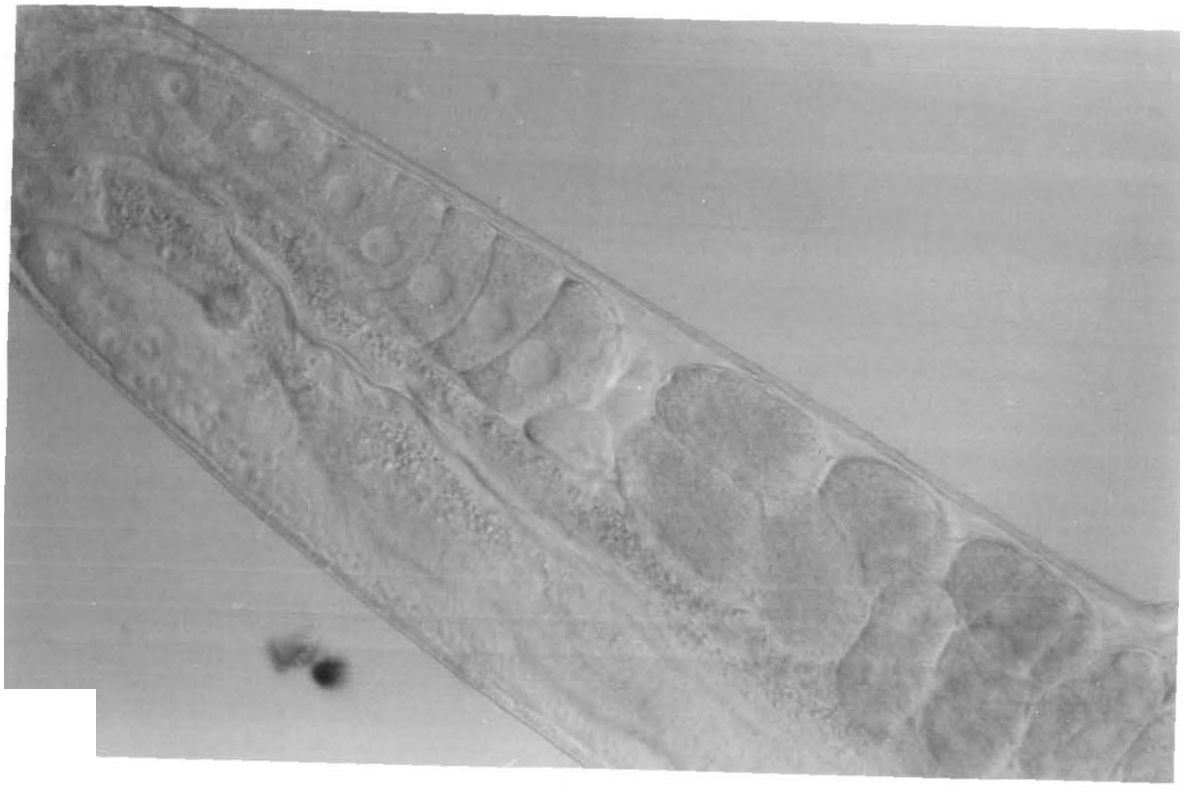
Frontispiece

Shown in this photograph (taken with Nomarski optics) is the gonad of a dumpy 11 hermaphrodite. Nuclei and nucleoli can be seen inside the developing oocytes. Embryos can can be seen inside the uterus.



STUDIES ON THE DEVELOPMENT OF CAENORHABDITIS ELEGANS USING TEMPERATURE SENSITIVE MUTANTS ON CHROMOSOME 5.

by

Kim R. C. Riekki

B.Sc., The University of Victoria, 1975.

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

January 1979

APPROVAL

Name: Kim R. C. Riekki

Degree: Master of Science

Title of Thesis: Studies on the development of Caenorhabditis elegans using temperature sensitive mutants on chromosome 5

Examining Committee:

Chairman: Robert C. Brooke

D. L. Baillie, Senior Supervisor

C. L. Kemp

R. Reeves, Assistant Professor,
Department of Zoology,
University of British Columbia

A. T. Beckenbach, Non-Supervisory Committee Examiner

Date approved March 6 1979

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/Project/Extended Essay

Studies on the development of Caenorhabditis elegans

using temperature sensitive mutants on chromosome 5

Author: _____
(signature)

Kim R. C. Riekki
(name)

March 6, 1979
(date)

Abstract

In the nematode Caenorhabditis elegans, six ethylmethanesulphonate (EMS) induced temperature sensitive mutations on chromosome 5 have been isolated. These mutants were characterized and it was found that three mutants (ts 1, ts 2 and ts 7) arrest as embryos. Ts 7 and ts 2 are monophasic with respect to the lethal phase. Ts 1 is a polyphasic ts lethal, with lethal phases during embryonic development, larval development, and during oocyte production. Ts 7 has a temperature sensitive period (t.s.p.) which extends from oogenesis through embryogenesis. Ts 2 has a t.s.p. which extends throughout development, and the t.s.p.s of ts 1 extend throughout development also. Male rescue experiments demonstrated a maternal effect on the embryonic arrest of ts 1, ts 2 and ts 7. The maternal effects of these three mutations were found to be reversible in some heat pulse experiments. It was found that either the maternal or the embryonic exposure to the restrictive temperature was sufficient to cause embryonic arrest. Three other temperature sensitive mutations, including a larval arrest mutant (ts 4) a sterility mutant (ts 5) and a mutant with both sterility and embryonic arrest (ts 12) were also isolated. The mutations occupy different positions on chromosome 5 and are of interest with respect to patterns of gene expression during development.

Acknowledgements

I would like to thank Dr. D. L. Baillie for his guidance throughout this project. I would also like to thank Dr. C.L. Kemp and Dr. Ray Reeves for their helpful suggestions. The technical assistance of Ms. Raja Rosenbluth, Clara Salamanca and S. Lee Liner was also appreciated.

Table of Contents

	PAGE
Frontispiece	
Title Page	
Approval Page	ii
Abstract	iii
Acknowledgements	iv
Table of Contents	v
List of Tables	vi
List of Figures	vii
Introduction	1
Materials and Methods	12
Results	24
Discussion	59
Conclusions	74
Bibliography	77

List of Tables

Table 1 Chromosome 5 mutants used in the this study 14

Table 2 Linkage data for ts mutants 58

Table 3 The stage of embryonic arrest in the three embryonic arrest mutants 59

List of Figures

PAGE

Figure 1 Screen for chromosome 5 ts mutants 18

Figure 2 Lethal phases of ts 7 and development of ts 7 at the restrictive temperature 29

Figure 3 A test for larval temperature sensitivity in ts 7 larvae 30

Figure 4 Mating Experiments on ts 7 homozygotes 31

Figure 5 Heat pulse experiments during oogenesis on ts 7 32

Figure 6 Lethal phases of ts 2 and development of ts 2 homozygotes at the restrictive temperature 37

Figure 7 A test for larval temperature sensitivity among ts 2 larvae 38

Figure 8 Mating experiments on ts 2 homozygotes 39

Figure 9 Heat pulse experiments during oogenesis on ts 2 40

Figure 10 Lethal phases and development of ts 1 at the restrictive temperature 46

Figure 11 Shift up experiments on ts 1 larvae 47

Figure 12 A further test for larval temperature sensitivity among ts 1 larvae 48

Figure 13 Mating experiments on ts 1 homozygotes at the restrictive temperature 49

Figure 14 Heat pulse experiments during oogenesis on ts 1 50

Figure 15 Lethal phases and development of ts 5 at the restrictive temperature 53

Figure 16 Lethal phases and the development of ts 4 at the restrictive temperature 54

Figure 17 Approximate map positions of ts mutants studied 57

Introduction

This thesis reports on a study of conditional lethals in the free living nematode, Caenorhabditis elegans (Maupas).

Two strains of C. elegans have been kept in monoxenic culture for nearly two decades. These strains are referred to as C. elegans (Bergerac) and C. elegans (Bristol). Although the two strains appear morphologically identical, their sensitivities to heat differ: C. elegans (Bergerac) will grow and reproduce well only up to 18°C, while C. elegans (Bristol) is more heat tolerant (Nicholas, 1975). The Bristol strain was used for this work.

Maintenance of stocks of C. elegans is easy due to the small size and method of reproduction of the organism. The mature worm is about one millimeter in length. Thus, large numbers can be kept in a small laboratory space. C. elegans is a hermaphrodite in which sperm production is finished before oocyte production begins. The hermaphroditism is not complete, and males are present at a frequency of less than 1%. Hermaphroditism offers simplicity of stock maintenance. Crosses are not necessary to maintain stocks of many mutants. The occurence of males in C. elegans allows crosses to be made.

Sex inheritance, which is of the X/X, X/O variety, is such that populations containing males can be maintained for genetic use by crossing X/O males with X/X hermaphrodites.

The sexes are easily recognized under a dissecting microscope. The hermaphrodite body tapers at both ends, while the male body tapers anteriorly and have a prominant "hook" at the posterior end. This hook is part of the copulatory apparatus.

Directly observable internal anatomy offers advantages to those interested in metazoan development. In C. elegans, the cuticle is transparent, allowing direct observation of the organ systems with the dissecting microscope, although resolution of cellular and subcellular details requires the use of a compound microscope.

The number of somatic cells in C. elegans is small: the hermaphrodite has 810 somatic nuclei and the male has 900 (Sulston and Horvitz, 1976). The gonads add many more cells, so that a mature hermaphrodite has about 3000 cells (Hirsh et. al., 1976). The small cell numbers and invariant pattern of cell division simplify the analysis of any system. Thus, cell lineages in the intestine, the nervous system, gonad etc. can be followed with Nomarski optics (see frontispiece).

The hermaphrodite gonad is easily recognized under the compound microscope. Each hermaphrodite gonad has two reflexed arms. At the proximal end of each arm, stem cells give rise to many nuclei. These nuclei are not separated by cell membranes until they reach the loop of the gonad in each arm. As they round the loop and enter the distal arm, individual oocytes become delineated and clearly visible. The ultrastructure of oogenesis has been studied (Abi- Rachid and Brun, 1975). Nuclei (germinal vesicles) can be seen within the oocytes. After passing down the distal arm of the ovary, the oocyte enters the spermatheca (Hirsh et. al., 1976), where it is fertilized by amoeboid sperm (Ward and Miwa, 1977). The fertilized egg then enters the uterus in which the early embryonic stages of the worm occur.

Embryos of C. elegans have many features which make them interesting. They are highly determinate and are essentially a mosaic of self differentiating parts (White, unpublished; Balinsky, 1975; Berril, 1971). Holoblastic cleavage delineates the early blastomeres. These blastomeres undergo many unequal cleavages, each with its own "rhythm" of divisions which may be followed with the light microscope (Deppe et. al., 1978). As cleavage proceeds inside the egg case (maximum diameter of 55 microns), gastrulation occurs by invagination at the 24 cell stage (Riddle, 1978). Following gastrulation, proliferation of

cells continues inside the egg case. At hatching, differentiation is almost complete.

Eggs are laid through a muscular vulva at the mid-point along the anterior-posterior axis. Egg laying is usually over in two days at 26°C, while at lower temperatures, adult worms may lay eggs for a more extended period.

Larval development in C. elegans is direct; from an unhatched egg of 550 cells, the larvae molt four times with intervening larval periods (L 1 larvae through L 4 larvae). These larval stages are most easily characterized by the length of the nematode and stage of development of the gonad (Hirsh et. al., 1976). Under adverse conditions, an alternate pathway of development may ensue. Starved worms may enter a desiccation and heat resistant stage called dauer larvae after the second or third molt and emerge to resume growth when conditions are appropriate (Riddle, 1978). Simple, direct development through larval stages to the adult permits a straight forward analysis of differentiation in various systems (eg.: nerve, muscle, etc). The analysis is simplified by the short generation time of about three days at 20°C. Mature adults usually live from three days to more than a week, depending on the temperature. Hermaphrodites maintained on a good bacterial lawn are easily transferred to new plates with no apparent damage (see Materials and Methods).

The genetics of C. elegans.

The study of the genetics of Caenorhabditis began almost three decades ago. In 1950, it was reported that a spontaneous mutant appeared in cultures of C. briggsae. This mutant was shorter than normal and referred to as "micro". By performing crosses between "micro" hermaphrodites and wild type males, the mutant was found to segregate in a Mendelian fashion from F 1 hermaphrodites (Nigon and Dougherty, 1950). More recently, Fatt and Dougherty (1963) ,using the heat sensitive (Bergerac) and heat tolerant (Bristol) strains of C. elegans, showed that heat sensitvity of C. elegans (Bergerac) was inherited as a mendelian characteristic.

Genetic analysis of C. elegans has concentrated on induced mutations. Techniques of EMS mutagenesis are used, and methods for optimal mutant recovery in EMS experiments are currently being explored (Baillie, unpublished). Brenner (1974) isolated a large number of EMS induced mutants in C. elegans, and assigned the mutants to six linkage groups. These linkage groups include five autosomes and the X chromosome. Each linkage group is characterized by many mutants. A large number of the mutants lie in a cluster on each autosome. The visible alleles isolated by Brenner are varied, but most fall into two broad categories : the dumpy (short, fat) and uncoordinated

(poor or abnormal movement) mutants. Brenner notes the usefulness of C. elegans in the genetic analysis of the nervous , muscular, and developing systems, and mentions lethal genes (Brenner, 1974).

Temperature sensitive mutants in developmental studies.

Lethal mutations cause death of the organism before it reaches reproductive maturity. Lethal mutants can have 100% lethality, 50% lethality (semi-lethal) or less than 50% lethality (subvital). The penetrance and expressivity of unconditional mutants are not affected by the experimental conditions. Experimental conditions markedly affect the penetrance and expressivity of conditional lethals. The penetrance of conditional mutants may be dependant on such factors as chemical environment, nutrition, population density, and temperature (Hadorn, 1961). This study is limited to a class of conditional mutants known as temperature sensitive mutants.

Temperature sensitive mutations are important in the analysis of assembly and development. This is particularly true in the bacteriophage T4. During the infectious process, some genes are expressed earlier than others (thus, T4 genes are

referred to as either early or late genes). Temperature sensitive alleles in both gene categories were isolated, and a "ts linkage map" constructed. Stocks were easily kept because the assembly of the bacteriophage was normal at the permissive temperature. At the restrictive temperature, mutants in the early genes (ie DNA synthesis mutants) and the late genes (e.g. head and tail components) are expressed. These mutants were found to map to specific areas of the linkage map (Epstein et. al., 1963). Temperature sensitive mutants have been used in developmental research on at least three organisms: the slime mold (Dictyostelium), the fruit fly, (Drosophila), and the nematode (Caenorhabditis). In slime molds, temperature sensitive mutants have found potential use in studying cell aggregation during the life cycle (Loomis, 1969). More varied analysis has been performed using Drosophila (see Suzuki et. al., 1976 for a review).

In Drosophila, studies on temperature sensitive mutants have proved useful in analyzing developmental phenomena. Suzuki and Procunier (1969) investigated a "cluster" of dominant temperature sensitive lethals on chromosome 2. They found that several of the TS mutants had temperature sensitive periods (TSP's) during embryogenesis and subsequent lethality in third instar larvae (Suzuki and Procunier, 1969). The temperature

sensitive homeotic cluster on chromosome 3 also have temperature sensitive periods before the phenotype is expressed (Postlethwait and Schneiderman, 1973). Homeotic mutants have also led to theories on the mechanism underlying the determination of the fates of cells within the imaginal discs (Grigliatti and Suzuki, 1971). Although clustering studies are genetically interesting, mutant clusters may not conform to the physical reality of chromosome length. Thus Tasaka and Suzuki (1973) note that the clustering of recessive temperature sensitive mutants on chromosome 3 may simply be due to the position of the centromere (Tasaka and Suzuki, 1973). From large scale screens for autosomal temperature sensitive mutants, it was found that that some classes of temperature sensitive mutants seem to occur with a low frequency on some chromosomes in Drosophila, as is the case with dominant temperature sensitive lethals on chromosome 3 (Rosenbluth et. al., 1972).

