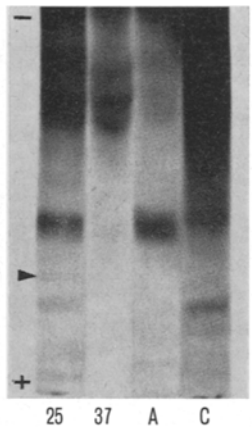

Fig. 2. Soluble protein profile on acrylamide gel electrophoresis during synchronous production of protoperithecia. 25 °C, note the band at Rf 0.78 with arrow mark which appeared in (step-down 37° to 25 °C) protoperithecial differentiating culture. 37 °C, absence of the band. A) actinomycin D treatment inhibits this band. C) cycloheximide treatment also inhibits the band.

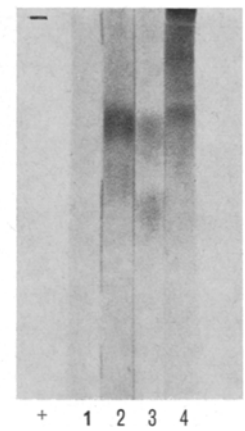

Fig. 3. Tyrosinase during synchronized development of protoperithecia. 1. 37 °C culture, no tyrosinase. 2. Step-down (37° to 25 °C) differentiating culture, 2 bands of tyrosinase. 3. Step-down (37° to 25 °C) actinomycin D treated, 2 bands of tyrosinase. 4. Step-down (37° to 25 °C) cycloheximide treated, 4 bands of tyrosinase.

is of interest. The possibility of this effect due to changes in cell wall permeability was checked by increasing the osmotic pressures of media with 1 to 10%, of NaCl, and polyethylene glycol '400'. There was no induction of ascogonia at 37°C. Thus the effect of polyols seems to be specific. Whether the induction of protoperithecia by polyols in non-permissive conditions (37°C cultures) is a phenomenon of derepression of genes or simply a metabolic effect providing an additional source of reduced nicotinamide coenzymes is under investigation.

Résumé. Une méthode pour la synchronisation de la différenciation des ascogones de *Neurospora* est présentée. Ce système de synchronisation basé sur la température facilitera l'analyse biochimique et génétique intégrée de la différenciation protopérithéciale. Il est déjà démontré qu'au moins une protéine est associée à ce processus et qu'il n'y a pas de corrélation entre la tyrosinase et cette différenciation.

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Sterilization of Paedogenetic *Heteropeza* Larvae with X-Rays

The gall midge *Heteropeza pygmaea* Winnertz (*Itonididae*, Diptera) can reproduce either bisexually or parthenogenetically in the adult stage, or paedogenetically[1–3]. Paedogenesis is parthenogenetic reproduction in the larval stage. It can be induced by defined nutritive conditions of the culture. In paedogenetically reproducing larvae, the ovaries produce small immature egg follicles which are released into the haemolymph. Within the larval blood the oocyte grows and after maturation embryogenesis goes to completion. Since no chorion is present the embryos can take up nutrients from the maternal haemolymph and grow continuously (see Figure). The increase in size of the embryos is at the expense of maternal tissues. At an age of 5 days a mother larva consists mainly of a stiffened body wall filled with offspring. The rearing conditions provided are such that the offspring, which hatch from the mother larvae, consist exclusively of daughter larvae which can reproduce paedogenetically.

The unusual mode of reproduction qualifies paedogenetic gall midges as interesting objects for in vitro culture of eggs and embryos in different culture media[4]. In vitro culture of *Heteropeza pygmaea* embryos is possible in a medium consisting of haemolymph and fat body cells obtained from *Heteropeza* larvae without progeny[5] (so-called sterile larvae). Such sterile larvae appear occasionally in standard cultures. For large scale culture experiments a convenient procedure for obtaining ample numbers of sterile larvae with large fat bodies and a maximum amount of haemolymph had to be developed. To this end we analyzed the effect of radiation on mother larvae, as well as on the eggs and embryos developing in their haemolymph. For all treatments we used 50 keV X-rays from a Müller-RT 100 machine.

In the first experiment mother larvae of different ages were irradiated. The age zero h is defined as the moment when a young larva hatches from its mother larva. With doses up to 3 kR no irradiated larvae died prematurely. But, with the exception of 96 h old larvae, the average of

