoculation Zone	Control
ether soluble fraction	21	13	41
water soluble fraction	71	6	11
ether-water interface	46	11	13

Table IX

Test of Response of C Males and C

Females to Pronase Treated Extract

Migrating	No. of Worms Found in:			
	Distilled Water	Inoculation Zone	Intact Water Extract	Pronase Tested Extract
C males		19	77	32
C females		14	20	16
C males	27	13		72

Figure 3. Effect of dilution of culture extract on migration of males to extract. Tests performed on maltese cross apparatus. Twenty males placed in inoculation zone in each experiment. Circles represent mean number of males migrating to each dilution. Closed circles (●———●) represent C males, open circles (o-----o) represent N males. Left hand graph show results using B culture extract, right hand curve uses extract from C cultures.

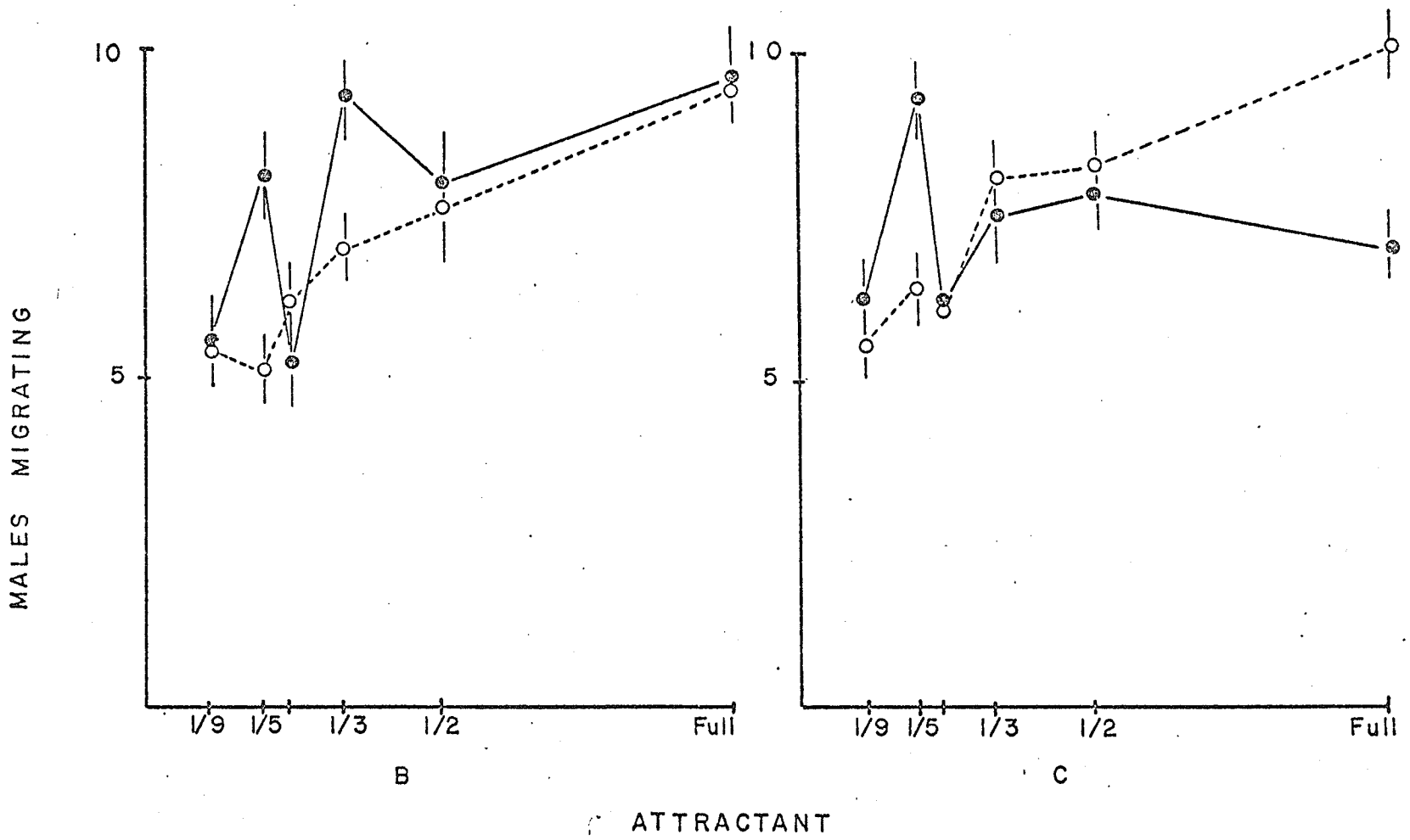

MALES MIGRATING
10
5
1/9
1/5
1/3
1/2
Full
B
C
ATTRACTANT

Figure 4. Effect of concentration of sex attractant on migration of males to attractant. Attractant from varying numbers of females was tested on maltese crosses. Circles represent mean numbers of males migrating to each attractant concentration. Closed circles (●——— ●) represent C males, open circles (o----- o) represent N males. Upper figure uses B females, lower figure uses C females.

