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Supporting experimental evidence of host specificity among southern African polystomes (Polystomatidae: Monogenea)

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Abstract Although monogeneans of anurans are generally regarded as host-specific, there is a lack of conclusive experimental evidence. Infection and cross-infection experiments were conducted with oncomiracidia of *Polystoma australis* and *P. marmorati*. In a series of experiments, oncomiracidia were given a choice between natural and substitute host tadpoles. Oncomiracidia of *P. australis* became established in substitute hosts but showed a preference for the natural host, whereas the oncomiracidia of *P. marmorati* showed a strong and statistically significant preference for the natural host. The results indicated that although the oncomiracidia of southern African polystomes showed a strong preference for their natural hosts, not all parasites exercised the same degree of host specificity.

Introduction

Monogeneans are primarily parasites of poikilotherm vertebrates. The vast majority are parasitic on freshwater and marine fishes, establishing themselves on the body surface or gills or even in the intestines of their hosts (Prudhoe and Bray, 1982). As adults, monogeneans of amphibians are found in the urinary bladder, mouth, stomach, or intestine or on the body surface of post metamorphic frogs, tadpoles, and salamanders (Prudhoe and Bray 1982).

Monogeneans of fish are known to be remarkably host-specific. Although monogeneans of amphibians appear to be highly host-specific, experimental evidence is insufficient and several researchers have emphasized the need for experimental studies on the host specificity of Monogenea (e.g., Hargis 1953, 1957; Llewellyn 1957; Tinsley 1974, 1978; Lambert 1981; Kok and Du Preez 1987).

Combes (1966, 1968) was the first investigator to point out the high degree of host specificity among European polystomes on the basis of experimental studies. He conducted experiments with *Polystoma integerrimum* in *Rana temporaria*, *P. pelobatis* in *Pelobatis cultripes*, and *P. gallieni* in *Hyla meridionalis*. Attempts to transfer these species to tadpoles of the wrong host were unsuccessful. At that stage, no attempt had been made to study host specificity among polystomes in Africa and in 1974, Tinsley stated that "the essential experimental studies of host specificity are at present lacking...." Shortly after this, Bourgat and Salami-Cadoux (1976) reported on the findings of the first experimental studies on host specificity involving African polystomes. They showed that *P. africanum*, which occurs naturally in *Bufo regularis*, could become established in tadpoles of the substitute hosts *R. galamensis* and *Hylarana albolabris* but that the parasites survived for only a few days, thus pointing strongly toward strict host specificity in this case. Kok and Du Preez (1987) conducted some infection and cross-infection experiments with *P. australis*. These were the first attempts at experimental investigation of the host specificity of polystomes from Africa since the work of Bourgat and Salami-Cadoux (1976). The results of that study emphasized the complexity of host-parasite associations within the African anuran polystomes and the need for further experimental studies on host specificity. On the basis of the verdicts of previous results and our own observations, we hypothesized that under experimental conditions, polystomes would establish better in natural hosts than

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in substitute hosts but that not all polystomes would exercise the same degree of host specificity. A series of experimental infections with infective stages of southern African polystomes and tadpoles of their natural and substitute hosts were conducted to test our hypothesis.

Materials and methods

Experimental infection

Oncomiracidia of *Polystoma australis* and *P. marmorati* were used in infection and cross-infection experiments with 11 species of tadpoles (see Tables 1, 2). Tadpoles available for this study were those of the natural hosts of the parasites used in this study, some from anurans known to harbor other species of polystomes and some from anurans not presently known to harbor polystomatid parasites.

Parasite eggs were obtained from infected adult hosts housed individually in 600-ml jars containing 50 ml of dechlorinated tap water. Eggs were collected by sieving of the water from the jars through plankton netting with a mesh size of 112 µm. Eggs were then kept in 25 ml of dechlorinated tap water in small petri dishes and hatched after 10–12 days.

Experiments were conducted as follows. Ten active oncomiracidia were placed in each of three 100-ml plastic containers (A, B, C), in each case with 25 ml of dechlorinated tap water. Two tadpoles of the natural host species were transferred to container A; two tadpoles of a substitute host species, to container C; and one tadpole each of both the natural and the substitute host species, to container B. Tadpoles of similar size were used as far as possible. Five repetitions were used in each trial when the availability of both parasite and host material allowed it. After 24 h, tadpoles were transferred to larger containers. Tadpoles were dissected after 5 days to determine the success of parasite establishment. Mean numbers and the location of parasites were registered and tadpoles were identified as follows: Nh in A, natural host tadpoles in container A; Nh in B, natural host tadpole in container B; Sh in B, substitute host tadpole in container B; and Sh in C, substitute host tadpoles in container C.

