in the skeleton photoperiod appear to be decreased transiently by both pulses in a similar manner. By contrast, it is remarkable that dTFs in the complete photoperiod show opposite responses to the onset (dawn) and offset (dusk) of the photoperiod, i.e., a transient decrease immediately after the dawn and a transient increase immediately after the dusk.

These opposite responses of the dTFs to the dawn and the dusk may be attributed to the decelerating effect of the continuing complete photoperiod, abrupt removal of which may provoke a rebound reaction in TFs such that they increase transiently. By contrast, in the skeleton photoperiod of 12, the TF rhythm may be abruptly reset to its new CT 0 identically by both the first and the second pulse, thus depicting identical hemicircle loops. [Indeed, recent experiments in our laboratory reveal that the TF rhythm previously entrained to the skeleton photoperiod of 12, when scheduled into DD after either the first or the second pulse, initiates the free-running oscillation with the first maximum in the rhythm occurring around 12 h after the pulse, no matter which was the first or the second pulse].

The present results, therefore, describe the roles of the four factors involved in the LD cycles to entrain the circadian rhythm of traverse frequency in the *Paramecium* population as follows: 1) the discrete onset of light acts principally to reset the rhythm to a new phase, causing an abrupt phase-advance in the rhythm; 2) the subsequent continuing action of light functions to decelerate the rhythmic oscillation of traverse frequency, which prevents the rhythm from being completely reset by the discrete onset of light and eventually decelerates the rhythm during the remainder of the light period; 3) the abrupt removal of the continuing light releases the rhythm from the decelerating effect, provoking a rebound reaction which transiently accelerates the rhythm; and 4) the rhythmic oscillation of traverse frequency gradually slows down so that it gradually returns to the innate DD free-run.

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Infection of frog tadpoles (Amphibia) by insect parasitic nematodes (Rhabditida)

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Summary. Infective stage juveniles of *Neoaplectana carpocapsae* (Steinernematidae) and *Heterorhabditis heliothidis* (Heterorhabditidae) were able to penetrate through the alimentary tract of young tadpoles of *Hyla regilla* (Hylidae) and *Xenopus laevis* (Pipidae) and enter the body cavity. Some infectives of *N. carpocapsae* were able to release their symbiotic bacterium, *Xenorhabdus nematophilus* inside the host and in two cases, the nematodes developed into adult females before they perished. Tadpole mortality was associated with foreign bacteria entering the penetration holes made by the invading nematodes. The infective stage juveniles of both nematodes frequently encountered a host defense reaction upon reaching the tadpole's coelom.
Key words. Neoaplectana; *Heterorhabditis*; frog; Amphibia; Nematoda; pathology.

Rhabditoid insect parasitic nematodes belonging to the genera *Neoaplectana, Steinernema* and *Heterorhabditis* are unusual in their ability to kill and develop in a wide variety of insects[2] as well as being able to penetrate, kill and at least partially develop in Symphylans[7], Isopoda[5], Areneae[3], Pseudoscorpionida[6] and millipedes[4]. The ability to infect arthropods other than insects was unusual but suggested that all arthropods shared certain structural and biochemical features to which nematodes could recognize and respond. However, it was perplexing when Kermarrec and Mauleon[1] reported that tadpoles of *Bufo marinus* could be killed by penetrating infectives of the 'Agriotos' strain of *Neoaplectana carpocapsae*. Since neoaplectanids and other rhabditoid nematodes are potential candidates for use against pest insects it was important to determine if the above results were applicable only to *Bufo* tadpoles because of some unknown combination of characters, or whether tadpoles of other amphibian species were also susceptible.

The present study was designed to test the effect of both *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* on the tadpoles of two frog species, one native and one exotic.

Materials and methods. The All strain of *Neoaplectana carpocapsae* Weiser (Steinernematidae) and the NC strain of *Heterorhabditis heliothidis* were used in this study. Tadpoles of the western tree frog *Hyla regilla* (Hylidae), a native of western North America were obtained from large earthen outdoor basins at the Magic Gardens Nursery in Berkeley, California. They were collected with a fish net and main-

tained in the laboratory in pond water with blue green algae as a source of food. Tadpoles of the clawed frog, *Xenopus laevis* (Pipidae) an experimental frog from South Africa, were obtained from Carolina Biological Supply Co. They were maintained in tap water on Wards No. 88W6534 Tadpole Food.