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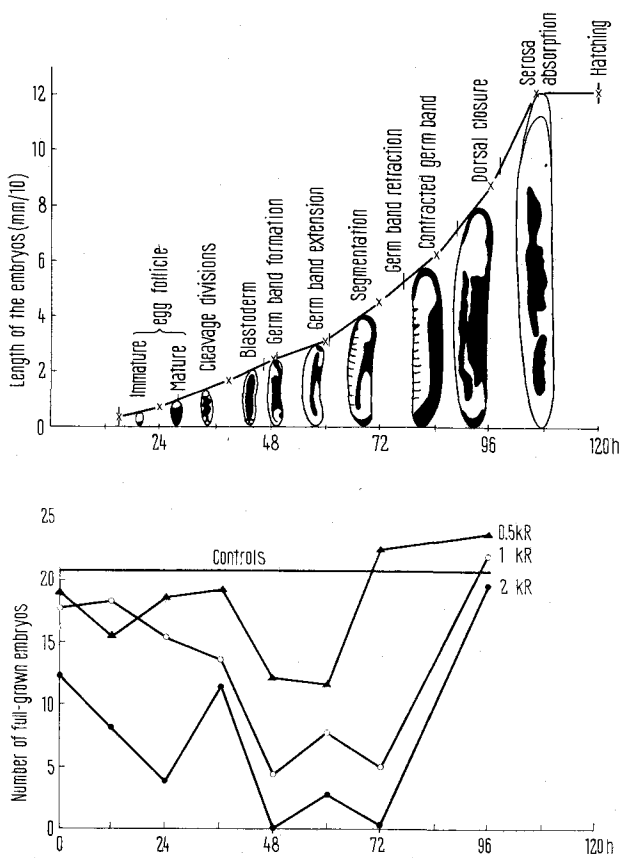
daughter larvae per mother larva was reduced (see lower figure). The reduction in the number of offspring per larva compared to untreated controls depends on the dose and the age of the larva at the moment of irradiation. Since within one mother as well as within a group of accurately timed mother larvae the embryos develop fairly synchronously, the age of the irradiated mother larvae is indicative of the developmental stage of the embryos irradiated. It is evident from the Figure that stages between blastoderm and germ band retraction (48 to 72 h age of the mother) are most sensitive to X-rays. With doses of 2 kR and higher, the majority of treated larvae can be sterilized. In an attempt to use this procedure to provide culture medium for the in vitro cultures, we found that this method has 2 disadvantages: a) The average length of the finally obtained sterile larvae after treatment at 48 h was less than that of the non-irradiated controls (see Table). b) The handling of a large number of mobile larvae for the X-ray exposure is not convenient.

X-ray irradiation of *Heteropeza* larvae, 9 h before or 48 h after hatching

Larval age at rradiation	Dose (kR)	No. of embryos per full-grown larva	Length of stiffened larvae (mm)	Duration of oogenesis + embryogenesis (h)
9 h before hatching	0	28.8	3.73	113
	3	0.02	3.66	192
	4.5	0	3.67	—
48 h after hatching	0	29.3	3.83	111
	1	11.6	3.17	138
	2	0.38	3.19	175

Each value is an average of values obtained from 25 treated larvae.



The upper diagram shows some stages of embryogenesis of paedogenetically developing embryos of *Heteropeza pygmaea*. Zero time on the abscissa is the time when the larval mothers of the embryos hatched. The lower diagram gives the average number of full-grown embryos per irradiated larva. The abscissa indicates the age of the larva at the moment of X-ray irradiation. Every point is based on 20 larvae analyzed.

In order to eliminate these disadvantages, we tried to sterilize larvae before egg follicles were released from the ovary. For this purpose we irradiated full-grown embryos before hatching. In this second experiment we used about 110 h old, immobile mother larvae which were filled with full-grown embryos. On an average about 9 h after the irradiation the daughter larvae hatched. As can be seen from the Table, somewhat larger doses are needed to sterilize the nonhatched larvae compared to 48-h-old larvae. In all cases in which any progeny developed in the irradiated larvae, the duration of oogenesis + embryogenesis was increased (see Table). The length of the stiffened larvae which are finally obtained about 6 days after treatment is not reduced in the irradiated series compared to the control series. The development and behaviour of X-ray sterilized larvae is very much the same as observed with the occasionally occurring spontaneous sterile larvae (KAISER[6,7]). In any case the sterile larvae feed and grow like normal fertile larvae. The histolysis of nearly all larval organs, with the exception of the hypertrophying fat body, takes place in aged sterile larvae. Since this histolysis is observed in fertile as well as in sterile larvae, it seems not to be induced by the offspring in fertile larvae as presumed by IVANOVA-KASAS[8]. It must be controlled by some maternal organ, perhaps the prothoracic glands, as suggested by KAISER[7,9].

Zusammenfassung. Durch Röntgenbestrahlung können die Ovarien oder die sich entwickelnden Embryonen in den paedogenetisch sich fortpflanzenden Gallmückenlarven *(Heteropeza pygmaea)* abgetötet werden. Die Embryonen sind besonders strahlenempfindlich in den Stadien zwischen Blastoderm und Keimstreif-Kontraktion. Nach Bestrahlung mit genügend hohen Dosen entstehen sterile Larven, die am Ende ihrer Entwicklung eine Histolyse fast aller larvalen Organe, mit Ausnahme des hypertrophierenden Fettkörpers, zeigen.

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