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STAGE SPECIFIC BEHAVIOUR OF NORMAL AND MUTANT

Panagrellus redivivus

by

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ABSTRACT

The stage specific behaviour of normal and mutant *Panagrellus redivivus* was investigated. Normal nematodes were tested for attraction to bacteria and response to osmotic stress. The behaviour of the nematodes depended on their stage of development. Larvae possessed a different array of behavioural patterns than adults.

The corkscrew mutant was generated by N, methyl-N-nitroso-N'-nitrosoguanidine. This mutation was a dominant characteristic which decreased the size of adults, lowered the fecundity and resulted in abnormal swimming behaviour. This corkscrew swimming behaviour was expressed most strongly in fourth stage larvae and adults indicating a genetic basis for the stage specific behaviour observed.

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I.

INTRODUCTION

Nematodes possess many characteristics which render them ideal for genetic studies (Dougherty, 1949). They are eutelic, have small numbers of chromosomes, can be grown axenically in large numbers, have a short generation time and high fecundity. In addition, they have a great variety of reproductive patterns including hermaphroditic, diecious and parthenogenic species. In spite of these advantages there have been few published reports of genetic mutations in nematodes.

The mode of reproduction of hermaphrodites makes them a logical choice for genetic studies. Spontaneously occurring dwarf mutants of *Caenorhabditis briggsae* and *Caenorhabditis elegans* have been described (Nigon and Dougherty, 1950; Dion and Brun, 1971). A genetic basis for infertility at 23°C in the Bergerac strain of *C. elegans* was found (Fatt and Dougherty, 1963). Two spontaneous mutants of the free-living diecious nematode *Panagrellus redivivus* have also been isolated (Lower, Hansen, Cryan and Yarwood, 1969; Lower, Willet and Hansen, 1970). One strain died when grown under deep culture conditions, the other was selected to survive 32°C (as compared with the standard growth temperature of 20°C).

The use of physical and chemical mutagens greatly enhances the frequency of mutation. Ethyl methanesulfonate (EMS) and 5-bromouracil (5-BU) have been used to induce mutations in *Panagrellus silusiae* (Samoiloff and Smith, 1971). A decrease in fecundity was associated with mutagenesis. Chemicals and X-rays were used to generate dwarf mutants in the diecious phytoparasite, *Aphelencoides composticola* (Person, 1973). Exposure to EMS has resulted in dwarf mutants of *C. elegans* (Cadet and Dion, 1973) as well as behavioural mutants (Brenner, 1973). Developmental and behavioural mutants of *C. elegans* have also been reported (Pertel, 1973). Some of these mutants express their abnormalities at specific points in the life cycle, particularly the third stage.

There is an obvious difference in the stages of the life cycle of parasitic nematodes; they often change from free-living to parasitic forms. In the free-living species these changes may not be as well defined. Indeed, many experiments have been reported where the stage of the nematodes was not considered, although many differences do occur. The cuticle of *P. silusiae* differs in structure in the various stages (Samoiloff and Pasternak, 1968). Isozymes are present in varying concentrations throughout the life cycle of *P. redivivus* (Chow and Pasternak, 1969).

In *P. silusiae* sex attraction is produced only by fourth larval stage and adult females and only fourth larval stage and adult males are competent to respond (Cheng and Samoiloff, 1971). The change in behaviour of *Ancylostoma tubaeforme* during its pre-infective larval stages has been documented (Croll, 1972). Developmental mutants of *C. elegans* which are expressed at the third larval stage as well as some developmental and behavioural mutants which are expressed only before or after the third larval stage have been isolated (Pertel, 1973). This suggests that there is stage specific gene action.

The concept of stage specific gene action is relatively novel. The mechanism by which genes do control the complex processes of development are gradually being elucidated. In insects, the initiation factors required for protein synthesis have been shown to be stage specific (Ilan and Ilan, 1971). Factors isolated from one stage of development will promote formation of the initiation complex only when the mRNA is from the same stage of development. The specificity lies either in the secondary structure of the mRNA or the possible existence of stage specific oligonucleotides preceding the AUG codon and recognizable only by a unique initiation factor.

Since insect and nematode development have often been compared it is likely that stage specific genetically regulated processes also occur in nematodes. If this is the case, it should be possible to generate a mutant which expresses its abnormal characteristic at specific stages. Several mutants were isolated, one of which, the corkscrew (CS) mutant, exhibited stage specific behaviour. This behaviour was analyzed throughout the life cycle. Coincident with this analysis other behaviour patterns in the stages of normal *P. redivivus* (such as attraction to various bacteria and response to osmotic stress) were examined.

II.

MATERIALS AND METHODS

Stock cultures of *Panagrellus redivivus* N strain were grown xenically in autoclaved medium consisting of three parts whole wheat flour to one part water. Mutants were grown monoxenically on *Escherichia coli* on Rothstein's medium (Heib and Rothstein, 1968) which consists of:

8.5g K_2HPO_4 , 4.2g KH_2PO_4 , 1.0g NaCl, 0.8g NaH_4Cl , 0.1g Na_2SO_4 , 0.05g $MgCl_2 \cdot 6H_2O$, 0.01g $CaCl_2$, 0.0005g $FeSO_4 \cdot 7H_2O$, 1.0g glucose, 16 g agar per litre distilled water, pH6.9-7.0.

Larvae were separated from adults by the method of Kriger and Samoiloff (in Press) using a Unichem Filter Sampler (Unichem Corp. Fairburn, Georgia).

A. Generation of Mutants

Larvae were removed from a culture of N strain *P. redivivus*. The remaining adults were washed, centrifuged at 6000g for 10 min and placed in 0.25 mg/ml N, methyl-N-nitroso-N'-nitrosoguanidine (K + K Laboratories, Inc.) for four hours. They were then washed in distilled water and centrifuged at 6000g for 10 min, three times. The mutagen treated nematodes were then placed on Rothstein's medium in 100 x 15 mm plastic petri dishes and allowed to mate

at random for two weeks (four generations). Cultures were then screened for any abnormally swimming animals. Abnormal nematodes were crossed with other nematodes with the same abnormality or with normal animals. Individual crosses were performed in 15 mm diameter depression spots containing Rothstein's medium. The offspring from each cross were examined. In some cases crosses between two abnormal or an abnormal and normal individual were made, in other cases the F_1 individuals of a pair were allowed to mate with each other. When offspring from the same parents exhibited the same abnormality it was considered to be a mutant line (Appendix I). Many of the mutations proved to be unstable and showed damping out effects. One mutant showed a corkscrew type of motion following a spiral path in fluid with increased movement of the anterior end. This mutant was called corkscrew (CS) and all subsequent discussion of mutants refers to this line.

Progeny of crosses of CS animals expressed the abnormal trait in ratios varying from 1:1 to all CS. The corkscrew trait was more pronounced in older larvae and adults. Inbreeding of CS animals, with the exclusion of all crosses that produced normal progeny, led, after six generations, to the establishment of a true-breeding

CS line. Breeding experiments using individuals from this stock were performed (Table I). When either parent was CS the progeny expressed the trait. Crosses of F_1 heterozygotes produced CS and normal offspring in a 3:1 ratio (Table II). Crosses of F_1 heterozygotes and normal animals produced a 1:1 ratio of CS and normal progeny (Table III).

CS animals show decreased fecundity as compared with normal animals or heterozygotes (Tables I and III). Larvae from CS parents have a lower mortality rate than larvae from normal or heterozygous parents.

B. Stage Specific Behaviour

1. Stage Specific Normal Behaviour

a) attraction to bacteria

The response of *P. redivivus* L 2's, males and females to *E. coli*, *Proteus morganii*, *Pseudomonas aeruginosa* and sterile nutrient agar was tested using the "Mickey Mouse Maze" (Samoiloff, McNicholl, Cheng and Balakanich, 1973). Nine ml of Czapex Dox Agar (Oxoid) was placed in the lid of a 100 x 15 mm plastic petri dish. The mold was placed in the liquid agar (Fig. 1). Into the test zone a 5mm² block of

nutrient agar containing a 24 hr bacterial growth was placed. Nothing was placed in the control zone. A gradient was established by incubation at room temperature for 18 hrs. The block of agar was removed before adding 10 nematodes to the inoculation zones of six mazes. After 1, 2, 3 and 4 hrs the number of nematodes migrating to the test or control zone (or in the connecting channels) was recorded.

b) Response to Osmotic Stress

Males, females and L2's were tested in each of:

- a) distilled water, b) 0.1 M NaCl c) 0.5 M NaCl
- d) 1.0 M NaCl e) 2.0 M NaCl f) a balanced salt solution, Zimmermann's medium (Dougherty, 1960) which consists of 0.075g MgSO_4 , 0.075g K_2HPO_4 , 0.275g NaCl, 0.3g KNO_3 per 100 ml distilled water pH 6.9-7.0. One nematode was placed in each of 40 depressions containing 0.2 ml of test fluid. After 10, 20, 40, 60, 90 and 120 min the behaviour was recorded as a) normal b) abnormal swimming c) stationary d) stretched out or e) coiled.

2. Comparison of Normal and Mutant Swimming Behaviour

a) frame by frame TV analysis

Single nematodes were placed in 0.2 ml of Zimmermann's medium in a 15 mm diameter depression spot and their behaviour recorded for 20 sec on a Panasonic Tape-A-Vision (Model NV-3020) or a Sony Videocorder (model AV-3650) with a Panaview TV Camera (model WV-400P) and a Sony Solid State television set using Memorex Chroma 1/4 in magnetic tape. The camera was mounted on an Olympus dissecting microscope and recordings were made at a magnification of 40X. Thirty each of L2's, L3's, L4's, females and males of both normal and CS were recorded. By slowly replaying the tape, the position of a nematode in succeeding frames (1 frame = 1/30 sec) was traced onto a transparency (Fig. 2). The length of the nematodes (measured with a Dietzen Plan Measure), the number of frames required for a wave of contraction to pass along the length of the nematode and the angle through which the head passed while the wave of contraction passed along the animal were obtained from this frame by frame analysis.

b) Analysis of tracks in agar

Tracks of nematode movement were produced as described by Croll (personal communication). Czapek Dox Agar (1.5%) was poured into and immediately out of a 60 x 15 mm plastic petri dish (the lid of a 35 x 10 mm dish for L2's). A nematode was placed in the center of the plate and allowed to migrate for 15 min (10 min for adults). In the darkroom a piece of Kodak Kodalith Ortho film type 3 was placed under the plate and parallel light shone through. Everywhere the nematode had been resulted in the refraction of the light and the production of a track. Between 20 and 30 tracks for each of L2's, L3's, L4's, females and males both normal and CS were obtained. Tracks were analyzed for the total distance travelled in 15 min (measured in mm) and the number of loops, reversals, turns and waves for every 10 cm travelled (Fig. 3).

III.

RESULTS

A. Stage Specific Behaviour of Normal Nematodes

1. attraction to bacteria

Males and females showed no preference for nutrient agar and larvae remained in the inoculation zone (Table IV, Fig. 4). Males were more attracted to *P.morganii* and *E. coli* than females while larvae remained in the inoculation zone (Figs. 5 and 6). Larvae showed a greater response to *P. aeruginosa* than either males or females (Fig. 7). When the attraction was strong the numbers migrating to the bacteria showed a steady increase over the four hours; weaker attraction resulted in a fluctuation in numbers found in the test zone during the four hours.

2. response to osmotic stress

Adult nematodes follow a specific pattern under adverse conditions - first they begin swimming abnormally, then they become stationary, and finally they stretch out, an indication of death. The higher the salt concentration the quicker they pass through the abnormal and stationary phases and stretch out. Females are best able to maintain

normal swimming behaviour under adverse conditions although after 10 min in 2.0 M NaCl all normal swimming behaviour ceases (Table V, Fig. 8). Once females begin swimming abnormally they pass through the stationary and stretched out phases faster than males or larvae (Figs. 9, 10, 11). Larvae show a response that is uncommon in adults - they coil up (Fig. 12). They can remain in this state for several hours and after removal from the salt solution begin swimming normally.