Experimental tests were conducted by placing the tadpoles in deep petri dishes (10 cm diameter × 2 cm deep) containing 40 ml of water. The nematodes were added to the water, together with food for the tadpoles. Controls consisted of placing the tadpoles in deep petri dishes with food but without nematodes. After adding the nematodes, the tadpoles were observed daily for 7 days. Dead individuals were removed, a small sample of their blood plated out on nutrient agar plates and the cadavers placed in wells in spot plates maintained in a moist chamber. The cadavers were examined daily for nematode development.

Hyla experiment I. The tadpoles collected from the field were divided into three size categories; small (10–12 mm in length); medium (15–18 mm in length) and large (20–25 mm in length). Ten small, ten medium and seven large tadpoles were placed in separate petri plates and challenged with both nematode species at concentrations of 16,000 nematodes/petri dish (400 nematodes per ml), respectively.

Hyla experiment II. Medium (15–18 mm in length) and large (20–25 mm in length) sized tadpoles were challenged with total nematode concentrations of 4000 (100 nematodes per ml), 8000 (200 nematodes per ml) and 16,000 (400 nematodes per ml), respectively. Seven medium and 6 large tadpoles were used for each concentration.

Xenopus experiment. Two-week-old *Xenopus* tadpoles (average length 0.8 cm, range 0.6–1.1 cm) that had been hatched from eggs were challenged with both nematode species at concentrations of 8000 (200 nematodes per ml), 16,000 (400 nematodes per ml) and 32,000 (800 nematodes per ml), respectively. Ten tadpoles were tested at each concentration.

Results. The results of all three experiments are shown in tables 1–3. The infective stages of both nematode species were ingested and could be found in the intestine of dissected tadpoles a few hours after being placed together in the same container (fig. 1). Entry into the body cavity of the tadpole was executed by direct penetration through the wall of the intestine (fig. 2). Once inside the body cavity, both nematode species could be found moving around inside the host (fig. 5). The infective stages that first entered the tadpoles would frequently be surrounded by host blood cells and connective tissue which immobilized and eventually killed some nematodes (fig. 4).

Examination of nutrient agar plates with blood drops of dead or dying tadpoles revealed the presence of *Xenorhabdus nematophilus* from 20% of all tadpoles challenged with *Neoaplectana carpocapsae*. In contrast, although all plates contained large numbers of coliform bacteria (mostly *Pseudomonas* spp.), there was no indication that *H. heliothidis* juveniles liberated their symbiotic bacterium, *X. luminescens*. Those cadavers which contained *X. nematophilus* also had nematodes in various stages of development. Most died before reaching the adult stage, probably due to the presence of other bacteria which made the environment inhospitable. However two infectives in separate cadavers (one in

Table 1. Mortality of small, medium and large *Hyla regilla* tadpoles challenged with infectives of *Neoaplectana carpocapsae* (All strain) and *Heterorhabditis heliothidis* (NC strain) at 16,000 nematodes/dish (400/ml), respectively.

Day	*N. carpocapsae* Small (N = 10)	Medium (N = 10)	Large (N = 7)	*H. heliothidis* Small (N = 10)	Medium (N = 10)	Large (N = 7)	Control Small (N = 10)	Medium (N = 10)	Large (N = 7)
1	0	0	0	0	0	0	0	0	0
2	7	0	0	9	5	1	0	0	0
3	1	0	0	1	3	2	0	0	0
4	1	2	0		2	3	0	0	0
5	0	3	1			1	0	0	0
6	1	5	2				0	0	0
7			4				1	0	0

Table 2. Mortality of medium and large *Hyla regilla* tadpoles challenged with three concentrations* of *Neoaplectana carpocapsae* (All strain).

Day	Medium 16,000* (N = 7)	8000 (N = 7)	4000 (N = 7)	Large 16,000 (N = 6)	8000 (N = 6)	4000 (N = 6)	Control Medium (N = 7)	Large (N = 6)
1	3	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	4	1	1	6	1	0	0	0
4		5	3		5	6	0	0
5		1	3				0	0

* Nematodes/dish.

