THE EFFECT OF TEMPERATURE AND PARENTAL AGE ON RECOMBINATION AND NONDISJUNCTION IN CAENORHABDITIS ELEGANS[1]

A. M. ROSE[2] AND D. L. BAILLIE

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

ABSTRACT

The effect of temperature and parental age on recombination frequency in C. elegans was studied between pairs of closely linked markers on linkage groups I and V. In the regions studied, recombination frequency varied three-fold over the temperature range 13.5° to 26°. Temperature-shift experiments indicated that a temperature-sensitive recombination event occurs approximately 50 oocytes prior to fertilization. Recombination frequency was observed to decrease with maternal age. The greatest decrease was observed in the first 24 hours of egg production. The frequency of male progeny, a measure of X-chromosome nondisjunction was also studied. This frequency increased with elevated temperature and age of the parent.

IN Caenorhabditis elegans, the effects of temperature and hermaphrodite age on recombination frequency have not been characterized. The effect of temperature on recombination frequency has been studied in a number of eukaryotic organisms including Drosophila (PLOUGH 1917; STERN 1926; GRELL 1966), Neurospora (RIFAAT 1959; McNELLY-INGLES, LAMB and FROST, 1966; TOWE and STADLER 1964; LANDNER 1970), Chlamydomonas (LAWRENCE 1965), Coprinus (LU 1974; LU and CHIU 1976), and many others. In Drosophila, maternal age has been shown to affect recombination frequency (STERN 1926; SCHULTZ and REDFIELD 1951) and nondisjunction (TOKUNAGA 1970). In humans, increasing age of the mother leads to increased X-chromosome nondisjunction (LENZ et al. 1959). In this paper, we report the effects of parental age and temperature on the recombination frequency between closely linked markers on linkage groups I and V. Documentation of these effects is not only essential for reproducibility in recombination mapping, but also may add to our understanding of the recombination process. The normal recombination phenomenon must be described before mutations that affect the recombination process in C. elegans can be characterized. The effects of temperature and parental age on X-chromosome nondisjunction rates are also described.

MATERIALS AND METHODS

The wild-type N2 strain and mutant strains of *Caenorhabditis elegans* var. Bristol were obtained originally from S. BRENNER. Mutants used in this study were of two types: uncoordinated behavioral mutants (Unc) and short fat morphological mutants (Dpy). Strains were maintained on 100 mm petri plates containing 30 ml of nematode growth medium, NGM, streaked with *Escherichia coli*, strain OP-50, as a food source (BRENNER 1974). Mating was done by placing 10 to 12 mutant hermaphrodites on a 35 mm petri plate with 12 to 16 males, and 24 hr later individual hermaphrodites were transferred to fresh plates.

The following strains were used to study regions on linkage group *I*: *dpy-5(E61)*, *dpy-14 (E188)*, *unc-15(E73)*, *unc-13(E51)* and on linkage group *V*: *dpy-11(E224)*, and *unc-42(E270)*. Double mutants carrying pairs of these markers were recovered from the segregants of the trans-heterozygote (BRENNER 1974). Figure 1 shows the region on linkage group *I* that was studied. *Unc-15* has been positioned 0.04 map units to the left of *unc-13* (WATERSTON, FISHPOOL and BRENNER (1977).

