Chapter 11

Mapping Genes in *C. elegans*

Robert C. Johnsen
and
Denise V. Clark

Department of Biological Sciences,
Simon Fraser University,
Burnaby, B.C., Canada, V5A 1S6

Robert C. Johnsen received his B.Sc. in Biology from Simon Fraser University in 1986. He is currently a Ph.D. student in genetics at Simon Fraser University. His research interests include genomic organization and the control of recombination.

Denise V. Clark received her B.Sc. in Biology from the University of British Columbia in 1983. She is currently a Ph.D. student in genetics at Simon Fraser University. Her research interests include the study of developmentally essential genes and their organization in *C. elegans.*

NEMATODE GENETICS

In the mid 1960's after the breaking of the genetic code using viral and bacterial systems, many molecular biologists began to turn their minds to an investigation of behaviour and the functioning of the nervous system. About this time, Sydney Brenner in Cambridge, England began to look for a suitable organism for genetic studies of this nature. In 1968, Brenner found an organism which has a relatively simple nervous system (about 300 cells). It has only six pairs of chromosomes and a short generation time (3 1/2 days at 20°C). It reproduces as a self-fertilizing hermaphrodite, giving about 300 progeny. It can also be out-crossed to males. It is small (1 mm. long as an adult) and transparent, thus enabling the morphological aspects of development to be observed under the microscope using living material. In addition, strains can be maintained frozen in liquid nitrogen. This organism is the free-living (non-parasitic) nematode *Caenorhabditis elegans*. By 1974, Brenner published a genetic map of *C. elegans* with 250 genes identified. Thus, this hermaphroditic nematode was shown to be a suitable organism for genetic analysis. Since 1974, several interesting mutations have been described and many lines of research have been followed using *C. elegans*. This work has been reviewed by Riddle (1978).

This laboratory exercise will use *C. elegans* to illustrate how a new mutation in a gene of an unknown location can be assigned to a specific chromosome. As well, the recombination distance between the unknown and a known marker, will be calculated from F_2 data. Unlike *Drosophila,* crossing-over occurs in both the male and female germ lines in *C. elegans.*

NOTES ON THE BIOLOGY OF *C. elegans*

C. elegans is normally maintained as a self-fertilizing hermaphrodite, producing both eggs and sperm. Spermatogenesis is complete before oogenesis. Developing oogonia move linearly along each arm of a two-armed gonad to become mature oocytes. At maturity they pass through a bag of sperm (spermatheca). It is here that fertilization takes place. Meiosis is completed after fertilization and the egg-shell forms. Cleavage begins shortly after fertilization. Since *C. elegans* has a transparent cuticle, it is possible to observe the developing eggs inside the mother. At about the 64-cell stage the "egg" is expelled to the outside through a mid-ventral opening, the vulva.

Life cycle

1. At 20°C, the average generation time is 3 1/2 days.
2. About 12-18h after an egg is laid, the shell ruptures and a first stage larva (L1) emerges.
3. The larva then goes through four larval molts L2, L3, L4 and adult. Essentially the stages differ in appearance only in size. However there are two distinguishing features to notice:

    a. Before the gonads are completely developed, the space of the vulva appears as a white crescent shape ("moon"). This is especially noticeable in the L4 larvae, which are referred to as "crescent stage." Although the vulva is ventral, the crescent appears to be on the side of the worm (since the worm crawls on its side). Identification of this stage is important for genetics, as it is a certain sign that no mating with a male has taken place yet (virginity).

    b. In the adult hermaphrodite developing eggs are visible.

4. An adult hermaphrodite will produce about 300 self-fertilized eggs over a period of 3 days at 20ºC.

Males

1. In order to perform crosses between different genotypes, males must be used.
2. As adults, males can be distinguished from adult hermaphrodites in that:

   a. they are generally thinner (carry no eggs).

   b. they have a copulatory tail apparatus (looks like a crochet hook). Larval males and hermaphrodites are not easily distinguished.