Another approach used with Drosophila involves a more in depth analysis of individual temperature sensitive mutations or loci. In the case of the Notch locus, which is necessary for embryonic development and normal wing structure, analysis of several temperature sensitive alleles showed the structural part of the gene to be located at one end of this complex locus (Shellenbarger and Moehler, 1975). In another study, several

temperature sensitive alleles of the mutant shibire were examined. Some of these were shown to have multiple temperature sensitive periods (Poodry et. al., 1973). A temperature sensitive lethal of the X-linked recessive, raspberry (ras), has been studied. It has been found that different tissues have different t.s.p.s (Grigliatti and Suzuki, 1970).

Maternal effects on development have also been studied with temperature sensitive mutants. Recessive maternal inheritance can be demonstrated using outcross experiments: one attempts to "rescue" (i.e. prevent) the zygotic lethality by introducing the wild type allele at fertilization. Maternal mutants giving few or no outcross progeny have been found in Drosophila (Tarasoff and Suzuki, 1970). Maternal effect mutants with temperature sensitive lethality have in some instances been shown to have localized embryonic defects (Fausto-sterling, 1977) or gross organizational defects as in the mutant bicaudal (Nusslein-Volhard, 1978).

Many temperature sensitive mutants have been isolated in C. elegans. For example, a temperature sensitive mutant affecting the assembly of muscle filaments has been isolated (Epstein and Thompson, 1974). Also, a temperature sensitive mutant causing transformation of hermaphrodites into males has been studied

(Klass et. al., 1976). A large number of temperature sensitive mutants have been isolated by Hirsh et. al. (1976). They have divided their mutants into several broad categories. These categories include larval arrest mutants, gonadogenesis mutants, spermatogenesis mutants, F 1 sterility mutants, ts morphological mutants, and embryo defective mutants (Hirsh and Vanderslice, 1976). Mutants with defective embryos include some with t.s.p.s near the stage of embryonic arrest. Some other mutants with embryonic arrest phenotypes had an additional temperature sensitive block in gonadogenesis. These mutants with two temperature sensitive phenotypes exhibited a maternal effect (Vanderslice and Hirsh, 1976).

The problem.

This thesis represents the first attempt to isolate and characterize temperature sensitive mutants of developmental interest on a specific linkage group of Caenorhabditis elegans. The approach taken is considerably different from the approach taken by other nematode workers, in that only temperature sensitive mutants linked to chromosome 5 were analysed.

Brenner (1974) initially established a linkage map of chromosome 5 using visibles. Aside from a couple of these visibles, no previous reports of mutants of developmental

interest on this linkage group have been made (Brenner, 1974). The main purpose of this thesis was to contribute to the understanding of chromosome 5 developmental genetics using temperature sensitive mutants.

Materials and Methods

1. Materials.

Worms were grown on petri plates containing nematode growth medium (NGM) and bacteria as described by Brenner (1974). NGM was prepared by autoclaving for 20 minutes at 225°C a mixture of agar (17 grams), peptone (2.5 grams), and 3 grams of NaCl dissolved in 975 milliliters of distilled water. The following chemicals were then added:

1 ml cholesterol (5mg/ml in ethanol)

1 ml of 1M calcium chloride

1 ml of 1M magnesium sulphate

25 ml of potassium phosphate (pH 6)

This mixture (NGM) was then poured in petri plates and allowed to gel. Then, OP-50 (uracil requiring) Eschericha coli were streaked in a cross-hatch pattern on the NGM, and the plates left one or two days before use. Two incubators were used; one was calibrated to an average temperature of 17°C +/-

.5°C (the permissive temperature), while the other was calibrated to 26°C +/- .5°C (the restrictive temperature).

Observations on worms and embryos were usually made using a Zeiss Jena dissecting microscope (Zeiss, Germany).

2. Stocks and Stock Maintenance.

The Simon Fraser lab holds stocks of the following mutants on chromosome 5 (table 1) that were used for mutagenesis and mapping as indicated later in the Methods section:

Table 1 Chromosome 5 mutants used in this study.

Table 1

Mutant	allele	description
dpy 11	e224	short, fat.
unc 76	e911	nearly immobile
unc 34	e117	slow mover

N-2-S is the strain originally obtained from England, and may be considered wild type for all characteristics.

Stocks were maintained at 17°C for most of this study, although 20 C was sometimes used as the permissive temperature. Stocks at the permissive temperature were maintained on large petri plates (100 x 15 mm), while restrictive temperature experiments were done on small petri plates (35 x 15 mm). An extra set of stocks was maintained in a different laboratory in a 15°C incubator. In total, three permissive temperature stocks of each ts mutant were maintained for most of the experimental work. For experimental purposes, worms were transfered to new plates two or three times a week to ensure that the results at the restrictive temperature were not confused with the effects of previous starvation. Worms used for experimental purposes were always transfered between petri plates by means of 6 inch wooden applicators sharpened at one end with a razor blade.

3. Mutagenesis.

Stocks of dpy 11 (e224) were used for mutagenesis. Ten hermaphrodites were placed on each of several large petri plates, and the young adults (visible oocytes) were washed from the plates with M-9 buffer. (M-9 buffer contains 6 g Na_2HPO_4, 3 g KH_2PO_4, .25 g of $Mg_2SO_4 7H_2O$, and 5 g NaCl per liter). Once suspended in M-9 buffer, the worms were subjected to mild

centrifugation and the excess buffer removed to bring the final volume to 2 ml.

In a separate test tube, 20 ul of EMS was dissolved in 2 ml of M-9 buffer. This mixture was then added to the 2 ml of M-9 buffer containing the worms. The final volume was 4 ml of approximately .05 M EMS. The test tube was covered with parafilm and then left in the fume hood for 4 hours. Worms were then removed to large petri plates and left to recover from the anaesthetizing effect of the mutagen. Progeny of mobile hermaphrodites were then screened for temperature sensitive mutants.

4. Screening.

Three screening procedures were used. Screen 1 involved placing mutagenized dpy 11 hermaphrodites on small petri plates with three wild type males. Mating was allowed to proceed for several hours before the parents were transfered. From each of a series of egg layings, several heterozygous dpy 11 hermaphrodites were placed on seperate petri plates. The progeny of these F 1 heterozygotes were then screened for hermaphrodites not giving a 3:1 ratio (wild types : dpy 11) at 26 C. Stocks giving less than the expected numbers of dpy 11 were then rechecked at the permissive temperature. To accomplish this, two or three F 2 heterozygotes from the

restrictive temperature were screened for aberrant segregation at the permissive temperature. If these hermaphrodites gave 3:1 ratios at the permissive temperature, a stock of dpy 11 from the plate was saved as a putative ts. Continued ratio distortion at the permissive temperature indicated a nonconditional lethal.

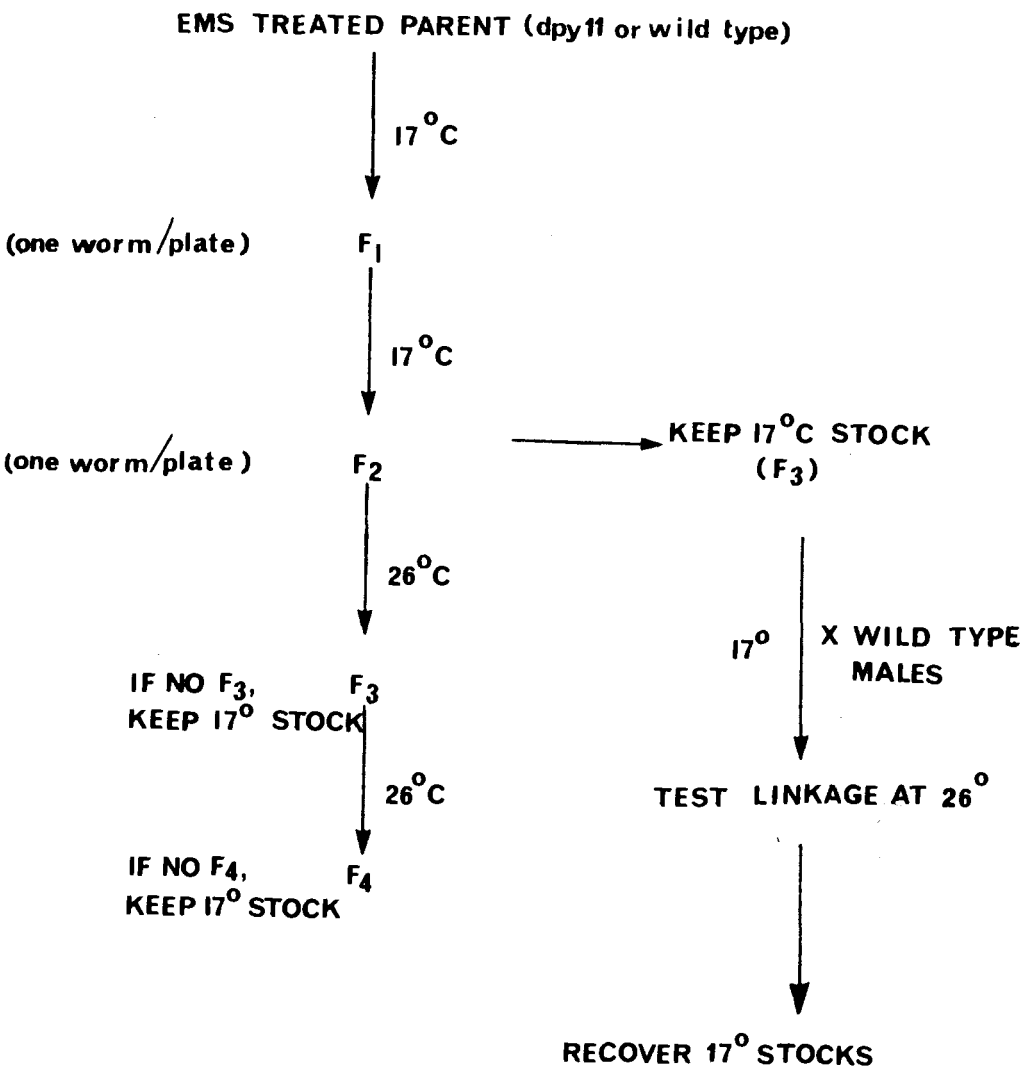
In screen 2, mutagenized dpy 11 homozygotes were selfcrossed at permissive temperature and the individual F 1 hermaphrodites allowed to self on separate plates at the permissive temperature. Two or three F 2 worms per F 1 were put on separate plates at the permissive temperature and allowed to self-cross for 12 hours. The F 2 hermaphrodites were then removed to new plates at the restrictive temperature, and screened for plates where 1) no F 3 progeny were produced or 2) F 3 progeny produced did not give any F 4. If either of these criteria were fulfilled, the permissive temperature stock was saved (see figure 1).

In screen 3, previously isolated ts mutants (isolated by Dr. Baillie on N-2-S) were screened as in screen 2 -ie, for hermaphrodites which either gave no progeny, or hermaphrodites whose progeny did not give progeny.

5. Mutant Characterization.

Figure 1 Screening protocol for ts mutations (see screen 2).

SCREEN FOR TS MUTANTS



Mutants were first characterized according to the following criteria : 1) time of developmental arrest when placed at the restrictive temperature as first stage larvae; 2) stage of developmental arrest when placed at the restrictive temperature as crescent stage worms; and 3) stage of developmental arrest when placed at the restrictive temperature as gravid adults. Other experiments included shift down experiments, outcross experiments, heat pulse experiments and segregation from heterozygotes as indicated in the results. Shift down experiments refer to shifts of C. elegans to the permissive temperature from 26 C. Shift up experiments refer to shifts of C. elegans to 26 C from the permissive temperature.

All experiments at 26 C were done on small plates preheated to the restrictive temperature for 20-30 minutes. Parental worms were transfered at regular intervals (12 hours) at 26 C. This time interval allowed a large number of eggs to be laid at 26 C, and a reasonable number of egg laying intervals. In all experiments where egg hatchability was tested, the eggs were counted at transfer time and the resultant progeny counted several days later.

In several experiments, the 12 hours of egg laying was modified to fit the experiment. For example, a short egg laying period of three hours was used to synchronize developing worms.

Also, to test for embryonic temperature sensitivity, two or three hours of egg laying were used.

Although most observations on eggs and worms were made with a dissecting microscope, some compound microscope observations were made. In these cases, larval or adult worms were paralyzed in 1% nicotine. M-9 buffer was used as a mounting medium for bright field microscopy in some cases.

Experiments to characterize ts mutants were performed on the progeny of a minimum of 5 hermaphrodites. The experiments were then repeated.

6. Mapping

For mutants originally isolated by mutagenesis performed on N-2-S stocks, ts/+ males were constructed and mated with dumpy 11 (dpy 11 is a mutation on chromosome 5). Several ts +/+ dpy 11 hermaphrodites were then selfcrossed at the restrictive temperature. Hermaphrodites which gave ratios approaching 2:1 (wild type : dpy 11) at 26^{o}C indicated that the mutation was linked to chromosome 5, and further mapping was done. Following isolation of homozygotes for the ts mutant and dpy 11, males heterozygous for unc mutations were mated with homozygotes for dpy 11 and the ts mutation. From the F 1,

progeny were obtained which were screened for dpy-unc double mutants at 17°C. These dpy unc hermaphrodites were then tested at 26°C for the presence of the ts phenotype. The frequency of the recovery of dpy-unc-ts triple homozygotes could be used to right-left position the ts with respect to the markers. Segregation analysis of the progeny from triple heterozygotes (all mutations in the cis position) was also used to position the mutant. Uncoordinated mutants used were unc 34 and unc 76 (see table 1).

Mutants isolated by mutagenesis performed on dpy 11 worms were easily assigned linkage to chromosome 5 by obtaining heterozygotes at the permissive temperature and then performing segregation analysis at the restrictive temperature. Worms that did not give a 3:1 ratio of wild type to dpy 11 indicated linkage to chromosome 5. The mutants were then right-left positioned by recording the frequency of recovery of dpy 11-unc 76 and dpy 11-unc 34 worms which were homozygous for the ts mutation at the restrictive temperature. As with the other mutants, segregation analysis of the progeny from triple heterozygotes (where the mutations were cis linked) was also used in mapping. The uncoordinated mutants used were usually unc 34 and unc 76.

In all segregation tests, the progeny of the hermaphrodites were counted until no more eggs were laid. In some cases, the

segregation included testing of the progeny for the ts phenotype at the restrictive temperature. Progeny were counted on small petri plates. Adult worms were transfered every 24 hours during segregations at the restrictive temperature.

7. Terminology

The parent (Po) in each experiment was the original worm collected at the permissive temperature and placed on a petri plate for experimental purposes. The terms "egg" and "embryo" are synonymous in this thesis. Both terms refer to developmental stages after fertilization and before hatching. The term "comma stage" refers to a postgastrular embryo. The "pretzel stage" (Von Erhenstein, 1977) refers to a vermiform embryo ready to hatch. Hatched eggs are L 1 larvae. These were collected for experimental purposes by leaving embryos 24 hours at 17^{o}C. Crescent stage worms are probably L 4 larvae, and are recognized by a clear, crescent shaped area in the region where the vulva will form in the adult. Gravid adults were defined as crescent stage worms left 24 hours at the permissive temperature before the shift to the restrictive temperature.

Leaky mutants are mutants where the phenotype does not occur in 100 % of the progeny obtained from homozygous hermaphrodites. In these mutants, the individual worms that do not express the

phenotype are refered to as leakers or escapers.

