Mutual exclusion of congeneric monogenean species in a space-limited habitat

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SUMMARY

Adults of the monogenean genus *Protopolystoma* infecting *Xenopus* species occur in an extremely space-limited habitat, the urinary bladder. *Xenopus wittei*, from a population in Rwanda naturally infected with *Protopolystoma fissilis* and *Protopolystoma simplicis*, were exposed to reinfection in captivity (for 1–3 months post-capture) and then monitored in the laboratory for up to 5 months in transmission-free conditions. The two parasites co-occurred in individual bladders less frequently than expected if they were dispersed randomly. Distribution of bladder infections was significantly non-independent ($n = 157$) and gravid worms of both species were never found in the same host. This pattern might be explained by interference competition between the parasites or by genetic differences in susceptibility within the host species, which is of allopolyploid origin. Other distributional data for sympatric polystomatid species pairs, including *P. fissilis* and *P. ramulosus*, show concurrent infections at frequencies consistent with random distributions (i.e. no evidence of interspecific competition or variability in species-specific susceptibility of the hosts). Interference between *P. fissilis* and *P. simplicis* (assuming host genetic factors are not involved) may therefore result from a mechanism specific to this species pair. Observations on infection turnover in captive hosts suggest that loss of adult worms may be related to the arrival of juveniles (of either species) in the urinary bladder. Ectopic infection of the host urinary ducts by adult and subadult *P. fissilis* was observed in some single-species infestations and may be density related. However, the use of an ectopic-site 'refugium' has never been observed in concurrent polystomatid infections.

Key words: *Protopolystoma*, host distribution, interference competition, *Xenopus*, allopolyploid host, ectopic infection.

INTRODUCTION

Adults of the polystomatid monogenean genus *Protopolystoma* occur in the urinary bladder of aquatic anurans (*Xenopus* species) in sub-Saharan Africa (Tinsley & Jackson, 1998*a*). These parasites have a direct life-cycle: eggs are passed to the exterior when host urine is voided and oncomiracidia subsequently invade the cloaca of new host individuals. Post-larval development takes place in the kidneys, with juvenile worms normally migrating, via the urinary ducts, to the urinary bladder where they mature (Tinsley & Owen, 1975). The site of adult infection presents a simple, contractile, enclosed habitat which is of small surface area in relation to parasite body size. Intraspecific competition is likely to be important in bladder-inhabiting polystomatids (Tinsley, 1993), and Combes (1983) attributed a negative distributional relationship between a polystomatid and a gorgoderid digenean infecting the urinary bladder of *Rana temporaria* populations to competitive effects. As very close proximity between individuals of different *Protopolystoma* species from *Xenopus* would be inevitable in concurrent infections (Jackson & Tinsley, 1998*a*), this host–parasite assemblage provides a system for study in which interspecific interactions between parasites might be particularly intense.

Most monogeneans occur on the body surface or gills of fishes, substrata which are more likely to provide spatial refuges in concurrent infections, due either to their relatively great surface area or structural complexity. Many cases of possible competitive interactions between parasite species have been reported (e.g. Dobson, 1985), although the general significance of competition for the distribution and evolution of parasitic organisms has been an issue of contention (e.g. Holmes, 1973; Price, 1980; Rohde, 1991). Amongst monogeneans, interspecific exclusion (by interference) has been suggested to explain temporal infection patterns in dactylogyrid gill parasites (Paperna, 1964; Buchmann, 1988). In these cases the interaction may depend on a host tissue response induced by one species. Rohde (1991), however, considered that there was little distributional evidence (within local host populations) for competition between marine gill monogeneans, which often occur at low density.

Urinary bladder-inhabiting polystomatids from anurans typically show a high degree of host specialization (e.g. Maeder, 1973; Tinsley, 1974; Bourgat, 1977; Murith, 1981; Vaucher, 1990; Jack-

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Table 1. Host distributions of co-occurring urinary bladder polystomatids from anurans

Host	Locality	Date/Authority	Species	Prevalence (%)	Mean intensity	Mean abundance	Concurrent infections		
							Observed	Expected	*n*
Xenopus wittei	Southern Rwanda	Nov. 1991	*Protopolystoma fissilis*	46	3·0 (2·1)	1·4 (2·1)	1†	2	41
			P. simplicis	12	1·6 (0·5)	0·2 (0·6)			
		Nov. 1992	*P. fissilis*	46	2·6 (1·8)	1·2 (1·8)	0	5	116
			P. simplicis	9	1·4 (0·7)	0·1 (0·4)			
*X. fraseri**	Eastern DRC	Nov. 1991	*P. fissilis*	26	3·4 (3·9)	0·9 (2·4)	3	2	47
			P. ramulosus	15	2·0 (1·2)	0·3 (0·8)			
Ptychadena porosissima	KwaZulu-Natal, RSA	Du Preez & Kok (1992)	*Metapolystoma porosissimae*	50	2·2	1·1	5	4	26
			Polystoma sodwanensis	31	1·1	0·6			