Table 3. Mortality of *Xenopus laevis* tadpoles challenged with three concentrations* of *Neoaplectana carpocapsae* (All strain) and *Heterorhabditits heliothidis* (NC strain), respectively.

Day	*N. carpocapsae* 32,000* (N = 10)	16,000 (N = 10)	8000 (N = 10)	*H. heliothidis* 32,000 (N = 10)	16,000 (N = 10)	8000 (N = 10)	Control (N = 10)
1	0	0	0	1	2	1	0
2	0	0	2	2	1	2	0
3	4	5	5	5	3	4	0
4	4	4	1	0	3	1	0
5	2	1	2	2	1	2	0

* Nematodes/dish.

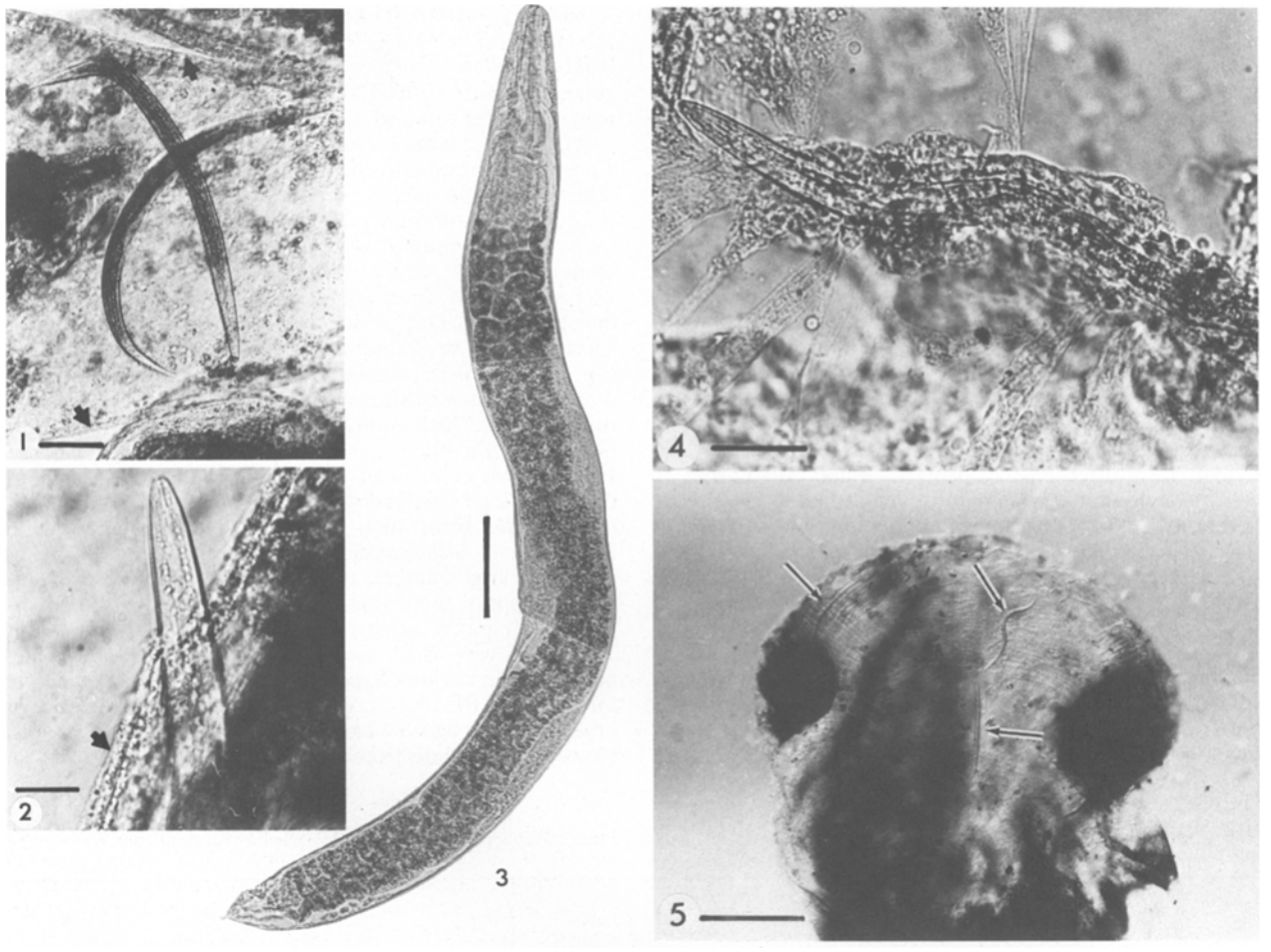

Figure 1. Two infective stage *Neoaplectana carpocapsae* in the intestinal lumen of a *Hyla regilla* tadpole (line = 90 µm) (arrows show intestinal epithelium).

Figure 2. Infective stage *Neoaplectana carpocapsae* penetrating through the intestinal wall of a *Hyla regilla* tadpole (line = 23 µm) (arrow shows intestinal epithelium).

Figure 3. Adult female *Neoaplectana carpocapsae* removed from the body cavity of a dead *Hyla regilla* tadpole (line = 100 µm).

Figure 4. Defense reaction of a *Hyla regilla* tadpole against an infective stage *Neoaplectana carpocapsae*. Note surrounding host cells and connective tissue (line = 46 µm).

Figure 5. Infective stage *Neoaplectana carpocapsae* (arrows) within the body cavity of a *Xenopus laevis* tadpole (line = 600 µm).

H. regilla and one in *X. laevis*) did reach the female stage before dying (fig. 3).

The experiments suggest that with *H. regilla*, there was an inverse relationship between size and susceptibility (table 1); however there was no obvious relationship between mortality of either tadpole species and nematode concentration at the dosage range used (tables 2 and 3).

Discussion. Tadpole mortality in these experiments is considered to be caused by intestinal microflora (bacteria) being carried into the body cavity during nematode penetration of the gut wall or passing into the coelom later through the nematode's entrance hole. Since the tadpole mortality rate with *H. heliothidis*, which did not liberate *X. luminescens*, was approximately the same as the mortality rate with *N. carpocapsae*, death cannot be attributed to the release of the symbiotic bacteria in the case of the latter species.