Several abbreviations appear in the text, especially with regard to gene designations. Dumpy 11 (e224) is typically abbreviated dpy 11. Uncoordinated mutants are abbreviated "unc" followed by the gene number. Allele isolation numbers are found only in Table 1. Temperature sensitive mutants are designated "ts" followed by the gene number. For ts 4, ts 5, and ts 7, all experiments were done on a dpy 11 background.

In several graphs, the abcissa is labeled "Po transfer time". At these times, individual worms were transfered to a separate, preheated plate. Thus, the interval between two successive transfer points represents an egg laying interval.

Heat pulses refer to experiments in which the parent is shifted to the restrictive temperature and then back to the permissive temperature.

The first developmental stage at which a shift down results in phenotypic expression is the start of the temperature sensitive period (t.s.p.) while the first developmental stage at which a shift up results in no phenotypic expression is the end of the t.s.p..

Results

A. Isolation of Linkage Group 5 ts Lethals

Some general statements can be made about screens for ts mutants. First, in screens for chromosome 5 linked lethals at the restrictive temperature (26^{o}C) (see screen 1 in methods), about 5% of the chromosomes tested carried a lethal within 5 map units of dpy 11. Among these lethals, only one was found to be temperature sensitive. This mutant was designated ts 12.

In screens designed for isolation of lethal mutants (see screen two in methods), several temperature sensitive mutants were found which gave either no F 3 or no F 4 progeny. Three of these mutants, ts 4, ts 5 and ts 7 were found to be on linkage group 5.

Among previously isolated ts mutants (isolated by Dr D. L. Baillie) two were found to be linked to chromosome 5. These mutants were designated ts 1 and ts 2.

B. Characterization of Mutants

Ts 7

Lethal phases (shift up experiments)

Lethal phases of ts 7 were determined by shifting homozygotes to the restrictive temperature at different stages of development. As seen in figure 2, shifting homozygous first stage larvae of ts 7 to the restrictive temperature resulted in growth of the larvae into gravid adults. These adults, when left at the restrictive temperature, laid eggs which failed to hatch. Older larvae of ts 7 placed at the restrictive temperature also became egg laying adults. As indicated in figure 2, homozygous L 4 larvae of ts 7 placed at the restrictive temperature became adults which produced developmentally arrested embryos.

Shifting gravid hermaphrodites of ts 7 homozygotes to the restrictive temperature also resulted in the production of developmentally arrested embryos (see figure 2) Among the first few eggs that were laid by gravid ts 7, some were found to develop through larval stages at the restrictive temperature (see figure 2). These escaper ts 7 became adults laying developmentally arrested embryos at the restrictive temperature (see figure 2).

Lethal phases of ts 7 were determined by shifting homozygotes to the restrictive temperature at different stages of development. The temperature sensitive lethality of ts 7 was restricted to the period of embryonic development.

To more precisely determine the lethal phenotype of ts 7, gravid adults were shifted to the restrictive temperature and transferred at 12 hour intervals for 36 hours. Each 12 hour egg laying interval was then left for 12 hours at the restrictive temperature before observation under the compound microscope. As shown in table 3, the embryonic arrest phenotype is uniformly "precomma" stage. Cell number estimates obtained from photographs indicated about 100 cells in the arrested embryos of ts 7.

A test for larval temperature sensitivity
(shift down experiments)

To determine if there was any larval temperature sensitive contribution to ts 7 lethality, worms grown to crescent stage at the restrictive temperature were shifted to the permissive temperature. Subsequent egg hatchability at the permissive temperature was then scored. As indicated in figure 3, nearly all of the eggs laid at the permissive temperature following L 4 shift down hatched and developed to adults. Thus, temperature

sensitivity prior to L 4 (when a large part of gonadogenesis has taken place) cannot be demonstrated in ts 7 homozygotes.

Mating experiments at the restrictive temperature

Homozygous ts 7 hermaphrodites were mated to homozygous wild type males at the permissive temperature. Figure 4 shows the result of hermaphrodites premated at the permissive temperature and shifted to the restrictive temperature with males. Outcross progeny were only obtained during the first 12 hours at the restrictive temperature. Eggs laid by mated hermaphrodites after the first 12 hours at the restrictive temperature failed to hatch. Virgin hermaphrodites placed directly at the restrictive temperature with males failed to give any outcross progeny.

Heat pulse experiments during oogenesis
(shift up, then down)

Heat pulse experiments on ts 7 homozygotes during oogenesis demonstrated maternal effects on embryogenesis. Egg hatchabilty profiles for ts 7 homozygotes at the permissive temperature following 12, 24 and 36 hour heat pulses during oogenesis are shown in figure 5. Some reversiblity of the ts 7 lethality was seen following 12 and 24 hour heat pulses . Nevertheless, the

lethality encountered during these time intervals suggested a maternal component to embryonic lethality. For example, the second broods following 12 and 24 hour heat pulses did not give 100% hatching, despite a lack of embryonic exposure to the restrictive temperature. Following 36 hours of exposure to the restrictive temperature during oogenesis, all eggs failed to hatch (see figure 5).

Segregation analysis of progeny from heterozygotes

Segregation from ts 7 heterozygotes was performed at the restrictive temperature. Worms heterozygous for a dpy 11-ts 7 chromosome gave segregation ratios at the restrictive temperature that were not Mendelian 3:1 (see table 2). The segregation pattern indicating linkage was observed when segregating from adults arising from L 1 larvae placed at 26 C, but not when segregating from parents placed at 26 C as L 4 larvae. Embryonic death from ts 7 heterozygotes is suggested since segregation did not give rise to the expected number of progeny homozygous for chromosome 5 markers (see table 2). Thus, embryonic temperature sensitivity and lethality were demonstrated.

Figure 2 Lethal phases of ts 7 and development of ts 7 at the restrictive temperature. Each line represents development of 5 homozygotes at 26°C from the stage indicated at the bottom of the table. The bars on the left indicate time of shift to the restrictive temperature, while the bars at the right end of each horizontal line indicate the lethal phase. The solid lines indicate the development of the majority of worms, while the dotted lines indicate the development of escapers.

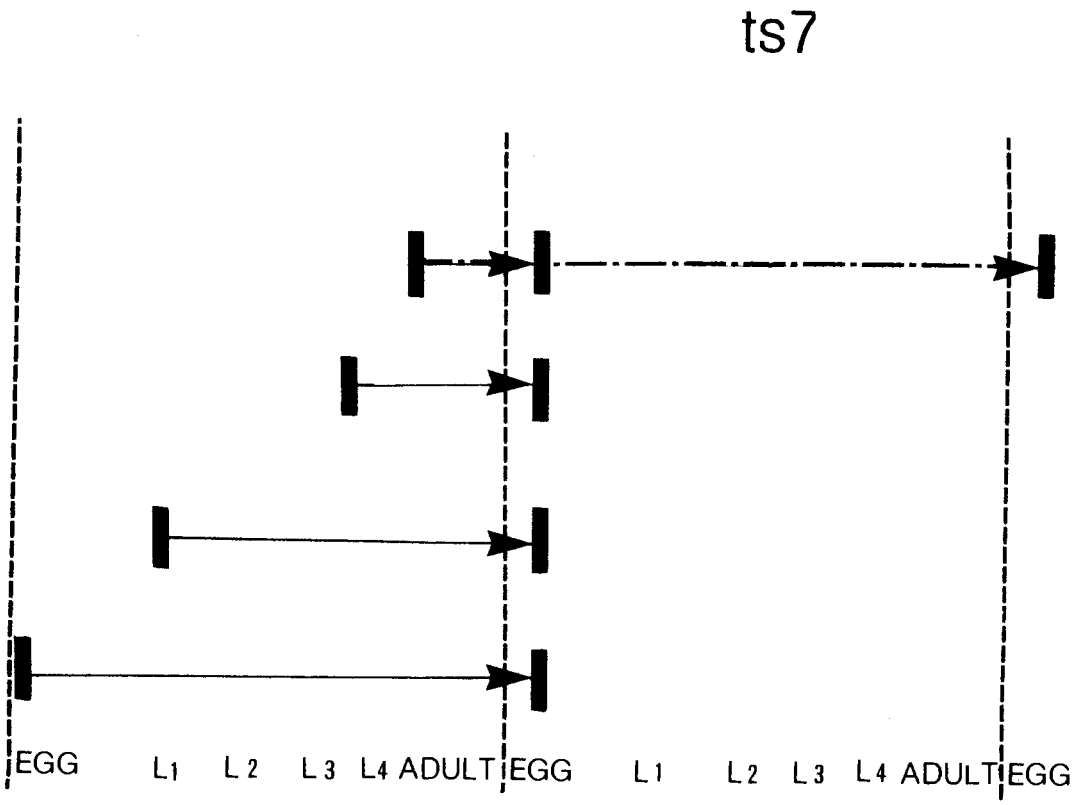

ts7
EGG
L1
L2
L3
L4
ADULT
EGG
L1
L2
L3
L4
ADULT
EGG

Figure 3 A test for larval temperature sensitivity in ts 7 larvae. Homozygous ts 7 eggs were placed at the restrictive temperature as eggs in the uterus, and the eggs that hatched were allowed to develop into cresent stage worms. These larvae were then shifted to the permissive temperature and the resulting adults were transfered at the time intervals indicated. The eggs were counted at the transfer intervals and the progeny several days later.

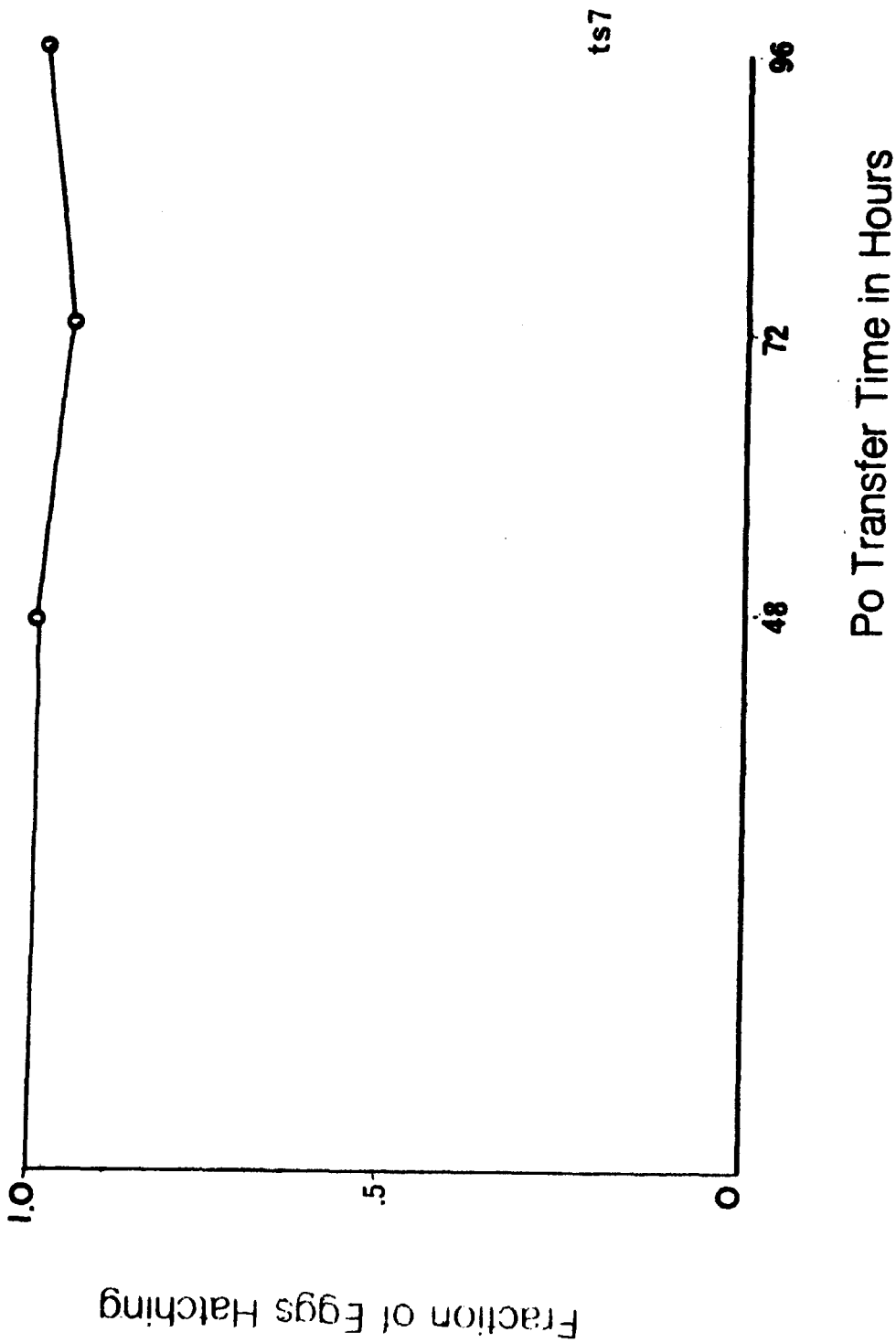

ts7
1.0
.5
0
48
72
96
Fraction of Eggs Hatching
Po Transfer Time in Hours

Figure 4 Mating Experiments on ts 7 homozygotes . Homozygous ts 7 hermaphrodites were mated to three wild type males at the permissive temperature for 24 hours. This made the result similar to the shifting of homozygotes without mating. Following this, the hermaphrodites and the males were transfered to the restrictive temperature. Each plate had one gravid hermaphrodite and three males. Results for 20^{o}C are also shown.