* Sample taken at 'Ebisha' locality of Tinsley & Jackson (1998*a*) and subject to same conditions as 1991 *X. wittei* specimens (see Materials and Methods section).
† Infrapopulation consisting of 1 adult *P. fissilis* and 1 juvenile *P. simplicis*.
Standard deviations in parentheses. (DRC, Democratic Republic of the Congo; RSA, Republic of South Africa.)

son & Tinsley, 1998*b*), and the presence of more than 1 species per host is rare. Bourgat & Murith (1980) reported 2 *Polystoma* species from the same population of *Ptychadena pumilio*, while Du Preez & Kok (1992) found concurrent infections with a *Polystoma* and *Metapolystoma* species in *Ptychadena porosissima*. Instances of concurrent infections with *Protopolystoma* taxa were reported by Tinsley & Jackson (1998*a*). *Protopolystoma fissilis* may co-occur alongside *Protopolystoma ramulosus* in *Xenopus fraseri*-like hosts in the Democratic Republic of Congo, and with *Protopolystoma simplicis* in *Xenopus wittei*-like hosts in Rwanda. The spatially limited microhabitat of these parasites could increase the potential for interspecific competition or reproductive interference (Jackson & Tinsley, 1998*a*) in concurrent infections.

The genus *Xenopus* contains a series of polyploid species, including the octoploid *X. wittei*, believed to have arisen by allopolyploidization (Tymowska, 1991; Kobel, 1996). These are derived from interspecific hybridization between distinct parental lineages. Such a mechanism of speciation may have led to parasite lineages previously specific to parental host taxa being brought into contact in hybrid host species (Tinsley & Jackson, 1998*a*, *b*). It is unknown whether speciation by allopolyploidization might also have consequences for intrapopulational variation in susceptibility to different parasite species.

This laboratory study investigates potential interactions and isolating factors between *P. fissilis* and *P. simplicis* by monitoring *X. wittei* from a population exposed to infection by both species. The distribution, within host samples, of these 2 parasites (and that of other co-occurring polystomatid species pairs) is examined for evidence of interspecific competition. Between-host variations in egg viability are also considered for evidence of reproductive interference between *P. fissilis* and *P. simplicis*.

MATERIALS AND METHODS

Hosts and parasites

Samples of octoploid clawed toads morphologically comparable to *X. wittei* Tinsley, Kobel & Fischberg were collected at Cyamudongo Forest, Cyangugu Prefecture, southern Rwanda by H.H.H. in November, 1991 and 1992 and transported to the UK within 1 (1991) and 3 (1992) months of capture. In the period before export they were maintained together (at ambient temperatures), in conditions which would have allowed parasite transmission. After arrival in the UK, all animals were maintained in a controlled temperature room (at 22 ± 1 °C) in dechlorinated tapwater, and fed twice weekly *ad libitum* on chopped ox-heart and tropical fish flake. When not being screened, groups of 20–30 toads

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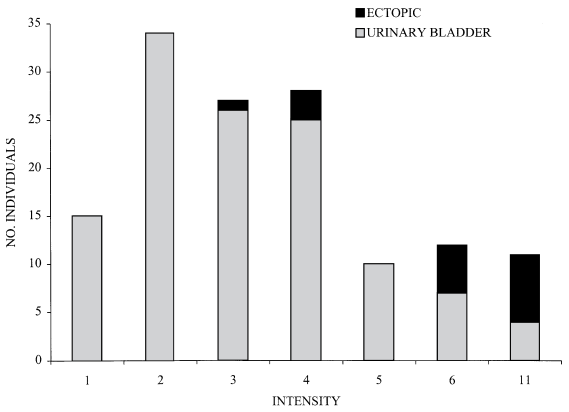

Fig. 1. Numbers of adult and subadult *Protopolystoma fissilis* occupying normal (host urinary bladder) and ectopic sites of infection (urinary ducts) at different infection intensities (*X. wittei*, 1992 sample).