The frequency of recombination between linked markers in the hermaphrodite was determined by counting progeny produced from *cis-heterozygotes* (e.g., ++/dpy-5 unc-15). All
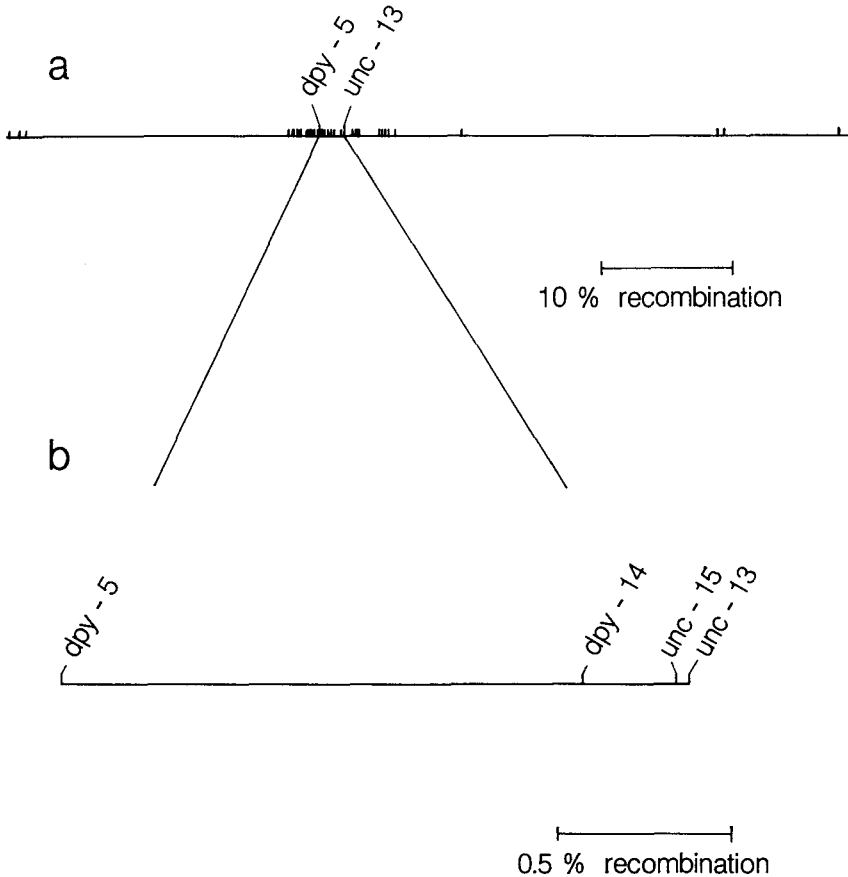

FIGURE 1.—(a) Map of Linkage Group *I*. The position of known genes is shown by vertical bars. (b) The positions of markers used in this study are illustrated on an expanded map of the region between *dpy-5* and *unc-13*. The positions of seven additional genes that map to this region have not been illustrated.

progeny were counted and removed, and the recombination frequency, p, was calculated according to the formula $p = 1 - \sqrt{1 - 2R}$, where R is the fraction of recombinant phenotypes (BRENNER 1974). The total number of progeny was calculated at $4/3 \times$ (the number of wild type plus half the recombinants) to correct for inviability of the segregating double mutant. The number of recombinant phenotypes was calculated as twice the number of dumpy progeny, since their viability is comparable to wild type.

The effect of temperature on recombination frequency in the hermaphrodite was studied. Heterozygotes were raised at the following temperatures: $13.5° \pm 0.3°$, 15.5 ± 0.3, $20° \pm 0.3°$, and $26.0° \pm 0.3°$. The recombination frequency for heterozygotes raised at room temperature was determined by raising the heterozygotes outside the incubators. The room temperature varied from $21°$ to $23°$ during the course of the experiment. In the case of temperature-shift experiments, heterozygotes that had been raised at $13.5°$ were shifted to $26°$ immediately after the first eggs were laid. In each case, successive 12-hr broods were collected.

In order to minimize the effect of temperature on development and survival of the various phenotypic classes, the heterozygotes were allowed to lay eggs at the experimental temperature, while their progeny to be scored were raised at $20°$.

The effect of maternal age on recombination was studied by comparing recombination frequencies in successive egg broods. Fourth-stage larval heterozygotes were raised on individual plates and transferred every 12 or 24 hr after the first eggs were laid. Since egg-laying rate increases with increasing temperature, 12-hr broods were used for the warmer temperatures ($20°$
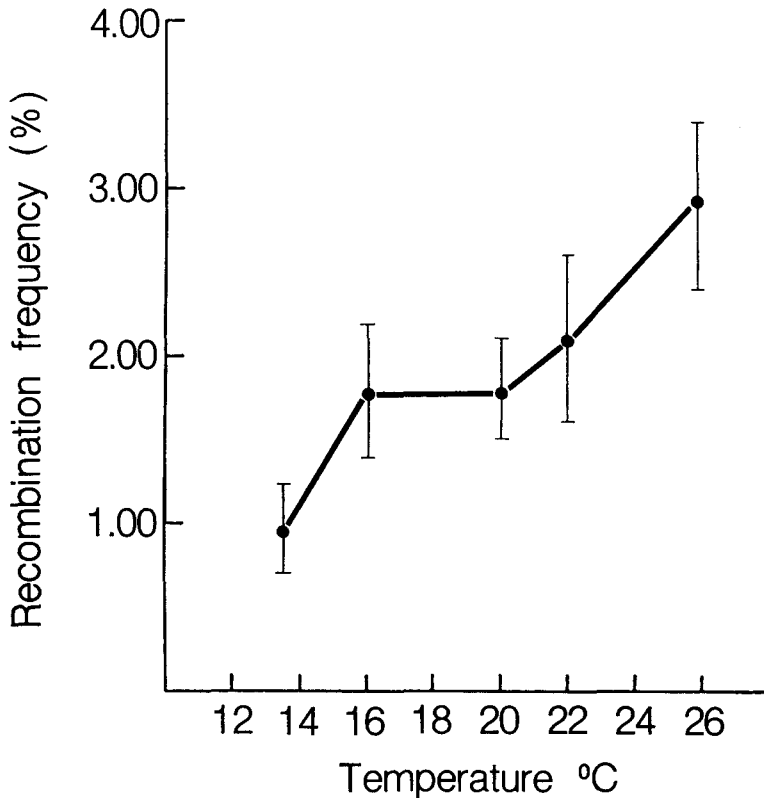

