Can parasites use predators to spread between primary hosts?

JOANNE CABLE[1]*, GABRIELLE A. ARCHARD[1], RYAN S. MOHAMMED[2],
MARK MCMULLAN[3], JESSICA F. STEPHENSON[1], HAAKON HANSEN[4]
and COCK VAN OOSTERHOUT[3]

[1] *School of Biological Sciences, Cardiff University, Cardiff CF10 3AX, UK*
[2] *Department of Life Sciences, The University of West Indies, St. Augustine, Trinidad and Tobago*
[3] *School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, UK*
[4] *Norwegian Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway*

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SUMMARY

Parasites typically have low reproductive fitness on paratenic hosts. Such hosts offer other significant inclusive fitness benefits to parasites, however, such as increased mobility and migration potential. The parasite fauna of the guppy (*Poecilia reticulata*) is dominated by the directly transmitted ectoparasites *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli*. In the wild, close predatory and competitive interactions occur between the guppy and the killifish *Rivulus hartii*. Previous observations suggest that these fish can share gyrodactylids, so we tested experimentally whether these parasites can use *R. hartii* as an alternative host. In aquaria, *G. bullatarudis* was the only species able to transmit from prey to predator. Both parasite species transferred equally well to prey when the predator was experimentally infected. However, in semi-natural conditions, *G. bullatarudis* transmitted more successfully to the prey fish. Importantly, *G. bullatarudis* also survived significantly longer on *R. hartii* out of water. As *R. hartii* can migrate overland between isolated guppy populations, *G. bullatarudis* may have an enhanced ability to disperse and colonize new host populations, consistent with its wider distribution in the wild. To our knowledge, this is the first empirical study demonstrating a predator acting as a paratenic host for the parasites of its prey.

Key words: *Gyrodactylus*, heterospecific interactions, paratenic, *Poecilia reticulata*, predator–prey, transmission.

INTRODUCTION

Specialism in parasites is normally described in terms of host range rather than specific resource dependency (Combes, 2001). This definition is, however, unsatisfying when a 'generalist' can infect many host species that are closely related, or when a 'specialist' infects two phylogenetically distinct host species that represent very different resources (Bakke *et al*. 2002). Brooks and McLennan (2002) therefore identified 'faux generalists' and 'faux specialists'. A 'faux specialist' is a parasite that is restricted to limited resources by ecological factors, but one that is able to exploit multiple hosts. 'Faux generalists', on the other hand, are specialists that utilize a specific resource that is phylogenetically widespread. With these caveats in mind, the concept of host specificity is still an extremely useful framework for understanding how host–parasite interactions are influenced by other organisms (Lafferty *et al*. 2006; Orlofske *et al*. 2012) and how, in turn, such interactions affect parasite virulence and distribution.

In this study we consider a specific relationship, where a predator acts as the paratenic host for the parasites of its prey. The use of paratenic hosts is known to promote parasite transmission and survival under certain conditions (Marcogliese, 1995), and prey may share parasites with their predators (e.g. Valtonen and Julkunen, 1995). Here, we investigate differential effects of the predator on the parasites of its prey, using the well-studied Trinidadian guppy (*Poecilia reticulata*). This freshwater tropical fish has become a model species in the study of evolution. Wild guppy populations are isolated by migration barriers and each experiences a unique set of selection pressures. The most commonly researched of these is predation, which has resulted in marked morphological, behavioural and genetic variation (Magurran, 2005). Even within a river, guppies exposed to different predation pressures vary in their colour patterns, shoaling behaviour, courtship and generation times (reviewed by Houde, 1997; Magurran, 2005). Upstream populations, which are above waterfall barriers, are characterized by low predation pressure and low genetic diversity, whereas downstream populations are exposed to greater predation pressure and have higher genetic diversity (e.g. Liley and Seghers, 1975; Reznick and Endler, 1982; Barson *et al*. 2009). Typically, female guppies select large bright showy males, but in the presence of predators, such as the cichlids *Crenicichla* and *Aequidens* spp., selection favours less conspicuous fish (e.g. Godin and McDonough, 2003) and females tend to mate