were kept in large plastic tanks, approximately $1 \times 0{\cdot}6 \times 0{\cdot}6$ m. Tanks were cleaned and water changed 1–3 times each week: this precluded parasite transmission as *Protopolystoma* eggs take more than 20 days to hatch at 22 °C and are not adhesive (Tinsley & Owen, 1975). All toads were adult and showed limited variation in size: males measured 31–42 mm and females 35–49 mm snout–vent length. During the course of this study none of the males was in breeding condition, lacking nuptial gloves; most of the females dissected did not contain mature ova, although these were present in a minority. The 1991 *X. wittei* sample was dissected 1–5 months after capture. Fifteen toads from the 1992 sample were dissected shortly after arrival in the laboratory (3 months post-capture). Parasite egg production from the remaining sample was detected by water screening (Jackson & Tinsley, 1988; Jackson & Tinsley, 1998*c*). Toads were isolated in 2 l jars 2/3 full of dechlorinated water for 7–10 days. After this time sediment from each jar was concentrated by decantation and searched for eggs under a stereo-binocular microscope with fibre optic illumination. All toads were screened 3–4 months after capture and hosts with patent infections dissected after varying time-intervals (during which they were intermittently screened). Animals not showing parasite egg production at 3–4 months were re-screened 6–7 months after capture: specimens with patent infections were dissected. Remaining animals were still negative for parasite egg production when screened again at 8 months post-capture and are counted as zero infections in the following analyses. Infection statistics given below are based on parasites found in the urinary bladder and distal region of the urinary ducts. Parasite species were identified by discrete differences in large hamulus point length and gut morphology and by the presence or absence of lobing of the large hamulus roots, following Tinsley & Jackson (1998*a*).

Parasite eggs

Each batch of parasite eggs collected at water screening (see above) was transferred, by Pasteur pipette, to a 100 ml crystallizing dish 2/3 full of a commercial brand of natural spring water. Batches never exceeded 150 eggs. Dishes were fitted with a loose-fitting lid and kept in a controlled environment room at 22 ± 1 °C (12L: 12D photoperiod); water lost through evaporation was periodically replaced. After 20 days, eggs were examined at 2–3 day intervals, under a stereo-binocular microscope with fibre-optic illumination, and those which had embryonated (and contained a fully developed infective larva) were removed.

RESULTS

Distribution of infection

Forty-one *X. wittei* taken in November 1991 were dissected after up to 4 months in the laboratory under transmission-free conditions (i.e. 1–5 months post-capture). Twenty-three hosts harboured urinary bladder infections: 18 contained *P. fissilis* only, 4 contained *P. simplicis* only, and 1 was infected with both species (infrapopulation consisting of 1 adult *P. fissilis* and 1 juvenile *P. simplicis*).

One hundred and sixteen *X. wittei* were taken in November 1992, and monitored in the laboratory for parasite egg production 3–8 months post-capture (under transmission-free conditions). All toads showing patent infections in this sample were dissected. Fifty-three specimens (46 %) contained *P. fissilis* and 11 (9 %) *P. simplicis*: none was infected with both species at the time of dissection.

Several concurrent infections with *P. fissilis* and *P. simplicis* would have been expected if these species occurred randomly in the samples (see Table 1). Their distribution in the 1992 sample was significantly non-independent, as was the case when data from 1991 and 1992 were pooled (χ^2-tests, $P < 0{\cdot}005$).

There was no evidence that the pattern of infection was related to host sex. Distributions of both *P. fissilis* and *P. simplicis* amongst males and females were independent (χ^2-tests, $P < 0{\cdot}05$) (1991 and 1992 samples pooled) and worm burdens (either species) were not significantly different between infected male and female hosts (Mann–Whitney, $P > 0{\cdot}05$).

Parasite development

Tinsley & Owen (1975) found that *P. xenopodis* in *X. laevis* shows a pre-patent period of 3–4 months at 22 °C. In *P. fissilis* and *P. simplicis* from the 1992 *X.*

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wittei sample, the maturation of young worms and recruitment of juveniles to the urinary bladder population continued during months 4–7 in captivity (up to 4 months after the opportunity for transmission was removed). Of 48 toads which did not show evidence of parasite egg production after 3–4 months, 8 (17 %) had developed patent infections by 6–7 months. Juvenile parasites were found in the urinary bladder of toads examined up to 7 months post-capture (3/20, 1/8, 7/26 and 6/24 for months 4–7, respectively).