FIGURE 2.—The effect of temperature on the frequency of recombination between *dpy-5* and *unc-15*. Vertical bars represent 95% confidence intervals.

and above) and 24-hr broods were used for the lower temperatures (13.5° and 15.5°). Parents were left on the last brood. In this way all progeny produced by an individual were scored.

In *C. elegans*, hermaphrodites normally produce only hermaphroditic (*XX*) progeny by self-fertilization. Occasionally males (*XO*) will occur, presumably the result of *X*-chromosome nondisjunction. The frequency of these males provides a straightforward assay for *X*-chromosome nondisjunction rate in the nematode.

<div align="center">RESULTS</div>

Effect of temperature: The recombination frequency for the region between *dpy-5* and *unc-15* was studied at five temperatures. Figure 2 illustrates the change in recombination frequency as a function of temperature. These results demonstrate that temperature can produce a wide variation in the rate of recombination. The increase above 20° obtained with *dpy-5 unc-15* has been verified with *dpy-5 unc-13*, and for a region on chromosome *V* marked by *dpy-11 unc-42*. As can be seen in Table 1, all of these strains showed nearly identical increases in recombination frequency when reared at 26°.

Temperature shift experiments: Since oogenesis begins during the larval stages and continues into adulthood past the onset of egg laying, the increase in recombination frequency at 26° can be used to study the timing of the temperature sensitive recombination event (ABI-RACHED and BRUN 1975; HIRSCH, OPPEN-HEIM and KLASS 1976). For example, heterozygotes raised at 13.5° and shifted to 26° at the beginning of egg laying are expected to produce recombinants first at a low frequency and later at the frequency characteristic of 26°. Figure 3 shows the results of this experiment. The recombination frequency for heterozygotes kept at 13.5° throughout the experiment showed the characteristic decrease

<div align="center">TABLE 1</div>

<div align="center">*Effect of temperature on recombination frequency*</div>

°C	N*	Progeny	Recombinants	% Recombination	95% C.I.†
dpy-5 unc-15/++ (I)					
26	26	4980	144	2.94	(1.63–3.29)
RT‡	17	3773	78	2.09	(1.63–2.55)
20	25	6292	112	1.79	(1.46–2.14)
15.5	18	4276	76	1.80	(1.39–2.20)
13.5	26	5223	50	0.96	(0.69–1.22)
dpy-5 unc-13/++ (I)					
26	25	2958	69	2.36	(1.74–3.06)
20	28	6980	105	1.51	(1.21–1.76)
dpy-14 unc-13/++ (I)					
26	18	1204	6	0.50	(0.25–0.99)
20	16	3069	9	0.30	(0.16–0.49)
dpy-11 unc-42/++ (V)					
26	10	1780	70	4.01	(3.03–4.83)
20	31	4624	101	2.21	(1.89–2.77)

