Mating behaviour of *Echinostoma trivolvis* and *E. paraensei* in concurrent infections in hamsters

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Abstract

Young adults of *Echinostoma trivolvis* and *E. paraensei* were recovered from hamsters previously infected with metacercarial cysts. Some worms of each species were exposed for 1 h to 3-H-tyrosine to label sperm and transplanted singly to uninfected hamsters with several unlabelled worms of the same or opposite species or both species. After 5 days, recovered worms were processed for paraffin sectioning and autoradiography. The resulting slides were observed for the location of radioactive sperm in the seminal receptacles of donor (labelled) and recipient (unlabelled) worms. When *E. trivolvis* was the donor with the recipient *E. paraensei*, self-insemination took place, but only one interspecies mating occurred out of 72 possible recipient worms. When *E. paraensei* served as the donor, self-insemination again occurred, but no cross-insemination was observed among the 59 *E. trivolvis* recipient worms. When single donor worms had a choice of either species of recipient worms, no interspecies mating took place, but both self- and cross-insemination occurred in the normal, unrestricted behaviour found in single species mating studies. Rates of both self- and cross-insemination were higher in concurrent infections of both recipient species than in single species mating studies. After transplant, both species localized in their natural habitat within the small intestine, with 1/3 overlapping in the duodenum, making interspecies mating a possibility. The correlation between mating and electrophoretic studies on the genetic relationship between 37-collar-spined echinostomes is discussed.

Introduction

The mating patterns of several closely related 37-collar-spined echinostome species have recently been studied. In each case, mating patterns for *Echinostoma caproni*, *E. trivolvis* and *E. paraensei* were found to be unrestricted in that they self-inseminated when isolated and both self and cross-inseminated in groups (Nollen, 1990, 1993, 1996a). These three species have also been recognized as being genetically closely related but definitely separate species by electrophoretic (Sloss *et al*., 1995) and DNA (Morgan & Blair, 1995) analysis.

Two studies have investigated possible mating barriers in concurrent infections between these species. In mice infected concurrently with *E. caproni* and *E. paraensei*, Nollen (1996b) found no cross-insemination between these species indicating definite mating barriers. A second study of mating between *E. caproni* and *E. trivolvis* in hamsters showed that interspecies mating took place at a low rate when *E. caproni* was the sperm donor (Nollen, 1997). No interspecies mating was found when *E. trivolvis* was the sperm donor.

The final mating study between these three species is reported herein, namely that between *E. trivolvis* and *E. paraensei*. Since Iorio *et al*. (1991) reported *E. trivolvis* adults grew poorly in mice but thrived in hamsters and *E. paraensei* can be maintained quite well in hamsters, the latter animals were used for concurrent infections with these species.

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Materials and methods

Syrian golden hamsters (Harlan-Sprague-Dawley), 5 to 6 weeks old, were infected with 60 metacercarial cysts of either *Echinostoma paraensei* or *E. trivolvis*. The *E. paraensei* strain was obtained from Dr E.S. Loker, University of New Mexico, Albuquerque, New Mexico, USA and maintained here in the laboratory. When large numbers of cysts were needed, they were supplied by Dr E.S. Loker or Lynn Hertel. Cysts of *E. trivolvis*, collected from naturally infected snails, were obtained from Dr B. Fried, Lafayette College, Easton, Pennsylvania, USA. After 10 days of growth, adults of both species were recovered.

The method of study was the same as used in previous mating studies on echinostome adults. Sperm were labelled in the donor worm with 3-H-tyrosine and then followed by autoradiography on sectioned worms for their distribution in adults of both species. Labelled sperm observed in the seminal receptacles of the donor adult indicated self-insemination, while labelled sperm in the seminal receptacles of unlabelled recipient worms indicated cross-insemination. Single labelled worms of both species were transplanted either with unlabelled adults of the opposite species or with both species to determine mating patterns between the species. Details of exposure to 3-H-tyrosine, transplant, and autoradiography procedures are given in Nollen (1996b).