B. Comparison of Normal and Mutant Swimming Behaviour

1. Frame by Frame TV Analysis

By tracing successive frames of nematodes swimming, a series of drawings which divided motion, a complex process, into its component events were obtained (Fig. 2). These component events could then be analyzed and quantitative comparisons of swimming behaviour made. The number of frames on the TV recording (1 frame = 1/30 sec) required for a wave of contraction to pass along the animal varied slightly throughout the life cycle in both CS and normal animals (Table VI, Fig. 13). The most striking difference occurs between normal and mutant females. The angle through

which the head passes is different in mutants, particularly in the second larval stage (Fig. 14). In normal animals the angle increases throughout the life cycle but in mutants it remains constant at a high value. The tracings also facilitate measurement of the lengths of the nematodes. Full-grown mutants are two-thirds the size of normal animals (Fig. 15). CS males and females are approximately the same length.

2. Analysis of tracks in agar

The total distance travelled by the nematodes in 15 min increases during the life cycle with the largest increment occurring between L4's and adults (Fig. 16). Mutants travel farther than normal animals except mutant adult males. The number of loops, reversals, turns and wavelengths were recorded for the distance travelled by each nematode and then converted to a standardized 10 cm path which was used for comparison of stages, sexes, mutant and normal animals. In the L2 and L3 normal nematodes there are more loops than in CS. There is a decrease in looping in L4 and adult normals but at the same time an increase in this characteristic in the mutants (Fig. 17). Reversals follow a similar pattern -

decreasing in normals and increasing in mutants, especially males (Fig. 18). The number of turns decreases in both CS and normal but the mutant adults turn more than their normal counterparts (Fig. 19). The number of waves per 10 cm follows a similar pattern, decreasing throughout the life cycle but consistently higher in mutant than in normal adults (Fig. 20).

IV.

DISCUSSION

Few nematode mutants have been described in detail. A dwarf mutant of *C. briggsae* was inherited as a monofactorial recessive characteristic (Nigon and Dougherty, 1950). It decreased fecundity and the viability of the offspring. Fertility at 23°C was dominant to sterility at that temperature in *C. elegans* (the Bristol strain was heat resistant, the Bergerac strain heat sensitive), (Fatt and Dougherty, 1963). These characteristics segregated as simple Mendelian factors giving rise to the expected numbers of sensitive and tolerant progeny. Two dwarf mutants of *C. elegans* are recessive and independent autosomal mutations (Dion and Brun, 1971). Seven other dwarfs of *C. elegans*, one of which also showed behavioural and morphological abnormalities, have been isolated (Cadet and Dion, 1973). These also exhibited monofactorial determination. Three recessive autosomal dwarf mutations of *Aphelencooides composticola* have been described; one was generated by X-rays and the other two, which are alleles of the same gene, by EMS (Person, 1973). Other authors have obtained mutants but have not published the genetics of the abnormalities (Samoiloff and Smith, 1971; Brenner, 1973; Pertel, 1973).

The CS mutant of *P. redivivus* is a dominant mutation as evidenced by the results of crosses of mutants and normals, crosses of F_1 hybrids and backcrosses. The number of abnormal animals is higher than expected because even in normal populations approximately 3% of the adults show some type of non-genetic behavioural abnormality. The CS characteristic does not appear to be sex-linked or sex limited since sex ratios were always close to 1:1, however some of the aspects of this behaviour may be more extreme in one sex than the other. Associated with the mutation is a decrease in fecundity which may have four possible causes:

- 1) ineffectiveness of males in swimming or responding to females
 - 2) inability of females to attract males
 - 3) premature death of females followed by *endotokia matricida* which occurs more frequently in mutants
 - 4) decreased viability of fertilized eggs within the female.
- The first possibility seems unlikely because the crosses of mutant males with normal females resulted in slightly higher fecundity than the reciprocal crosses. The second suggestion is also unlikely because if females are unable to attract males and mating is due to random collisions the CS ♀ x N ♂ and N ♀ x CS ♂ crosses should show a greater difference in progeny. It is possible that the

small size of the female as compared with normal females accounts for increased *endotokia matricida*. If this were the case a greater differential should exist between the fecundity of CS ♀ x N ♂ and N ♀ x CS ♂ crosses. It is also possible that the reduced fecundity is due to a decreased viability of fertilized eggs where both the male and the female play a deciding role. This is supported by the fact that larvae of two CS parents have a lower mortality rate than the larvae of mixed or normal parents, indicating that almost all eggs that complete embryogenesis and hatch will ultimately give rise to mature animals.

The stage and sex of nematodes used in an experiment are often not clearly stated. There is a distinct difference between males and females in life span, osmotic tolerance and nutritional requirements (Abdulrahman, in Press). The larvae of free-living nematodes are usually regarded simply as undeveloped adults and rarely as separate genetic entities. The response of the different stages to bacteria and their behaviour under stress indicate that larvae do possess some behavioural attributes unique to that stage. In response to all the bacteria tested except *Pseudomonas aeruginosa* males and females were attracted to slightly different extents but larvae tended to remain in the inoculation zone. When *P. aeruginosa* was the test organism not only did the larvae

respond, but they did so to a greater degree than either males or females. This can only be explained by the existence of larval receptors which are different and more discriminating than those of the adult. This is not surprising when one considers that in parasitic forms of nematodes the pre-infective and parasitic stages may be opposite in their environmental responses.

Larvae exhibit another response which is uncommon in adults - that of coiling up in an adverse environment - which enables them to survive the same conditions that kill adults. The dauer larvae forms of several parasites are also able to survive hostile environments.. Adults are able to coil up but they do so randomly and not as a specific response to the environment. The ability of larvae to coil up under adverse conditions infers that there are genetic instructions at this stage which are missing or inactive in adults.

The most impressive evidence for a special larval array of activities comes from the swimming behaviour of the stages of normal and CS nematodes. The results of the frame by frame analysis show that although the time required for a wave of contraction to pass over an animal is the same for all stages, the angle through which the head passes varies considerably. Normal larvae do not move their

heads much indicating that they are not actively sampling their environments whereas adults do. The mutants swing their heads actively from side to side in all stages of the life cycle. This corresponds to the activated state of behaviour as proposed by Samoiloff, Balakanich, and Petrovich, (1974). This characteristic of the mutant is the only one that is exhibited by larvae as well as adults.

Croll and Blair (1973) have demonstrated that the tracks produced by nematodes in agar are a reliable and predictable means of analyzing the movement of nematodes. The tracks obtained for normal and CS *Panagrellus* provide much information on their movement patterns. Consistent with the larval head inactivity obtained by the frame by frame analysis and their response in the Mickey Mouse Maze is the fact that larvae do not move a great deal on an agar plate. This is true for both mutants and normals. However, larvae do exhibit more looping, more reversing and turning than adults indicating that they are very active although they do not travel far. Since the wavelength is a direct function of the body length it is reasonable that larvae have more waves per 10 cm than adults, and mutants, since they are smaller, have more waves than normals. These results clearly demonstrate that, for most parameters tested, mutant and normal larvae are more similar whereas mutant and normal adults and in fact mutant males and females differ widely.

The CS mutation will be valuable as a marker gene for further genetic studies of *P. redivivus*. It is a dominant characteristic which segregates in a Mendelian fashion giving rise to expected numbers of normal and CS progeny. The expression of this behaviour is dependent upon the stage of the animal - second stage larvae very closely resemble normal larvae. Pertel (1973) has evidence that genetically, the crucial stage for free-living as well as parasitic nematodes is that of the third or pre-infective stage. This study supports this and gives further evidence that in free-living nematodes there may be a distinct set of genes which are activated specifically for larvae and another set which are active in the adult stage. The CS mutation becomes strongly expressed only when the animal is an L4 or adult indicating that it is at the third larval stage where the transition occurs.

The nature of this mutation which primarily causes the nematodes to swim in a strange manner could be due to a muscular or nervous defect, or both. Further experiments such as electron microscopy to see if there are any distinct lesions must be undertaken to determine the exact nature of the defect. Because the organism is eutelic neither the nerve nor the muscle cells are dividing. The means by which an abnormality affecting these cells can be expressed at one stage and not another presents a problem fundamental to developmental biology.

V.

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Table I Results of Crosses F₁ Generation

CROSS	CS ♀ x CS ♂	CS ♀ x N ♂	N ♀ x CS ♂	N ♀ x N ♂
No. Crosses	13	16	12	19
No. Abnormal Larvae	420	445	510	0
No. Normal Larvae	43	59	62	1593
Total Larvae	463	504	572	1593
No. Abnormal Adults	433	410	483	39
No. Normal Adults	0	0	0	1306
Total Adults	433	410	483	1345
Fecundity (avg. no. larvae/pair)	35.6 ± 5.6*	31.0 ± 4.3	48.2 ± 7.8	83.8 ± 6.6
Mortality	4%	19%	16%	16%

* standard error

Table II	Results of Crosses		F ₂ Generation	
Parental Cross	CS ♀ x CS ♂	CS ♀ x N ♂	N ♀ x CS ♂	
F ₁ self	CSCS x CSCS	CSN x CSN	CSN x CSN	
No. Abnormal progeny	835	2085	1926	
No. Normal progeny	0	643	628	
ratio A:N	-	3.2	3.1	
x ²	-	2.47	.23	
x ² theoretical at p = .05	-	3.84	3.84	

Table III Results of Crosses F_1 Backcross

Parental Cross	CS ♀ x N ♂	CS ♀ x N ♂	N ♀ x CS ♂	N ♀ x CS ♂
F_1 Cross	CSN ♀ x N ♂	N ♀ x CSN ♂	CSN ♀ x N ♂	N ♀ x CSN ♂
No. Crosses	12	7	10	10
No. Abnormal progeny	381	230	331	360
No. Normal progeny	352	262	268	344
Fecundity	61.0 ± 11.8	74.6 ± 13.2	61.7 ± 14.8	70.4 ± 12.7
Ratio A:N	1.1	.9	1.2	1.0
χ^2	1.14	2.08	3.28	.36
χ^2 theoretical at p = .05	3.84	3.84	3.84	3.84

Table IV Response of N strain *P. redivivus* to bacteria

Nutrient Agar				<i>P. morganii</i>				<i>E. coli</i>				<i>P. aeruginosa</i>			
Females	Time (Hrs)	No. in Test	No. in Control	No. in Innoculation zone	No. in Test	No. in Control	No. in Innoculation zone	No. in Test	No. in Control	No. in Innoculation zone	No. in Test	No. in Control	No. in Innoculation zone		
	1	28	17	106	38	16	86	34	15	86	32	13	77		
	2	25	20	84	30	16	56	39	15	56	43	9	48		
	3	23	25	78	34	14	46	44	16	46	43	8	38		
	4	28	26	82	29	9	39	51	14	39	49	5	37		
Males	1	18	17	120	31	18	92	30	13	92	20	16	97		
	2	20	25	100	42	13	67	43	18	67	35	5	77		
	3	14	24	96	52	3	57	42	12	57	33	5	68		
	4	24	20	79	61	3	38	60	16	38	45	5	54		
Larvae	1	0	0	154	2	0	129	2	4	129	25	7	70		
	2	2	4	146	4	2	117	8	1	117	40	7	49		
	3	5	7	146	9	2	109	6	4	109	57	7	38		
	4	6	4	139	7	1	104	8	3	104	62	5	40		

Table VI Stage Analysis of Swimming Behaviour

Stage	Distance /15 min	Loops /10cm	Turns /10cm	Reversals /10cm	Wavelengths /10cm	Length μ	Frames /wave	Angle head passes through (degrees)
Normal L2	17.9 ± 3.3*	15 ± 2	140 ± 10	20 ± 4	710 ± 22	280 ± 8	13.2 ± .4	53.6 ± 3.3
CS L2	24.4 ± 5.0	10 ± 2	130 ± 13	21 ± 4	774 ± 17	239 ± 6	12.9 ± .5	127.8 ± 7.7
Normal L3	31.8 ± 4.5	11 ± 2	117 ± 11	16 ± 4	561 ± 17	384 ± 8	12.8 ± .4	71.9 ± 4.8
CS L3	57.8 ± 7.5	9 ± 1	102 ± 9	13 ± 3	546 ± 15	314 ± 9	11.2 ± .4	143.6 ± 7.0
Normal L4	63.7 ± 7.8	6 ± 1	66 ± 6	14 ± 3	398 ± 11	645 ± 16	11.0 ± .3	93.4 ± 7.0
CS L4	70.3 ± 7.5	13 ± 2	77 ± 7	20 ± 4	429 ± 10	496 ± 13	8.3 ± .4	130.2 ± 6.9
Normal °	96.3 ± 8.5	4 ± 1	33 ± 3	9 ± 2	259 ± 9	1112 ± 24	13.4 ± .7	100.5 ± 6.6
CS °	126.9 ± 17.8	8 ± 1	60 ± 6	14 ± 3	377 ± 13	704 ± 14	8.5 ± .5	137.0 ± 7.1
Normal °	92.9 ± 9.6	4 ± 1	36 ± 3	11 ± 2	314 ± 10	910 ± 15	11.0 ± .4	113.4 ± 7.5
CS °	69.3 ± 10.7	10 ± 2	64 ± 8	31 ± 7	379 ± 12	673 ± 12	10.7 ± 1.1	150.2 ± 7.3

* standard error

Figure 1. Mickey Mouse Maze. a = inoculation zone,
b = test zone, c = control zone,
d = connecting channel.

