Induction, detection and isolation of temperature-sensitive lethal and/or sterile mutants in nematodes. I. The free-living nematode *Caenorhabditis elegans* [1]

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SUMMARY

Applying a series of techniques intended to induce, detect and isolate lethal and/or sterile temperature-sensitive mutants, specific to the self-fertilizing hermaphrodite nematode *Caenorhabditis elegans*, Bergerac strain (Abdulkader & Brun, 1976), 25 such mutants have been found. Optimal conditions for the application of mutagenic treatment and the detection of such mutations are discussed.

RÉSUMÉ

Introduction, detection et isolement de mutants thermosensibles létaux et/ou stériles chez les nématodes. I. Le nématode libre Caenorhabditis elegans.

Grâce à l'élaboration d'un protocole original de détection et d'isolement des mutations thermosensibles létales et/ou stériles, spécifique du nématode hermaphrodite autofécond *Caenorhabditis elegans*, souche Bergerac (Abdulkader & Brun, 1976), 25 d'entre elles ont été obtenues. Les conditions optimales de traitement par l'agent mutagène et de détection de telles mutations sont discutées.

The prolificity of nematodes, whether parasitic or free-living, is one of their most spectacular characteristics. Applied biologists cannot fail to be intrigued by this phenomenon : those who are interested in phyto or zooparasitic species seek to limit it, while those dealing with soil fauna wish to encourage it.

As a result, the study of nematode reproduction, and particulary of gametogenesis and development, is crucial. Thus, a comparative study of these functions, on the one hand in normal individuals, on the other hand in temperature-sensitive (ts) mutants, must prove extremely interesting. This type of analysis has, in fact, been most fruitful in the understanding of multiplication, not only in microorganisms (Edgar & Lielausis, 1964 ; Fattig & Lanni, 1965 ; O'Donovan, Kearney & Ingraham, 1965 ; Condon & Ingraham, 1967 ; Skarvronskaya *et al.*, 1971), but also in multicellular plants and animals. Among the latter, attention is drawn to the results of studies among the insects, eg. *Drosophila* (Suzuki *et al.*, 1967 ; Baillie, Suzuki & Tarassoff, 1968 ; Tarassoff, 1970 ; Holden, 1973 ; Wright, 1973 ; Gans, Audit & Masson, 1975), *Habrobracon* (Smith, 1967 ; 1968 ; 1971) and *Musca domestica* (McDonald & Overland, 1972).

This success is based particularly on the fact that ts mutants, whose reproduction at permissive temperature is the same as in the wild-type, show disrupted gametogenetic or developmental

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activities when they are exposed to extreme temperatures (non-permissive temperatures) at a certain stage in their life cycle (temperature-sensitive period). The effects of the non-permissive temperature may vary, leading to sterile and/or lethal organisms.

In this study, we describe the conditions which have enabled us to obtain and isolate, in the laboratory, sterile and/or lethal ts mutants of the free-living soil nematode *Caenorhabditis elegans*, Bergerac strain. The protocols to be described here are also applicable in the case of other nematodes of the soil fauna, and with appropriate modifications, to phytoparasitic nematodes.

Thermic conditioning of nematodes and selection of non-permissive temperature

The usual temperature for raising the wild-type (18 °C) was chosen as permissive temperature. At this temperature, the wild-type nematodes reach female sexual maturity when they are four day-old and then lay eggs for a least five days (Brun & Lebre, 1968). At 24 °C, they reach sexual maturity in only 2 1/2 days, but then do not lay for more than two days.

The choice between 24 °C and 26 °C as non-permissive temperature was made by incubating wild-type eggs, laid at 18 °C by four day-old hermaphrodites and raised at this temperature, at either 24 °C, or 26 °C. In each case, their viability was determined, then the fecundity of

TREATMENT WITH THE CHEMICAL MUTAGEN

To induce ts mutations, the alkylating-agent

or lay eggs which fail to hatch. The fact that other animals, brothers of these putative mutants, are kept in culture at 18 °C enables