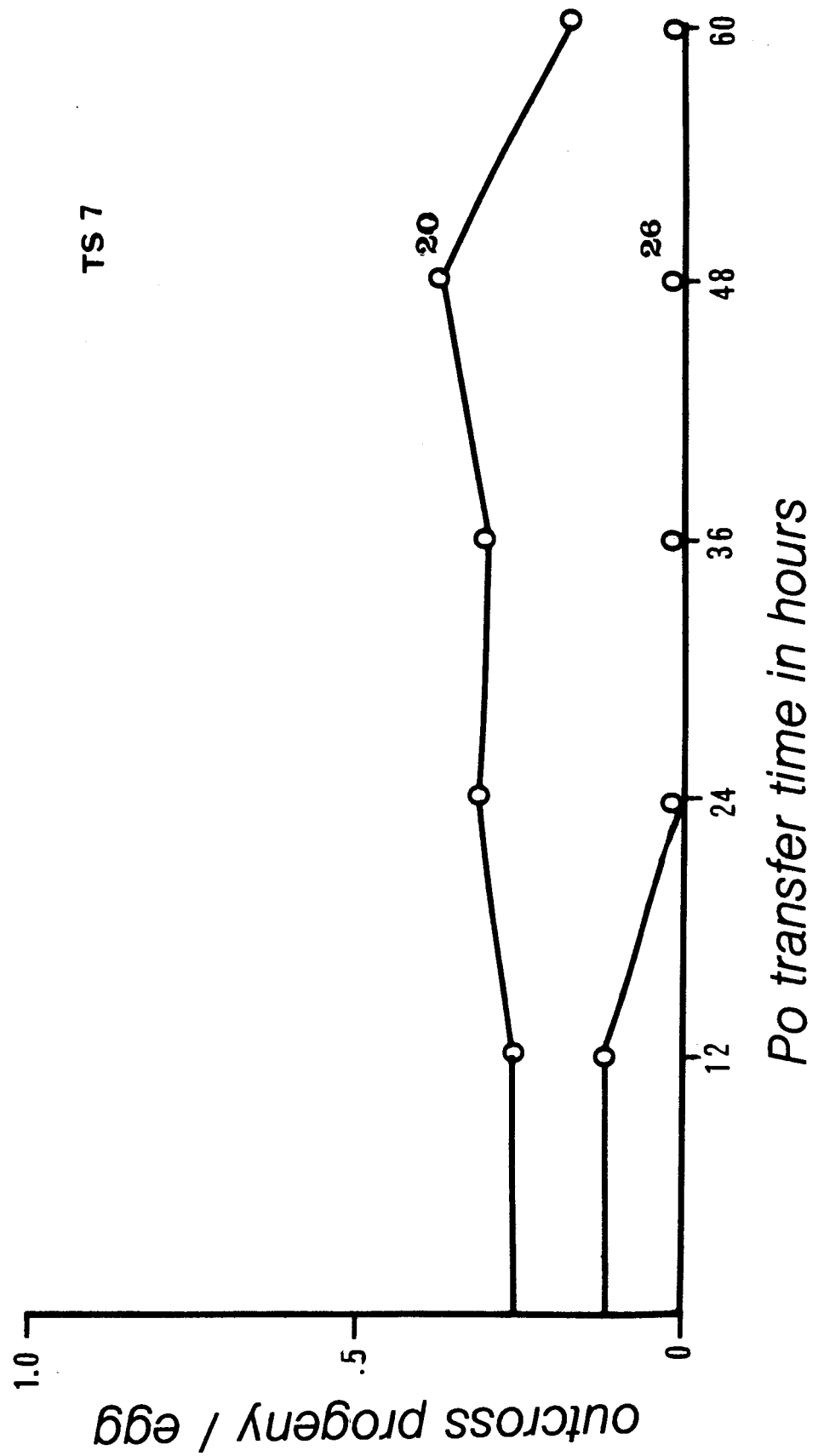
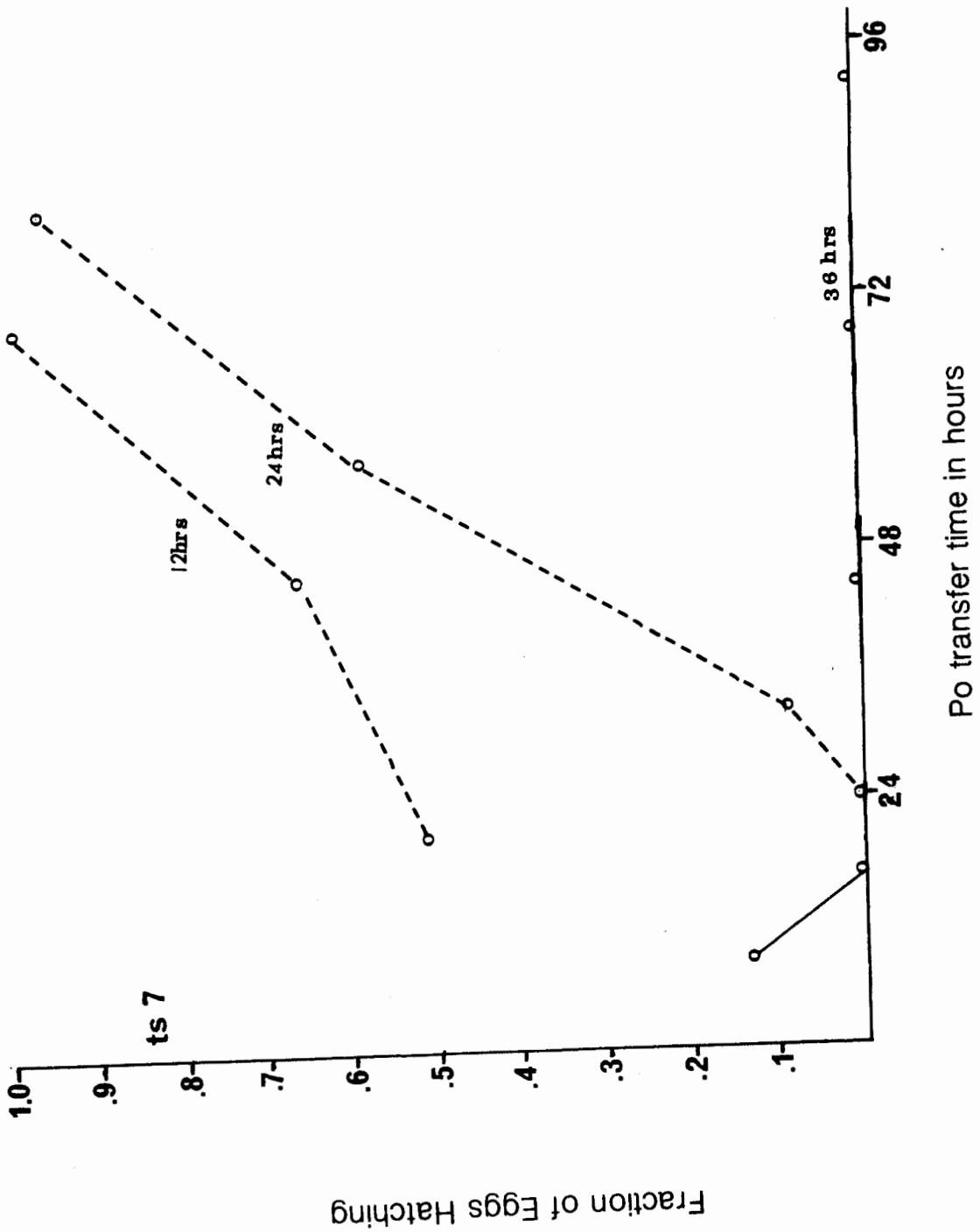

TS 7
20
26
1.0
.5
0
12
24
36
48
60
outcross progeny / egg
Po transfer time in hours

Figure 5 Heat pulse experiments during oogenesis on ts 7. The effect of 12, 24 and 36 hour heat pulses on ts 7 homozygotes during oogenesis are shown in this figure. Each parent was shifted to the restrictive temperature as a gravid adult.———o——— = the fate of eggs laid at the restrictive temperature and transfered to the permissive temperature . - - - -o - - - - = the fate of eggs laid at the permissive temperature following the heat pulse.


Fraction of Eggs Hatching
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
ts 7
12hrs
24hrs
36 hrs
24
48
72
96
Po transfer time in hours

TS 2

Lethal phases (shift up experiments)

The lethal phase of ts 2 was determined by shifting homozygotes to the restrictive temperature at different stages of development. As indicated in figure 6, shifting homozygous first stage larvae to the restrictive temperature resulted in the growth of the larvae into adults. These adults laid eggs which arrested as embryos. Shifting L 4 larvae to the restrictive temperature also resulted in the larvae becoming egg laying adults. Eggs laid by these worms , when left at the restrictive temperature, failed to hatch (see figure 6).

When compared to crescent stage worms, adult worms homozygous for ts 2 gave a different result when shifted to the restrictive temperature. Although a large number of developmentally arrested embryos were obtained from the gravid adults, the first 12 hours of egg laying contained a number of eggs which developed into adults. As with ts 7, "escapers" of ts 2 at the restrictive temperature became adults laying eggs which were developmentally arrested (see figure 6).

Thus, the result of experiments on ts 2 show it to be a monophasic embryonic lethal.

The lethal phase of ts 2 was more accurately determined by shifting gravid hermaphrodites to the restrictive temperature and observing embryos in each of three successive 12 hour egg laying intervals. Following a 12 hour period at the restrictive temperature, embryos were observed under the compound microscope (see table 3). The result indicated some variation in the type of embryonic arrest observed. There was a shift to earlier embryonic arrest as the parent spent more time at the restrictive temperature. Many ts 2 embryos were observed that had particularly large cells. These embryos, seen amongst precomma stage embryos, varied amongst themselves with respect to the size of the large cell(s). Often, dead ectodermal cells were seen on the embryo. Control experiments indicated the majority of N-2-S (wild type) embryos were hatching at this time.

A test for larval temperature sensitivity
(shift down experiments)

To determine if there was any larval temperature sensitivity contributing to the embryonic lethality of ts 2, leakers from gravid ts 2 homozygotes were shifted to the permissive temperature as L 4 larvae. As indicated in figure 7, only about 50% of the eggs hatched. This indicated that exposure of larvae to the restrictive temperature could result in subsequent embryonic lethality of ts 2.

To test the extent of larval temperature sensitivity , escapers of gravid ts 2 were shifted to the permissive temperature during successively later larval stages. Fewer hatching eggs were obtained as the larvae approached L 4 stage before the shift down. Thus, ts 2 was shown to be a monophasic embryonic lethal with a temperature sensitive period extending throughout gonadogenesis.

Mating experiments at the restrictive temperature

Outcross experiments were done with ts 2 at the restrictive temperature. Following mating at the permissive temperature, gravid ts 2 were shifted with males to the restrictive temperature. Most outcross progeny were obtained during the first 12 hours of egg laying at the restrictive temperature and fewer outcross progeny were obtained in later broods (see figure 8). Low numbers of outcross progeny were obtained when L 4 worms of ts 2 were mated at the restrictive temperature. In this case, the few outcross progeny that appeared were not restricted to the first eggs laid. Self-cross progeny were not obtained in mating experiments at 26^{o}C, indicating that embryos were developmentally arrested.

Heat pulse experiments during oogenesis
(shift up, then down)

Heat pulse experiments with ts 2 were performed during oogenesis. Egg hatchability profiles at the permissive temperature following 12, 24 and 36 hour heat pulses during oogenesis are shown in figure 9. Substantial reversibilty of the ts phenotype was observed following a 12 hour heat pulse. However, following 24 hours at 26^{o}C during oogenesis, egg hatching at the permissive temperature was reduced to below 50% throughout egg laying. 36 hours exposure to the restrictive temperature resulted in no egg hatching at the permissive temperature. As with ts 7, the second broods in ts 2 following 24 hour heat pulses were composed of embryos exposed to the restrictive temperature only as oocytes. These restrictive temperature oocytes became developmentally arrested embryos at the permissive temperature.

Segregation analysis of progeny from heterozygotes

Heterozygous hermaphrodites of ts 2 were placed at the restrictive temperature and the selfcross progeny were examined. As indicated in table 2, progeny homozygous for chromosome 5 markers were encountered less frequently than expected for a Mendelian ratio.

Figure 6 Lethal phases of ts 2 and development of ts 2 homozygotes at the restrictive temperature. The bars and lines have the some meanings as in figure 2.

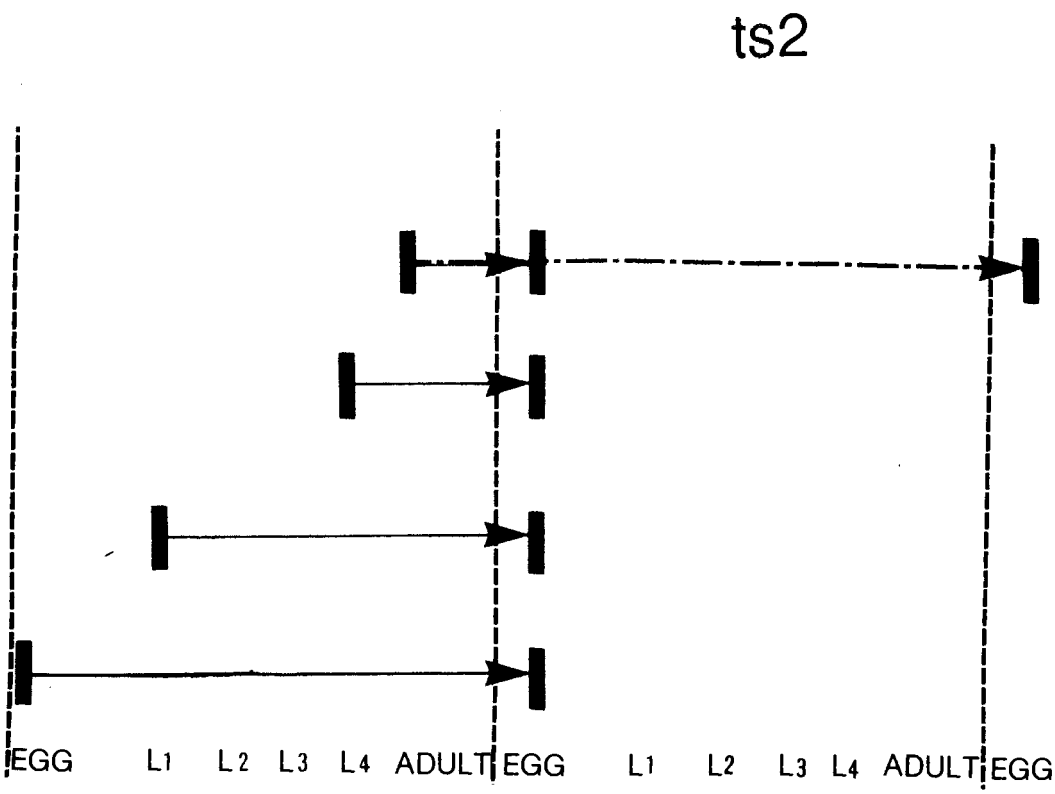

ts2
EGG
L1
L2
L3
L4
ADULT
EGG
L1
L2
L3
L4
ADULT
EGG

Figure 7 A test for larval temperature sensitivity among ts 2 larvae. The experimental design and the plot of these data is as in figure 3, except escapers from homozygous gravid ts 2 were used.

ts2
Fraction of Eggs Hatching
1.0
.5
0
48
72
96
Po Transfer Time in Hours

Figure 8 Mating experiments on ts 2 homozygotes. The experimental design and plot of these data is the same as in figure 4 for ts 7 except that the number of outcross progeny was estimated from twice the number of males obtained. Outcross progeny were counted this way in order to distinguish selfcross progeny from outcross progeny, since the experiment was not done on hermaphrodites carrying visable markers.

outcross progeny / egg
1.0
.5
0
12
24
36
48
60
Po transfer time in hours
TS 2
20
26

Figure 9 Heat pulse experiments during oogenesis on ts 2. The experiment was performed as indicated in figure 5 for ts 7.

Fraction of Eggs Hatching
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
ts 2
12 hrs
24 hrs
36 hrs
24
48
72
96
Po transfer time in hours

TS 1

Lethal phases (shift up experiments)

Lethal phases (developmental blocks) of ts 1 were determined by shifting homozygotes to the restrictive temperature (26°C) at successively later stages of development. As seen in figure 10, shifting homozygous first stage larvae (L 1) to the restrictive temperature resulted in an arrest of development of the larvae in mid-larval stages (L 2-L 3) (see figure 10). Older homozygous ts 1 larvae shifted to the restrictive temperature resulted in the appearance of a new phenotype: adult sterility. An increasing percentage of the larvae became sterile adults at the restrictive temperature as the larvae approach L 4 larvae at the permissive temperature (see figure 11). In shift up experiments on L 4 larvae, the L 4 worms became egg laying adults and the eggs failed to hatch (see figure 10).

Shifting gravid hermaphrodites homozygous for ts 1 to the restrictive temperature resulted in an embryonic arrest phenotype as well as some escapers which arrested in midlarval stages. These larvae came from the first eggs laid by gravid ts 1 hermaphrodites (see figure 10).

Thus, in simple shift up experiments on ts 1 at successively later stages of development, three periods of developmental arrest were observed: embryonic, larval, and adult sterility.

The lethal phases obtained from gravid hermaphrodites of ts 1 homozygotes at the restrictive temperature were more accurately determined by observation under a compound microscope. The larvae reached a length comparable to L 2 larvae, although some reached a length comparable to L 3 larvae (Hirsh et al, 1976). Observations on developmentally arrested embryos of ts 1 were tabulated in table 3. Not all of the embryos from gravid ts 1 hermaphrodites blocked at the same stage. There was a shift to a earlier embryonic arrest as the parent spent more time at the restrictive temperature.