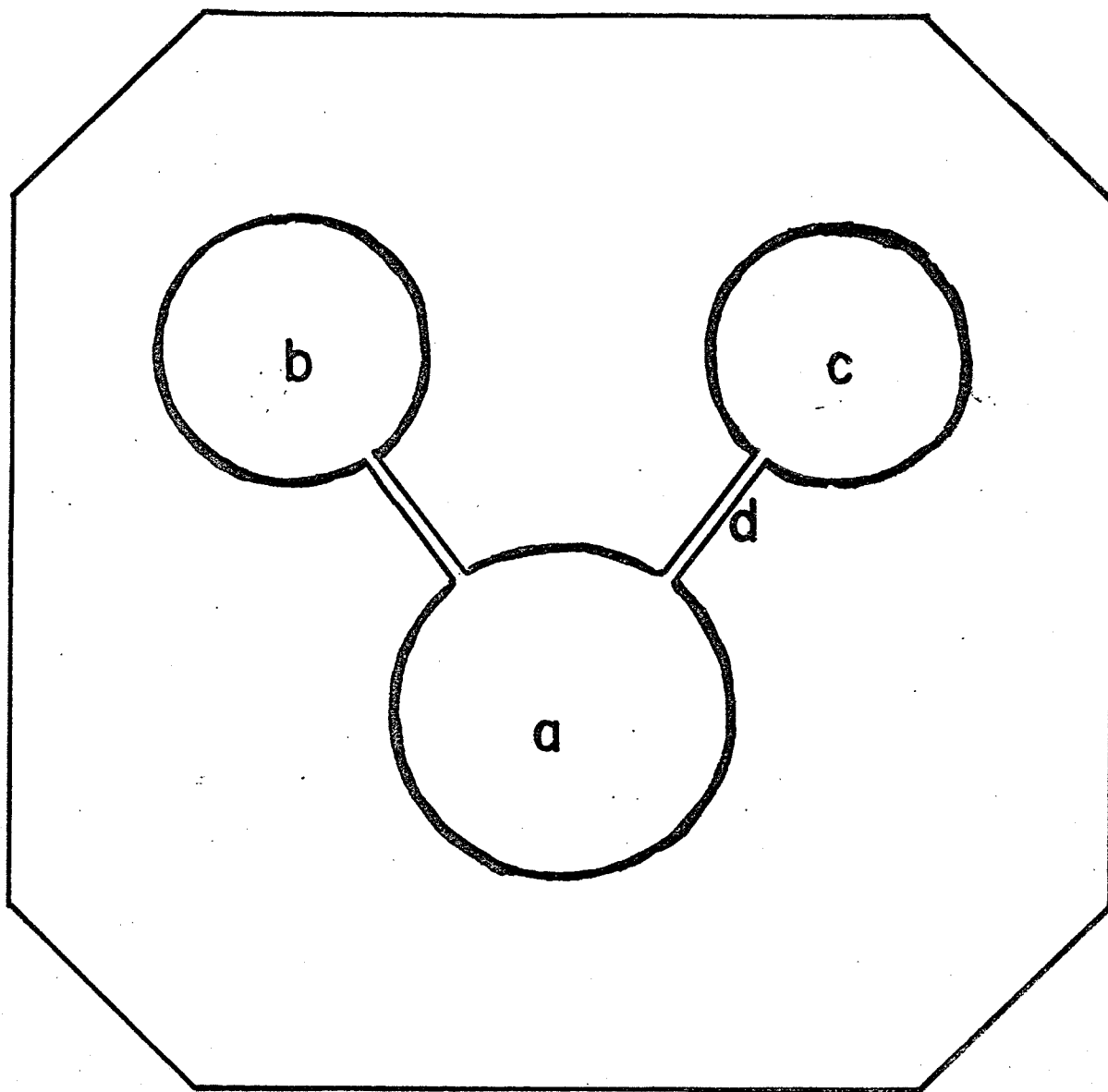
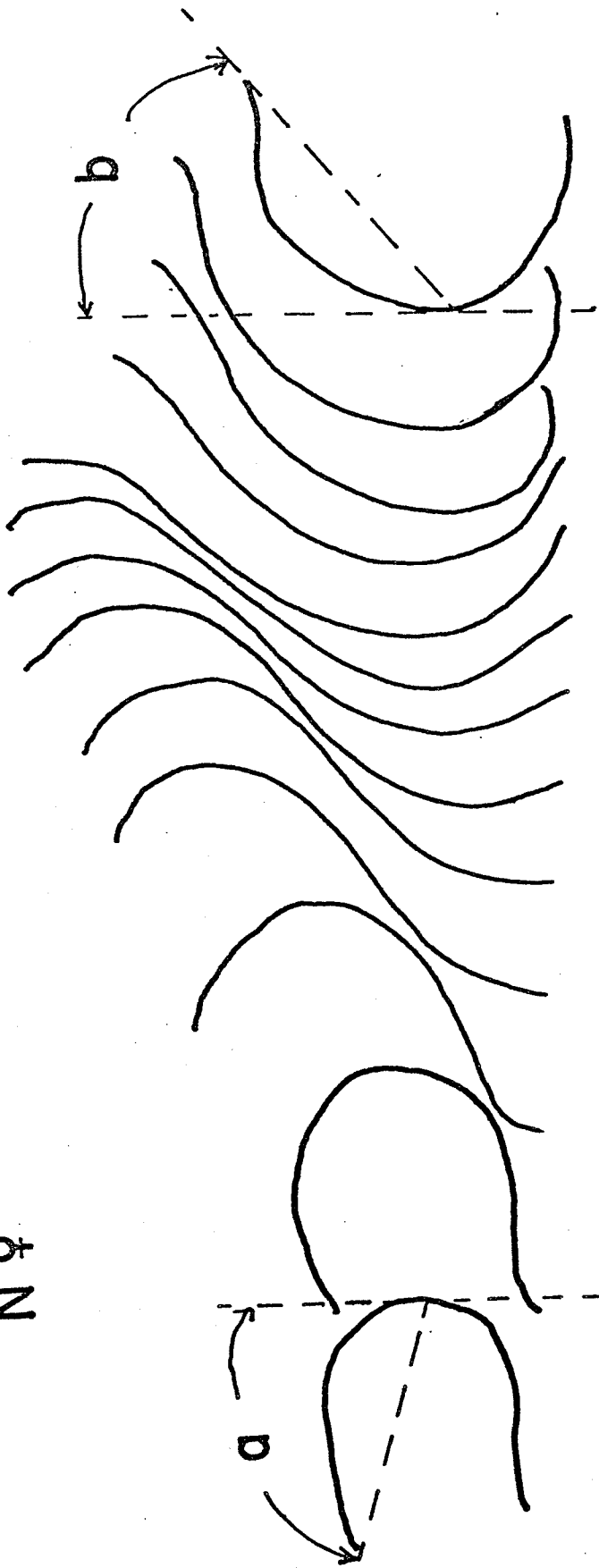


Figure 2. Frame by frame analysis of swimming behaviour.
1 frame = 1/30 sec. a = angle at beginning
of cycle, b = angle at end of cycle.
1cm = 110 μ

N ♀



CS ♀

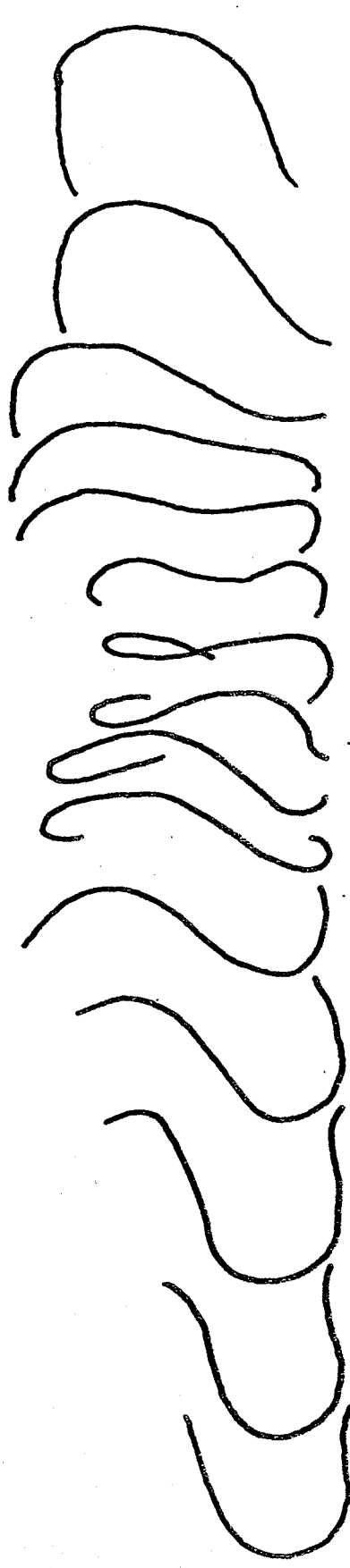


Figure 3. Analysis of track of normal female.
a = loop, b = turn, c = reversal,
d = wavelength, x = beginning of
track.

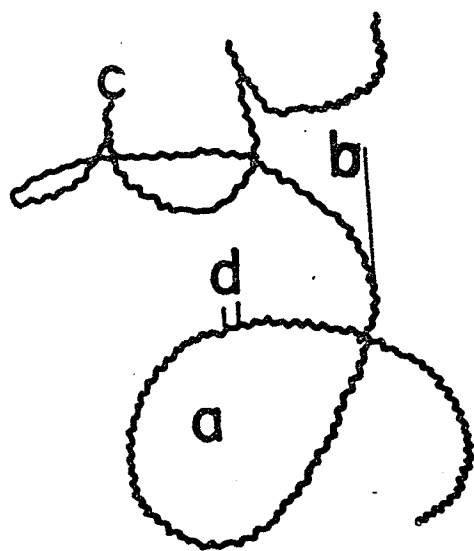


Figure 4. Attraction of *P. redivivus* to nutrient agar.

Figure 5. Attraction of *P. redivivus* to *Proteus morganii*.

Figure 6. Attraction of *P. redivivus* to *E. coli*.

Figure 7. Attraction of *P. redivivus* to *Pseudomonas*
aeruginosa.

Figure 8. Percent of nematodes swimming normally after exposure to various solutions.

▼ - ▼ = females, ▽ - ▽ = males,
● — ● = larvae.