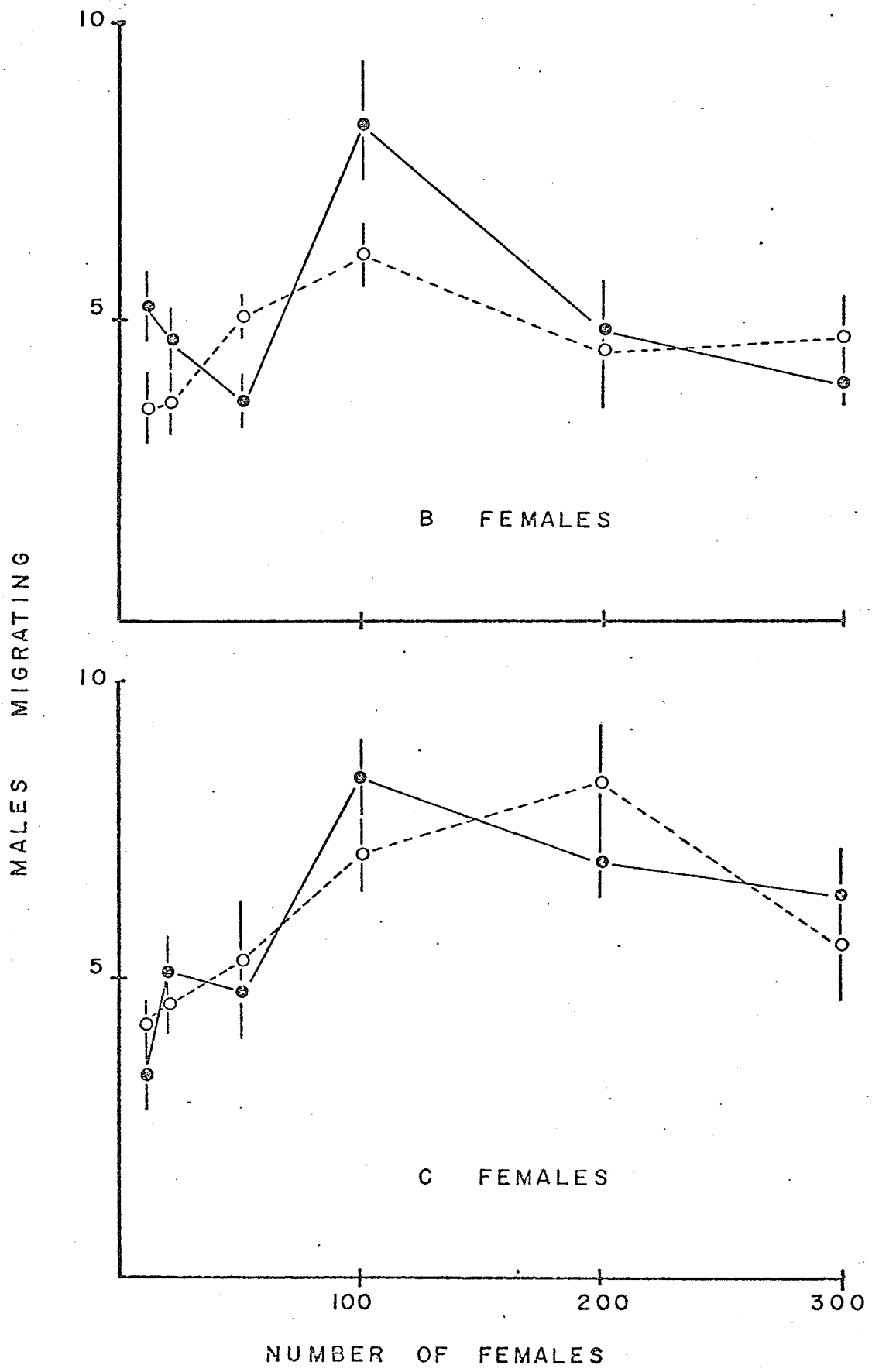

10
5
B FEMALES
10
5
C FEMALES
MALES MIGRATING
100
200
300
NUMBER OF FEMALES

Figure 5. Tracks of C males migrating in response to attractant from C females. Tracks represent 5 different males tested in separate plates. Source of attractant is rectangular block of agar over which females had been maintained.

