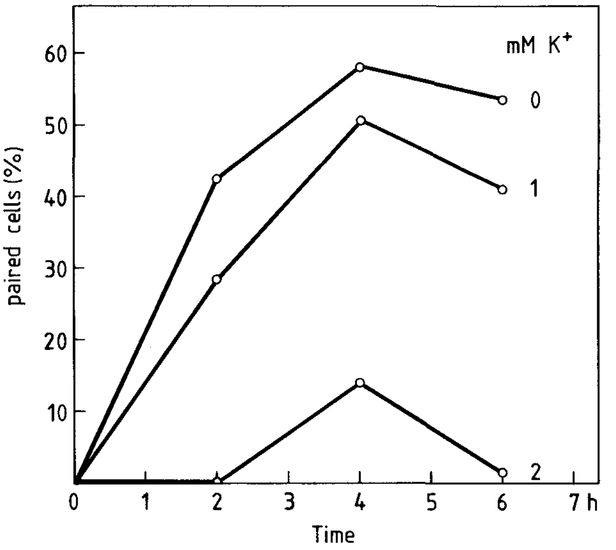

Time course of pair formation in clone B24 of *Euplotes vannus* at 0, 1 and 2 mM K^+.

out effects on the nuclear apparatus. None of the other ions were equally effective, even Rb^+ and Cs^+ required higher concentrations to inhibit pair formation. Li^+, NH_4^+, TEA up to 10 mM could not replace K^+. The ineffectiveness of TEA was particularly striking, since it strongly prevents conjugation between complementary mating types[4].

Heredity. The original *clk* clone B24 (mating type B) was crossed to the non-reactive A28 (mt A). None of the 18 surviving exconjugant clones formed pairs at 0 mM K^+, indicating recessiveness of the *clk* trait. In accordance with this assumption, backcrosses of these exconjugants to B24 yielded new clones with the *clk* phenotype, one of them being B26 (mt B). When this clone was backcrossed to its non-reactive parent, again *clk* clones segregated. However, in all crosses their number was significantly lower than expected for a single Mendelian gene, only 10% instead of 50%, from 64 clones tested. Thus the observations assure genetic determination and recessiveness of the *clk* trait, but leave genetic conditions somewhat uncertain. Monogenic heredity, on the other hand, is still not excluded, since the viability of exconjugants only was 20–40%. Lack of *clk* phenotypes therefore could be due to sublethal pleiotropic effects of the gene.

Occurrence of clk in natural populations. Observations on wild stocks are listed in table 2. In the Naples syngen several clones revealing *clk* were found. They represent all four mating types[10]. In other populations clones occur with a graduation from no reaction to nearly 100% engagement in conjugation at 0 mM $[K^+]_0$. Normally, sexual activity in *E. vannus-crassus-minuta* requires cell contact between different mating types[11]. Occasional intraclonal conjugation is explained by change of mating type expression in heterozygotes as a consequence of aging[12]. Since our mating type B is determined by the lowest allele in the dominance hierarchy[10,13], the B-clones can only be homozygous. Therefore pairing and induction of micronuclear division at low external potassium concentrations take place without a change in mating type expression. Until now this was reported only for *E. patella*[14] and *E. octocarinatus*[15], which both have a mating type determination (single locus, multiple codominant alleles) different from *E. vannus*. In *Paramecium* most of the mutations that affect reactions against potassium impede conjugation[2], or make the cells die in low K^+ concentrations[16]. Therefore the *clk* mutant might become a tool in electrophysiological[17] approaches to elucidate the functional role of potassium ions in conjugation.

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Effect of hyperthermia upon gamma-ray induced crossing-over in an excision repair deficient male *Drosophila melanogaster*

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Summary. Hyperthermia of 1 h at 38 °C did increase gamma-ray induced crossing-over in meiotic cells of male larvae and adults. However, there was considerably less effect of the heat treatment upon radiation induced crossing-over (a chromosome breakage event) in an excision repair mutant *y mei-9*^{a}.