P. redivivus Normal Behaviour

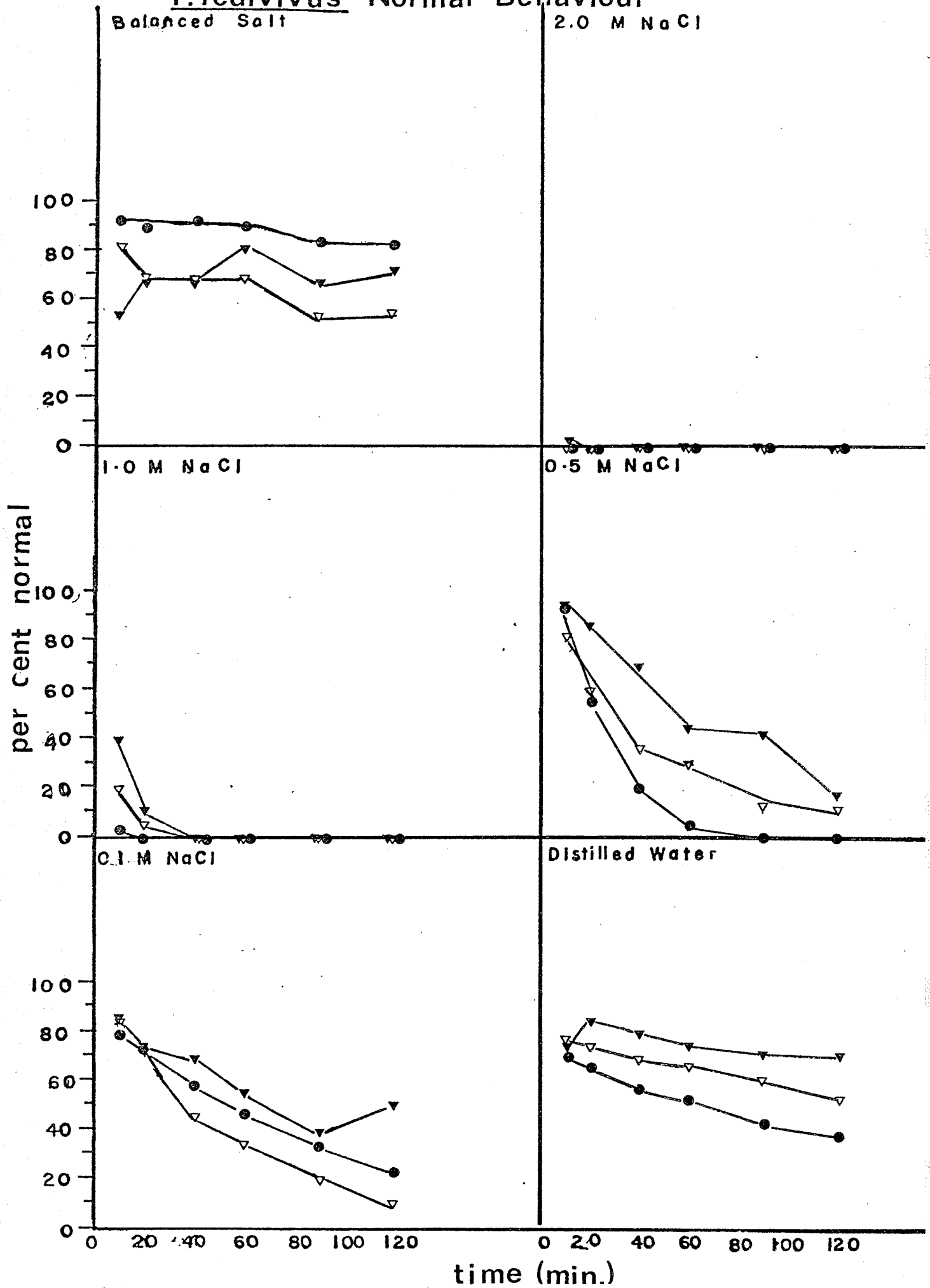


Figure 9. Percent of nematodes swimming abnormally after exposure to various solutions.

▼ - ▼ = females, ▽ - ▽ = males,

● — ● = larvae. -----

P. redivivus Abnormal Behaviour

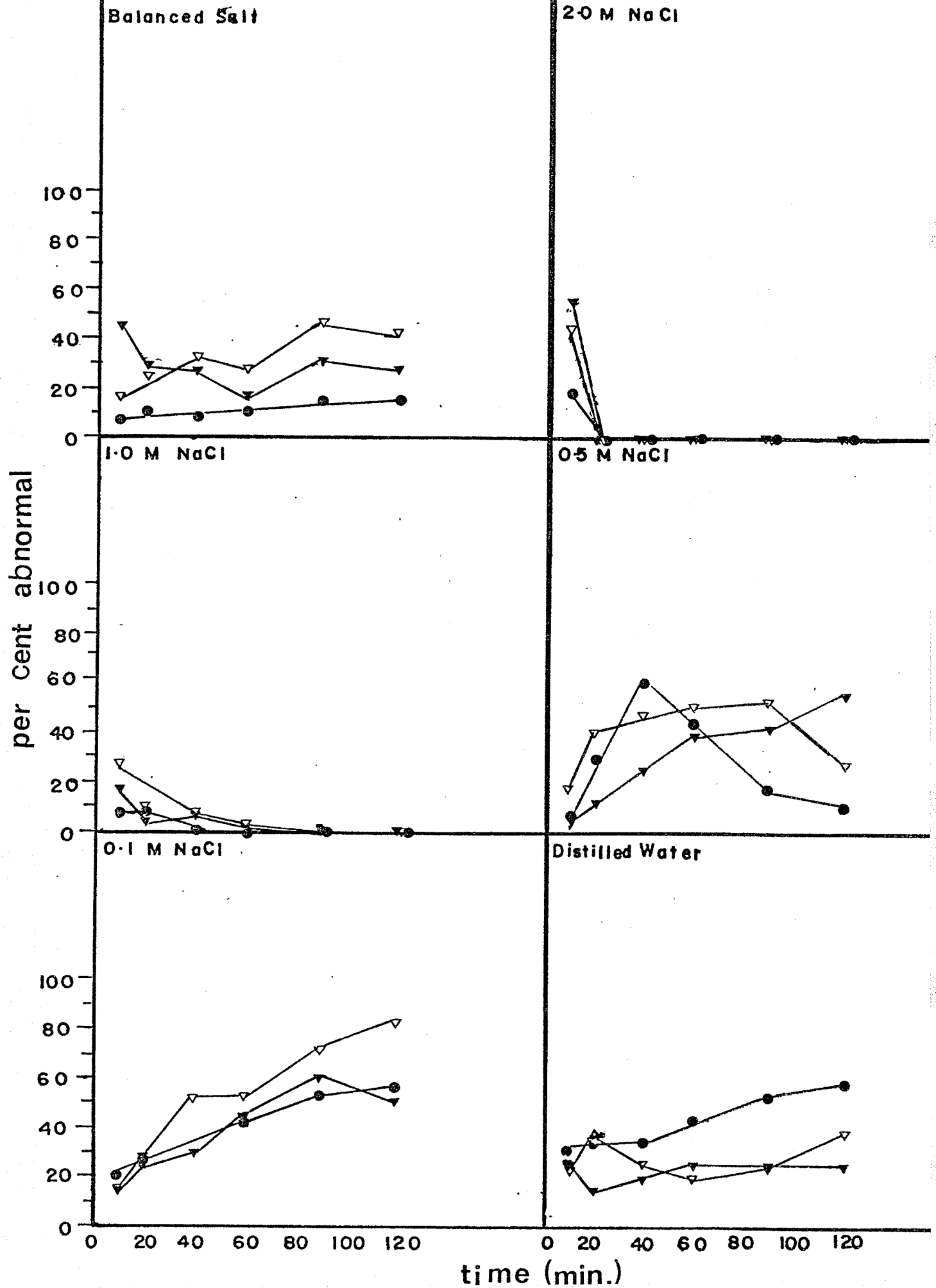


Figure 10. Percent of nematodes stationary after exposure to various solutions.

▼ - ▼ = females, ▽ - ▽ = males,

● — ● = larvae.

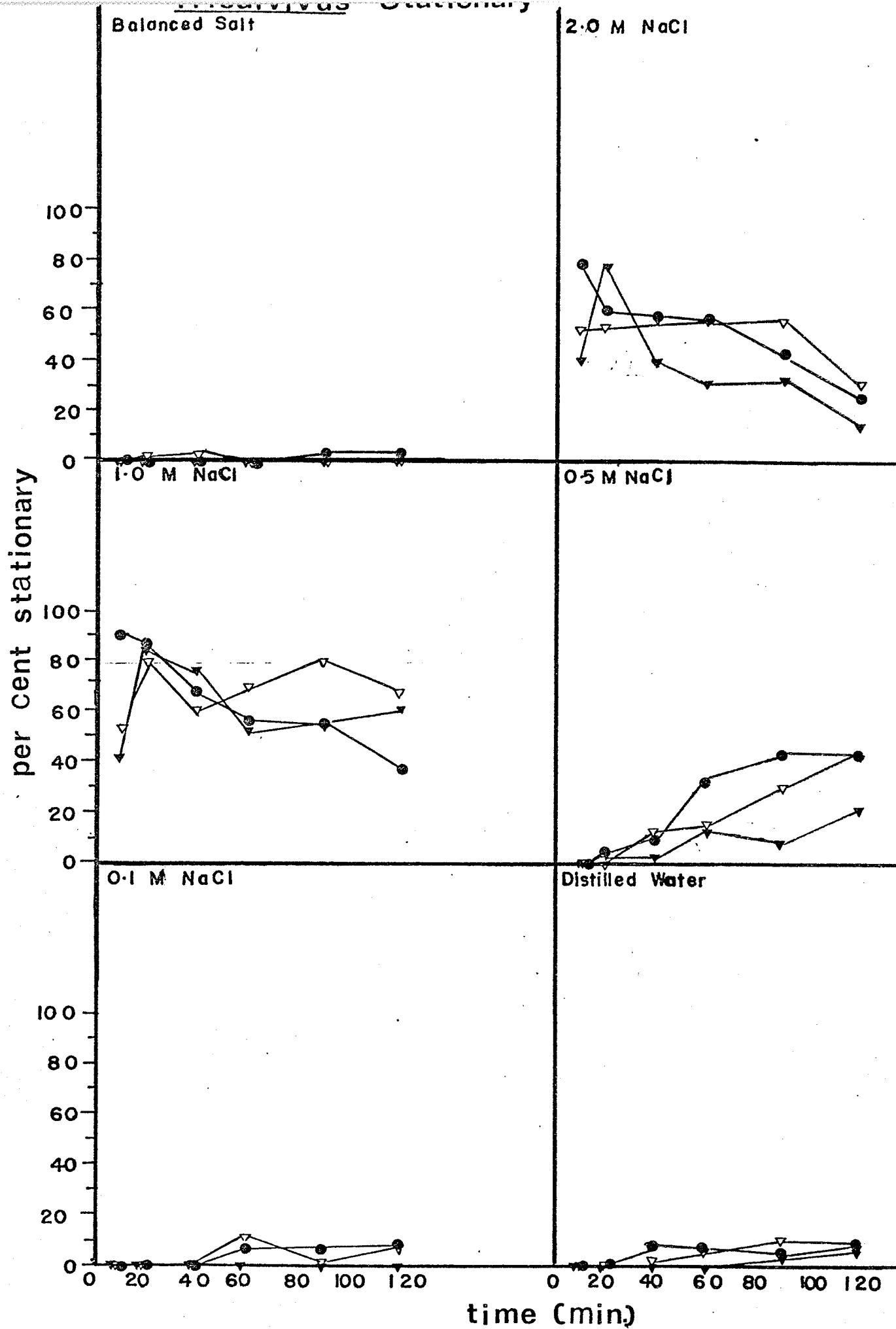


Figure 11. Percent of nematodes stretched out after exposure to various solutions.