A test for larval temperature sensitivity
(shift up, then down)

To further test for larval temperature sensitivity, overlapping heat pulse experiments were performed. If all stages of larval development were sensitive to the restrictive temperature, one would expect 24 hour heat pulses to result in expression regardless of the larval stage subjected to the restrictive temperature. Although all larval stages showed some

temperature sensitivity, two intervals of larval development were more sensitive to the restrictive temperature (see figure 12).

Mating experiments at the restrictive temperature

It is expected that some mutants with temperature sensitivity during oogenesis would give outcross progeny at the restrictive temperature. When hermaphrodites of ts 1 homozygotes were mated at the permissive temperature and shifted to the restrictive temperature as gravid adults along with males, large numbers of outcross progeny appeared only during the first 24 hours at the restrictive temperature (see figure 13). On the other hand, virgin hermaphrodites mated at the restrictive temperature gave rise to very few outcross progeny throughout the egg lay period.

Heat pulse experiments during oogenesis
(shift up then down)

Maternal effects may be demonstrated by temperature shift experiments. Provided the parent spends sufficient time at the restrictive temperature, embryonic arrest may be observed upon shift of the parent to the permissive temperature. Figure 14 shows the effect of 12, 24 and 36 hour heat pulses on gravid

ts 1 homozygotes, and resulting egg hatchability at the permissive temperature. The result was some reversibility of the temperature sensitive embryonic arrest of ts 1 (see figure 14). After the shifts of the adults to the permissive temperature, the first egg laying period was composed partially of eggs that were contained in the uterus before the shift down. Thus, at least part of the developmentally arrested embryos contained in the first 12 hours of egg laying at the permissive temperature may be explained by embryos exposed to the restrictive temperature before the shift down. Progeny in the second egg laying interval were exposed only as oocytes to the restrictive temperature. Egg hatching approached 100% in this second brood following both 12 and 24 hour heat pulses. In 36 hour heat pulses to gravid ts 1, some developmentally arrested embryos were obtained due only to exposure of oocytes to the restrictive temperature (see figure 14).

Segregation analysis of progeny from heterozygotes

Segregation from heterozygous hermaphrodites of ts 1 resulted in a ratio that did not approaoch a 3:1 ratio of wild types to mutant (see table 2). This result suggested that homozygotes of ts 1 from the heterozygous ts 1 parent were arresting in development.

Ts 1 may be described as a polyphasic temperature sensitive lethal with maternal effects on embryogenesis.

Figure 10 Lethal phases and development of ts 1 at the restrictive temperature. See figure 2 for ts 7 for explanation of this figure for ts 1 homozygotes.

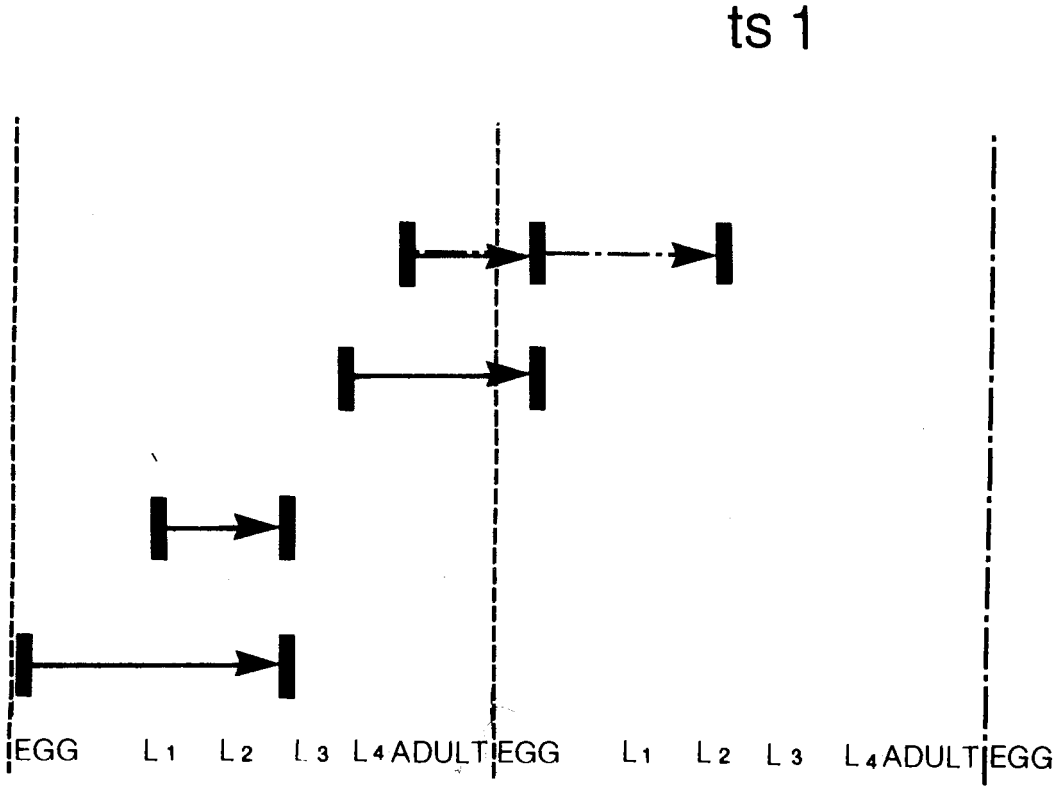

ts 1
EGG
L1
L2
L3
L4
ADULT
EGG
L1
L2
L3
L4
ADULT
EGG

Figure 11 Shift up experiments on ts 1 larvae. Ts 1 larvae were shifted from 17°C to the restrictive temperature at at the times indicated on the x-axis. Worms which did not arrest as larvae became sterile adults during these time intervals. 5 homozygous ts 1 larvae were use at each data point.

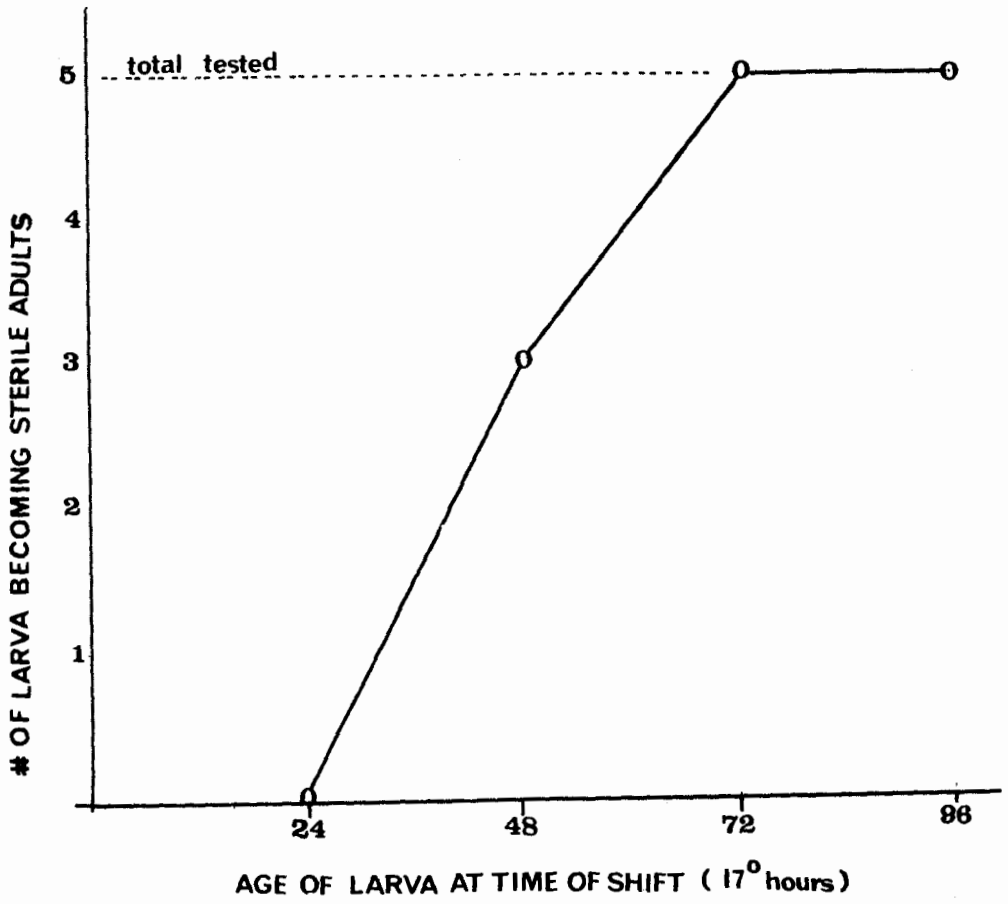

total tested
5
4
3
2
1
OF LARVA BECOMING STERILE ADULTS
24
48
72
96
AGE OF LARVA AT TIME OF SHIFT (17° hours)

Figure 12 A further test for larval temperature sensitivity among ts 1 larvae. Homozygous ts 1 larva were shifted to the restrictive temperature at the times indicated on the x-axis. The larvae were then left at the restrictive temperature for 24 hours and then again shifted to the permissive temperature. After 3-5 days, the number of worms laying eggs was scored, and plotted on this graph as % larvae becoming fertile adults (the Y-axis).

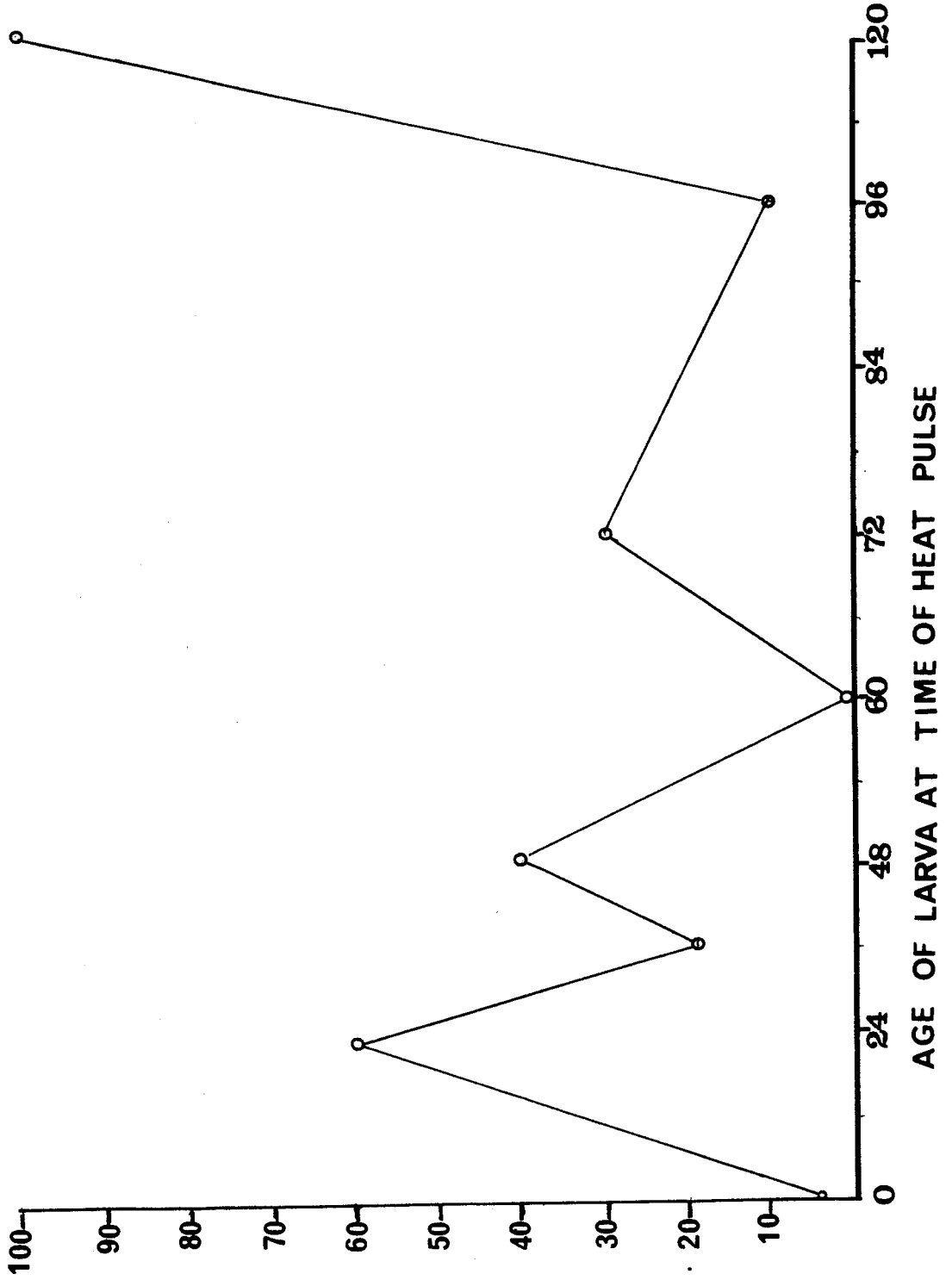

% larvae becoming fertile
100
90
80
70
60
50
40
30
20
10
0
24
48
60
72
84
96
120
AGE OF LARVA AT TIME OF HEAT PULSE

Figure 13 Mating experiments on ts 1 homozygotes at the restrictive temperature. This experiment was performed on ts 1 homozygotes not carrying other markers, and thus, the number of outcross progeny was indicated as twice the number of males. See figure 8 on ts 2 for explanation of this.