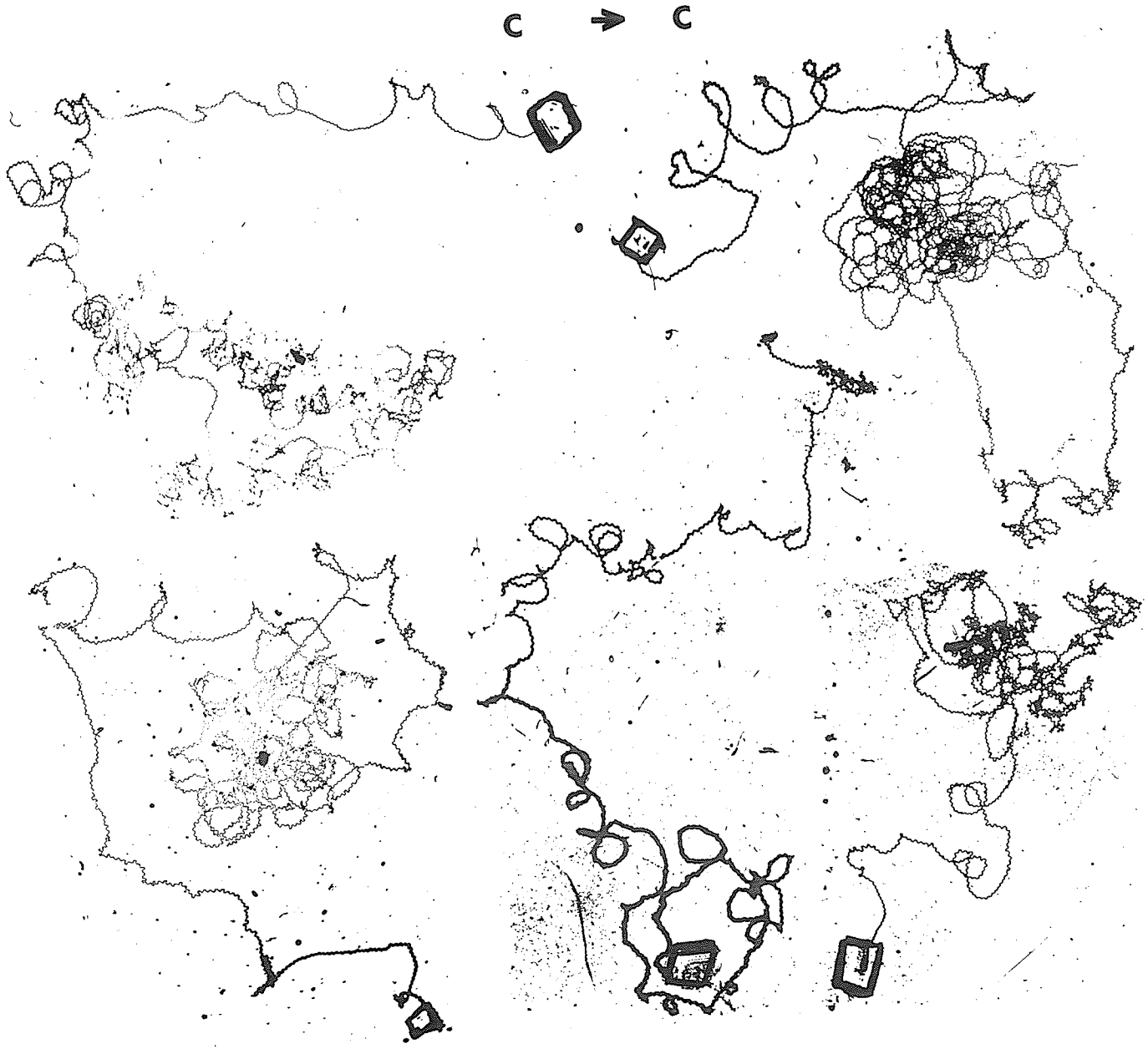

C → C

Figure 6. Tracks of C males migrating in response to attractant from B females.

C → B

Figure 7. Tracks of N males migrating in response to attractant from C females.