▼ - ▼ = females, ▽ - ▽ = males,

● — ● = larvae.

P. redijivus Stretched Out

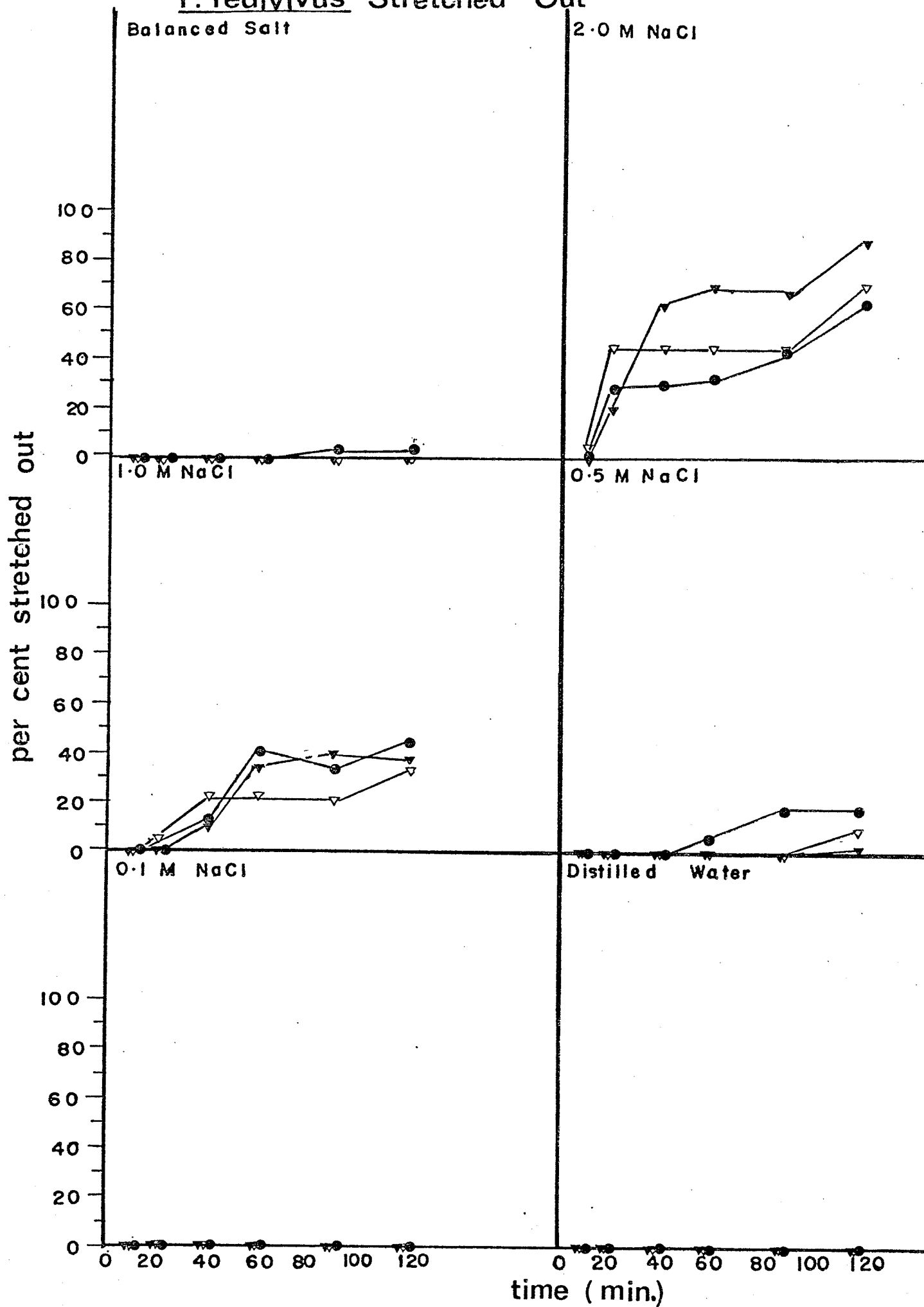


Figure 12. Percent of nematodes coiled after exposure to various solutions.

▼ - ▼ = females, ▽ - ▽ = males,

● — ● = larvae.

P. redivivus Coiled

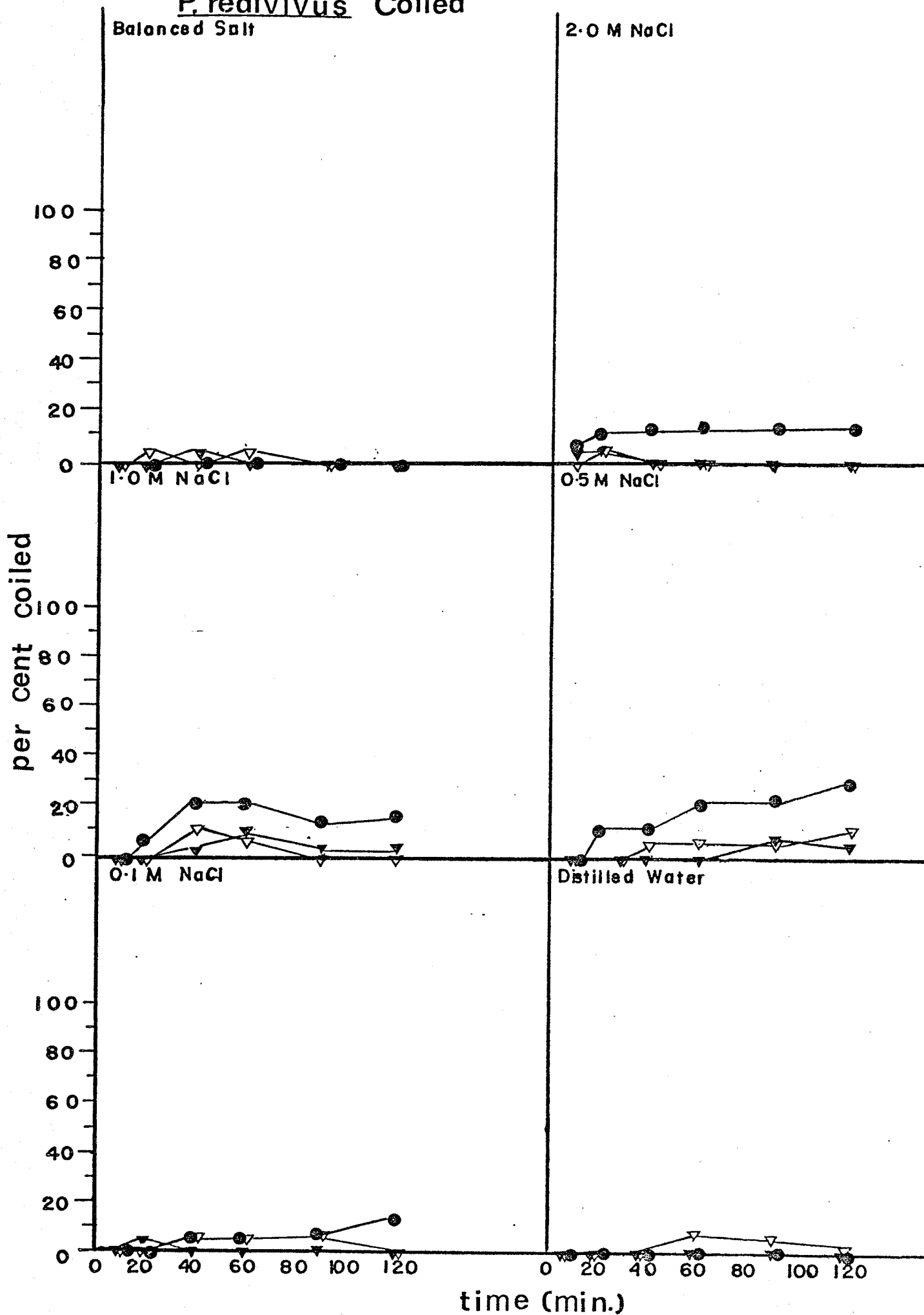


Figure 13. Number of frames required for a wave of contraction to pass over the body of the nematode. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

No. of frames required for a wave of contraction
to pass over the body of P. redivivus

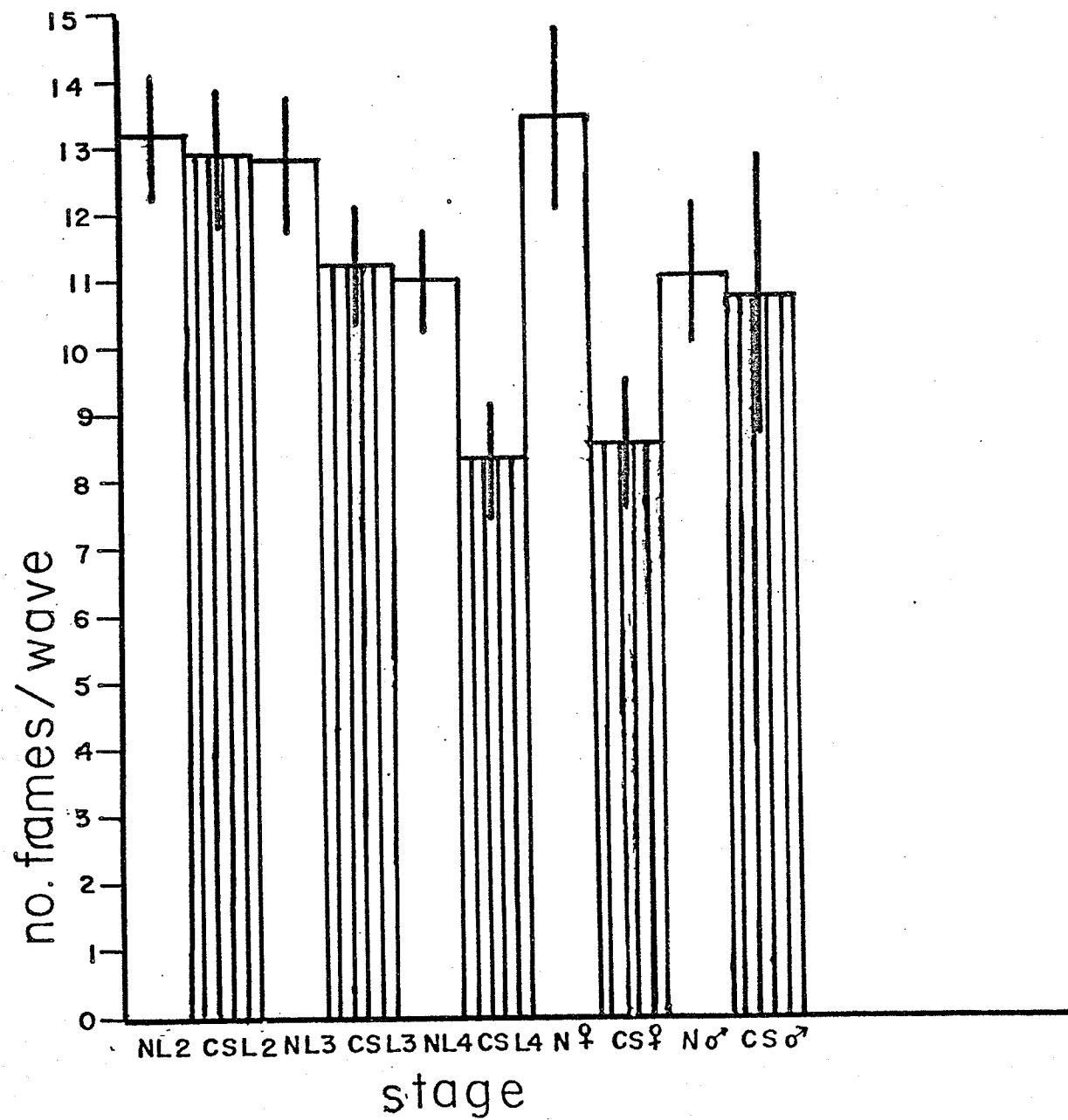


Figure 14. The angle that the head passes through while a wave of contraction passes over the body of the nematode. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