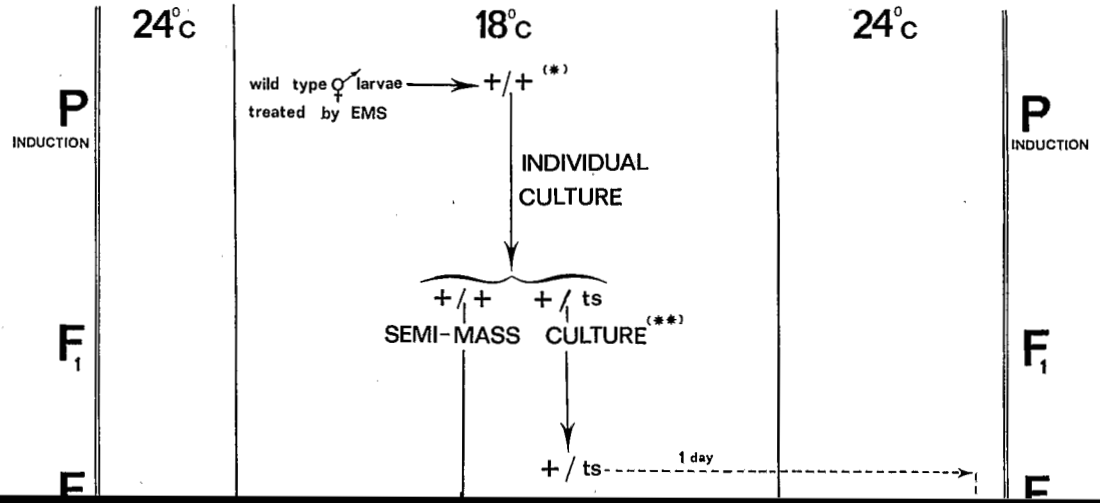
24°c
18°c
24°c
P
INDUCTION
wild type ♂ larvae
treated by EMS
+/+ (*)
INDIVIDUAL
CULTURE
+/+ +/ts
SEMI-MASS CULTURE (**)
F1
+/ts
1 day
P
INDUCTION
F1

Table 1

Viability of the eggs of *C. Elegans* Bergerac wild-type strain at 26 °C and 24 °C.

Average viability of an egg at 26 °C $= \frac{766 + 13}{1\,050} = 0.742$. Confidence interval : 0.742 ± 0.053.

Average viability of an egg at 24 °C $= \frac{336 + 4}{450} = 0.755$. Confidence interval : 0.755 ± 0.039.

Temperature	*Number of eggs*	*Observation after a time equal to :*	*Number of survivors*		*Observations of the descendants at 26 °C of the adults*
			Larvae	*Adults*	
26 °C	150	2 d. 12 h	2	099	Very few abnormal eggs in the body.
	150	2 d. 12 h	1	123	Some abnormal eggs in the body.
	150	2 d. 15 h	4	095	Rare abnormal eggs in the body + some abnormal eggs on the drops of nutritive medium.
	150	2 d. 15 h	2	107	
	150	2 d. 15 h	3	116	idem
	150	2 d. 22 h	1	122	idem
	150	2 d. 22 h	0	104	idem
	Total 1 050		13	766	
24 °C	150	2 d. 16 h	1	118	Numerous laid eggs.
	150	2 d. 18 h	2	107	Numerous laid eggs + some larvae.
	150	2 d. 18 h	1	111	Numerous laid eggs + some larvae.
	Total 450		4	336	

Indeed, the small number of eggs laid never hatched. Besides, examination with dissecting microscope (× 160) revealed that they were all abnormal. One category appeared to be a cytoplasmic mass, lacking in consistency and enclosed in a membrane, while others were spherical. At the centre of all these eggs was a dark brown zone surrounded by a paler ring.

At 24 °C

As far as the viability is concerned (Tab. 1), the result was similar to that obtained at 26 °C, of the order of 75%. A close analogy also holds between the development times, egg → adult, which in both cases was 2 1/2 days (Tab. 1).

On the other hand, at 24 °C, the animals were still fertile. Fecundity of F_0 hermaphrodites was of the order of 55, and those of the descendance F_1, F_3 and F_4, cultivated at 24 °C, were 34.5, 48.9 and 49.3 respectively (Tab. 2). In addition, these animals could be maintained in culture at 24 °C.

Table 2

Fecundity of *C. Elegans* Bergerac of wild-type strain at 24 °C. F_0 = 1st generation at 24 °C (eggs laid at 18 °C, then shifted to 24 °C → adults F_0) ; F_1 = 2nd generation at 24 °C ; F_3 = 4th generation at 24 °C ; F_4 = 5th generation at 24 °C

Generation	*Sample size*	*Average fecundity*
F_0	81	55.00 ± 2.75
F_1	70	34.51 ± 3.59
F_3	44	48.91 ± 6.21
F_4	31	49.37 ± 9.94

Detection and isolation of sterile and lethal ts mutants

Sixty putative mutants have been detected following a systematic examination of the F_3 descendants of 1404 F_2 adults, taken in varying numbers depending on the different treatments. A retest of these 60 strains after two generations has enabled the definitive isolation of 25 ts mutants : 13 steriles and 12 lethals.

