To study the pharmacology of levamisole on nematodes further, freshly collected whole *A. lumbricoides* were held horizontally in an open Perspex bath containing Tyrode at 37 °C and connected by rubber collars to an isotonic recorder. Solutions in Tyrode were injected into the worms (0.1 ml solution into a 3 g worm). Although tubocurarine, and atropine (all tested up to 20 μg/g) failed to prevent contractions caused by levamisole (0.3 μg/g), both the ganglion blockers mecamylamine and pempidine (5 μg/g) blocked levamisole induced contractions. We consider that these results confirm the suggestion of VAN NEUTEN[1] that levamisole is acting as a ganglion stimulant in nematodes.

In contrast to the report that paralysis of *A. lumbricoides* by levamisole is irreversible[1], our experiments have shown reversible paralysis with 3 species of nematodes, *A. lumbricoides*, *Nippostrongylus brasiliensis*, and *Nematospiroides dubius* when they were continuously maintained in levamisole. Adult *N. brasiliensis* maintained in levamisole solutions in Morgan, Morton and Parker saline with antibiotics (NaCl, 6.8 g/l; KCl, 0.4 g/l; $CaCl_2$, 0.2 g/l; $NaHCO_3$, 2.2 g/l; $MgSO_4 \cdot 7H_2O$, 0.2 g/l; $Fe(NO_3)_3$ $9H_2O$, 0.1 g/l; $NaH_2PO \cdot H_2O$, 0.14 g/l; glucose, 1 g/l; penicillin, 400,000 U/l; dihydrostreptomycin, 0.4 g/l; mycostatin, 100,000 U/l) regained motility. Both the rate of paralysis and de-paralysis was dependent upon the concentration of levamisole. Using approximately 20 worms per 10 ml in 100 ppm of levamisole, all worms paralysed within 10 min and all resumed active movements within 8 h; most worms kept in levamisole maintained these movements for 72 h. The reversibility of paralysis was not however observed with free living infective larvae of *N. brasiliensis*. Similar experiments with *A. lumbricoides* maintained in Tyrode (1 worm/50 ml) containing 100 ppm of levamisole demonstrated that some worms resumed movement after paralysis, though the movements were less vigorous than in normal worms. Both the time taken for recovery and the percentage of *A. lumbricoides* resuming active movements varied widely from batch to batch of worms. The suggestion that levamisole may act by inhibition of fumarate reductase[3] in addition to its function as a ganglion stimulant is difficult to reconcile with the reversible nature of levamisole paralysis.

The connection between the mode of action of levamisole and 2 other anthelmintics, methyridine (2-[2-methoxyethyl]pyridine) and pyrantel (*trans*-1-methyl-2-[2-(2-thienyl)vinyl]-1, 4, 5, 6-tetrahydropyrimidine) was first suggested by experiments with *N. brasiliensis*. Adult worms that had resumed active movements after incubation in 100 ppm of levamisole were washed and transferred to Morgan, Morton and Parker saline or saline plus 100 or 1000 ppm of methyridine. Even after 16 h in 1000 ppm of methyridine, some of the worms which had recovered from levamisole remained active although normal worms in methyridine were inactive. Worms were also incubated in either 100 ppm pyrantel (as tartrate), or 100 ppm levamisole or 100 ppm of pyrantel plus levamisole. Significantly more worms were showing activity in levamisole or levamisole plus pyrantel than in pyrantel on its own.

Both methyridine, bephenium[4] (benzyl dimethyl-2-phenoxyethyl ammonium) and pyrantel[5] cause contraction in normal *A. lumbricoides* muscle. However, when injected into *A. lumbricoides* that had recovered motility after incubation in levamisole, methyridine (30 μg/g), pyrantel (30 μg/g) and 40 μg/g bephenium (as hydroxynaphthoate) failed to cause contractions; acetyl choline (1 μg/g) and choline phenyl ether (0.6 μg/g) however caused contractions. It would appear that levamisole can cause a tachyphylactic type of response at the nerve ganglion receptor site of nematodes permitting muscular activity to resume. The presence of levamisole at the receptor site blocks the activity of certain other anthelmintics, which therefore presumably act at the same or closely adjacent site in the ganglion. The variation in ability of the 4 anthelmintics to paralyse nematodes will depend on both the variation in their affinity for the receptor sites as well as differences in rate of entry into the nematodes.

Résumé. Les *Ascaris* qui se sont «déparalysés» après une incubation prolongée dans 100 ppm de levamisole, ne se contractent pas après injection des anthélminitiques béphenium, méthyridine et pyrantel. Etant donné que la mécamyline et la pempidine bloquent les contractions des *Ascaris* provoquées par le levamisole, il paraît probable que chez les Nématodes ces 4 anthélmintiques sont des stimulateurs des ganglions.