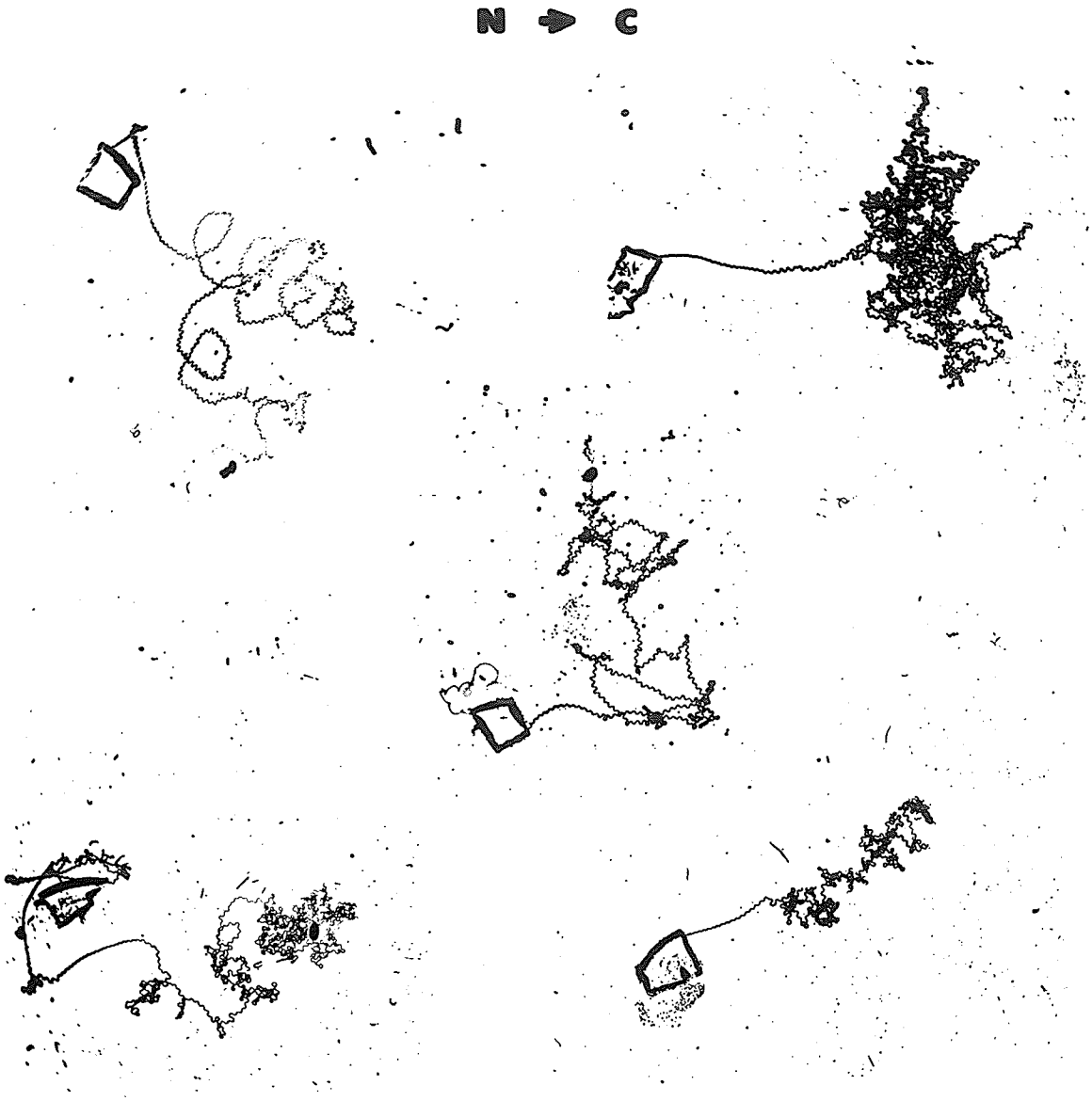

N → C

Figure 8. Tracks of N males migrating in response to attractant from B females