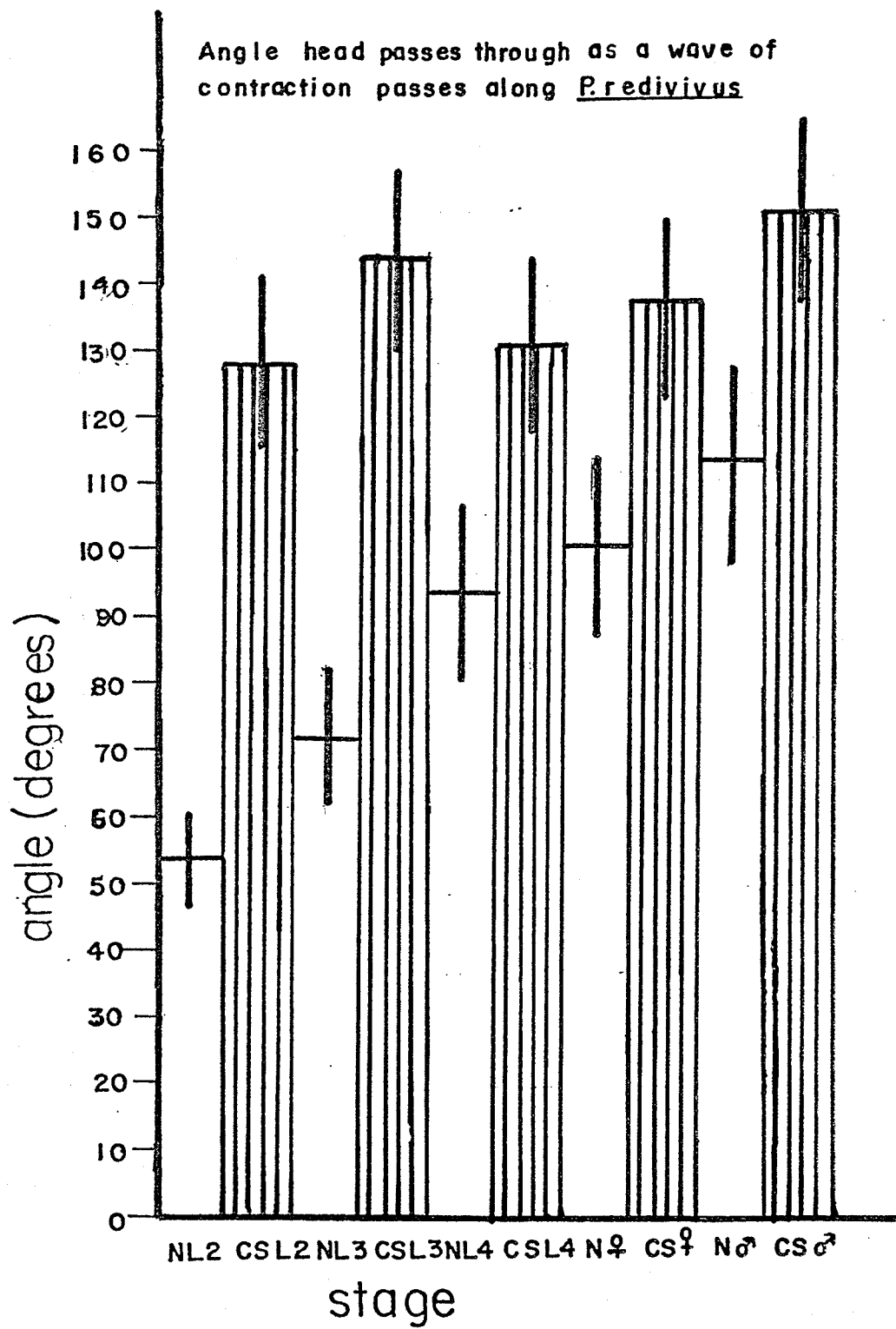


Figure 15. The length of normal and CS nematodes. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

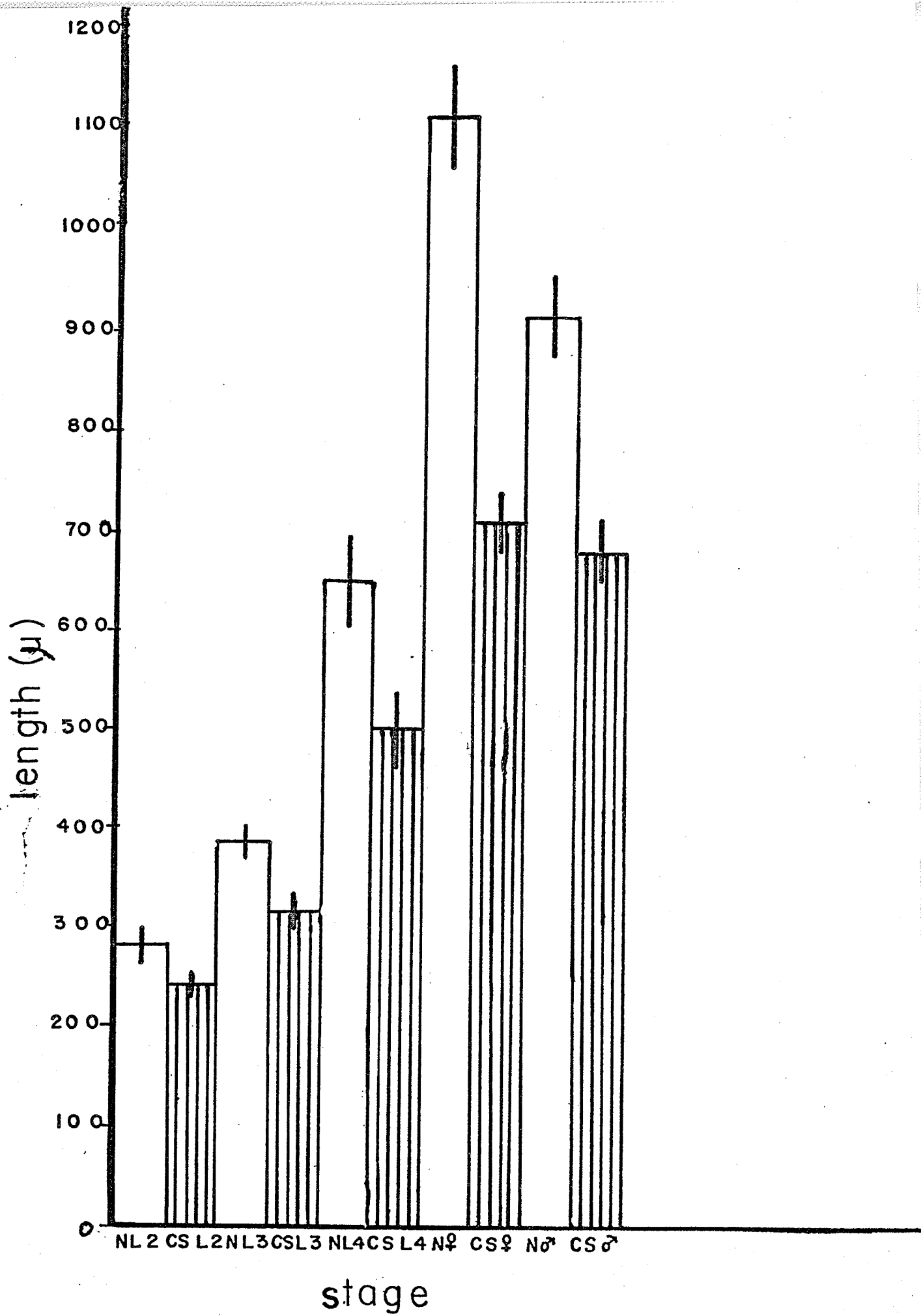


Figure 16. The distance travelled over an agar plate in 15 min. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

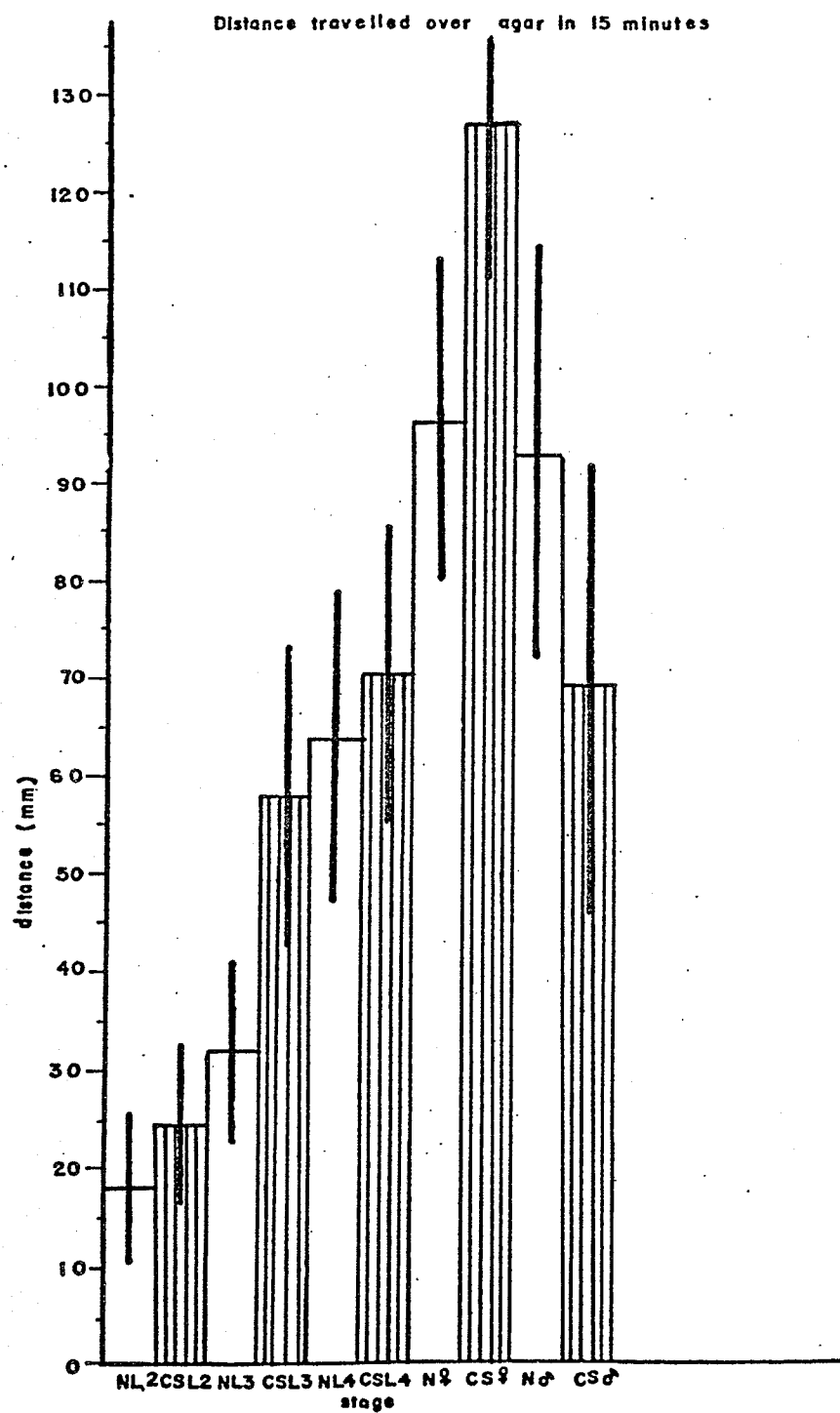


Figure 17. The number of loops made for each 10 cm travelled over an agar plate. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