* N = no. of heterozygotes.
† Confidence interval.
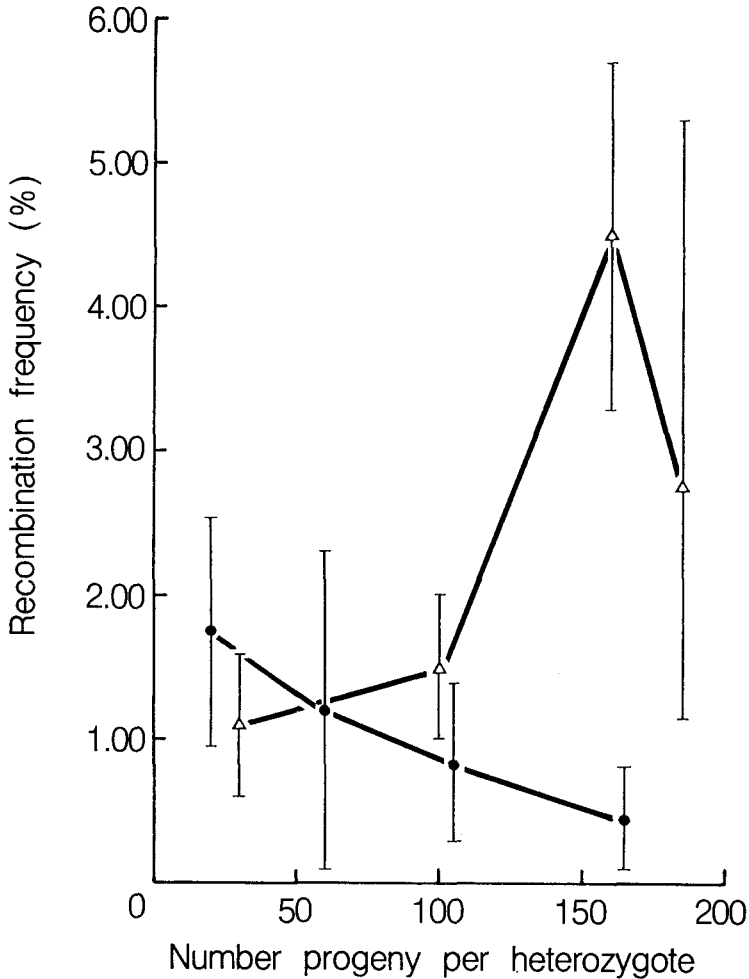‡ Room temperature = 22 ± 1°.

FIGURE 3.—Recombination frequency for shifted and nonshifted parents as a function of number of progeny. △ Brood analysis for heterozygotes raised at 13.5° and shifted to 26° when egg laying began. ● Brood analysis for heterozygotes that remained at 13.5°. Vertical bars represent 95% confidence intervals.

with parental age (see below). On the other hand, heterozygotes raised at 13.5°, but shifted to 26° for egg laying showed an increase in recombination frequency in the later broods. Table 2 shows that after 100 progeny had been produced the recombination frequency began to increase dramatically. This increase indicates the appearance of an increased number of recombination events at high temperature as a result of shifting the adult heterozygote.

Effect of parental age: A brood analysis was done as described in MATERIALS AND METHODS in order to determine the effect of parental age on recombination frequency. Table 3 shows the average number of progeny produced per brood at the three temperatures studied. At 26°, *dpy-5 unc-15/+ +* hermaphrodites laid eggs for four successive 12-hr broods (2 days) and produced half as many

TABLE 2

Temperature-shift analysis of recombination frequency

Brood	Progeny	Recombinants	% Recombination
Raised at 13.5°; Eggs laid at 26° (N = 31)			
1–12 hr	1805	20	1.10
13–24 hr	2413	36	1.49
25–36 hr	1204	54	4.48
37–48 hr	299	8	2.68
Maintained at 13.5° (N = 26)			
1–24 hr	1031	18	1.75
25–48 hr	1163	14	1.20
49–72 hr	1208	10	0.83
73–96 hr	1822	8	0.44

progeny as at 20°. The result agrees with that of HIRSCH, OPPENHEIM and KLASS (1976), who found that the greatest number of progeny are produced between 16° and 20° and a decreased number are produced at 26°. Our data indicate that at 13.5°progeny production decreased.

The variation in recombination frequency with parental age is shown in Figure 4. Because the total number of progeny produced differs at the three temperatures studied, brood sizes are expressed as a fraction of the total progeny. At all three temperatures, recombination frequencies decreases with parental age. At 20, the greatest numbers of recombinants are produced in the first 12 hr. This result has been confirmed with *dpy-5 dpy-14(I)*, and with *dpy-11 unc-42(V)*, as shown in Table 4.

Rate of nondisjunction: The effect of temperature on X-chromosome nondisjunction is recorded in Table 5. At 20°, males occur in the progeny of self-crossing hermaphrodites at a frequency of about 1/1000. The rate of X-chromosome nondisjunction as a function of parental age is shown in column 5 of Table 4. These data show that the older hermaphrodites produce more exceptional male progeny than younger ones.