It is interesting to note that the 'penetration instinct' of the infective stage nematodes is so strong that they will even push through the intestinal wall of some vertebrates. On the basis of alimentary tract observations, it is felt that the higher susceptibility of young tadpoles of *H. regilla* is primarily the result of their relatively thin intestinal wall in comparison to older tadpoles, thus allowing the nematodes easier access to the body cavity. A greater ability to produce a defense reaction in larger tadpoles may also be a factor once the coelom is invaded.

As soon as the tadpoles died, their bodies were filled with bacteria, fungi and sometimes protozoa, thus creating an environment that is not favorable for nematode development. Those infectives of *N. carpocapsae* that did initiate development were probably successful in releasing their symbiotic bacteria soon after entering the coelom. It is doubtful whether populations of the symbiotic bacterium (*Xenorhabdus nematophilus*) ever dominated in the tadpole cadaver. Those few neoaplectanids that did develop certainly did so in a population of mixed bacteria, as evidenced by the small numbers of colonies of *Xenorhabdus* on the nutrient agar plates in comparison to other bacterial species.

That young tadpoles of some amphibians can die as a result of penetrating infective stage *Neoaplectana* and *Heterorhabditis* nematodes is clear from these experiments and those reported earlier [1]. Although the latter authors did not discuss

the manner of infection of *Bufo* tadpoles with *N. carpocapsae*, it is probably similar to the results presented here, with death resulting from bacteria that entered the host's coelom through penetration holes made by the nematodes. These nematodes are not being used or recommended to control aquatic insects at this time; however, if they are so in the future, then their effect on young tadpoles should be taken into consideration.

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Identification and electroantennographic activity of sex-specific geranyl esters in an abdominal gland of female *Agriotes obscurus* (L.) and *A. lineatus* (L.) (Coleoptera, Elateridae)[1]

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Summary. Both geranyl hexanoate and geranyl octanoate were identified by GC/MS as the major volatiles in a hitherto uncharacterized abdominal gland in females in *Agriotes obscurus*. Only geranyl octanoate was found in *A. lineatus*. In EAG tests performed on *A. obscurus* males, geranyl butanoate and geranyl hexanoate elicited the strongest antennal responses.
Key words. Electroantennogram; GC/MS; geraniol; geranyl hexanoate; geranyl octanoate; opalescent gland; nerol.