Statistical analysis

A Kruskal-Wallis test was used to test for differences among all samples, followed by the Games and Howell test to determine specific significant differences between pairs of samples. In cases where parasites did not become established, variances were equal to nil and the Games and Howell procedure could not be followed. In these cases the Kruskal-Wallis test was used to indicate differences between two samples (Sokal and Rohlf 1981). Probability values (*P*) of less than 0.05 were regarded as significant.

Results

Polystoma australis

When given a choice between tadpoles of its natural host *Semnodactylus wealii* and a substitute host, *Bufo gutturalis* (container B), or simply the opportunity to infect one host without choice (container A or C), *P. australis* became established in both the natural host tadpoles and the substitute host tadpoles with equal success (Table 1). When substitute host tadpoles of *Natalobatrachus bonebergi* were used, oncomiracidia established equally well in both natural and substitute host tadpoles if no choice was given (container A or C). When given a choice (container B), more oncomiracidia established in the natural host tadpoles (Table 1), but the difference was not significant. When substitute host tadpoles of *Hyperolius marmoratus* were used, more oncomiracidia became established in natural host tadpoles and hardly any oncomiracidia established in the substitute host tadpoles (Table 1). The difference was significant.

P. marmorati

In all experimental infections conducted with oncomiracidia of *P. marmorati* the pattern of establishment in the natural host *H. marmoratus* (container A) was very similar. Oncomiracidia readily became established, with numbers averaging between 2.5 and 3.9 oncomiracidia/host tadpole (Table 2). When given a choice between the natural host and a substitute host tadpole (container B), more tadpoles became established in the natural host tadpoles, and for all but *Rana angolensis*

Table 1 List of substitute hosts used in experiments with *Polystoma australis*, numbers of tadpoles used in each experiment, and mean numbers of *P. australis* oncomiracidia established in natural host tadpoles (*Semnodactylus wealii*, Nh in A and Nh in B) as opposed to tadpoles of substitute hosts (Sh in B and Sh in C). Different lower-case alphabetical letters (a, b) indicate statistically significant differences

Hosts	Sample size (tadpoles)				Mean number of parasites			
	Nh in A	Nh in B	Sh in B	Sh in C	Nh in A	Nh in B	Sh in B	Sh in C
S. wealii	10	5			1.9	2.2		
Bufo gutturalis			5	10			1.8	1.5
					a	a	a	a
S. wealii	10	5			2.8	3.8		
Natalobatrachus bonebergi			5	10			1.0	2.1
					a	a	a	a
S. wealii	10	5			3.0	4.6		
Hyperolius marmoratus			5	10			0.2	0
					a	a	b	b

Table 2 List of substitute hosts used in experiments with *P. marmorati*, numbers of tadpoles used in each experiment, and mean numbers of *P. marmorati* oncomiracidia established in natural host tadpoles (*Hyperolius marmoratus*, Nh in A and Nh in B) as opposed to tadpoles of substitute hosts (Sh in B and Sh in C). Different lower-case alphabetical letters (a, b) indicate statistically significant differences

Hosts	Sample size (tadpoles)				Mean number of parasites			
	Nh in A	Nh in B	Sh in B	Sh in C	Nh in A	Nh in B	Sh in B	Sh in C
H. marmoratus	12	7			3.9	4.1		
Xenopus laevis			7	12			0	0
					a	a	b	b
H. marmoratus	10	5			3.1	1.6		
Bufo rangeri			5	10			0	0
					a	a	b	b
H. marmoratus	8	4			3.6	5.0		
Rana angolensis			4	8			2.5	1.4
					a	a	ab	b
H. marmoratus	9	4			3.6	4.8		
Cacosternum boettgeri			4	9			1.0	1.9
					a	a	b	ab
H. marmoratus	10	5			2.6	3.4		
Strongylopus fasciatus			5	10			0.2	0
					a	a	b	b
H. marmoratus	28	14			3.4	4.4		
Natalobatrachus bonebergi			14	28			0.6	0.9
					a	a	b	b
H. marmoratus	12	7			3.0	3.0		
Semnodactylus wealii			7	12			0.1	0.3
					a	a	b	b
H. marmoratus	10	5			3.1	3.6		
Kassina senegalensis			5	10			0.2	0.4
					a	a	b	b
H. marmoratus	12	5			2.5	1.8		
H. pusillus			5	12			0	0
					a	a	b	b

the difference was statistically significant. No oncomiracidia of *P. marmorati* became established in tadpoles of *Xenopus laevis*, *B. rangeri*, *H. pusillus* (Table 2). When substitute host tadpoles were used (container C) the same phenomenon was observed for all species. Very few oncomiracidia became established, with numbers averaging between 0 and 1.9 oncomiracidia/host tadpole (Table 2). As compared with the pattern of establishment in natural hosts (container A) the difference was significant for all species but *Cacosternum boettgeri* (Table 2).