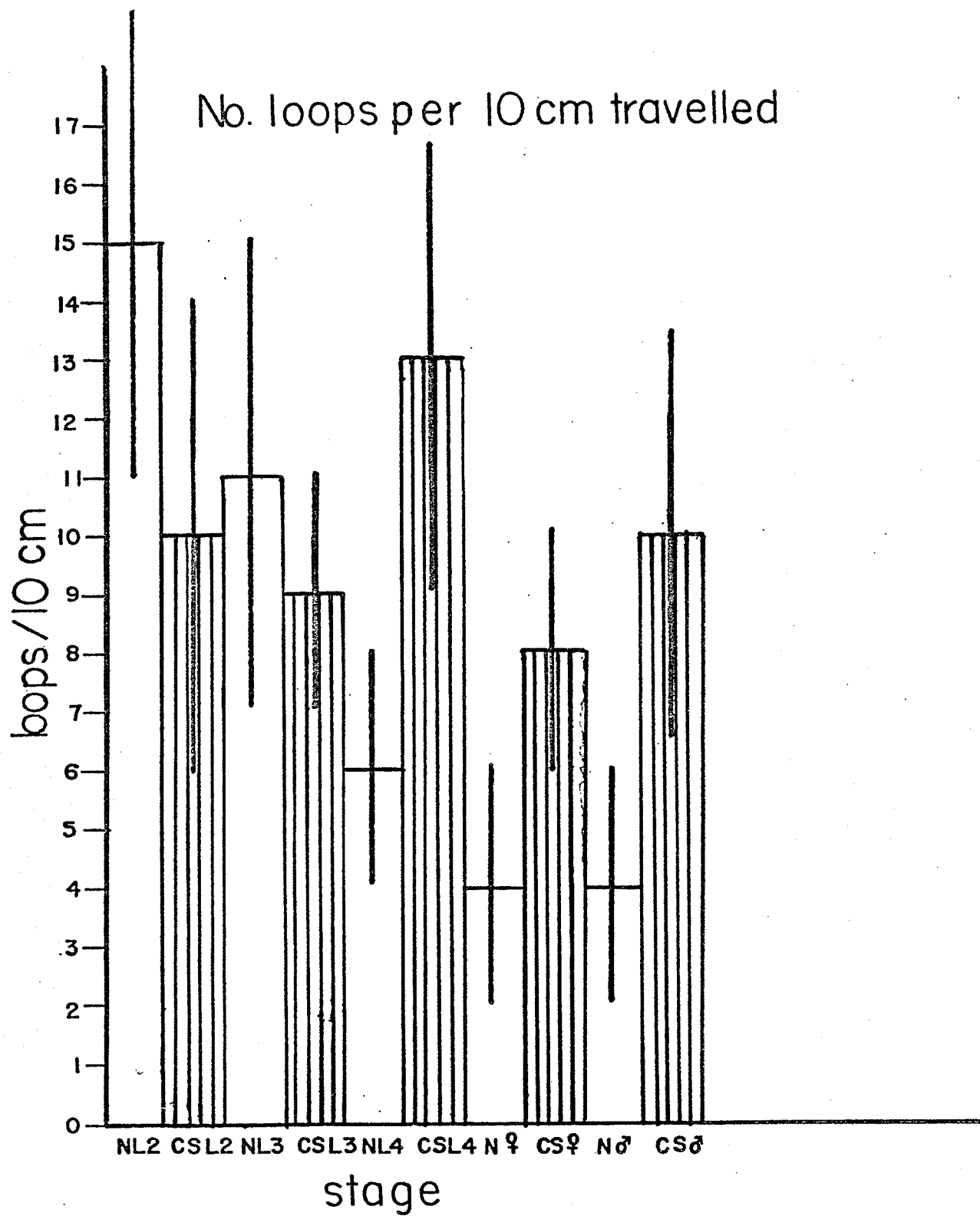


Figure 18. The number of reversals made for each 10 cm travelled over an agar plate. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

No. reversals per 10cm travelled

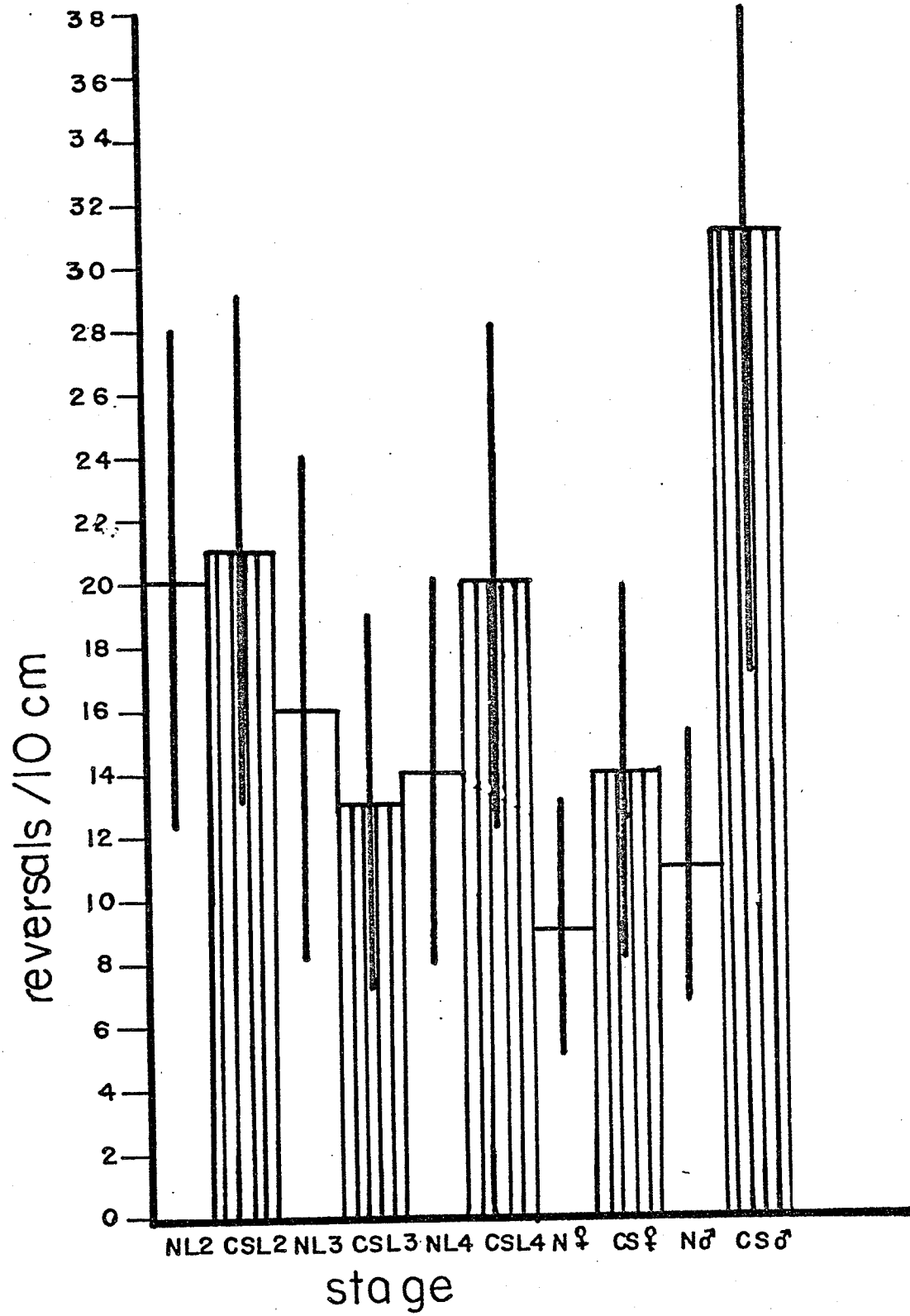


Figure 19. The number of turns made for each 10 cm travelled over an agar plate. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

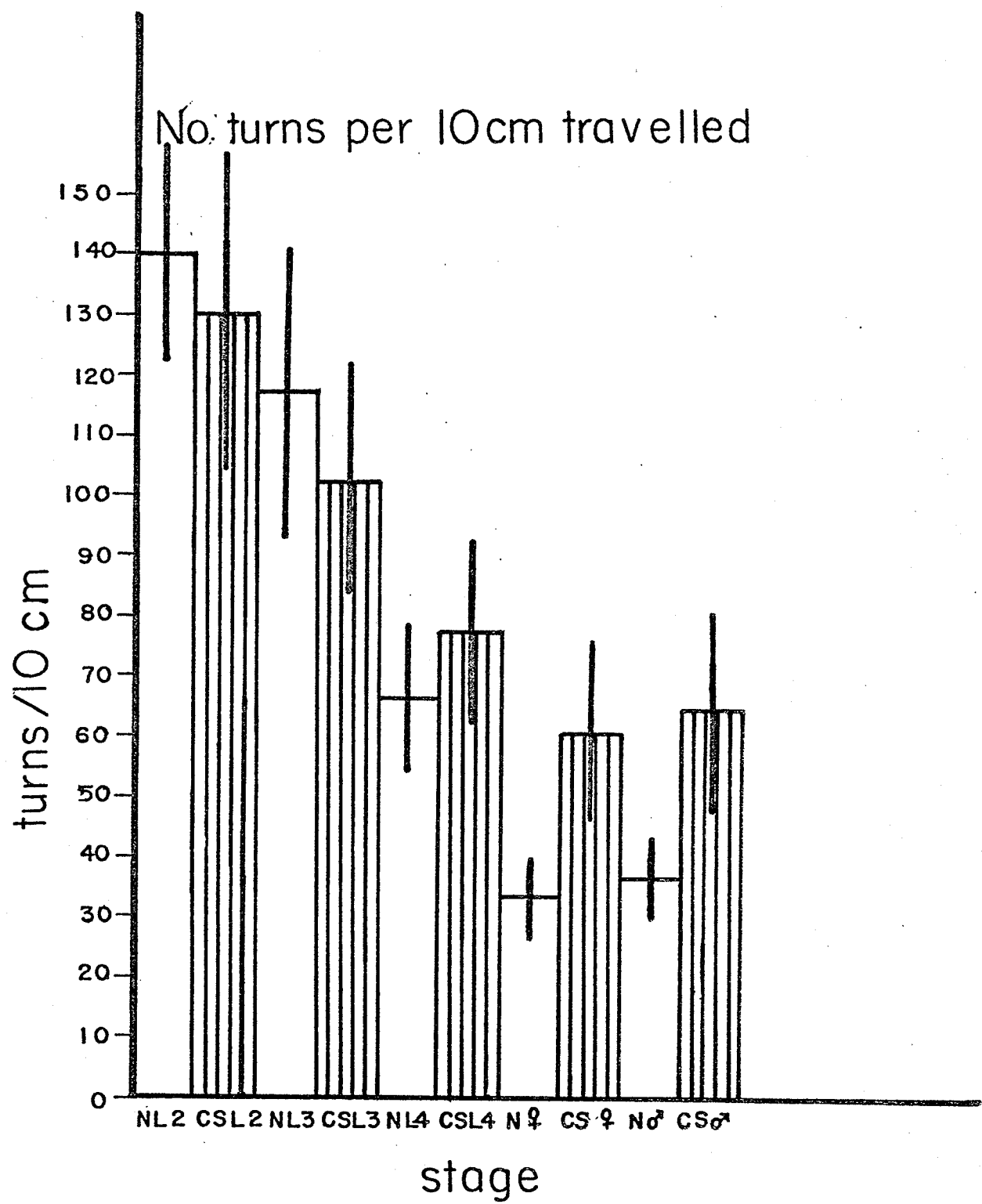
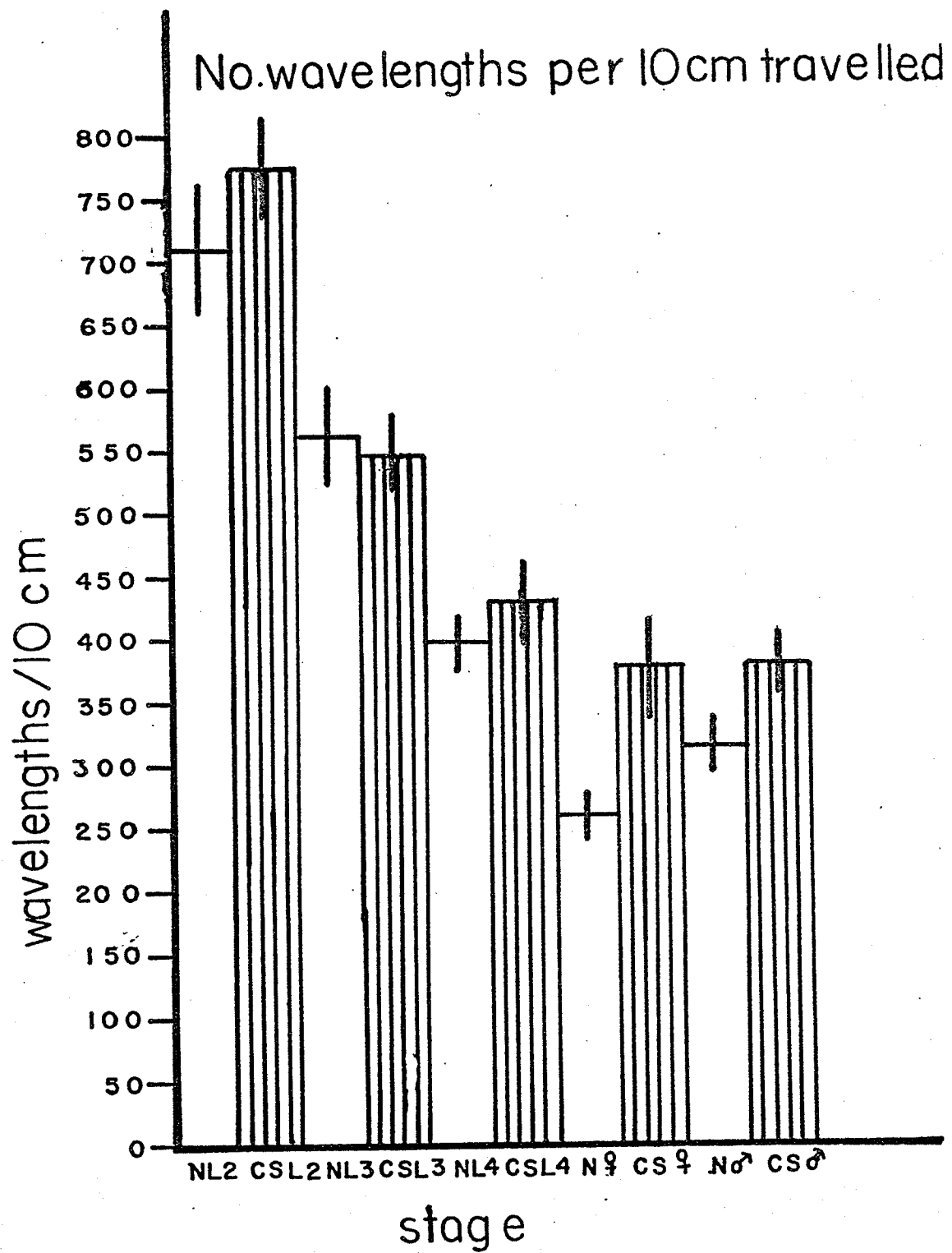
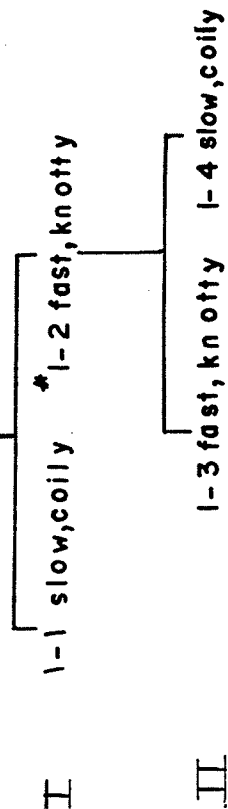