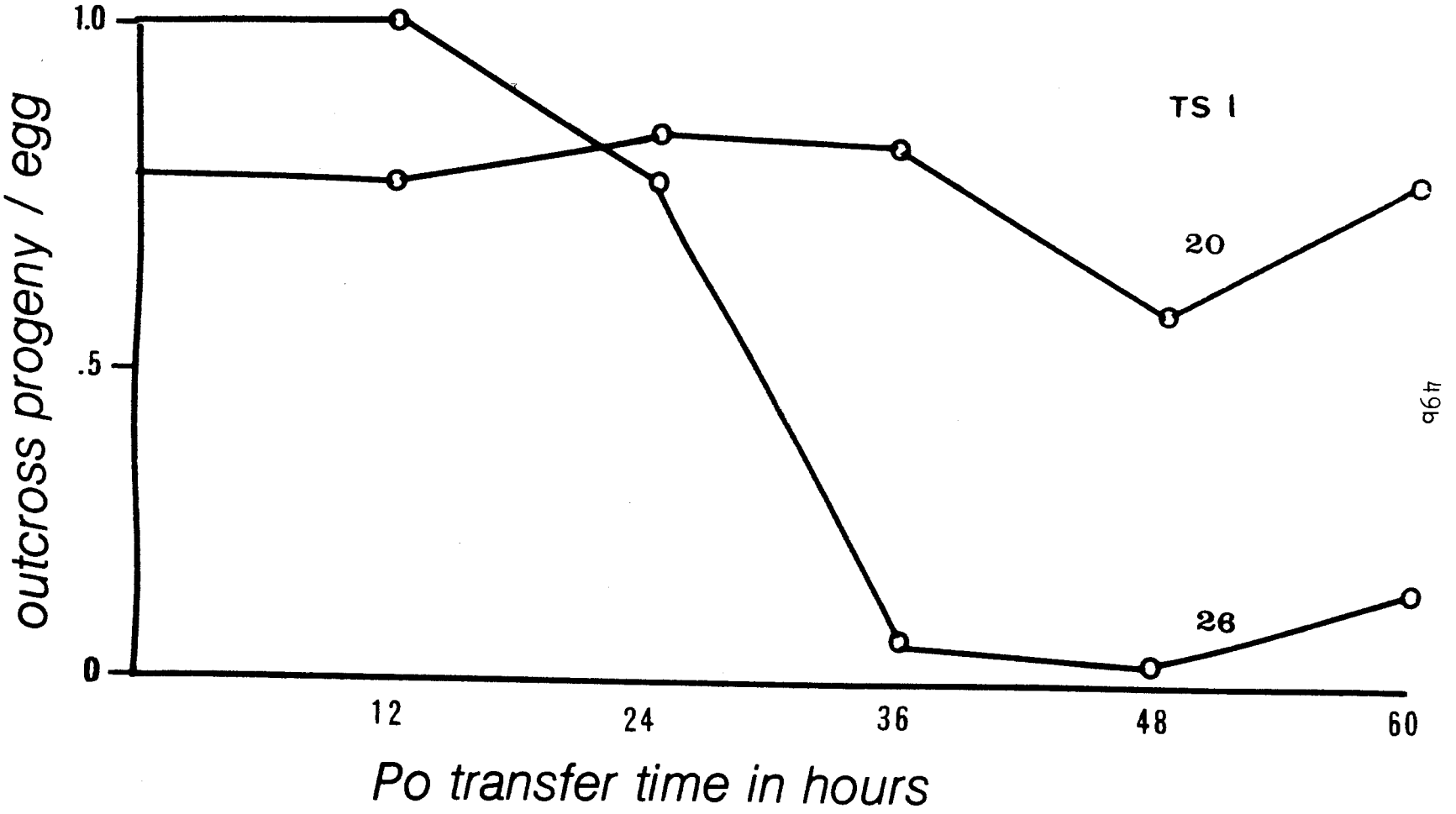
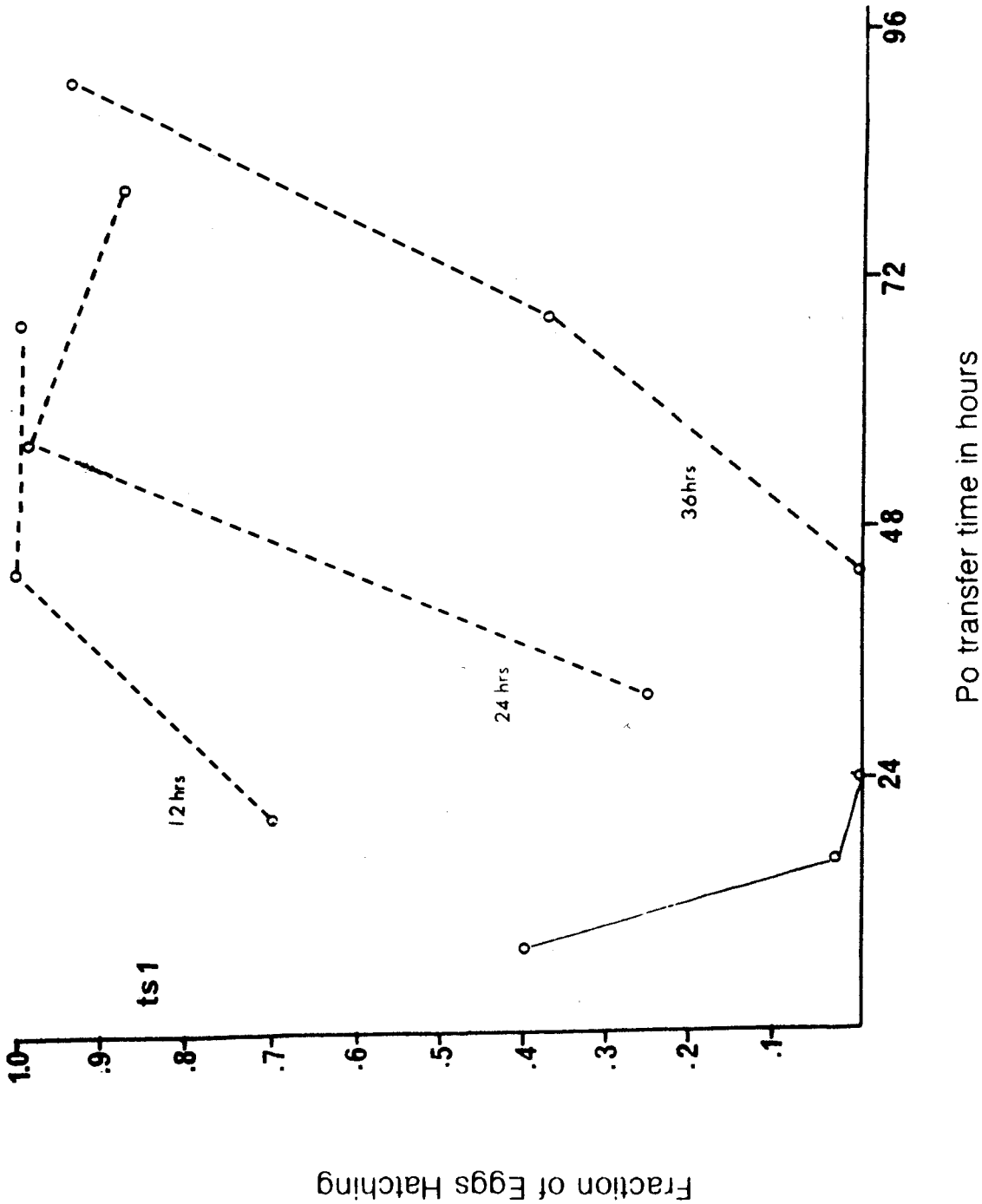

1.0
.5
0
outcross progeny / egg
TS I
20
26
12
24
36
48
60
Po transfer time in hours

Figure 14 Heat pulse experiments during oogenesis on ts 1 . See figure 5 for an explanation of this figure on shift ups, then down on ts 1 homozygotes during oogenesis.


Fraction of Eggs Hatching
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
ts1
12 hrs
24 hrs
36hrs
24
48
72
96
Po transfer time in hours

Other Chromosome 5 Ts Mutants.

Three other mutants were found which were linked to chromosome 5. These mutants were designated ts 4, ts 5 and ts 12. Properties of the mutants indicated that they were considerably different from the mutants already discussed, and so, the mutants were not considered in many experiments done with ts 1, ts 2 and ts 7.

When compared to the other mutants studied, ts 5 displayed a different temperature sensitive phenotype. The mutant is characterized by almost complete sterility. Many more progeny were obtained at the permissive temperature than were obtained at the restrictive temperature. In shift up experiments at successively later stages of development, homozygotes would grow to adults which laid very few eggs. The eggs that were laid would develop to adults (see figure 15). In mating experiments with ts 5 at the restrictive temperature, very few outcross progeny were obtained. The number of outcross progeny do not approach normal levels expected for worms not carrying ts 5. Segregation from heterozygous ts 5 hermaphrodites at the restrictive temperature indicated that ts 5 was linked to chromosome 5 and that progeny homozygous for ts 5 and other chromosome 5 mutants were not produced (see table 2).

Another mutant, ts 4, was isolated in screens for ts mutants and then assigned linkage to chromosome 5. By means of shift up experiments, the phenotype of ts 4 was found to be midlarval lethality (see figure 16). Male rescuability was found to be low. Segregation from ts 4 dpy 11/+ + at the restrictive temperature resulted in fewer dpy 11 progeny than expected if ts 4 was not linked to chromosome 5 (see table 2).

Ts 12 was isolated in screens for chromosome 5 linked lethals at the restrictive temperature and behaved as a lethal at the restrictive temperature. Homozygous larval stages and adults of ts 12 at the restrictive temperature gave about 30% dead embryos. Among embryos hatching at the restrictive temperature, about 50% became sterile adults. The remaining eggs hatching at the restrictive temperature became egg laying adults. Nevertheless, segregation from hermaphrodites heterozygous for ts 12 and other chromosome 5 markers indicated progeny homozygous for ts 12 and other chromosome 5 markers were lethal.

Figure 15 Lethal phases and development of ts 5 at the restrictive temperature. Refer to figure 2 for an explanation of this figure.

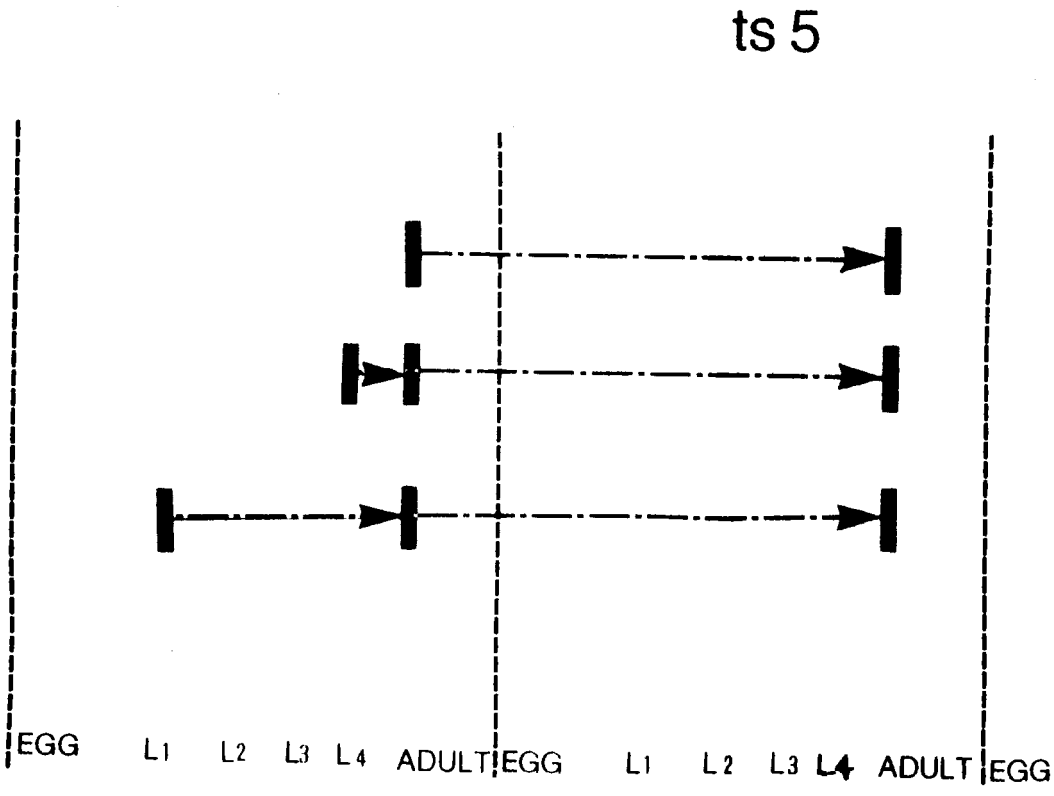
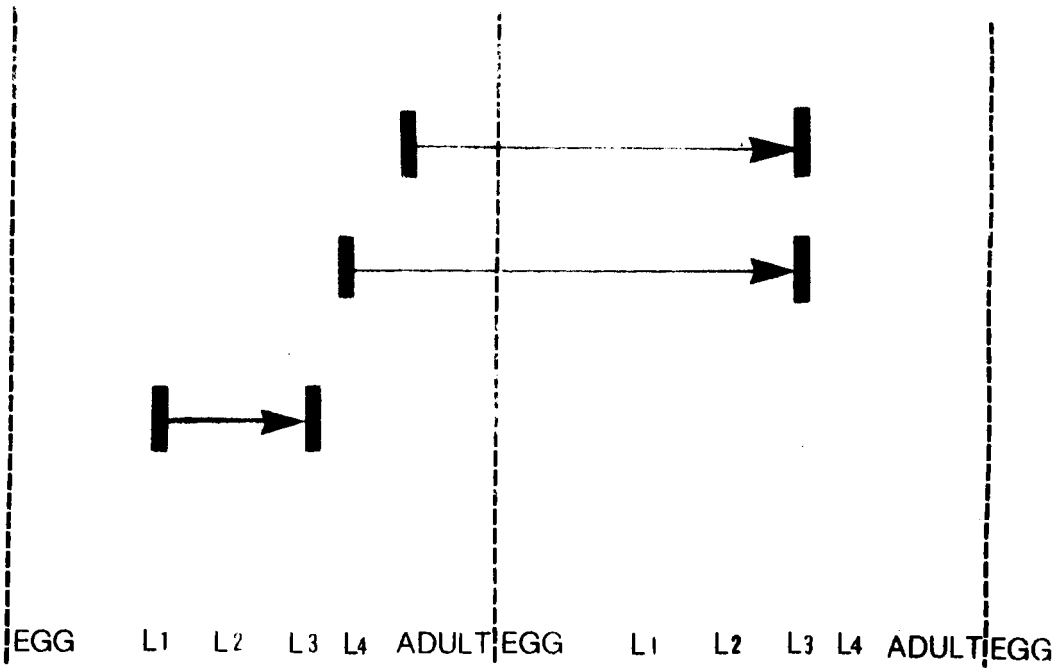

ts 5
EGG
L1
L2
L3
L4
ADULT
EGG
L1
L2
L3
L4
ADULT
EGG

Figure 16 Lethal phases and the development of ts 4 at the restrictive temperature. Refer to Figure 2 for details on the meaning of this figure.

ts 4



C. Mapping

Ts 7 was mapped to chromosome 5 by segregation analysis of progeny from dpy 11 ts 7/+ + at the restrictive temperature. By segregation from unc 34 dpy 11 ts 7/+ + + and dpy 11 unc 76 ts 7/+ + +, ts 7 was mapped to the right of unc 76 on chromosome 5 (see figure 17).

Ts 2 was mapped to chromosome 5 by segregation from ts 2 +/ dpy 11 + and ts 2 dpy 11/+ +. Segregation analysis of progeny from ts 2 unc 34 dpy 11/+ + + and ts 2 dpy 11 unc 76/+ + + indicated that ts 2 was to the left of unc 34 on chromosome 5 (see figure 17).

Ts 1 was assigned linkage to chromosome 5 by segregation analysis of progeny from from + ts 1/dpy 11 + and dpy 11 ts 1/+ + at the restrictive temperature. Segregation analysis of the progeny from dpy 11 unc 76 ts 1/+ + +, as well as unc 76 ts 1/+ + indicated that ts 1 was to the left of dpy 11 on chromosome 5 (see figure 17).

Ts 5 was assigned to chromosome 5 by the restrictive temperature segregation analysis of progeny from ts 5 dpy 11/+ +. By segregation from ts 5 dpy 11 unc 76/+ + + at 26^{o}C, as well as segregation from unc 34 ts 5 dpy 11, ts 5 was found to map between dpy 11 and unc 34 (see figure 17).

Ts 12 was assigned linkage to chromosome 5 following segregation from ts 12 dpy 11/+ + at the restrictive temperature. Following segregation analysis of the progeny from ts 12 dpy 11 unc 76/ + + +, and unc 34 ts 12 dpy 11/+ + + , ts 12 was positioned to the left of dpy 11 (see figure 17).

Ts 4 was assigned linkage to chromosome 5 following segregation analysis of progeny from dpy 11 ts 4/+ +, but experiments to position the mutant on the chromosome have not been done.

Figure 17 Approximate map positions of ts mutants studied This figure shows approximate positions of some chromosome 5 ts mutants relative to some of the visable markers used. The bar below the figure represents 5% recombination, as determined for the visables on chromosome 5 by Brenner (1974). The approximate position of each mutant is indicated by the line with the arrows.

Table 2 Linkage Data and segregation from heterozygotes at the restrictive temperature. This table shows segregation data for each ts mutant . Progeny testing refers to the testing of F 1 segregants by placing them at the restrictive temperature.