3. During mating, the male sperm are passed to the hermaphrodite via the vulva.
4. Once the male sperm have entered the hermaphrodite, they are used for fertilization (out-cross) in preference to the hermaphrodite's own sperm (self-cross).

Sex Determination

1. *C. elegans* has five autosomes (A) and one sex-chromosome (X).
2. Hermaphrodites, 5(AA),XX, produce 5A,X eggs and 5A,X sperm, while males, 5(AA),XO, produce 5A,X sperm and 5A,O sperm.
3. Thus self-cross progeny from hermaphrodites are all hermaphrodites. Consequently, hermaphrodite strains can be maintained indefinitely (assuming enough food is available) without mating.
4. Out-cross progeny, from hermaphrodite X male matings will be 50% males and 50% hermaphrodites. To maintain males, matings must be done at every generation.

NOTES ON GENERAL TECHNIQUES:

Culture Medium

1. *C. elegans* is grown on petri plates which have an agar base (nematode growth medium or NGM). The agar is streaked with a strain of *E. coli* which does not grow too thickly (OP50). The nematodes feed on the *E. coli.*
2. Occasionally, contaminating bacteria also grow and make visual observation of the worms more difficult. Plates should therefore be kept covered when you are not manipulating worms.
3. To avoid rapid evaporation, store plates upside down.

Handling Nematodes

1. Worms can be manipulated under a dissecting microscope at 12-25 X magnification, lighting should be from below and should be diffuse.
2. Worms are transferred from one plate to another by lifting up a single one with a sharpened wooden applicator stick, shifting the second plate into view, focusing on the *E. coli* and gently resting the point of the stick on the agar (try to avoid poking holes into the agar). Within a few seconds the worm will crawl off the stick onto the agar.
3. Be sure to use a FRESHLY sharpened stick whenever a different strain is handled (eggs and small larvae may be on the used stick).
4. Eggs and larvae may also cling to adult worms. Therefore, when isolating males for a cross, always transfer from the source plate to an intermediate plate first. After a few minutes the males will have moved around enough to be free of clinging eggs and larvae, and can be transferred to the mating plate.

Maintaining a Hermaphrodite Strain

1. One or two young adult hermaphrodites are placed on a fresh plate.
2. After 3-4 days at about 20°C (7 days at 15°C), some of the progeny worms are put onto fresh plates.
3. This procedure is continued about every generation.

