4 Michelson, A.M., in: The Chemistry of Nucleosides and Nucleotides. Academic Press, New York 1963.
5 Goldberg, N.D., and Haddox, M.K., A. Rev. Biochem. *46* (1977) 823.
6 Anderson, T.R., Mayer, G.L., and Nicholl, C.S., J. cycl. Nucl. Res. *7* (1982) 225.
7 Vardanis, A., J. biol. Chem. *255* (1980) 7238.
8 Canas, P.E., and Congote, L.F., Can. J. Biochem. *60* (1982) 1.
9 Wei, J.W., and Hickie, R.A., Int. J. Biochem. *15* (1983) 789.
10 Cheng, Y.C., and Bloch, A., J. biol. Chem. *253* (1978) 2522.
11 Chan, P.J., Packin, G.S., Slotnick, E.S., Chodos, W.S., Manara, L.R., and Belsky, D.H., J. In Vitro Fert. Embryo Transfer *3* (1986) 268.
12 Wordinger, R.J., and McGrath, M., Experientia *35* (1979) 1683.
13 Johnson, G.S., Morgan, W.D., and Pastan, I., Nature, New Biol. *236* (1972) 247.
14 Witschi, E., Fedn Am. Soc. exp. Biol. *1* (1972) 178.
15 Hsu, Y.-C., Devl Biol. *76* (1980) 465.

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A mutation of *Euplotes vannus* causing induction of intraclonal conjugation (selfing) in low $[K^+]_o$

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Summary. A newly discovered recessive mutation, *clk*, in the marine ciliate *Euplotes vannus* is responsible for induction of intraclonal *c*onjugation at *l*ow, i.e. < 2 instead of 10 mM $[K^+]_o$. Heredity was assured by crosses to standard clones and by backcrosses. Pairing in *clk* clones is prevented by K^+ at 2 mM, by Rb^+ and Cs^+ at 4 mM, not by Li^+, NH_4^+, and TEA^+ up to 10 mM. Clones with the *clk* phenotype were found in several natural populations.
Key words. Intraclonal pairing; low potassium concentration; *Euplotes vannus*.

Potassium ions apparently play crucial roles in conjugation processes in ciliates[2]. High extracellular concentrations affect pair formation in two opposite directions: induction of intraclonal conjugation in *Paramecium*[3], and inhibition of conjugation between opposite mating types in *Euplotes*[4]. In this report, an effect of reduced external potassium concentration on sexual behavior is described: a newly found mutant *(clk)* of the marine *E. vannus* is induced to intraclonal conjugation when transferred to medium with low (< 2 mM) $[K^+]$.

Materials and methods. All clones of various origins (table 2) are members of the *vannus* morphotype of the *Euplotes vannus-crassus-minuta* group[5-7]. Mutant clones are descendents of a stock from Naples/Italy; for designation see 'Heredity'. Cells were bred in artificial seawater (mM): NaCl 465.3; $NaHCO_3$ 2.4; KCl 10; $CaCl_2$ 10.4; $MgCl_2$ 24.8; $MgSO_4$ 28.1 in petri dishes[8] resulting in populations of 8–16000 cells in 8 ml medium. They were transferred to low K^+-medium by sieving. K^+ analogues were applied as chlorides. Ion compositions were altered by addition of appropriate adjusting media in 1:1 proportion. Changes in the nuclear apparatus were visualized by a modified orcein method[8], and Con A binding site fields on the cell membrane by means of horseradish peroxidase[9] (during the latter treatment, cells were partly damaged.) Experiments were performed at 24.5 °C in a constant temperature room.

Results and discussion. Description of the phenotype. Pairs appeared 2–3 h after transfer to 1 mM K^+, and in a still higher proportion at 0 mM (fig.). The percentage increased rapidly, but decreased after 5–6 h. All details were in accordance with normal conjugation, including susceptibility to 5 μM cycloheximide. Courtship behavior, adherence of two mates in angled position, and quick rotation[9] were observed. Only the pre-conjugation phase (lag phase) was longer. Also the typical field of Con A binding sites in the pairing region[9] occurred. Aceto-orcein preparations revealed performance of micronuclear divisions together with macronucleus destruction[8]. The most advanced cytological stage, however, was that of 8 micronuclei; the majority of pairs progressed only to the 2 or 4 micronuclei stage. Thereafter the partners separated without having exchanged pronuclei. Consequently, exconjugants with macronuclear anlagen were not found. Although the series of cytogenetic events was thus not completed to synkaryon formation, all traits indicate that conjugation was induced in the mutant cells, but continued only to a certain point. The inability to terminate conjugation could be due to the detrimental effects of lack of K^+ for so many hours.