TABLE 3

Number of progeny per brood per dpy-5 unc-15/++

°C	N*	Successive broods					Total
		1	2	3	4	5	
26†	26	40 ± 3.86	37 ± 3.89	19 ± 2.06	23 ± 2.37		119
20†	25	53 ± 3.73	59 ± 3.58	60 ± 3.70	62 ± 5.60	15 ± 8.85	249
15.5‡	18	53 ± 5.42	77 ± 5.06	70 ± 6.19	33 ± 6.47	2	235
13.5‡	26	39 ± 3.65	44 ± 2.89	46 ± 2.83	38 ± 3.50	32 ± 3.13	199

* N = number of heterozygotes.
† Successive broods taken every 12 hours.
‡ Successive broods taken every 24 hours.

TABLE 4

Brood analysis at 20°

Brood	No. progeny	No. recombinants	% Recombination	Males/1000
dpy-5 unc-15/++ (N = 25)				
1–12 hr	1347	38	2.86	0.7
13–24 hr	1419	24	1.62	0
25–36 hr	1520	24	1.58	0
37–48 hr	1555	22	1.42	1.3
49–60 hr	380	4	1.05	5.3
Total	6221	112	1.80	1.1
dpy-5 dpy-14/++ (N = 30)				
1–12 hr	1427	32	2.26	2.1
13–24 hr	2295	36	1.58	0.4
25–36 hr	1485	20	1.35	1.4
37–48 hr	1205	12	1.00	3.3
49–60 hr	936	10	1.07	4.3
Total	7348	110	1.81	2.4
dpy-11 unc-42/unc-68 (N = 18)				
1–12 hr	744	22	3.00	0
13–24 hr	1770	52	2.98	0
25–36 hr	1114	22	2.00	0
37–60 hr	1144	14	1.26	4.4
Total	4772	110	2.31	1.1

DISCUSSION

It has been observed for many eukaryotic organisms that recombination frequency is affected by changes in temperature and maternal age. The effects of temperature and age on recombination frequency make the construction of reliable recombination maps impossible unless these affects are controlled. We have investigated these two variables in the nematode, *C. elegans*, in order to emphasize the need for standardized recombination mapping. Our studies show a threefold increase in recombination frequency with increasing temperature. This variation in recombination frequency with temperature must be considered in any experimental study measuring map distance between genes. Increases in recombination frequency with elevated temperature have been observed in Drosophila by PLOUGH (1917), in Neurospora by RIAFAAT (1959) and in Coprinus

TABLE 5

Effect of temperature on nondisjunction

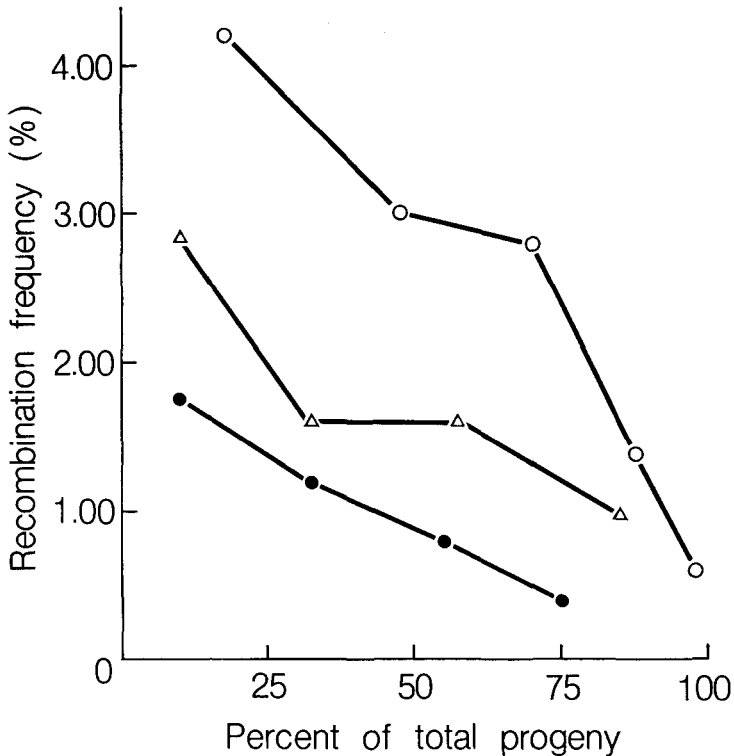| °C	Wild types	Wild-type males	Males/1000	C.I.
26	1933	17	6.05	(7.5–10.1)
RT	2791	4	1.43	(0.9– 4.7)
20	4663	5	1.07	(0.6– 3.2)
15.5	3169	2	0.63	(0.5– 2.4)
13.5	3682	6	1.63	(0.9– 4.7)