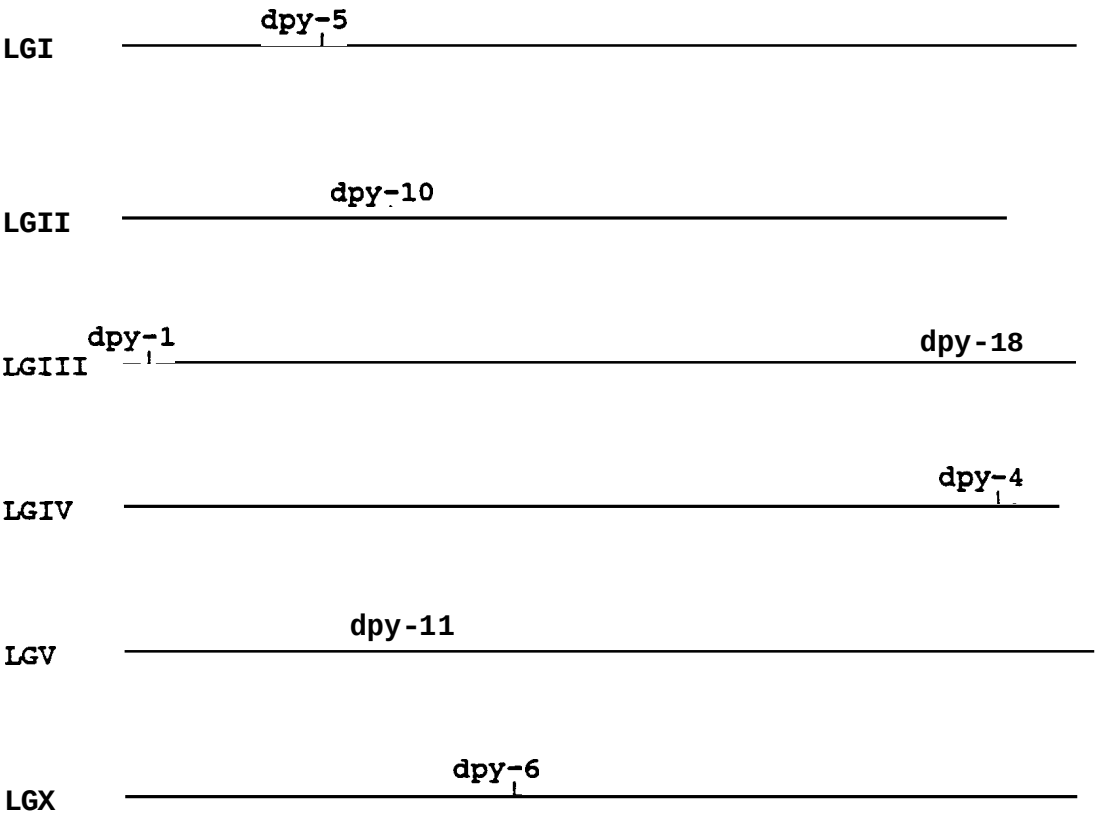
Mating Hermaphrodites and Males

1. Place at least 7 males on an intermediate plate.
2. Place about 4 young adult hermaphrodites onto a mating plate.
3. Transfer the isolated males to the mating plate. Keep this at about 20°C for 24h (males won't mate at 15°C).
4. 24h later, transfer single mated hermaphrodites to separate plates.
5. About 48h later start looking for progeny (remember, there may be some self-cross progeny).

Labelling of Plates

1. Use a permanent marking pen.
2. Label the bottom plate (in case plates are dropped and tops get mixed up).
3. Use small writing at the edge so that the writing does not obscure the microscope light.

Location of some of the C. ***elegans*** dpy genes:



NOTES ON THE GENETIC NOMENCLATURE OF *C. elegans*

1. *C. elegans* has six chromosomes (linkage groups):
   LG's I, II, III, IV and V are the autosomes
   LG X is the sex-chromosome.

2. A large number of genes have been identified and mapped. Among the genes that affect the visible morphology of the worm, there are two major classes: Dpys and Uncs.
3. Mutations in dpy genes produce "dumpy", short fat worms. Mutations in unc genes produce "uncoordinated worms that cannot move in the elegant sine wave of the wild-type (Wt).
4. 28 Dpy and 110 Unc genes have been mapped. They are named dpy-1, dpy-2, . . . and unc-1, unc-2, . . . etc.
5. Mutant genotypes are written with small letters (underlined or in italics): e.g. dpy-1. Mutant phenotypes are written with the first letter capitalized: e.g. Dpy.

GENETICS LABORATORY EXERCISE

Purpose: To map an unknown recessive Unc mutation of *C. elegans* (unc-?) to a particular linkage group, and to do a two-factor cross to map its distance from a linked Dpy mutation.

General approach (see Experimental Procedures below)

1. Prepare a male carrying a recessive dpy-m marker as a heterozygote (dpy-m/+), by mating Dpy-m hermaphrodites X Wt males.
2. Po cross: Mate Unc-? hermaphrodites X dpy-m/+ males
3. F_1 x F_1 cross: Select individual heterozygous F_1 hermaphrodites onto separate plates (using virgins) and allow these to produce self-cross F_2 progeny.
4. F_2 progeny: Score the phenotypes of the F_2's to establish whether dpy-m and unc-? linked.
5. Two Factor Cross: A two factor cross will be conducted to determine the recombination distance of ync-? from the linked Dpy.