Figure 20. The number of wavelengths for each 10 cm travelled over an agar plate. Vertical lines represent 95 percent confidence limits. Clear bars represent normal animals, striped bars represent CS animals.

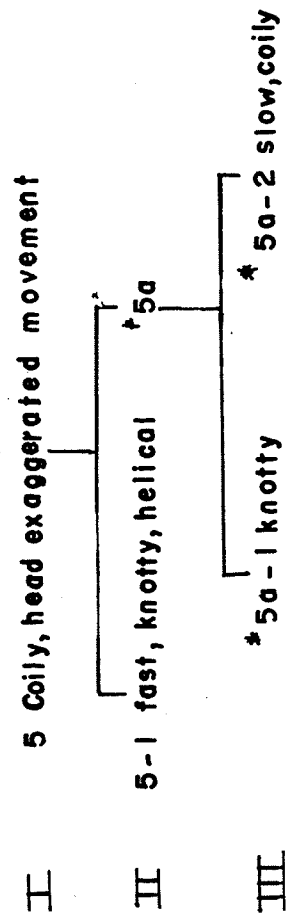


Appendix I Mutants Generated

Cross 1 Tail stiff ♀ X Tail stiff ♂

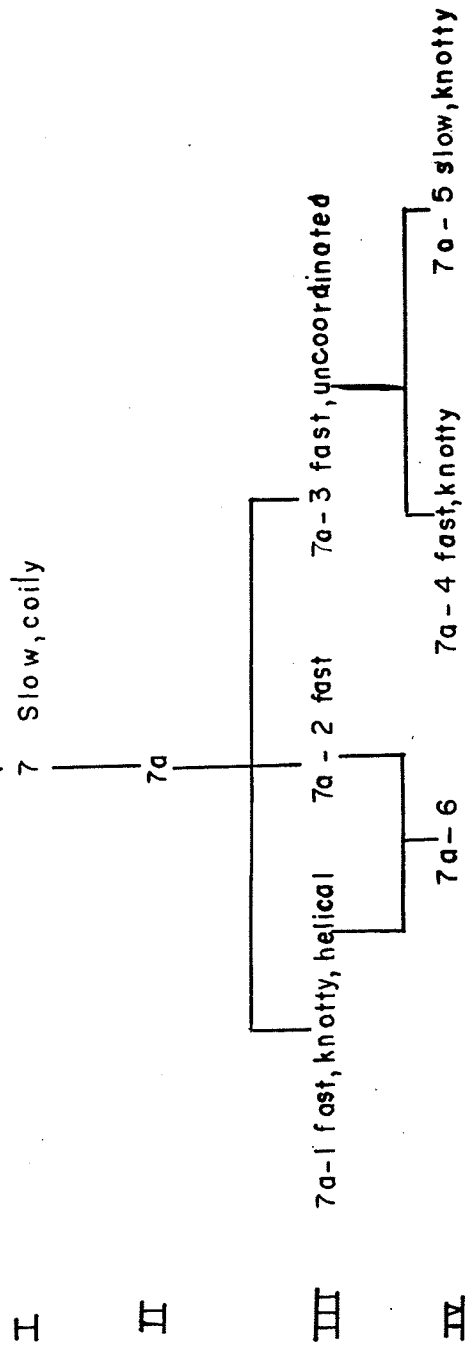


Cross 5 Head twitchy, tailstiff ♀ X Fast, middle stiff ♂

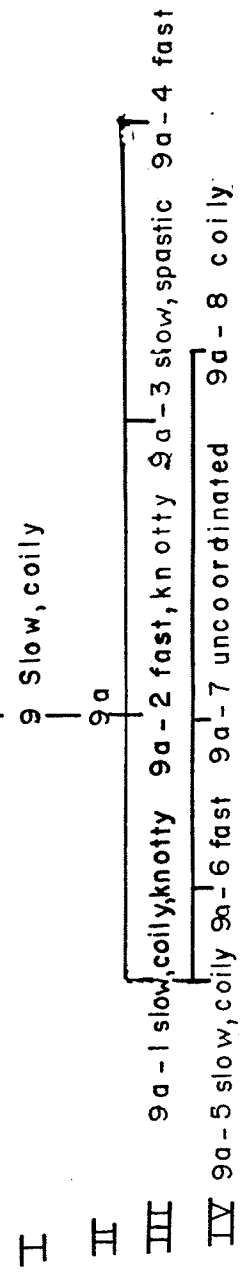


* Maintained as a stock

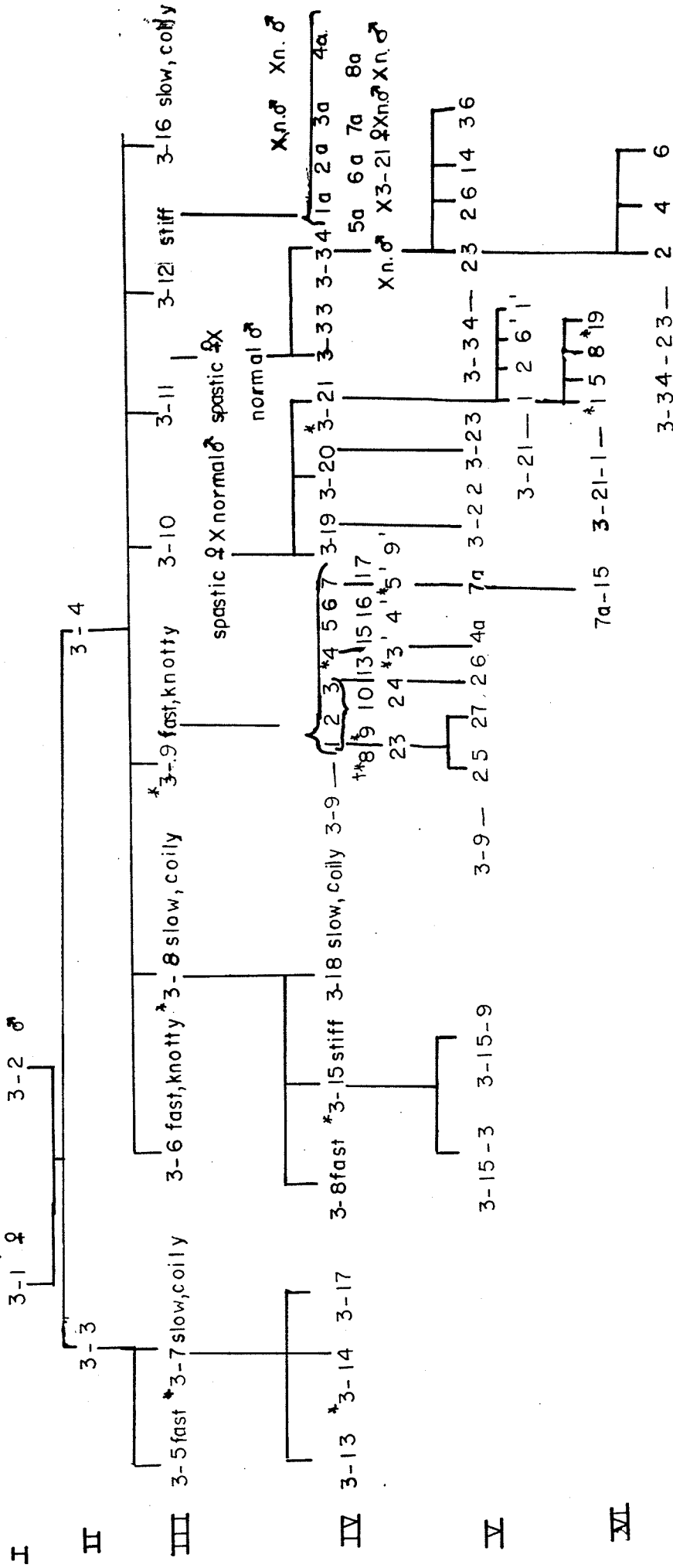
Cross 7 Tail stiff, uncoordinated ♀ X Middle stiff, uncoordinated ♂



Cross 9 Fast, knotty ♀ X Fast, knotty, tail drags ♂



Cross 3 Fat, middle stiff ♀ X Fat, tail stiff ♂



† 3-9-8 — Corkscrew