Key words. Hyperthermia; gamma ray; crossing-over; *Drosophila;* repair mutant.

The emergence of hyperthermia as a radiosensitizer has been rapid because of its application in cancer therapy. Although the exact mechanism in enhancing the damage of ionizing radiation is unknown, hyperthermia has become useful in the clinic. Several possible mechanisms have been suggested among them that hyperthermia may interfere with repair processes[1-3]. Mittler reported that hyperthermia may affect excision repair in radiation induced breakage and loss of chromosome[4,5]. In an attempt to obtain more information on whether hyperthermia does interfere with excision repair processes, the effect of hyperthermia upon radiation induced crossing-over in *Drosophila* was selected. Normally, crossing-over does not occur in *Drosophila melanogaster* males. However, there are reports in which radiation has induced crossing-over in male meiotic cells and the consensus of the researchers is that a 2-hit event which breaks the chromatids may be involved[6–8]. The hypothesis to be tested was whether hyperthermia increases radiation induced damage by affecting the excision repair system. If this indeed is one of the actions of hyperthermia, then there should be an increase in radiation induced damage in a repair proficient organism and less damage induced by hyperthermia in a mutant with limited excision repair capabilities. There was some evidence for it has been reported that excision repair mutant *y mei-9*^{a} was the strain in which hyperthermia caused the least increase in radiation induced chromosome loss[4] and additional data have been presented that repair excision defective mutants *cn mus(2)201*^{D1} and *mus(3)308*^{D2} did respond weakly to hyperthermia sensitization of radiation induced chromosomal damage[5].

Material and methods. To study the effect of hyperthermia upon radiation induced crossing-over in male *Drosophila,* 1-day-old adult males and 6-day-old larvae heterozygous for third chromosome roughoid (*ru,* 0.0), hairy (*h,* 26.5), thread (*th,* 43.2), scarlet (*st,* 44.0), curled (*cu,* 50.0), stripe (*sr,* 62.0), ebony (*e*^{s}, 70.7), claret (*ca,* 100.7) were placed in vials and heated in a water bath at 38 °C for 1 h. The adult flies were irradiated with 14 Gy of gamma rays from ^{137}Cs at 3.65 Gy/min while the larvae received 8 Gy. The males which served as the repair proficient control carried *y* (yellow) *w* (white) on the X chromosome, while the excision repair mutant males *y mei-9*^{a} on the X chromosome, and both strains were heterozygous for *ru h th st cu sr e*^{s} *ca.* To obtain the spermatoza in which induced crossing-over could be detected, the 1-day-old adult males were irradiated and then mated 5 days and these offspring were discarded. On days 6–8, 8–10, and 10–12 the males were mated at a ratio of one male to 3 virgin *ru th st cu sr e*^{s} *ca.* The reduction division has been reported[9] to occur about day 7, thus broods day 6-8, included late spermatogonia at the time of irradiation, while the later broods represented progressively earlier spermatogonia. Some of the broods that represented early spermatogonia at the time of irradiation may have undergone further divisions and thus produced clusters of crossovers and noncrossovers. In previous work it was reported that there was no difference in the analyses of induced crossing-over whether the data were obtained from pooled or single males. Thus, the crossing-over was presented as the percentage of

Table 1. Induced crossing-over in $\frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{+\ +\ +\ +\ +\ +\ +\ +}$ 1-day-old adult males