Theory

1. Diagram the crosses.
2. What F_1 progeny do you expect from the Po cross? Genotypes? Phenotypes? Sexes? Consider the 2 possibilities: either unc-? is on an autosome or unc-? is on the X chromosome.
3. From those F_1 heterozygous hermaphrodites that received the dpy-m marker, what ratios of F_2 (i.e. Wt : Dpy : Unc : Dpy Unc) do you expect if dpy-m and unc-? are (a) linked and (b) unlinked. If linked, assume 10% linkage.

Experimental Procedure:

1. Examine and practice handling *C. elegans*. Adjust microscope lighting (light must come from below and be diffuse).

   a. Compare different ages. A plate with wild-type hermaphrodites will be provided. Keep also for 2.a. 1) below. Note: eggs, larvae, and adults. Try to identify crescent stage L4 larvae, and transfer them to a fresh plate for practice.

   b. Compare hermaphrodites and males. A plate with wild-type males and hermaphrodites will be provided. Sort the two sexes onto fresh plates for practice.

c. Compare the phenotypes. A plate with a mixed hermaphrodite population will be provided (i.e. with Dpy, Unc, and Wt worms). Sort the different phenotypes onto fresh plates and see how they respond to gentle prodding on the head and the tail.

2. Your strains:

a. Each group will receive 3 homozygous hermaphrodite strains to be maintained throughout the experiment:

1) wt — from 1a. above.
2) unc-? — this will be the same strain for all groups.
3) dpy-m — this is a marker strain. Each group will receive a different dpy-m.

b. You will need these strains for the crosses below, and as reference phenotypes.

c. For each strain place 2-3 young adult hermaphrodites on a fresh plate. Remember to use a FRESHLY sharpened stick for each strain. Repeat this every generation.

3. Mapping the unknown unc-?:

a. Prepare the dpy-m/+ males by mating Dpy-m hermaphrodites X Wt males.

1) Place at least 7 wild-type males onto an intermediate plate.
2) Place 4 Dpy-m hermaphrodites (L4 or young adult) onto another plate, the mating plate.
3) Transfer the Wt males from the intermediate plate to the mating plate. Keep the mating plate at 20°C (or room temperature).
4) 24h later, transfer the mated hermaphrodites to fresh plates (1 hermaphrodite per plate). Keep the mating plate as well.
5) As the adult progeny emerge (2-3 days later), set up your Po cross.

b. Po cross:

1) From (3a) above screen the progeny. Self-cross progeny will be Dpy-m hermaphrodites, while out-cross progeny will be phenotypically Wt males and hermaphrodites.
2) Isolate at least 7 dpy-m/+ males onto an intermediate plate.
3) Place 4 Unc-? hermaphrodites (L4 to young adult) onto a mating plate.
4) Transfer 7 dpy-m/+ males from the intermediate plate to the mating plate.
5) 24h later, transfer the mated hermaphrodites to fresh plates (1 hermaphrodite/plate). Keep the mating plate as well.
6) As the F_1's mature (about 2 days later) set up your F1 self-cross. Also, inspect the phenotypes of the F_1 males. Is unc-? on LG X?

c. F1 x F1 self-cross:

1) Select 7 phenotypically Wt crescent stage F_1 hermaphrodites onto individual plates. If a F_1 hermaphrodite is left too long on any one plate, the F_2 progeny will be quite crowded and asynchronous. This would make the F_2 scoring very difficult. To avoid this problem, the F_1 hermaphrodites will be transferred to fresh plates periodically, i.e sequential broods of F_2's will be collected ("Brood A", and "Brood B" etc.). To keep track of each individual F_1 hermaphrodite (remember they do not all have the same genotype), number these first 10 plates "1A","2A"... "10A".

2) After 18-24h, transfer the F_1 hermaphrodites to fresh plates (labelled correspondingly "1B", "2B" etc.) to collect the B brood. Note whether there are any progeny eggs on the A brood plates. 12h broods are advisable.