Infection turnover

Thirteen hosts from the 1992 sample lost patent infections prior to dissection (4–7 months after capture). In 6 of these (46 %) the original infection was found to have been replaced by juveniles of *P. fissilis* (4 cases, 31 %) or *P. simplicis* (2 cases, 15 %). Juvenile bladder worms were less frequent in toads which contained patent infections at dissection during the same time period ($n = 43$): 8 (19 %) harboured immature *P. fissilis* (7 cases, 16 %) or *P. simplicis* (1 case, 2 %). If the same per host probability of juvenile infection observed in the 'adult-infected' group, was applied to the group in which patent infections had been lost, then the overall probability (calculated from a binomial distribution) of finding 6 or more such infestations in a sample of 13 would be 0·02. The sequence of dissections may have allowed some juveniles in infected toads to mature prior to examination. However, there was no indication of a fall in the number of young specimens present from months 4 to 7 (see above). The high proportion of young worms in toads which had lost an infection was therefore unexpected.

The identity of a 'replaced' infection was determined in 1/6 cases: a large, adult *P. simplicis* was shed between 14 and 4 days before the recovery of a post-migratory *P. fissilis*. Over this period the infected toad had been voiding 9·3 parasite eggs/day (minimal estimate, assuming oviposition ceased 4 days before host dissection).

Ectopic infections

Observations of *Protopolystoma* spp. juveniles in their hosts' urinary ducts are extremely rare (unpublished data). This is presumably due to the rapidity of parasite migration between the kidneys and urinary bladder. However, 11 adult and 5 subadult *P. fissilis* specimens occurred in the distal urinary ducts of 5 *X. wittei* from the 1992 sample. These toads contained relatively large *P. fissilis* infrapopulations (including the maximum observed worm burden of 11) and represented 24 % of individuals containing 3 or more worms in the distal urinogenital system (see Fig. 1). In hosts with total infections of 11, 6, 6, 4, and 3 parasites, 7, 1, 4, 3, and 1 individuals, respectively, occurred in the urinary ducts. The distribution of worms in ectopic sites was significantly non-independent between 'low' (1–2 parasites/toad) and 'high' (> 3) density infections (expected frequency of ectopic infection site in 'low density' group = 5, observed = 0; χ^2-test, $P < 0{\cdot}005$).

Egg viability

Percentage embryonation was 99 % in 418 *P. simplicis* eggs from 5 hosts and 97 % in 2657 *P. fissilis* eggs from 35 hosts. Eggs shed by 1 host from which a large, ovigerous *P. simplicis* was expelled and replaced by a juvenile *P. fissilis* (see above), showed 99 % embryonation ($n = 93$). These had been collected 14–4 days prior to host dissection. Eggs from another host in which a patent infection of unknown identity was lost (and also replaced by a *P. fissilis* juvenile) showed 100 % embryonation ($n = 44$). They had been collected over the 14 days prior to host dissection (the original infection having been lost within the final 4 days).

DISCUSSION

Distributions of *P. fissilis* and *P. simplicis* were significantly non-independent in samples of *X. wittei* taken from the same local population in 1991 and 1992. These were naturally infected but had also been exposed to reinfection in captivity (for 1–3 months post-capture) before maintenance in transmission-free conditions (for up to 5 months). The low frequency of concurrent urinary bladder infections (compared to expectation for a random distribution of both species) may indicate that interference competition occurs when both parasites occupy this site in the same host individual. In fact, no concurrent infections with adult parasites were found: the only observed case (occurring in the 1991 sample) involved an adult *P. fissilis* and a juvenile *P. simplicis* (which may only have been present in the urinary bladder for a short time). Distributional data for 2 other sympatric polystomatid species pairs do not show evidence of interaction: *P. fissilis* and *P. ramulosus*, and *Polystoma sodwanensis* and *Metapolystoma porosissimae* (see Du Preez & Kok, 1992) both show host distributions consistent with random infection. A specific mechanism of exclusion may therefore exist between *P. fissilis* and *P. simplicis*, which is not simply a consequence of space limitation or of some general aspect of polystomatid behaviour. Direct antagonistic interactions or indirect competition via the host immune system could be involved. Combes (1983) studied an assemblage of 2

gorgoderid digeneans and 1 polystomatid occurring in the urinary bladder of *Rana temporaria*. The only negative association (attributed to competition) was between the polystomatid and one of the gorgoderids, suggesting that the extent of any interspecific interference may depend on particular biological characteristics in each species pair, irrespective of their phylogenetic relatedness.