Results

Adults of *E. paraensei* were recovered from their natural habitat in the small intestine of hamsters, the first few centimetres of the duodenum. *Echinostoma trivolvis* adults, on the other hand, were found scattered throughout the duodenum and upper jejunem. Contact between the two species was possible in concurrent infections since 69 *E. trivolvis* adults of the total 207 (33%) recovered were within 1 cm of *E. paraensei* adults in the duodenum.

Worm recovery and mating data are given in table 1. When *E. trivolvis* served as the sperm donor (labelled worms), three of nine adults self-inseminated and only one cross-inseminated out of a possible 72 unlabelled *E. paraensei*. When *E. paraensei* served as the sperm donor, four of ten adults self-inseminated but none cross-inseminated with 59 possible unlabelled *E. trivolvis* adults.

Data from a second series of experiments, where one labelled worm of one of the species was transplanted with

Table 1. Insemination in concurrent infections of *Echinostoma trivolvis* (ET) and *E. paraensei* (EP) labelled with tritiated tyrosine and returned to uninfected hamsters with unlabelled worms.

Number of worms/experiment					Insemination		
Labelled		Unlabelled		Number of experiments		Cross	
EP	ET	EP	ET		Self	EP	ET
	1	5		1	0	1	
	1	7		2	1	0	
	1	8		2	1	0	
	1	9		3	1	0	
	1	10		1	0	0	
Totals		72		9	3	1	
Percentages					33 (3/9)	1 (1/72)	
1			3	2	0		0
1			4	4	2		0
1			5	1	1		0
1			10	1	0		0
1			11	2	1		0
Total			59	10	4		0
Percentages					40 (4/10)		0 (0/59)
	1	5	5	1	1	0	0
	1	7	2	1	1	0	1
	1	10	2	2	1	0	3
	1	11	2	1	1	0	0
Totals		43	19	5	4	0/43	4
Percentages					80 (4/5)		21 (4/19)
1		5	3	1	1	3	0
1		6	3	1	1	3	0
1		7	4	1	1	4	0
1		7	3	1	0	4	0
1		12	4	1	1	4	0
Totals		35	17	5	4	18	0/17
Percentages					80 (4/5)	51 (18/35)	

(Number of adults inseminated/total adults).

unlabelled adults of both species, are also given in table 1. Here, when *E. trivolvis* was the sperm donor, but self- (4/5) and cross-insemination (4/19) were observed with worms of the same species. None of the 43 unlabelled adults of *E. paraensei* showed labelled sperm and no mating was detected.

The same pattern was observed when *E. paraensei* served as the sperm donor species. In this case four of the five labelled worms carried out self-insemination and these five sperm donors also cross-inseminated with 18 of 35 possible unlabelled adults of *E. paraensei*. No evidence for cross-insemination with the 17 unlabelled *E. trivolvis* adults was found.

Data in table 2 compare the percentages of self- and cross-insemination from previous reports of interspecies mating with results found in this study. For *E. paraensei*, self-insemination rates in single species studies and with *E. trivolvis* present compare favourably (44% (4/9) vs. 40% (4/10)). However, when both unlabelled *E. paraensei* and *E. trivolvis* were present with *E. paraensei* as sperm donor, the percentage of self-insemination was 80% (4/5).

When *E. trivolvis* was the sperm donor, self-insemination rates were again much higher in transplants with both recipient species present. Here 80% (4/5) self-insemination was observed but 55% (6/11) in a single species study and 33% (3/9) when *E. paraensei* was the recipient species. More trials would give a better picture of the significance of these data.

Cross-insemination rates were much lower in single species studies with *E. paraensei* (21% (8/39)) than when unlabelled *E. trivolvis* were present (51% (18/35)). A similar picture was found, although not as great, when *E. trivolvis* was the donor species (14% (5/35)) in a single species study than when unlabelled *E. paraensei* were present (21% (4/19)).