3) After 18-24h, remove the F_1's, or transfer them once more for an 18h C brood if there were no progeny on the A plates.

4) As soon as the F_2's reach adulthood and you can recognize their phenotypes, score them.

d. NOTE: It is important to brood the worms in order to:

1) have a small number of worms on any one plate

2) have a synchronous population

e. Score the F_2 phenotypes.

1) Remove the adults as you score them.

2) As soon as enough F_2's have matured so that you can tell which of the F_1's carried the dpy-m marker, continue scoring only from these F_1's.

3) Continue scoring a plate until all the F_2's have been removed.

4) Aim for scoring about a total of 300 F_2's (from dpy-bearing F_1's). The data you use should come only from plates that have had all their F_2's scored. It is therefore better to score the total progeny from a few F_1's, rather than the early, partial progeny from many F_1's.

f. Record the data and have it available for collection and distribution to the class.

4. Two Factor Mapping:

From the compiled data, it should be apparent to which chromosome the Unc mutation maps. By using the linked dpy-m unc ?, it will be possible to map the unc-? -dpy-m distance. The procedure involves out-crossing the appropriate Dpy Unc to wild-type males and picking heterozygotes in the F_1 (these will be wild-type).

a. Determine to which chromosome unc-? maps and then obtain 5-6 young adult Dpy Uncs from the appropriate group (i.e. that which shows linkage).

b. Place 10-12 wild-type males onto an intermediate plate for 5-10 minutes.

c. Place 5-6 dpy-m unc-? hermaphrodites on to a fresh mating plate.

d. Transfer the males from the intermediate plate to the mating plate. Keep the mating plate at 20°C (or room temperature).

e. After 24 hrs, transfer the mated hermaphrodites to fresh plates (1/plate). Keep the mating plate as well. You may transfer a few males to each plate if you wish.

f. When the F1's begin to develop, select wild-type L4 (virgin) hermaphrodites and transfer them (1/plate) onto fresh plates (2 per individual in your group will suffice). Remember, the presence of many males indicates out-crossing took place.

g. The hermaphrodites must be brooded at 12-18h intervals (five 12h broods is preferable).

h. Score the progeny on the brood plates to completion (usually 2-3 days). You do not want to score the next generation.

i. Record the data and have it available for collection and distribution to the class.

QUESTIONS FOR CLASS
(Include these in your discussion)

1. Why do we not test to a dpy-m on the X-chromosome?
2. If hermaphrodites are true breeding, how do males arise and why are they maintainable?
3. Why do we not use the scoring data from the linkage determination, to determine the distance between the two markers?

FOR THE TECHNICIAN

Inquiries and stock requests should be directed to Mark Edgley, CGC, Division of Biological Sciences, 110 Tucker Hall, University of Missouri, Columbia, MO, 65211. We recommend the dpy's shown on the genetic map for markers and unc-42 for the unknown. You will also need the wild-type N2 male stock.

Recipe for NGM (for 2L - we use 60mm petri plates):

6g NaCl
34g Agar (Sigma)
5g Bactopeptone
make up to 1.95L with distilled water
Autoclave and then add:
2 ml cholesterol (5 mg/ml in 95% EtOH)
2 ml 1M calcium chloride
2 ml 1M magnesium sulphate
50 ml 1M potassium phosphate pH 6
when set, streak with 1/2 ml liquid culture (10g tryptic soy broth/L) of OP50 (available from the CGC)

MISCELLANEOUS NOTES ON KEEPING WORMS

1. For long term maintenance of stocks, keep hermaphrodite strains at 15ºC with parafilm to prevent desiccation. A dauer larva form survives for several months. Healthy, femle worms can be recovered by placing a piece of the old agar on a fresh NGM plate and leaving it for a few days.
2. Worms can be frozen in liquid nitrogen for indefinite lengths of time.
3. It is important to realize that generally only phenotypically wild-type males will successfully mate

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