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Reproductive Incompatibility between Similar Genotype Flies (*Drosophila melanogaster*) Raised at Two Different Temperatures

In *Drosophila melanogaster*, the usual growth temperature is 25 °C, although it is possible to get complete development from egg to adult at temperatures ranging from 12° to 32 °C[1]. A biometrical analysis[2] showed important morphological modifications in adults grown at low temperature. Thus, we decided to look for possible correlative physiological differences in such flies. As 13° reared males proved to be sterile[3], 13° females were crossed with normal (25°) males in order to measure egg production and fertility. It was then discovered that in this cross copulation often resulted in premature death of females. Moreover, the survivors produced poorly hatching eggs, suggesting alteration of the fertilization process. From these observations we concluded that a kind of reproductive incompatibility arose between adults of the same genotype raised under different environmental conditions.

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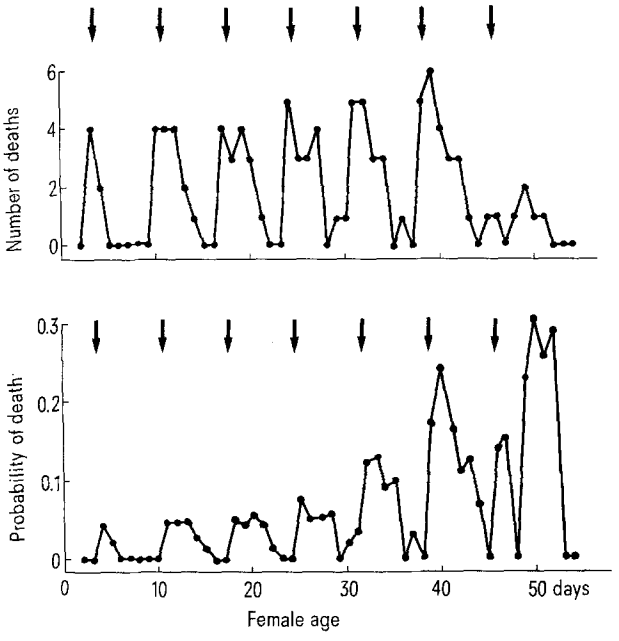

Fig. 1. Mortality of 13 °C reared females in intermittent presence of 25 °C reared males (1 day every week). Arrows indicate days males were present. (The experiment started with 100 females; probability of death is the ratio of death to survivals.)

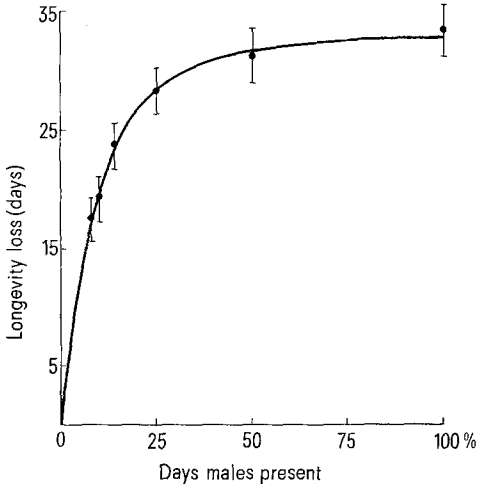

Fig. 2. Reduction of mean longevity (compared to virgins) of 13 °C reared females in the presence of 25 °C reared males.

Experiments were made on F_1 heterozygous flies, obtained from a cross between two laboratory inbred lines. Such flies are highly vigorous and give more reproducible results than do inbred flies. Laying always took place at 25°. Eggs, aged 0 to 6 h, were placed in vials containing axenic-killed yeast medium[4] and were then either kept at 25° or transferred to 13°. All emerging adults were studied at 25°. In a first experiment, 4 types of crosses were considered: females 25° × males 25°; females 25° × males 13°; females 13° × males 25°; females 13° × males 13°. Groups of 10 males and 10 females were placed in plastic cages[5] and their longevities at 25° measured. The mean values for females are given in Table I.

In both kinds of females, longevity dropped when crossed with 25° reared males. The shortening was slight for 25° females but great for 13° females. Such females appear, therefore, highly sensitive to copulation.

The phenomenon was further investigated by varying the ratio of males to females while the population density in each cage was kept approximately constant (20 or 21 flies per cage). The presence of males always reduced the lifespan of females, as shown in Table II. In the case of 13° females crossed with 25° males, the strong reduction increased with proportion of males.