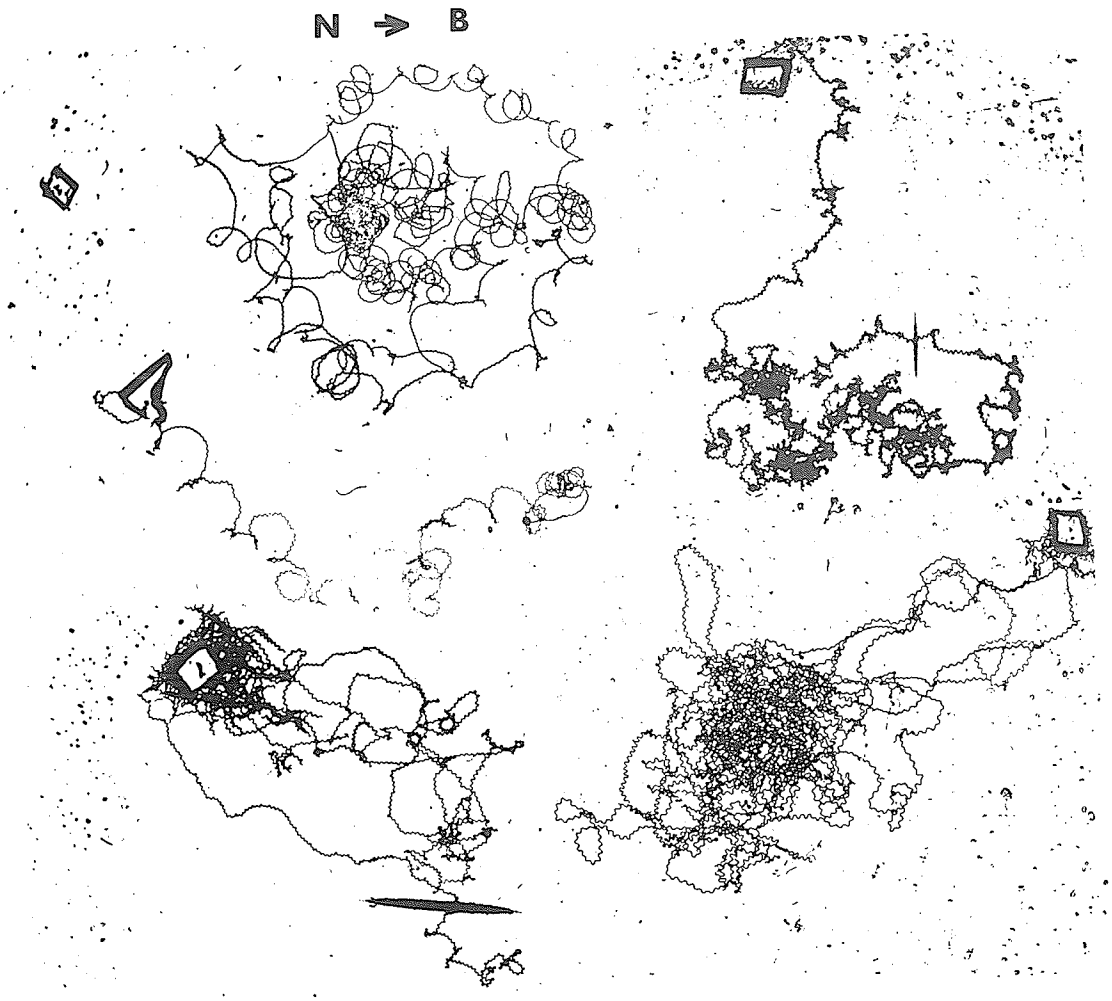

N → B

Figure 9. Tracks of N males in the absence of sex attractant