Chromosome V :Recombination Analysis

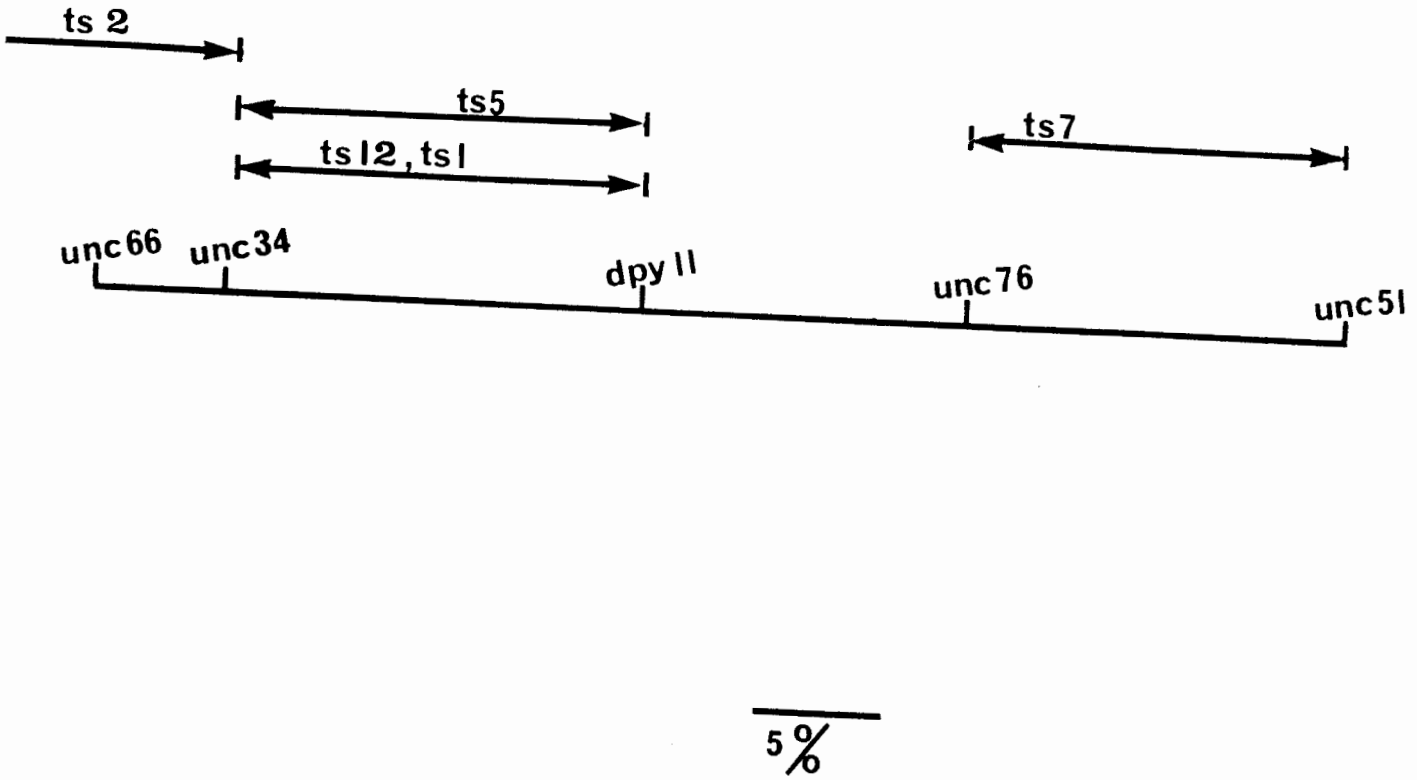


Table 2

Genotype	Dpy	unc	dpy-unc	wild type
ts 7 dpy 11 unc 76/+++	15	1	5	249
unc 34 dpy 11 ts 7/+++	23	27	13	413
dpy 11 unc 76 ts 1/+++	7	12	29	285
ts 2 unc 34 dpy 11/+++	60	26	91	517
ts 2 dpy 11 unc 76/+++	11	29	37	276
unc 34 ts 5 dpy 11/+++	37	5	3	607
ts 5 dpy 11 unc 76/+++	30	67	35	1045
ts 12 dpy 11 unc 76/+++	2	18	61	406
unc 34 ts 12 dpy 11/+++	35	87	58	604
ts 4 dpy 11/++	36			478

Table 3 Stage of embryonic arrest in the embryonic arrest mutants during diferrent time regemes at the restrictive temperature. - = no data collected.

STAGE OF ARREST	tsl			ts2			ts7		
	initial 12 hr egg lay	12-24 hr egg lay	24-36 hr egg lay	initial 12 hr egg lay	12-24 hr egg lay	24-36 hr egg lay	initial 12 hr egg lay	12-24 hr egg lay	24-36 hr egg lay
Unfertilized	0	0	0	0	0	0	0	0	0
Precomma(with large cell)	0	0	0	20	28	-	0	0	0
Precomma(no large cell)	11	44	42	22	4	-	114	36	12
Comma stage	4	21	5	3	0	-	1	0	0
Pretzel stage	28	25	0	6	0	-	0	0	0

Discussion

Since the control of many developmental phenomena is probably at the level of regulation of gene expression, patterns of gene expression have become a focal point in developmental studies. The mutations discussed in this thesis are temperature sensitive mutants. These have been used as a probe to study when gene products (often proteins in the case of ts mutants; see Whitmann and Whitmann-Liebold, 1965) are active during development. The time of gene action in ts mutants can be approximated with the determination of lethal phases, and more accurately described with the determination of the t.s.p. (Suzuki, 1970; Suzuki et. al., 1976). In the following discussion, I have assumed that there is a gene product for each of the mutants studied.

Lethal phases and the t.s.p.

Monophasic mutants offer the simplicity of determination and interpretation of lethal phases and t.s.p.'s. Ts 2 and ts 7 may both be described as monophasic temperature sensitive embryonic lethals with maternal effects on the embryonic lethality. Although the t.s.p. of ts 7 only extends from oogenesis through early cleavage, the t.s.p. of ts 2 extends

from first stage larvae through part of embryonic development. The t.s.p. of ts 7 is easily understood in biochemical terms: many examples are known of macromolecules synthesized during oogenesis and utilized during embryogenesis (see Davidson, 1977). A monophasic lethal with a very extended t.s.p., as seen with ts 2, is less well understood. As mentioned with ts 1, examples are known of developmentally important substances which are present during most of development (Mahowald, 1971B). However, in the case of ts 2, it is not clear whether the ts gene product is specific to the reproductive and embryonic tissues, or is of general occurrence in all tissues but only essential in embryonic development.

Ts 1 is an example of a polyphasic ts lethal; the ts 1 gene product is essential during several stages of development. Although polyphasic lethals such as ts 1 present difficulties in the interpretation of the multiple lethal effects, interesting data can be obtained on the development of these mutants at the restrictive temperature. One problem with the polyphasic lethals such as ts 1 is that of demonstrating that all the phenotypes are due to a mutation in a single gene. Although the phenotypes of ts 1 are all observed on several types of recombinant chromosomes, the possiblity remains that two or more tightly linked mutations are responsible for the phenotypes. This possiblity is lessened by the fact that all phenotypes of

ts 1 are temperature sensitive, and it is unlikely that more than one temperature sensitive mutation on chromosome 5 would be recovered in one screen. Other workers have found mutants in C. elegans with more than one temperature sensitive lethal phase. For example, the "zygote defective" mutants of Hirsh et. al. (1976) were shown to have gonadogenesis blocks as well as blocks in embryogenesis, and these developmental blocks are thought to be due to a point mutation in single genes (Vanderslice and Hirsh, 1976).

In polyphasic lethals like ts 1, it is not known if all effects of the gene are restricted to reproductive and embryonic cells. It is possible that other tissues are affected by the mutation, but not as severely so as to cause lethality. This possibility is indicated by the fact that L 1 larvae placed at the restrictive temperature block in midlarval stages rather than becoming gonadless adults. Since gonadogenesis mutants are found in C. elegans (Hirsh et. al., 1976) it is possible for some tissues of C. elegans to develop while others are arrested. Nevertheless, homozygous L 1 larvae of ts 1 do arrest as larvae, indicating that other tissues, such as the gut, may be affected by ts 1. A more complete analysis of the tissues affected by ts 1 might be obtained through mosaic analysis. Ts 1 is not a general cell lethal mutant since crescent stage worms, unfertilized oocytes, embryonic arrest before 22 cells,

and developmentally arrested L 1 larvae are not obtained at 26 C.

The blocks in gonadogenesis, oocyte production and embryogenesis in ts 1 may be explained, at least partially, on the basis of developmental phenomena. Normal gonadogenesis produces structures essential for production of a normal oocyte. In ts 1, the mutant gene product is functional during gonadogenesis as indicated by the larval lethality. It is likely that many events during gonadogenesis would affect the culminating event of gonadogenesis: formation of the oocyte. The embryonic lethality noted for ts 1 may be explained on the basis of a maternal effect (discussed later), thus relating the embryonic lethality to the oogenetic defect. Examples of developmentally important substances being present throughout the life cycle of an organism can be seen in the polar granules of Drosophila, which are limited to the reproductive system (Mahowald, 1971). Perhaps similar developmentally important substances are present in the development of C. elegans, and are essential during several phases of development.

In polyphasic mutants such as ts 1, several developmental blocks are found in the reproductive (and embryonic) tissue. One may ask why all stages of development of the reproductive system are not found arrested during an appropriate shift up

experiment for the stage in question. One possible explanation for this is that the ts gene product either is not present, or is not fully active in the molecular milieu of certain stages of development of the reproductive system. A similar hypothesis has been constructed by Grigliatti and Suzuki, (1970). Working with a temperature sensitive lethal allele of the sex-linked Drosophila mutant, raspberry, it was found that different tissues had different t.s.p.'s for the mutant in question. This might be due to molecular differences in the different tissues studied (Grigliatti and Suzuki, 1970). Polyphasic mutants previously isolated in Drosophila include a sex linked lethal (Tarasoff and Suzuki, 1970), alleles of the shibire series (Poodry et. al., 1975), and alleles of the Notch locus (Shellenbarger and Moehler, 1978).

In the work with the ts allele of raspberry, it was noted that the t.s.p.'s of the different tissues did not overlap. With ts 1, adequate experimentation has not been done to determine any overlap in t.s.p.'s. However, these may not be demonstrable by simple shift down experiments, and may require more complex methodology. A simpler analysis of gene action is possible with monophasic temperature sensitive mutants.

The embryonic arrest in ts 1 and ts 2 showed some variation in expression. The longer the parents spent at the restrictive

temperature, the earlier the embryonic arrest. In both ts 1 and ts 2 there is a variation in embryonic arrest from precomma embryos through differentiation within the egg case. Some of the dead embryos of ts 2 deserve special mention. The embryos with disproportionately large cells may mean that certain cell lineages are lethally affected before others in these embryos of ts 2. This could reflect a biochemical event limited to certain blastomere lineages. In gastropods, the progeny of the first two blastomeres synthesize qualitatively different proteins (Donnahoo and Kafatos, 1973). Similarly, Drosophila mutants are known which have maternally inherited localized defects in the blastoderm (Fuasto-Sterling, 1977). Athough many differences between the cell lineages exist, the interpretation of the embryonic arrest of ts 2 requires a more complete analysis of the embryonic arrest phenotype. This is complicated by the variation observed. As with ts 1, the time related variation in ts 2 embryonic arrest could reflect an accumulation of temperature sensitive molecules or damage accumulation during successive intervals at the restrictive temperature. A simpler analysis of embryonic arrest can be made with ts 7, where no time related variation in gene expression is seen. An interesting contrast between ts 7 and the other mutants with embryonic phenotypes is seen. Ts 7 has a rather short t.s.p. (oogenesis and embryogenesis) and has little variation in its pattern of embryonic arrest. Ts 1 and ts 2, both with extended

t.s.p.'s, also have variation in the pattern of embryonic arrest. The variation in embryonic arrest may be due to the time necessary for the adults to accumulate the ts gene product in the oocytes. Since ts 7 is not functionally active during gonadogenesis, it may be that enough ts gene product is functional during oogenesis to result in uniform embryonic arrest, while in ts 1 and ts 2, the effect of the ts gene product must be accumulated in the oocytes via action during gonadogenesis. Thus, the earlier embryonic arrest in ts 1 and ts 2 with increasing adult exposure to 26^{o}C may be the result of the accumulation of gene product inactivation which would normally occur during gonadogenesis.

Maternal effect mutants

In maternal effect mutants, changes due to the mutation are present during oogenesis, and affect subsequent development. Using this definition of a maternal effect, the isolated ts mutants were subject to further analysis.

The experiments with exposure to the restrictive temperature only during oogenesis demonstrate maternal effects in ts 1 ts 2, and ts 7. However, some reversibility of the ts lethality is noted. The reversibility of the lethality following 12 and 24 hour heat pulses increases with successive

egg laying intervals at the permissive temperature, especially in ts 1 and ts 7. Thus, the result appears to be the consistent with "accumulation" mediated shift to earlier embryonic arrest at 26°C in shift up experiments.

The simplest model to account for the results of the oogenetic heat pulse experiments on the embryonic mutants considers developmental phenomena. The first brood at the permissive temperature includes eggs that were in utero at the time of the shift, these were exposed to the restrictive temperature as oocytes and embryos, thus acounting for the low amount of hatching. The second brood following the heat pulse consists of embryos that were exposed to the restrictive temperature primarily as membrane delimited oocytes. The presence of arrested embryos in this second brood indicated that the temperature sensitive molecule was functional during that stage of oogenesis (in the distal arm of the gonad). The third brood at the permissive temperature is composed of embryos which were not exposed to the restrictive temperature as membrane delimited oocytes, and can develop to adults at the permissive temperature.

This simple model does not work for ts 2, since there is not a continual increase in egg hatching in successive broods following a 24 hour oogenetic heat pulse. Thus, in ts 2,

oocytes which have not become membrane delimited (in the proximal arm of the gonad) may contain a "store" of gene product inactivation which prevented continuous increase in egg hatchability in successive broods at the permissive temperature following shift down. Thus, the egg hatching profiles of ts 2 following oogenetic heat pulses may reflect accumulated damage due to gene product inactivation at different stages of oogenesis. Alternative models involving gene product resynthesis or reactivation of the molecule may be constructed. It remains unclear whether the inactivated gene product for each mutant is a product of the oocyte nucleus or accessory gonadal cells.

In ts 1, ts 2 and ts 7, embryonic arrest due only to exposure to the restrictive temperature during oogenesis has been demonstrated. Two possibilities exist for the embryonic lethality. The first is that the temperature sensitive molecule could be shunted into the cytoplasm of the cleavage cells and cause embryonic arrest due to inactivity during cleavage. Alternatively, the embryonic arrest may be due to a inactivity of the ts gene product during oogenesis. The heat pulse experiments on the embryonic arrest mutants indicate that the ts gene product is present during oogenesis and alterable by heat during that period so as to cause inactivity during either oogenesis or embryogenesis.

Since the embryonic arrest mutants studied here all showed embryonic arrest due only to exposure as embryos, the ts gene products must be functional during part of embryogenesis. With this in mind, the question becomes whether the ts product in embryos is a contribution from the maternal cytoplasm, or is a result of synthesis from the embryonic genome.