The lethal effect of males on 13° reared females was also clearly demonstrated in experiments of intermittent copulation. Groups of ten 13° females were mixed temporarily with groups of fifteen 25° reared young males. Results obtained with females allowed to copulate with males 1 day each week are shown in Figure 1. Death of females mainly occured during the 4 or 5 days following copulation. Surviving females were, however, not protected nor insensitive to the killing effect, since most of them died later as a consequence of further copulations.

Different periodicities for male and female confrontations were used and the general curve is presented in Figure 2. Reduction of longevity was proportionnal to duration of presence of males. Maximum reduction was approximately attained when males were present $^1/_4$ of the time.

Interesting results concerning the female physiology were also obtained from the study of egg hatchability. Data from various crosses are indicated in Figure 3. As the 13° reared males were sterile on emergence, they were kept at 25° for a week before crossing to gain normal sperm production[3]. (Interestingly, 13° virgin females staged a week at 25° did not acquire normal resistance to copulation.) The rearing temperature of both males and females was important to egg hatchability: 25° reared females were always more fertile than 13° females: 25° females gave better hatching when crossed with 25° males than with 13° males. When crossed with 13° males, 13° females produced numerous viable larvae even when a month old (see Figure 3). But the same females crossed with 25° males lost fertility quickly. Examination of the unhatched eggs showed that most of them were unfertilized and did not contain dead embryos. Therefore, the rapid fertility decrease in the 13° female × 25° males cross

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Fig. 3. Effect of age on fertility (percentage of eggs hatching) of females reared at 13° or 25° and crossed with 13° or 25° reared males.

Table I. Mean longevities (days at 25 °C) of females crossed with males reared at 13° or 25°

		Growth temperature of female			
		13 °C		25 °C	
		m	*n*	m	*n*
Growth temperature of males	13 °C	42.04 ± 1.99	70	58.88 ± 1.66	67
	25 °C	20.49 ± 0.98	71	52.23 ± 0.87	69
Differences (days)	21.55 ± 2.21	21.55 ± 2.21		6.65 ± 1.86	
Reduction (%)		51.3		11.3	

m, mean in days; *n*, number of females.

Table II. Percentage of reduction of female longevity (compared to virgins) when the proportion of males was increased

Growth temperature		Reduction of longevity (%)					
♀	♂	(0)	(0.5)	(1)	(1.5)	(2)	(3) [a]
25 °C	25 °C	0	18.48	18.00	18.27	21.99	22.33
	13 °C	0	12.18	14.36	19.70	21.63	
13 °C	13 °C	0	18.56	14.78	19.98	20.93	21.12
	25 °C	0	40.05	46.78	46.70	52.34	65.92

[a] Values in parentheses are numbers of males per female.

appeared as another harmful effect of copulation. This decrease probably reflected a complex physiological alteration in the surviving females.

Why are only 25° reared males clearly harmful to females? Direct observations reveal that they are much more active and much more able to copulate than are 13° individuals, even those staged a week at 25°[6,7]. The noxious influence of the males thus seems mainly correlated with their general vitality, sexual activity and aggressivness.

Life duration is longer in virgin than in mated females[8–12]. Egg production is less in virgin females, hence a lower metabolism could allow them a greater lifespan. This explanation was first contested by KUMMER[10], who suggested a direct, noxious effect of copulation. It was also demonstrated that decreasing the egg production by lowering the amount of ingested food did not result in increase of lifespan[13]. It is thus probable that, for a *Drosophila* female, copulation is always somewhat deleterious. The dramatic effect observed in the 13° reared females could be merely an exaggeration of the usual process, such females having increased sensitivity to copulation. Death of the females could be produced by mechanical traumatism, nervous stress or toxic influence of the male seminal fluid. These different hypotheses were tested but results remain inconclusive.

The reproductive incompatibility between flies of the same genotype described here appears as a new phenomenon constituting a new kind of sexual isolation or selection in mating (syngamic isolation). Study of this phenomenon in other species is desirable. Analogous results have already been obtained with other *Drosophila* strains. It is possible this is a particular case of the general effect of environmental factors on reproductive compatibility[14].

Résumé. La température subie au cours du développement modifie de façon profonde et irréversible la physiologie des drosophiles adultes. Lorsque des femelles élevées à 13°C sont croisées avec des mâles élevés à 25°C, on observe une véritable incompatibilité reproductrice, qui se traduit par une diminution rapide du pourcentage d'éclosion des œufs et par la mort prématurée des femelles. Seuls les mâles élevés à 25° et sexuellement très actifs exercent cet effet nocif. Réciproquement, seules les femelles élevées à basse température présentent cette hypersensibilité à la copulation. Plusieurs hypothèses peuvent actuellement être envisagées pour expliquer le phénomène.

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