Frequency of ts mutants in the F_2 : estimation of the ts mutation rate

As is shown in Table 3, the mutation frequency in the F_2 varied from 0.7 to 2.13%, according to the different mutagenic treatments. It was not possible, however, to establish whether the differences in these frequencies were statistically significant, since any decision would be of doubtful value because of the low sample size (378) for the test at the 0.05 M E.M.S. concentration.

As far as estimation of the gametic mutation rate P is concerned, where :

$$P = \frac{\text{Total number of ts mutated gametes,}}{\text{total number of gametes produced}}$$

where f is the frequency of ts/ts individuals in the F_2, i.e. :

$$f = \frac{\text{number of mutants obtained.}}{\text{number of worms tested}}$$

Indeed, if one admits that :

a. treatment of L_2 larvae induces mutations essentially in the gonia and that it is equally likely that these give rise to spermatozoa or oocytes ;

b. mutations affect the chromosomes ;

c. probability of induction of 2 ts mutations in the same gamete is extremely small, thus negligible ; *a fortiori*, in the same chromosome ;

d. F_1 individuals are all equally fertile ;

e. eggs of ts/ts individuals have the same viability at 18 °C as other genotypes, and

f. fusion of 2 F_2 ts-mutated gametes is very rare, thus negligible,

then the frequency of ts/ + heterozygous animals in the F_1 is 4 times that of ts/ts homozygous animals in the F_2 and therefore equal to 4 f. But, on the other hand, the frequency of ts/ + heterozygous nematodes is only twice that of ts-

RESEARCH INTO OPTIMAL CONDITIONS FOR THE ... the high concentration 0.1 M for 7-10 hours

Table 4 A

Comparison of the visibility of lamps treated by E.M.S.

Table 5 A

Comparison of the fecundity of animals treated by E.M.S.
t-test (comparison of means). Critical value for 5% significance level = 1.96
* Significant difference.

	Type of treatment and fecundity						
		E.M.S. concentration and treatment time					
Type of treatment and fecundity	*Control: 13 h in E.M.S.-free buffer solution*	*0.05M × 7 h*	*0.05M × 10 h*	*0.05M × 13 h*	*0.1M × 4 h*	*0.1M × 7 h*	*0.1M × 10 h*
	139 ± 11.09	80.26 ± 9.90	81 ± 8.02	76.50 ± 10	88.15 ± 12.33	52.76 ± 10.82	36.59 ± 9.75
Control 139 ± 11.09		11.176*	12.076*	11.858*	8.705*	15.713*	19.406*
0.05 M × 7 h 60.26 ± 9.90			0.168	0.755		5.340*	
0.05 M × 10 h 81 ± 8.02				0.994			10.146*
0.1 M × 4 h 88.15 ± 12.32						6.069*	9.190*
0.1 M × 7 h 52.76 ± 10.82							3.140*

Nevertheless, even though 26 °C cannot be used as non-permissive temperature in the detection of ts mutants, due to the complete sterility of the wild-type strain, the viability of this strain is still very high (74%) at 26 °C enabling genetic and physiological studies of the lethal mutants obtained to be carried out.

Induction and detection of ts mutations in nematodes

At 0.05 M and 0.1 M concentrations, and for treatment times from 4 to 13 hours, E.M.S. treatment of *C. elegans* larvae induces lethal and/or sterile ts mutations at 24 °C, by its action on the L_2 larvae. The mutation rate is always highest for the 0.1 M concentration (3 to 4%). Although at this concentration there are not significant differences in the mutation rate, whether the E.M.S. treatment is used for 4, 7 or 10 hours, short treatments (4 hours and 7 hours) are to be recommended for nematodes.

Indeed, the greatly reduced fecundity of the animals subjected to long treatments on the one hand (Table 4), and of their F_1 descendants on the other, prompts us to think that, in such conditions, numerous non-conditional lethal and/or sterile mutations are induced in the genome. Inevitably, this must lead to the death of a certain number of ts mutants.

In contrast with experimental protocols used

to obtain ts mutants in *Drosophila* (Suzuki, 1967 ; Fattig, 1972 ; Ayles, 1973 ; Gand, 1975), our methods (Abdulkader & Brun, 1976), summarized in Figure 1, allow detection in a single step of all sterile and/or lethal ts mutations occurring in any part of the genome. However, this technique requires a complementary genetic study to define, for each mutation, the chromosomal localization and precise genetic determinism.

Influence of EMS concentration and of the difference between permissive and non-permissive temperatures on the mutant frequency and detection possibilities

perature, "sister" cultures transferred to 24 °C would consist not only of senile F_3 but also of F_4 and F_5.

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