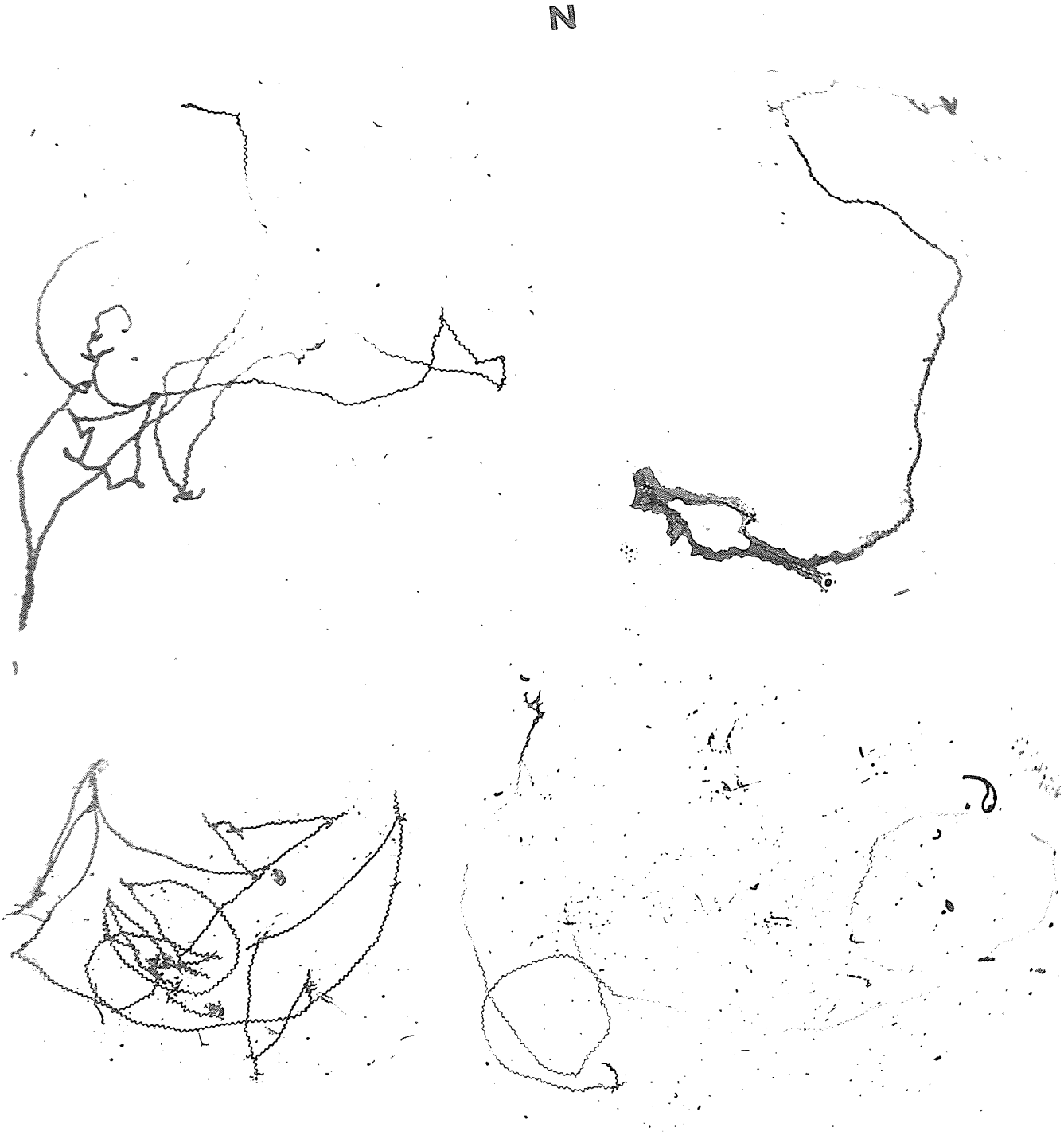

N

Figure 10. Tracks of C males migrating in the absence of sex attractant.