Effects of K^+ analogues. Specificity of action of potassium ions on *clk* clones was tested by comparison with other monovalent cations (table 1). K^+ inhibited pair formation at 2 mM, the few exceptional pairs (fig.) being temporary, with-

Table 1. Effects of ions on intraclonal conjugation in a *clk*-clone (B26) of *Euplotes vannus*

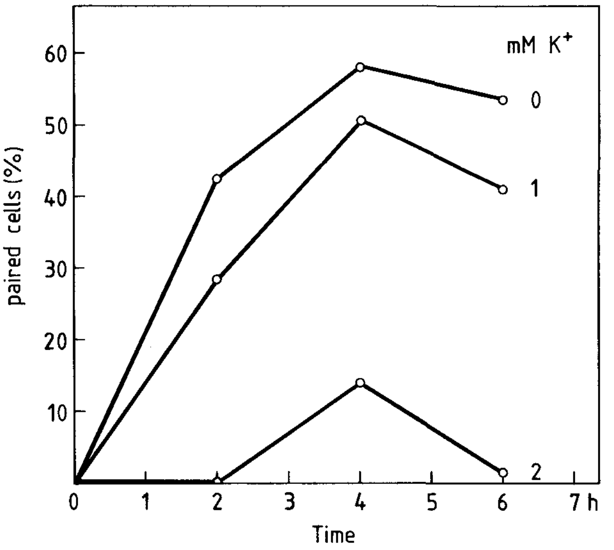
Ion	Observations* at mM			
	1	2	4	10
K^+	+	–	–	–
Rb^+	+	+	–	–
Cs^+	+	+	–	–
Li^+	+	+	+	+
NH_4^+	+	+	+	+
TEA^+**				+

* + = conjugation; – = no pairs; ** tetraethylammonium.

Table 2. Reaction of clones of *Euplotes vannus* from different origins to medium without K^+

Origin	Clones tested	Observations*		
		–	(–)	+
Naples/Italy	31	17	7	7
Tropea/Italy	1	0	0	1
Brindisi/Italy	2	0	1	1
Vrsar/Jugoslavia	3	1	2	0
Barcarès/France	2	0	0	2
Quiberon/France	7	7	0	0
Patras/Greece	2	2	0	0
Nafplion/Greece	2	2	0	0
Mombasa/Kenya	18	18	0	0
Morehead/North Carolina	3	0	2	1

* – = no reaction; (–) = single pairs; + = conjugation, including induction of micronuclear divisions.



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$^+$]$_o$. 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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2 Hinrichsen, R. D., Amberger, E., Saimi, Y., Burgess-Cassler, A., and Kung, C., Genetics *111* (1985) 433.
3 Kitamura, A., and Hiwatashi, K., J. exp. Zool. *231* (1984) 303.
4 Lueken, W., J. Protozool. *31* (1984) 61A.
5 Génermont, J., Machelon, V., and Demar, C., Atti Soc. tosc. Sci. nat., Mem. Serie B *92* (1985) 53.
6 Fernandez-Leborans, G., Arch. Protistenk. *131* (1986) 215.
7 Valbonesi, A., Ortenzi, C., and Luporini, P., Atti Soc. tosc. Sci. nat., Mem. Serie B *92* (1985) 67.
8 Lueken, W., J. Protozool. *20* (1973) 143.
9 Lueken, W., and Oelgemöller, B., J. exp. Zool. *236* (1985) 193.
10 Lueken, W., Gaertner, M., and Breer, H., J. exp. Zool. *226* (1983) 11.
11 Dini, F., and Luporini, P., J. Protozool. *32* (1985) 111.
12 Heckmann, K., J. exp. Zool. *165* (1967) 269.
13 Heckmann, K., Arch. Protistenkd. *106* (1963) 393.
14 Akada, R., J. Protozool. *33* (1986) 235.
15 Heckmann, K., and Kuhlmann, H.-W., J. exp. Zool. *237* (1986) 87.
16 Richard, E. A., Hinrichsen, R. D., and Kung, C., J. Neurogenet. *2* (1985) 239.
17 Krüppel, T., and Lueken, W., Verh. dt. zool. Ges. *79* (1986) 339.

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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