FIGURE 4.—Recombination frequency between *dpy-5* and *unc-15* at three temperatures as a function of the fraction of progeny produced. O 26°; △ 20°; ● 13.5°.

by LU (1969). Unlike Drosophila (PLOUGH 1917) and Coprinus (LU 1969), our lowest temperature did not show an increase in recombination frequency, although increases in recombination frequency at temperatures lower than those studied by us are possible.

The temperature upshift experiment indicates a stage in meiosis sensitive to increased temperature. A three-fold increase in recombination frequency was observed after the production of 100 to 150 progeny. Since *C. elegans* has two gonads, this increase corresponds to a temperature-sensitive stage 50 to 75 oocytes prior to fertilization. The oocytes of *C. elegans* are linearly arrayed in the gonad such that examination of this linear array reveals that 50 oocytes prior to fertilization corresponds to pachytene (ABI-RACHED and BRUN 1975; HIRSCH, OPPENHEIM and KLASS 1976). Since we studied a temperature shift and not a pulse of high temperature, we could observe only the latest temperature-sensitive events. Earlier temperature-sensitive stages (McNELLY-INGLES, LAMB and FROST 1966; LU 1969; 1974; LANDNER 1970; GRELL 1978) would not be observed. It is noteworthy that a temperature downshift experiment did not produce interpretable results, since shifting heterozygotes from 26° to 13.5° resulted in few progeny. About 20 progeny were produced that showed the expected high recombination frequency; however, no low recombination frequency progeny survived the shift. This observation could be interpreted as consistent with the conclusions of

Lu and Chiu (1976) that cold temperature prevents the repair of DNA nicks accumulated during the recombination process.

We observed that in *C. elegans* as in *D. melanogaster* (Stern 1926; Schultz and Redfield 1951) recombination frequency decreases with maternal age. In the hermaphrodite, spermatogenesis occurs prior to and is completed before oogenesis begins (Brenner 1974). This implies that the decrease in recombination frequency with successive broods is a reflection of changing oocyte recombination rate. It is difficult to imagine how variations in recombination frequency in the spermatocyte could account for the observed brood pattern. It follows that the rates for the oocyte and the spermatocyte must differ in the hermaphrodite. Furthermore, the observed recombination frequency can never fall below that of the spermatocyte. Since the oocyte rate decreases with age, the possibility of an observed similarity in the two rates (Brenner 1974) over short broods is not ruled out. This means that recombination frequencies measured by counting the progeny of any 24-hr egg-laying period will most likely be anomalously high or low compared to the recombination frequency if all the progeny had been counted. The only way to insure consistency when determining map distances is to exhaust the parent of progeny.

In Drosophila, Grell (1971; 1973) observed that *X*-chromosome nondisjunction was increased by elevated temperature. Similarly, we observed an increase in the rate of *X*-chromosome nondisjunction at temperatures above 20°. At 22° our observations agree with the reported rate of spontaneous males (1/700) (Hodgkin 1974). At 26°, our data show a six-fold increase. At even higher temperatures, heat shock has been reported to produce 5% male progeny (Herman, cited by Riddle 1978). Nondisjunction increase with maternal age in *C. elegans* as it does in Drosophila (Tokunaga 1970) and humans (Lenz *et al.* 1959). The mechanisms responsible for this effect are not known.

This paper emphasizes the fluctuation in recombination frequency caused by variations in temperature and parental age. It is our hope that documentation of these effects will persuade *C. elegans* geneticists to report the temperature and brood conditions under which recombination experiments are conducted. Furthermore, the adoption of standard mapping conditions at 20° where parents are exhausted of progeny is recommended for recombination mapping. The documentation of increased nondisjunction with age in *C. elegans* suggests that this organism is useful for studying this meiotic phenomenon. We have characterized the effect of two parameters that influence the recombination and nondisjunction processes. This characterization makes the study of mutations in *C. elegans* that alter the recombination process more feasible. We are currently studying a strain with increased recombination frequency.

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