Some statements can be made on the synthesis of the ts gene product from observations made during male rescue experiments. Since eggs fail to hatch during outcrosses of ts 1, ts 2, and ts 7 at the restrictive temperature, these eggs are probably dying due to a maternal contribution to embryonic development. The low numbers of outcross progeny obtained indicates that the ts gene product is not synthesized (then utilized) in sufficient quantity between fertilization and embryonic arrest to cause the embryos to escape the developmental block. Few conclusions can be drawn from the male rescue experiments when discussing oocyte and embryo contributions to lethality. For example, experiments to test the expression of homozygous ts embryos from homozygous wild type mothers and vice versa have not been performed, although future isolation of attached autosomes in C. elegans could lead to isolation of homozygous wild type embryos from homozygous lethal hermaphrodites. Other aspects of the male rescue experiments need explaining. For example, in ts 1 and ts 2, some outcross progeny are obtained from homozygotes at the

restrictive temperature when they are not expected. These embryos may represent the class of embryos blocking late at the restrictive temperature due to the small interval of exposure of the parent. They may have reached a stage of development where the synthesis of the ts gene product is sufficient to allow the embryos to develop past the embryonic arrest. In ts 7, where little or no variation in the embryonic arrest phenotype of the mutant was found, outcross progeny were also generally absent (excluding the escapers in the first 12 hour brood) at the restrictive temperature. Experiments designed to test if paternal genome exposure to the restrictive temperature is sufficient to cause embryonic arrest have not been done. One may also question the mating experiments in this thesis by asking for proof of mating at the restrictive temperature. These data were obtained by shifting mated hermaphrodites to the permissive temperature and noting the increase in outcross progeny (Hirsh, unpublished).

The demonstration of a maternal component to the lethality of ts 1, ts 2 and ts 7 does not exclude the possible synthesis of the ts gene product from the embryonic genome between fertilization and embryonic arrest. Two sets of data indicate that the temperature sensitive molecule is formed as a result of zygote genome activity. First, the dead embryos obtained from the heterozygous hermaphrodites indicate embryonic lethality

independent of the maternal genome. Second, when mated hermaphrodites are shifted to the permissive temperature, some increase in outcross progeny (fewer dying eggs) occurs. This increase can only be due to the heterozygous embryonic genome. Many examples of maternal effects on embryonic development are known, both biochemical (Davidson, 1977) and genetic (Rice and Garren, 1975; Nusslein-Volhard, 1977; Fuasto-Sterling, 1977). As mentioned by Hadorn (1961) and demonstrated by several workers (Romans et. al., 1976) they may be either male rescuable or not be male rescuable. Male rescuable maternal effects in Drosophila include the mutants involved in pyrimidine synthesis (Lindsley and Grell, 1967) while in fowl the ts mutant frizzle fowl (ff) is also a male rescuable maternal mutant (Hadorn, 1961). Perhaps the level of synthesis of the ts gene product on the embryonic genome between fertilization and embryonic arrest also effects the number of outcross progeny obtained from ts embryonic lethals at the restrictive temperature. This hypothesis may also explain some of the outcross progeny obtained from ts 1 and ts 2 homozygotes at the restrictive temperature.

From experiments on the embryonic lethals, it has become clear that maternal exposure to the restrictive temperature is sufficient cause embryonic arrest at the permissive temperature. The proportion of arrested embryos depends on the

length of the oogenetic heat pulse and the mutant being studied.

The three other mutants isolated were ts 4 (larval lethal) ts 5 (sterile) and ts 12 (embryonic and sterile). These mutants did not give 100% lethality, making experiments similar to the ones performed on the embryonic arrest mutants difficult to interpret. Nevertheless, stocks of these mutants have been kept. One good example of a mutant of low penetrance that is important in development is the maternal ts mutant, bicaudal, in Drosophila (Nusslein-Volhard, 1977). Mutants such as ts 5 may be important in the study of oocyte production , which in Drosophila has accumulated a large amount of data (King, 1970). Mapping of the temperature sensitive mutants was easiest when the ts mutant was isolated on worms carrying a chromosome 5 marker. Thus, ts 4, ts 5, ts 7 and ts 12 were easily assigned linkage by outcrossing worms of the genotype ts-dpy11/++ at the restrictive temperature. Mapping was then done as outlined in "Results". The exact positions of the mutants requires further experiments, since true recombination frequencies are different at 26^{o}C (Rose, personal communication). Also, with large genetic distances, frequencies of the recombinant classes (dpy, unc and dpy-unc) are obscured due to double recombinant events. This difficulty may be overcome in future experiments by choosing unc markers which are closer to dpy 11. Further, by

screening mutagenized chromosomes already having visible markers, several steps in the mapping procedure can be eliminated.

Little can be said regarding the distribution of ts mutants on chromosome 5 from these few mutants. Although ts mutants were found mapping near both ends of the linkage group, the frequency distribution along the linkage group would require many more mutants. This is adequately demonstrated in the literature where it was reported that no "clustering" of 9 third chromosome ts lethals was found (Fattig and Rickol, 1972). Later, when sampling a larger group of ts lethals, it was found that a "cluster" was present in one particular area of the linkage group (Tasaka and Suzuki, 1973). Although the isolated ts mutants proved useful in the elucidation of patterns of gene expression, the more basic question of how genes important in development are arranged on the chromosome would require a larger number of very closely linked mutants. Presumably, by isolating a large number of closely linked lethals between two visibles 5 or less map units apart, the organization and regulation of developmentally important genes may be elucidated. Such screens for chromosome 5 linked mutants, if performed at the restrictive temperature, could yield ts mutants. This thesis represents a foundation for the analysis of such mutants. Such tightly linked ts mutants might be used to determine if

there is any temporal regulation of gene activity using the techniques outlined in this thesis.

Further experiments with the mutants discussed in this thesis could follow biochemical or genetic approaches. Biochemical identification of the ts gene product of each mutant could be useful to compare the presence of the molecule with its time of action. Genetically, one might wish to explore cell interactions in early development by generating mosaic ts lethals. This thesis may be the foundation of these and other future experiments.

Conclusions

Three screening methods for chromosome 5 linked temperature sensitive mutants are operational.

Temperature sensitive mutants have been found which block in embryogenesis, during larval development and as sterile adults at the restrictive temperature. All mutants with embryonic phenotypes had a maternal effect. Two embryonic lethals were monophasic while one was polyphasic.

Male rescue experiments, segregation from heterozygotes, and temperature shift experiments can be used to determine developmental phases in which the gene product is functionally active.

By shifting the ts mutants to the restrictive temperature at different stages of development, lethal phases were determined. Ts 1 had lethal phases during larval development, as adults (ie-sterility) and as embryos. The lethal phases of ts 2 and ts 7 were restricted to embryogenesis while the (leaky) phenotypes of ts 12, ts 4 and ts 5 were embryonic/adult, larval and adult sterility respectively.

The three mutants ts 1, ts 2, and ts 7 were treated as a unit in experiments designed to characterize the pattern of gene expression more fully. Several criteria indicated maternal effects on embryonic development in these mutants. First, male recuability was low at the restrictive temperature, and decreased as the parents aged at 26°C. Second, the use of virgin hermaphrodites decreases the number of outcross progeny at 26°C. Third, the sterility of ts 1 may reflect oogenetic involvement, and thus suggests a maternal effect. Fourth, heat treatments during oogenesis reveal the presence of developmentally arrested embryos in the absence of embryonic exposure to the restrictive temperature. Finally, the temperature sensitive period for all three embryonic arrest mutants extends back from embryogenesis at least as far as oogenesis.

Twelve hour transfer times were successful in most experiments at the restrictive temperature, since it allowed a complete fractioning of the egg laying with a minimum of inconvenience. The following outline is suggested for future research on chromosome 5 linked temperature sensitive mutants. 1. Collect ts mutants from mutagenized worms carrying two chromosome 5 markers five or less map units apart. This will aid in mapping. 2. Determine lethal phases by shifting

homozygotes to the restrictive temperature at different stages of development. 3. Investigate maternal inheritance via shifts to the restrictive temperature during oogenesis. Check the finding with outcross experiments at the restrictive temperature.

Bibliography

Abi-Rachid, M. and Brun, J.L. 1975. Etude Ultrastructuale Des Relations Entre Ovocytes Et Rachis Au Cours De L'Ovogenese Du Nematode Caenorhabditis elegans. Nematologica 21:151-162.

Balinsky, B.I. 1975. An Introduction To Embryology (4th edition) W.B. Saunders and Company. Toronto.

Berril, N.J. 1971. Developmental Biology. McGraw-Hill Book Company, Toronto.

Brenner, S. 1974. The Genetics of Caenorhabditis elegans. Genetics 77: 71-94.

Davidson, Eric H. 1977. Gene Activity In Early Development (2nd edition). Academic Press, N.Y.

Deppe, U. Schierenberg, E., Cole, T., Kreig, C., Schmitt, D., Yonder, B., and Von Ehrenstein, G. 1978. Cell Lineages of the embryo of the Nematode Caenorhabditis elegans. Proc. Nat. Acad. Sci. U.S.A. 75(1): 376-380.

Donohoo, P. and Kafatos, F.C. 1973. Differences in the proteins Synthesized by the Progeny of the First Two Blastomeres of Ilyanassa, a "Mosaic" Embryo. Dev. Biol. 32: 224.

Epstein, H. F. and Thomson, J. N. 1974. Temperature Sensitive Mutation Affecting Myofilament Assemby in Caenorhabditis elegans. Nature 250: 579-580.

Epstein, H. F., Bolle, A., Steinberg, C.M., Kellenbarger, E., Boy de la Tour, E., Chevalley, R., Edgar, R.S., Sussman, M. Denhardt, G. and Leilausis, A. 1963. Physiological Studies of Conditional Lethal Mutation in Bactteriophage, T4D Cold Spring Harb. Symp. Quant. Biol. 28: 375-392.

Fatt, H.V. and Dougherty, E.C. 1963. Genetic Control of Differental Heat Tolerance In Two Strains of the Nematode Caenorhabditis elegans. Science 141: 266-267.

Fattig, W.D., and Ricoll, W.L. 1972. Isolation of Recessive Third Chromosome Temperature Sensitive Mutations in Drosophila melanogaster. Genetics 71: 309-313.

Fauso-Sterling, A., Wiener, A.J. and Digan, M.E. 1977. Analysis of a Newly Isolated Temperature Sensitive Maternal Effect Mutation of Drosophila melanogaster. J. Exp Biol. 200: 199:210.

Grigliatti, T. and Suzuki, D.T. 1970. Temperature Sensitive Mutations in Drosophila melanogaster. V. A Mutation Affecting the Concentrations of Pteridines. Proc. Nat. Acad. Sci. U.S.A. 76: 1101-1108.

Grigliatti, T. and Suzuki, D.T. 1971. Temperature Sensitive Mutations in Drosophila melanogaster. VII. The Homeotic Mutant ss a40a . Proc. Nat. Acad. Sci. U.S.A. 68: 1307-1311.

Hadorn, E. 1961. Developmental Genetics and Lethal Factors. Wiley, N.Y.

Hirsh, D., Oppenheim, D., and Klass, M. 1976. Development of the Reproductive System of Caenorhabditis elegans. Dev. Biol. 49: 220-235.

Hirsh, D., and Vanderslice, R. 1976. Temperature Sensitive Developmental Mutants of Caenorhabditis elegans. Dev. Biol. 49: 220-235.

Klass, M., Wolf, N. and Hirsh, D. 1976. Development of the Male Reproductive System and Sexual Transformation in the Nematode Caenorhabditis elegans. Dev. Biol. 52: 1-18.

King, R. C. 1970. Ovarian Development in Drosophila melanogaster Academic Press, N.Y.

Lindsley, D. and Grell, E. H. 1967. Genetic Variations in in Drosophila melanogaster. Carnagie Institution of Washington.

Loomis, W.F. 1969. Temperature Sensitive Mutants of Dictyostelium discoideum. J. Bact. 99: 65-69.

Mahowald, A.P. 1971a. Polar Granules of Drosophila IV. Cytochemical Studies Showing Loss of RNA from Early Stages of Embryogenesis. J. Exp. Zool. 176: 345.

Mahowald, A.P. 1971B. Polar Granules of Drosophila III. The Continuity of Polar Granules During the Life Cycle of Drosophila. J. Exp. Zool. 176: 329-344.

Nicholas, W.L. 1975. The Biology of Free Living Nematodes. Clarendon Press, Oxford.

Nigon, V.and Dougherty, E.C. 1950. A Dwarf Mutant in a Nematode. A Morphological Mutant of Rhabditis briggsae Dougherty and Nigon, 1949 (Nematoda: Rhabditidae). J. Hered. 41:703-709.

Nusslein-Volhard, C. 1977. Genetic Analysis of Pattern Formation in the Embryo of Drosophila melanogaster: Characterization of the Maternal-Effect Mutant Bicaudal. Wilhelm Roux Archives 183: 249- 268.

Poodry, C.A., Hall, L. and Suzuki, D.T. 1973. Developmental Properties of Shibere ts 1.: A Pleiotropic Mutation Affecting Larval and Adult Locomotion and Development. Dev. Biol. 32: 373-386.

Postletwait, J. and Schneiderman, H.A. 1973. Developmental Genetics of Drosophila Imaginal Discs. in Annual Review of Genetics volume 7: 381-434.

Rice T. B. and Garren, A. 1975. Localized Defects in Blastoderm Formation in Maternal Effect Mutants of Drosophila. Dev. Biol. 43: 277-286.

Riddle, D. 1978. The Genetics of Development and Behavior in Caenorhabditis elegans. J. of Nematology 10 (1): 1-16.

Romans, P., Hodgetts, R. B., and Nash, D 1976. Maternally Influenced Embryonic Lethality: Allele Specific Genetic Rescue at a Female Fertility Locus In Drosophila melanogaster. Can. J. Genet. Cytol. 18 (4): 773-781.

Shellenbarger, D. L. and Moehler, J.D. 1975. Temperature Sensitive Mutations of the Notch Locus in Drosophila melanogaster. Genetics 81: 143-162.

Shellenbarger, D.L., and Moehler, J.D. 1978. Temperature Sensitive Periods and Autonomy of Pleiotropic Effects of L (1) N TS 1, a Conditional Notch Lethal in Drosophila. Dev. Biol. 62 (2):432-446.

Rosenbluth, R., Ezell, D. and Suzuki, D.T. 1972. Temperature Sensitive Mutations in Drosophila melanogaster IX. Dominant Cold Sensitive Lethals on the Autosomes. Genetics 70: 75-86.

Suzuki, D.T. and Procunier, D. 1969. Temperature Sensitive Mutations in Drosophila melanogaster, III. Dominant Lethals and semilethals on Chromosome 2. Proc. Nat. Acad. Sci. U.S.A. 62: 369-376.

Suzuki, D.T. 1970. Temperature Sensitive Mutations in Drosophila melanogaster. Science 170: 695.

Suzuki, D.T., Kaufman, T., Falk, D. and the U.B.C. Drosophila Research Group 1976. Conditionally Expressed Mutations in Drosophila melanogaster in the Biology of Drosophila, volume 1 (Ashburner and Novitski, editors).

Sulston, J. E. and Horvitz, B.R. 1976. Post-Embryonic Cell Lineages of The Nematode Caenorhabditis elegans. Dev. Biol. 56 (1) :110-156.

Tarasoff, M. and Suzuki, D.T. 1970. Temperature Sensitive Mutations in Drosophila melanogaster, VI. Temperature Effects on the Development of Sex-Linked Recessive Lethals. Dev. Biol. 23: 492-509.

Tasaka, S.E. and Suzuki, D.T. 1973. Temperature Sensitive Mutations in Drosophila melanogaster XVII. The Genetic Properties of Heat and Cold Sensitive Lethals on Chromosome 3. Genetics 74: 509-520.

Vanderslice, R. and Hirsh, D. 1976. Temperature Sensitive Zygote Defective Mutants of Caenorhabditis elegans. Dev. Biol. 49: 236-249.

Von Erhenstein, G. 1977. Personal Comunication.

Ward, S. and Miwa, J. 1978. Characterization of Fertilization Defective Mutants of the Nematode Caenorhabditis elegans. Genetics 88:285-303.

Whittman, H.G.and Whittman-Liebold, B. 1963. Protein Chemical Studies of Two RNA Viruses and Their Mutants. Cold Spring Harb. Symp. of Qaun. Biol. 31: 163-172.