		$\frac{\text{Gametes with crossovers}}{\text{Total gametes}}$		
Brood in days	Control	38°C for 1 h	14 Gy of γ rays	38°C + 14 Gy
		y w		
6– 8	$\frac{0}{2263}$	$\frac{0}{2496}$	$\frac{14}{10075} = 0.139\%$*	$\frac{24}{8032} = 0.299\%$*
8–10	$\frac{0}{2184}$	$\frac{0}{2590}$	$\frac{30}{8974} = 0.334\%$*	$\frac{171}{11483} = 1.489\%$*
10–12	$\frac{0}{2429}$	$\frac{1}{1518} = 0.066\%$	$\frac{57}{9537} = 0.598\%$*	$\frac{92}{9375} = 0.98\%$*
		y mei-9^a		
6– 8	$\frac{6}{11200} = 0.054\%$	$\frac{16}{12801} = 0.124\%$	$\frac{58}{18359} = 0.316\%$	$\frac{46}{11353} = 0.405\%$
8–10	$\frac{1}{6263} = 0.016\%^{+}$	$\frac{13}{10291} = 0.126\%^{+}$	$\frac{39}{11828} = 0.33\%$*	$\frac{54}{7227} = 0.747\%$*
10–12	$\frac{3}{5903} = 0.051\%^{+}$	$\frac{27}{7907} = 0.342\%^{+}$	$\frac{137}{11114} = 1.232\%$	$\frac{184}{16169} = 1.138\%$

$^+$ Significant differences between control and H broods utilizing 2 × 2 contingency tables with Yates' correction analysis. *Significant difference between R and H+R broods.

Table 2. Induced crossing-over in $\frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{+\ +\ +\ +\ +\ +\ +\ +}$ male 6-day-old larvae

		$\frac{\text{Gametes with crossovers}}{\text{Total gametes}}$		
Brood in days	Control	38 °C for 1 h	8 Gy of γ rays	38 °C+8 Gy
		y w		
0–3	$\frac{0}{571}$	$\frac{1}{2653} = 0.0377\%$	$\frac{34}{9006} = 0.378\%$*	$\frac{67}{8990} = 0.745\%$*
3–6	$\frac{0}{582}$	$\frac{0}{2767}$	$\frac{32}{8208} = 0.39\%$*	$\frac{107}{10907} = 0.98\%$*
6–9	$\frac{0}{714}$	$\frac{0}{922}$	$\frac{14}{6292} = 0.223\%$*	$\frac{67}{10239} = 0.654\%$*
		y mei-9^a		
0–3	$\frac{0}{937}$	$\frac{7}{6763} = 0.104\%$	$\frac{10}{2449} = 0.408\%$	$\frac{7}{1945} = 0.359\%$
3–6	$\frac{0}{608}$	$\frac{1}{3197} = 0.0313\%$	$\frac{20}{1989} = 1.01\%$	$\frac{28}{2346} = 1.19\%$
6–9	$\frac{0}{517}$	$\frac{1}{1614} = 0.062\%$	$\frac{15}{1527} = 0.98\%$	$\frac{27}{2572} = 1.05\%$

* Significant differences between R and H+R broods utilizing 2 × 2 contingency tables with Yates' correction analysis.

the number of male gametes which produced offspring. Since the spermatozoa produced 12 days after irradiation of 1-day-old adult males yielded less crossovers[9], the broods produced by irradiated adults were limited to 12 days.

A dose of 14 Gy was found to be lethal to larvae and the amount of radiation was lowered to 8 Gy which enabled some of the larvae to survive and become adults. The adult males which emerged from irradiated larvae were mated as soon as possible to *ru h th st cu sr e*^{s} *ca* homozygous females for three broods 0–3, 3–6, and 6–9 days. Crossovers were scored when at least two mutant or wild type alleles were involved in the induced recombination, for the presence or absence of a single mutant could represent a deletion or mutation[10]. If the phenotype of the induced recombinant flies involved two breaks, these were scored twice.