Discussion

Recovery sites for *E. paraensei* in the duodenum and *E. trivolvis* in the duodenum and jejunem were consistent with previous studies on concurrent infections of echinostomes (Nollen, 1996b, 1997). Since 1/3 of the *E. trivolvis* were within 1 cm of *E. paraensei*, interspecies mating was a possibility. However, results of these transplant studies showed a very low rate of less than 1% (1/72) cross-insemination between donor *E. trivolvis* and recipient *E. paraensei*. The reciprocal cross with *E. paraensei* as the donor and *E. trivolvis* as the recipient indicated no interspecies mating (0/59).

When both species were sperm donors in the presence of other species, they carried on their normal unrestricted mating behaviour exhibited in single species transplants (Nollen, 1996a, 1997). When the donor species was transplanted with the opposite species it always self-inseminated and at a rate similar to that found in single species studies. When in the presence of both recipient species, self- and cross-insemination took place by the donor species at a higher rate than in single species studies. In both instances the donor species carried on its normal, unrestricted mating pattern in spite of the opposite species being present. This same behaviour was found in other concurrent transplant studies between *E. caproni* and *E. paraensei* (Nollen, 1996b) and *E. caproni* and *E. trivolvis* (Nollen, 1997).

In the previous study, Nollen (1996b) showed that neither *E. paraensei* nor *E. caproni*, when used as sperm donors, would carry out interspecies mating. A similar study utilizing concurrent infections of *E. caproni* and *E. trivolvis* showed a moderate rate of intraspecies mating (13% (5/39) when *E. caproni* was the donor species, but not when *E. trivolvis* were labelled) (Nollen, 1997).

Strength or weaknesses of mating barriers should indicate species relationships if species are defined as interbreeding populations. In these studies of concurrent infections of echinostome species (Nollen, 1996b, 1997), *E. paraensei* never mated with *E. caproni* or *E. trivolvis*. On the other hand, *E. caproni* would mate with *E. trivolvis* but not *E. paraensei*, while *E. trivolvis* would mate with *E. paraensei* but not *E. caproni*. Thus, according to these mating barrier studies, *E. paraensei* was more distant from both *E. caproni* and *E. trivolvis* than the latter species were to each other. A similar genetic relationship was found by electrophoresis of isozymes (Sloss *et al.*, 1995) between these three echinostome species, with *E. paraensei* being more distant from *E. caproni* and *E. trivolvis* than they were to each other.

It is unlikely that these three species will ever be found in overlapping natural habitats and thus capable of hybridization. *Echinostoma caproni* is of African origin being first described by Richard (1964) from snails collected in Madagascar, while *E. trivolvis* (formerly *E. revolutum*) has been collected throughout the North American continent. *Echinostoma paraensei* was first described from snails collected in Brazil (Lie & Basch, 1967) and also recently reported from Australia in the cercarial stage analysed for mitochondrial ND1 sequences by Morgan & Blair (1998). Mating studies then not only reveal mating patterns in these different species but also may give some indication of genetic relationships through the strengths and weaknesses of mating barriers found in these artificial concurrent infection studies.

Table 2. Percent insemination of *Echinostoma paraensei* (EP) and *E. trivolvis* (ET) in single species and concurrent infections.

Labelled		Unlabelled		Percent insemination		
					Cross	
EP	ET	EP	ET	Self	EP	ET
X		X		75 (6/8)[1]	21 (8/39)[1]	
X				44 (4/9)[1]		
	X		X	58 (7/12)[2]		14 (5/35)[2]
	X			55 (6/11)[2]		
X			X	40 (4/10)		0 (0/59)
	X	X		33 (3/9)	1 (1/72)	
X		X	X	80 (4/5)	51 (18/35)	0 (0/17)
	X	X	X	80 (4/5)	0 (0/43)	21 (4/19)

(Number adults inseminated/total adults)
[1] Nollen, 1996a.
[2] Nollen, 1993.

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