Discussion

The results of the present study demonstrate that oncomiracidea of both *Polystoma australis* and *P. marmorati* show a preference for their natural host tadpoles. Although oncomiracidia of *P. australis* showed a preference for the natural host, a substantial number nonetheless became established in substitute hosts. Infection and cross-infection experiments with *P. marmorati*, on the other hand, revealed a significant preference for the natural host, and hardly any parasites became established in the substitute hosts.

All of the results as described above were obtained from infections induced under laboratory conditions. Tadpoles were exposed to abnormally high numbers of oncomiracidia in small volumes of water, thus forcing repeated contact between oncomiracidia and tadpoles. Du Preez et al. (1997) have reported that host specificity among southern African polystomes is determined by host recognition during first contact between the parasite and the potential host tadpole. They found that if the oncomiracidium would make contact with a substitute tadpole, the parasite would not normally remain on the tadpole.

Under natural conditions in the eastern Free State, *P. australis* infects two anuran hosts, namely, *Semnodactylus wealii* and *Kassina senegalensis*. Kok and Du

Preez (1987) confirmed this during infections induced under experimental conditions. *S. wealii* apparently supports the larger part of the natural *P. australis* supra population, but at localities where the two hosts occur together, *P. australis* become established in both host species with equal success (Du Preez, unpublished observations).

Under experimental conditions, Kok and Du Preez (1987) successfully established *P. australis* in pre- as well as postmetamorphic *Natalobatrachus bonebergi* substitute hosts. In all, 2 groups of 5 and 4 *N. bonebergi* tadpoles were exposed to 5 and 20 *P. australis* oncomiracidia/tadpole, respectively. In the first group the tadpoles varied in age between 15 and 30 days and were premetamorphic. Three tadpoles from this group became infected, but the neotenic parasites were poorly developed. In one tadpole a parasite started to produce eggs after 16 days, but at a very slow rate as compared with the neotenic parasite in natural host tadpoles (Kok and Du Preez 1987). In the second group of four tadpoles, all within days of the onset of metamorphosis, predestined bladder parasites became established and bladder parasites were recovered from two of the three surviving postmetamorphic frogs. Three parasites were recovered from one frog at 8 days after metamorphosis and one parasite was recovered from the other frog on day 28, when the host died.

The pattern that emerges is therefore one of host specificity for both *P. australis* and *P. marmorati*, expressed both in the behavior of the infective stages (Du Preez et al. 1997) and in their establishment in host tadpoles (present study). Host specificity is evidently stronger in *P. marmorati* than in *P. australis*, which can be deduced from experimental infections (Kok and Du Preez 1987; present study) and from the occurrence of *P. australis* in two host species under natural conditions.

Combes and Channing (1979) accepted a high degree of host specificity as the rule for all polystomes but stated that exceptions to this rule could occur, although this was considered to be unlikely. Murith (1982) claimed that substitute host species could support neither the development of predestined bladder parasites nor the establishment of parasites in the urinary bladder of postmetamorphic substitute hosts. The experimental establishment of *P. australis* in postmetamorphic *N. bonebergi* (see Kok and Du Preez 1987) and the natural establishment of *P. australis* in *K. senegalensis* contrast sharply with Murith's (1982) findings. Although the infestation of *N. bonebergi* was carried out under experimental conditions, one could argue that if this is possible under laboratory conditions, there is also the possibility that it can occur under natural conditions.

This phenomenon, i.e., that not all polystomes exercise the same degree of host specificity, could explain the recording of *P. aethiopiense* from three different host species, namely, *Rana angolensis*, *Bufo regularis*, and *Ptychadena mascareniensis* (cf. Tinsley 1974); *P. gabonensis* from three host species, namely, *R. albolabris* (cf. Euzet et al. 1966), *R. amnicola*, and *R. longipes* (cf. Murith et al. 1978); *P. grassei* from three host species, namely, *Leptopelis calcaratus* (cf. Euzet et al. 1966), L. hyloides (cf. Maeder 1973), and *L. occelatus* (cf. Murith et al. 1978); *P. llewellyni* from two host species, *Afrixalus leptosomus* (cf. Euzet et al. 1974) and *A. fulvovittatus brevipalmatus* (cf. Murith et al. 1978); and *P. ragnari* from two hosts, *Phrynobatrachus alleri* (cf. Maeder et al. 1970) and *P. acraensis* (cf. Bourgat 1977). On the other hand, careful reexamination of these parasites and cross-infection experiments may reveal strict host specificity and demonstrate that some of the known polystomes represent more than one species.