Results and discussion. As previously reported[9], the most frequently induced crossovers involved the breaks in the region surrounding the centromere between *st* and *cu* with appearance of *ru h th st* and *cu sr e*^{s} *ca* offspring. The hyperthermia treatment alone did not induce crossing-over in the adult males of the control *yw*, however, there was a significant increase in cross-overs induced in the repair deficient *y mei-9*^{a} males (table 1). The hyperthermia treatment also increased the radiation-induced cross-overs in the three broods of repair proficient *yw* but only in one 8–10-day brood of *y mei-9*^{a} (table 1). These data support the hypothesis that one of the methods that hyperthermia increases radiation induced damage is that heat may affect a repair process, hence more damage, but if the repair process is already defective the hyperthermia cannot increase the radiosensitivity. The gamma-ray irradiation alone induced more crossing-over in the *y mei-9*^{a} strain compared to *yw* and this may be due to the mutagen sensitivity[11] of *mei-9*^{a}.

The 1-h hyperthermia treatment alone had no effect upon induced crossing-over in the 6-day-old larvae (table 2) in both *yw* and *y mei-9*^{a} experiment. However, the heat treatment significantly increased the radiation induction of crossovers in all broods from the *yw* male larvae but none in the broods produced by the *y mei-9*^{a} males (table 2). Again more support for the hypothesis that one of the actions of hyperthermia in inducing radiosensitivity may be due to the interference of excision repair process, hence more radiation induced damage.

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Thermoperiodically induced diapause in a mite in constant darkness is vitamin A dependent

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Summary. By rearing the predacious mite *Amblyseius potentillae* in a daily temperature cycle in constant darkness it could be shown that diapause may be thermoperiodically induced. When the same experiments were performed using diets without vitamin A it appeared that vitamin A is necessary to achieve a state of reproductive diapause in this mite.

Key words. Thermoperiodism; photoperiodism; diapause; carotenoids; vitamin A; predacious mites; *Amblyseius potentillae.*

The perception of environmental cues such as photoperiod and the transduction of photoperiodic 'information' into a neuroendocrine signal in insects and mites is still poorly understood at a physiological level; the photoperiodic mechanism has so far been described only in terms of formal models, dealing mostly with the possible kinetics of the photoperiodic clock[1-3]. From genetic studies with spider mites[4,5] and from dietary studies with predacious mites[6-8], two moth species[9,10] and a parasitoid wasp[11] it appeared that carotenoids are somehow involved in the photoperiodic response. In the case of the predacious mite[8] and the wasp[11], vitamin A could be substituted for β-carotene, from which it was concluded that a rhodopsin might function as the photoreceptor for the photoperiodic clock. However, direct evidence for a photoreceptor function is still lacking. From action spectrum studies in an aphid[12] and a whitefly[13] it was concluded that possibly a carotenoprotein, but not rhodopsin, might function as the photoperiodic photoreceptor.

Apart from photoperiod in some insects daily fluctuating temperatures (thermoperiods) were found to be capable of inducing diapause in the complete absence of light[14-21]. Temperature may have various direct as well as indirect effects on the photoperiodic mechanism, but apparently it may also act as a 'zeitgeber' in its own right[1,2,22]. Although very little concrete information is available on thermoperiodic induction of diapause it has been argued that the same clock mechanism may be involved in both photoperiodism and thermoperiodism in insects[16,19,22]. Here we report for the first time a thermoperiodic response in a mite in constant darkness; moreover, this response proved to be dependent on the presence of vitamin A in the diet of these mites, just as shown previously for the photoperiodic response in the same species.

Materials and methods. The strain of the phytoseid mite *Amblyseius potentillae* used for the experiments originated from an apple orchard in the Netherlands[23]. *A. potentillae* exhibits a facultative reproductive diapause which is expressed only in the females and which is induced normally by short-day photoperiods experienced during the immature stages[6-8]. Mites were kept on rectangles of black plastic (8 × 15 cm), surrounded by a barrier of moist filter paper. Cultures were supplied with pollen of the broad bean, *Vicia faba,* as the only source of food, which constitutes a carotenoid-depleted diet for these mites[7,8]. β-Carotene and vitamin A acetate (retinol acetate) were added in crystalline form (5% w/w) and mixed with the pollen. The maternal generation of mites