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Research Note

Intra- and Interspecific Chemoattraction in *Echinostoma caproni* and *E. trivolvis* Adults In Vitro

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ABSTRACT: Intra- and interspecific chemoattraction was studied in 14-day-old *Echinostoma caproni* and *E. trivolvis* from domestic chicks. Experiments were carried out at 38.5 ± 1°C in petri dishes with an agar substratum overlaid with Locke's solution. *Echinostoma trivolvis* exhibited significantly greater intraspecific attraction than *E. caproni*; this attraction was significantly greater than the interspecific attraction.

KEY WORDS: *Echinostoma trivolvis, Echinostoma caproni,* attraction, pairing, intraspecific, interspecific, in vitro.

In accord with the recent review of Christensen et al. (1988), this study uses the names *Echinostoma caproni* and *Echinostoma trivolvis* for 2 related species of 37-collar-spined echinostomes, previously referred to as *E. liei* and *E. revolutum,* respectively.

Larval and adult stages of closely related 37-collar-spined echinostomes are morphologically quite similar, yet differ physiologically. Thus, Fried and Emili (1988) noted only subtle morphologic differences between the metacercarial cysts of *E. trivolvis* and *E. caproni,* but more obvious specific differences in both the percent and rate of excystation of cysts in vitro.

To document further physiologic differences between these 2 closely related species, we examined behavior and pairing patterns of the echinostomes in vitro. Although numerous studies on the in vitro pairing tendency of *E. trivolvis* are available (Fried, 1986; Haseeb and Fried, 1988), similar studies on *E. caproni* are not; interspecific pairing in echinostomes has not been demonstrated. The present study compares intra- and interspecific pairing between *E. caproni* and *E. trivolvis* adults in vitro.

Echinostoma trivolvis and *E. caproni* were grown for 14 days in domestic chicks (Fried and Weaver, 1969; Fried et al., 1988). Worms were quickly removed from the ileum at necropsy, washed in several changes of Locke's solution, and maintained individually in petri dishes containing 10 ml Locke's solution for about 0.5 hr prior to use.

The bioassay consisted of a petri dish (6 cm diameter) with a nutrient agar substratum and a 10-ml Locke's overlay (Fried and Roberts, 1972). Two worms were placed 20 mm apart in each dish and incubated at 38.5 ± 1°C under subdued light. The distances between worm centers were measured in millimeters at 15-min intervals for 90 min without removing the worms from the incubator. All worms were alive at the end of the experiments. Observations were based on 16 different pairs of worms for each combination (Fig. 1). The unmodified data were compared using a 2-tailed Kolmogorov–Smirnov test (TRUE EPISTAT®, Epistat Services, Richardson, Texas). The percent attraction was determined by the

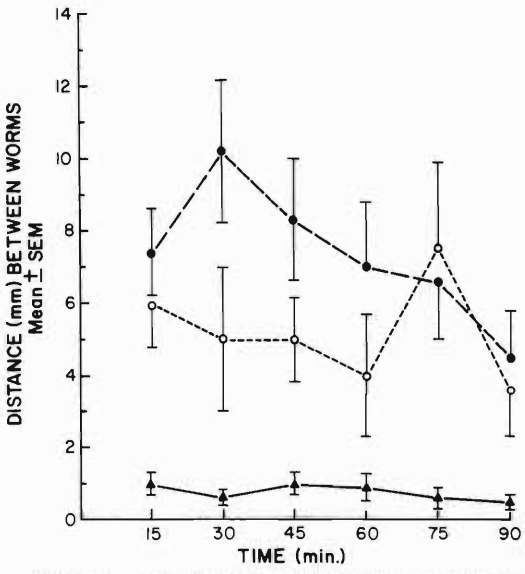

Figure 1. The distance (mm) between worm pairs is shown as mean ± SEM. Open circles, *Echinostoma caproni* vs. *E. caproni*; triangles, *E. trivolvis* vs. *E. trivolvis*; closed circles, *E. caproni* vs. *E. trivolvis*.

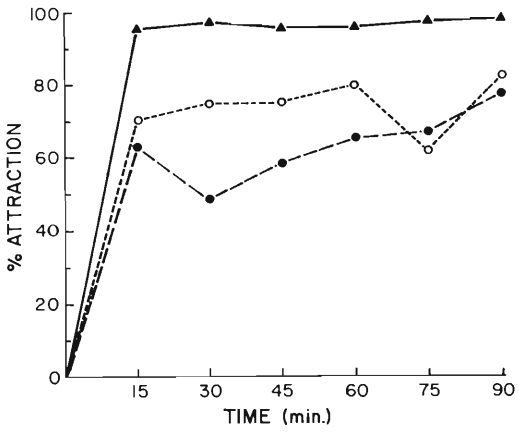

Figure 2. Intra- and interspecific attraction in *Echinostoma caproni* and *E. trivolvis* is shown as percent attraction, which was calculated by the formula: ([20 − D]/20) × 100, where 20 is the starting distance and D is the distance between a worm pair at an observation time. Open circles, *E. caproni* vs. *E. caproni*; triangles, *E. trivolvis* vs. *E. trivolvis*; closed circles, *E. caproni* vs. *E. trivolvis*.

formula: ([20 − D]/20) × 100, where 20 represents the initial distance between worms and D is the actual distance between a worm pair at a particular time point. Thus, by this formula 0% attraction would indicate that the worms remained at their original distance (20 mm) or moved further apart and 100% attraction would indicate that a worm pair was in contact.

The distance between worms averaged about 1 mm for *E. trivolvis*, 5 mm for *E. caproni*, and 8 mm for *E. trivolvis* vs. *E. caproni*. The mean distances at each time between worms are shown in Figure 1. Contact pairing (worms in physical contact along any 2 surfaces) was observed 60% of the time in *E. trivolvis*, 25% of the time in *E. caproni*, and only 9% of the time in the interspecific trials.

Intraspecific attraction of *E. trivolvis* was significantly greater ($P < 0.05$) than that of *E. caproni* at all time points except 30 and 60 min. The intraspecific attraction of *E. trivolvis* was significantly greater ($P < 0.05$) than interspecific attraction at all time points except at 90 min. The intraspecific attraction of *E. caproni* was significantly greater ($P < 0.05$) than interspecific attraction at 30 and 60 min. The percent attraction in the intraspecific and interspecific studies is shown in Figure 2.

The results of these experiments clearly show that *E. trivolvis* and *E. caproni* each has its own in vitro pairing pattern and that the interspecific pattern is yet different from either intraspecific pattern. In a previous study, interspecific pairing between *Zygocotyle lunata* and *E. trivolvis* had a pattern identical to the intraspecific pattern of *Z. lunata* (Fried and Wilson, 1981). Differences in the results between these 2 studies are not clear at this time. Moreover, the significance of in vitro pairing is not well understood; protrusion of cirri was never seen in the present study, suggesting that this pairing is not related to cross-copulation.

Lipophilic factors, particularly sterols, are presumably responsible for mediating intraspecific pairing of *E. trivolvis* (Fried et al., 1980; Fried, 1986). It is not known if a similar mechanism exists for intraspecific pairing in *E. caproni* or for interspecific pairing between *E. trivolvis* and *E. caproni*.

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