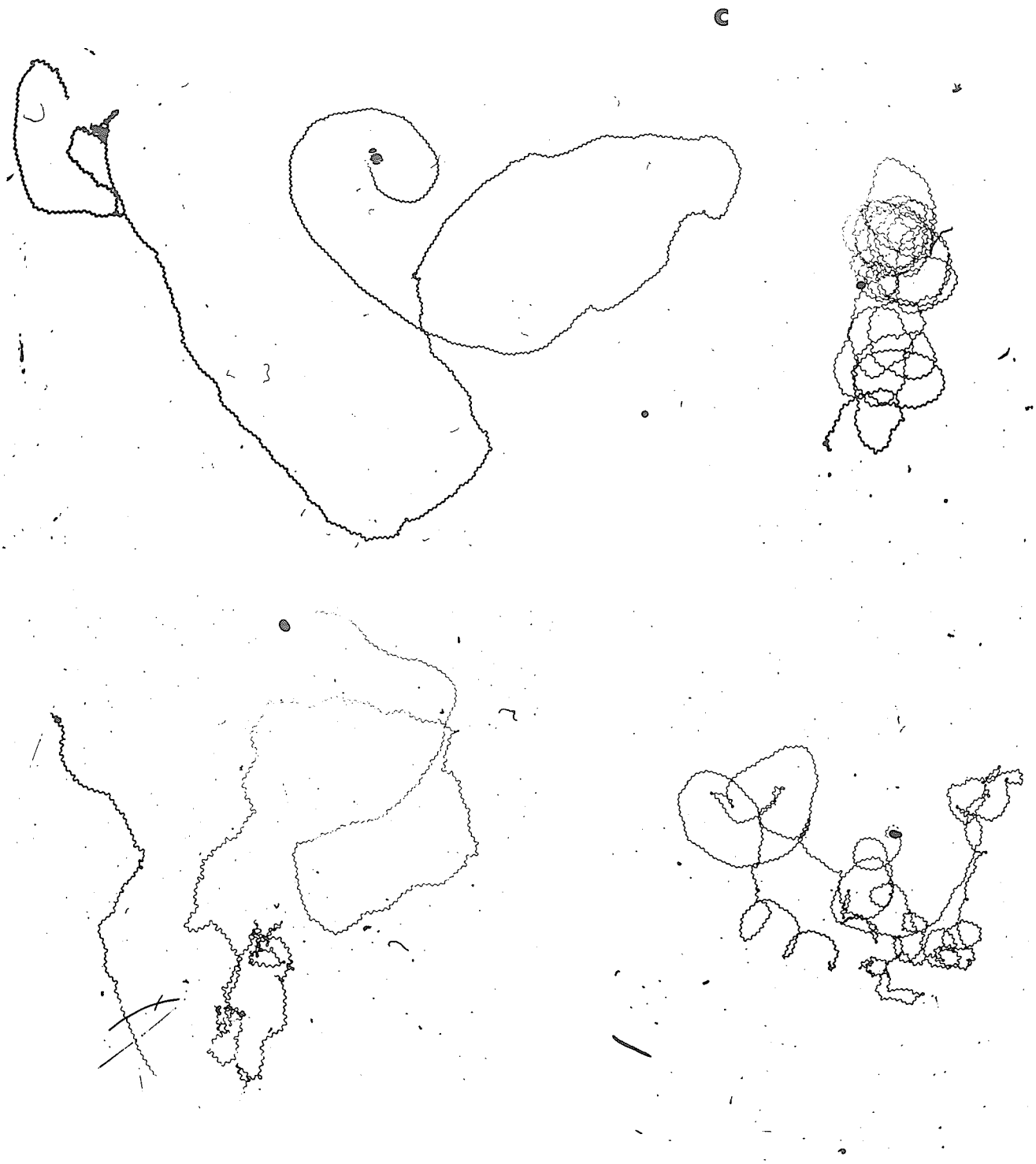
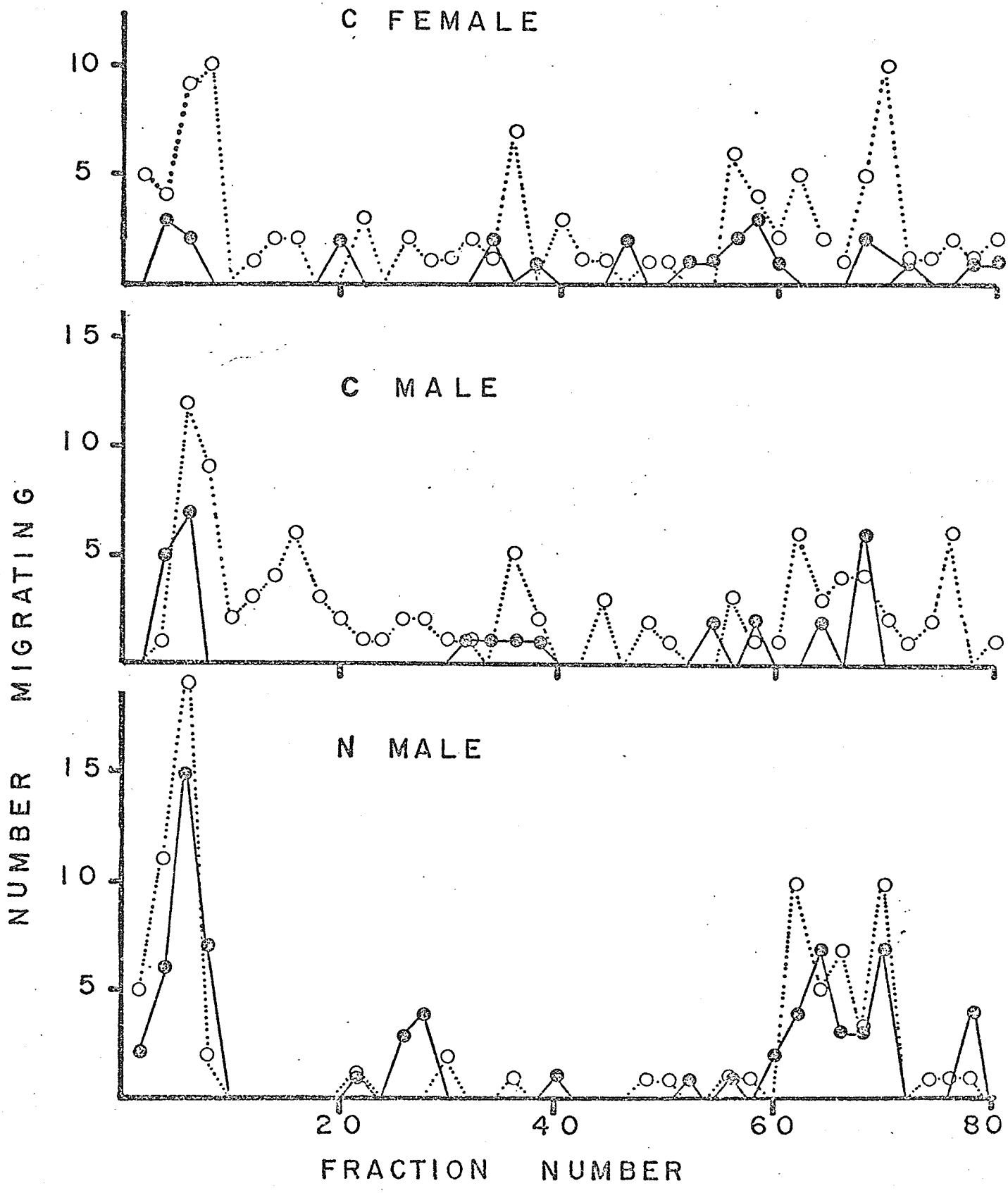


Figure 11. Test for attraction of fractions of C strain culture extract by C males, N males and C females. Closed circles (●——●) represent the number of animals migrating to the source after 1 hour. Open circles (O---O) represent the number of animals migrating to the source after 14 hours. High molecular weight fractions are to the left, low molecular weight fractions are to the right.


C FEMALE
C MALE
N MALE
NUMBER MIGRATING
FRACTION NUMBER
10
5
15
10
5
15
10
5
20
40
60
80