The pattern of occurrence of juvenile worms in the 1992 *X. wittei* sample indicated a maximum prepatent period of 4 months or more at 22 °C. Observations on infection turnover in this group of toads suggested that expulsion of adult worms and migration of juveniles (of either species) into the urinary bladder may be related. An unexpectedly high number of young parasites was recovered from hosts which had lost a patent infection. However, the identity of expelled worms was not determined except in 1 case where a *P. fissilis* juvenile replaced an adult *P. simplicis*. There was no evidence of senescence from the egg production rate of the adult fluke: a minimal estimate showed this to be relatively high over the 10-day period prior to detachment (comparison with unpublished data for *P. simplicis*).

The non-random distribution of parasite species in a host population might result from factors other than competition (Simberloff, 1990). It is assumed here that environmental heterogeneity in host exposure to oncomiracidia of *P. fissilis* and *P. simplicis* had not been significant in the field, although this is not known. The period of exposure in captivity (under spatially confined conditions) would have been expected to produce a relatively even per host risk of contact with parasite larvae of either species. Variability in host genetic predisposition to infection by different parasite species might also be significant, although in two instances parasites were observed to develop, at least to the urinary bladder stage, in the same host individual. *Xenopus wittei* is an octoploid species arising from hybridization between distinct tetraploid lineages (Tymowska, 1991). It would have inherited putative genes affecting host–parasite compatibility from both parental forms. However, allopolyploid clawed toads, such as *X. wittei*, have undergone a process of diploidization, and not all immune system genes which are inherited may be expressed (Du Pasquier *et al*. 1977; Tymowska, 1991). The consequence of this evolutionary process for population-level variability in susceptibility to parasitic infection are not known, and merit further study.

Previous work on potential interspecific interactions amongst monogeneans has focused on assemblages from fish gills. Paperna (1964) suggested that *Dactylogyrus vastator* excluded *Dactylogyrus extensus* from carp gills by the stimulation of a host response. Possible cases of interactive site segregation and/or negative interspecific effects on infection levels have also been reported more recently (e.g. Ramasamy *et al*. 1985; Buchmann, 1988; Koskivaara, Valtonen & Vuori, 1992). However, other authors have found little evidence that species infecting the same host influence the microhabitats or numbers of their neighbours (e.g. Dzika & Szymanski, 1989; Rohde, 1991; Rohde *et al*. 1994; Geets, Coene & Ollevier, 1997; Hayward, Perera & Rohde, 1998). For co-occurring *Protopolystoma* species the limited space (relative to parasite size, see Jackson & Tinsley, 1998*a*) and lack of structural complexity of the urinary bladder may increase the likelihood of competitive or reproductive interactions.

The cases of ectopic infection, of the host urinary ducts, by adult and subadult *P. fissilis* may be density related, occurring in infrapopulations of 3 or greater. All individuals from this site were morphologically normal, indicating that they had developed to their existing size *in situ* or migrated here secondarily from the bladder (specimens of other *Protopolystoma* species which, on rare occasions, mature in the host kidneys show abnormal growth with stunted haptoral structures, Tinsley & Jackson, 1998*a*). Tinsley (1993) considered that intraspecific competitive interactions may be significant in urinary bladder polystomatids. The urinary ducts might therefore serve, at least temporarily, as a refugium from competition with conspecifics in *P. fissilis*. However, such site shifts have not yet been observed in concurrent polystomatid infections.

Jackson & Tinsley (1998*a*) reported reductions in the viability of parasite eggs from *X. fraseri*-like hosts concurrently infected with *P. fissilis* and *P. ramulosus*, suggesting that reproductive interference may occur between these species. In the present study, egg samples from 2 hosts in which *P. fissilis* juveniles replaced *P. simplicis* or unidentified infections showed 99–100 % viability. The juveniles had functional male reproductive systems (monogenean development is typically protandrous) and could potentially have cross-inseminated an adult. However, it is impossible to know how long juveniles and adults coincided in the host urinary bladder, if at all.

Differing interactions between *P. fissilis* and *P. simplicis* or *P. ramulosus* might reflect the ages or frequencies of their sympatric relationships. Thus, reproductive interference may occur in the more recent (or less frequent) pairing, while an antagonistic interaction could have evolved as a response to gamete wastage or competition for resources between *P. fissilis* and *P. simplicis*. An alternative hypothesis, that the non-random occurrence of these two species may result from genetic variation in the allopolyploid host population, is possible but is not supported by the ability of both to develop to the urinary bladder stage in the same host individual, or by evidence for a link between dislodgement of adult parasites and arrival of juveniles in the urinary bladder. The present study provides rare evidence

that antagonistic interactions may occur between monogenean congeners co-existing in the same host population.

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