The click beetles *Agriotes lineatus* and *A. obscurus* are widely distributed in Europe[4], with large populations typically in fallow fields. During the larval stage, which may extend over five years, these species are saprophagous[4,5], or feed on roots; as adults, they are thought to feed on rye and wheat, particularly the pollen[5]. Click beetle larvae (commonly called wireworms) can reach numbers high enough to cause economic damage, and many species, including *A. lineatus* and *A. obscurus*, are important pest insects. Control of elaterids has received considerable attention[6], and one biological control approach consists of capturing the males by luring them to the odor of live females[7]. The odor signals of elaterids are not well known, although sex attractants have been reported for several species (see review by Jacobson[8]). In *Agriotes*, evidence suggests that male attractants are produced in the female abdomen[9–11], but the precise site has not been established. To determine both the source and chemical identity of these volatile substances released by female *A. lineatus* and *A. obscurus*, extracts of isolated abdominal glands and intact abdomina were compared by GC/MS. The physiological response of males and females to individual components was evaluated by electroantennography (EAG)[12].

Material and methods. Chemical analyses. Seven *Agriotes lineatus* (Linnaeus, 1767) and 21 *A. obscurus* (Linnaeus, 1758) females were collected in May–June, at the peak of their activity season[13], at the Ecological Research Station on Öland, Sweden. Intact abdomina, isolated abdominal accessory glands[14] and hitherto undescribed (see Results) abdominal opalescent glands from single insects were extracted separately in redestilled hexane (Merck; 0.1 ml for glands, 1 ml for abdomina). For comparison, intact abdomina of 5 *A. lineatus* and 12 *A. obscurus* males were similarly extracted. Each extract was analyzed by GC or GC/MS.

Separations of volatile constituents were carried out on an HP 5880 gas chromatograph using a Superox FA 30 m fused-silica capillary column (i.d. 0.25 mm), temperature programmed at 50 °C for 4 min, followed by 4 °C/min to 200–220 °C. Identifications of components were made using an LKB 2091 mass spectrometer combined with a PYE gas chromatograph, equipped with a WG11 50-m glass capillary column (i.d. 0.25 mm); mass spectra and retention times were compared with those of authentic samples and references. Mass spectra and voucher beetle specimens are deposited at the Ecological Research Station.

Electrophysiological studies. EAG tests were performed on 57 *A. obscurus* and 2 *A. lineatus* males, 26 *A. obscurus* and 1 *A. lineatus* females. Two procedures were followed in the preparation of test samples: 1) most substances were diluted in redistilled hexane; 10 µl samples were then pipetted onto pieces (size found not to be critical) of Whatman No. 1 filter paper, which were placed in vials within disposable plastic syringes; and 2) a few substances were diluted in paraffin (Merck) and put directly into vials within syringes. After equilibrating the gas/liquid phases for a minimum of 5 min, 7.3 ml were injected with a hydraulic piston for 1.0 s into a steady air flow (4.0 l/min) aimed towards the antennae 10 mm away. Seventeen different substances (fig. 3) were tested at 1-min intervals, and in duplicate on the majority of the insects. EAGs were amplified with a purpose-built amplifier (Murphy Developments, Hilversum, The Netherlands, type CD 83-1 b), which also displayed the initial fast DC deflections and was equipped with an automatic base-line drift compensator.

Depolarization data, expressed in mV, were treated according to van der Pers[15]. Several error factors were minimized by a step-wise procedure. These factors include: 1) the decreasing sensitivity of the antenna (accounted for by presenting a standard as every third stimulus and evaluating responses to test substances in relation to the standard); 2) small differences between test runs, i.e. setup and animal conditions (accounted for by expressing each relative value above as a fraction of the sum of relative values for each animal). Evaporation rates (molecules/s) of each test substance were computed volumetrically according to a method to be described in detail elsewhere[16].

Significant differences in responses were determined using the Wilcoxon rank sum test ($p < 0.05$).