As a conclusion to our studies we can state that the polystomes used in this study are host-specific, and we expect this to be the case for other polystome species as well. However, not all polystomes exercise the same degree of host specificity. A similar phenomenon has been observed for *Diplozoon gracile* (Monogenea; Diplozoidae). Le Brun et al. (1986) reported experimental infections with four species of *Diplozoon*. *D. nipponicum*, *D. paradoxum*, and *D. homoion* were found to be strictly host-specific, but *D. gracile* established and became mature on substitute hosts, indicating a lesser degree of specificity.

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References

Bourgat R (1977) Étude comparative des polystomes (Monogenès) de ranidés (Anoures) du sud Togo. Description de *Polystoma togoensis* n. sp. Bull Mus Natl Hist Nat Zool 312: 447–463

Bourgat R, Salami-Cadoux ML (1976) Recherches experimentales sur la spécificité parasitaire des polystomes de *Bufo regularis, Rana galamensis* et *Hylarana albolabris* au Togo. Rev Sci Med Biol Togo 1: 41–42

Combes C (1966) Recherches expérimentales sur la spécificité parasitaire des polystomes de *Rana temporaria* et de *Pelobates cultripes* (Cuv.). Bull Soc Zool Fr 91: 439–444

Combes C (1968) Biologie, écologie des cycles et biogéographie de digènes et monogènis d'amphibiens dans l'est des Pyrénées. Bull Mus Natl Hist Nat Zool 51: 1–195

Combes C, Channing A (1979) Polystomatidae (Monogenea) d'amphibiens d'Afrique du Sud: *Polystoma natalensis* n.sp., parasite de *Strongylopus grayii* (Smith 1849). Vie Milieu Ser C 28–29: 61–68

Du Preez LH, Kok DJ, Seaman MT (1997) Host recognition behaviour of polystome oncomiracidia (Polystomatidae: Monogenea) in contact with natural and substitute anuran hosts. J Afr Zool 111: 47–55

Euzet L, Combes C, Knoepffler L-PH (1966) Polystomes des amphibiens du Gabon. Biol Gabonica 2: 215–233

Euzet L, Bourgat R, Salami-Cadoux M-L (1974) *Polystoma galamensis* (Monogenea) parasite de *Rana galamensis* Duméril et Bibron, 1841, au Togo. Ann Parasitol Hum Comp 49: 63–68

Hargis WJ Jr (1953) Monogenetic trematodes of Westhampton Lake fishes. III. Part 2. A discussion of host-specificity. J Helminthol 20: 98–104

Hargis WJ Jr (1957) The host-specificity of monogenetic trematodes. Exp Parasitol 6: 610–625

Kok DJ, Du Preez LH (1987) *Polystoma australis* (Monogenea): life cycle studies in experimental and natural infections of normal and substitute hosts. J Zool 212: 235–243

Lambert A (1981) Sensors and effectors in the behaviour of oncomiracidia: ciliated epidermis and sensilla. Parasitology 82: 59–60

Le Brun N, Lambert A, Justine J-L (1986) Oncomiracidium, morphogenèse du hapteur et ultrastructure du spermatozoide de *Pseudodactylogyrus anguillae*. Ann Parasitol Hum Comp 61: 273–284

Llewellyn J (1957) Host-specificity in monogenetic trematodes. Bull Soc Neuchatel Sci Nat 32: 199–212

Maeder A-M (1973) Monogènes et trématodes parasites d'amphibiens en Côte d'Iviore. Rev Suisse Zool 80: 267–322

Maeder AM, Euzet L, Combes C (1970) Espèces nouvelles du genre *Polystoma* (Monogenea) en Afrique occidentale. Z Parasitenkd 35: 140–155

Murith D (1982) Etude in vivo de la nature des relations hôte-parasite dans le complexe amphibien-polystome (Monogenea). Rev Suisse Zool 89: 957–965

Murith D, Miremad-Gassmann M, Vaucher C (1978) Contribution à l'étude des polystomes d'amphibiens du Cameroun. Rev Suisse Zool 85: 681–698

Prudhoe S, Bray RA (1982) Platyhelminth parasites of the Amphibia. Oxford University, Oxford

Sokal RR, Rohlf FJ (1981) Biometry. Freeman, San Francisco

Tinsley RC (1974) Observations on *Polystoma africanum* Szidat with a review of the inter-relationships of *Polystoma* species in Africa. J Nat Hist 8: 355–367

Tinsley RC (1978) Oviposition, hatching and the oncomiracidium of *Eupolystoma anterorchis* (Monogenoidea). Parasitology 77: 121–132