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* Corresponding author: School of Biological Sciences, Cardiff University, Cardiff CF10 3AX, UK. E-mail: cablej@cardiff.ac.uk

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Table 1. Prevalence, mean intensity and range of *Gyrodactylus* spp. recovered from *Rivulus hartii* and *Poecilia reticulata* in Trinidad

		Rivulus hartii (Predator)				*Poecilia reticulata* (Prey)			
Year	Location[a]	No. of host specimens	Prevalence (%)	Mean intensity	Range	No. of host specimens	Prevalence (%)	Mean intensity	Range
2003, 2006 and 2008	Upper Aripo	30	0	0	0	244	42·6	3·3	1–19
2003	Drainage Ditches (flood plain)	4	0	0	0	162	19·1	1·7	1–7
2003	Lower Caura	5	0	0	0	237	0	0	0
2003	Upper Caura	15	13·3	1	1	252	9·9	1·5	1–5
2006	Mayaro	3	33·3	1	1	40	97·5	13·6	1–62

[a] The Upper Aripo, flood plain drainage ditches and Lower Caura are all within the Caroni drainage. The Mayaro is within the South eastern drainage.

earlier to maximize reproductive success (Reznick, 1982). Downstream predators also include freshwater prawns, *Macrobrachium* spp., and the killifish *Rivulus hartii*. However, this killifish is ubiquitous in the water bodies inhabited by guppies and, probably because of its ability to disperse over land (Reznick, 1995), is often the only fish species present in more isolated river habitats (Walsh *et al*. 2011). Its predation on small, immature guppies has been well documented in the laboratory (Mattingly and Butler, 1994) and in the wild (Magurran, 2005). When size-matched however, these fishes shoal together and both species appear to benefit from this behaviour (Fraser *et al*. 2011), so the relationship is more complicated than one of predators and prey.

An often-overlooked aspect of the interaction between the guppy and its predators is the potential effect of parasites (Magurran, 2005; Cable, 2011). Guppies are subject to natural and sexual selection via parasitism. Uninfected females prefer males with fewer parasites (Kennedy *et al*. 1987) and parasitized females are less discriminatory in their choice of males than healthy fish (López, 1999). Parasites increase the probability of guppies being lost downstream during spate conditions (van Oosterhout *et al*. 2007), and can be a significant cause of mortality under aquarium conditions (Cable and van Oosterhout, 2007). The ectoparasitic monogeneans *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* are the dominant guppy parasites, and they vary in abundance between different guppy populations (Cable, 2011). *Gyrodactylus bullatarudis* is less well-studied than *G. turnbulli*, but tends to be more virulent (Cable and van Oosterhout, 2007). Although gyrodactylids are fairly host specific, both *G. bullatarudis* and *G. turnbulli* were found experimentally to infect a wider range of hosts than predicted, with *G. bullatarudis* being more of a generalist than *G. turnbulli* (see King and Cable, 2007; King *et al*. 2009).

We investigated the interaction between guppies, their predator (*R. hartii*) and their dominant parasites (gyrodactylids). Specifically, we tested (i) whether *R. hartii* naturally acquires infections of *Gyrodactylus* spp. in the wild, (ii) susceptibility of isolated *R. hartii* to infection, (iii) frequency of gyrodactylid transmission between guppies and *R. hartii*, and (iv) whether gyrodactylids have the potential to survive on *R. hartii* when the host is out of water.

MATERIALS AND METHODS

Field data

Between 2003 and 2008, we collected *P. reticulata* and *R. hartii* using a seine net from various sites in Trinidad (Table 1). These fish, carefully scooped out of the water using small buckets (to avoid dislodging ectoparasites), were anaesthetized in MS222 and either preserved individually in 90% molecular grade ethanol at the time of capture, or screened for ectoparasites on the day of capture using a stereo-microscope with fibreoptic illumination. Where three or more fish of the same species were collected ($n = 5$ sites) we recorded gyrodactylid prevalence, mean intensity and range (*sensu* Bush *et al*. 1997). *Ieredactylus*, a parasitic genus recently described from *R. hartii*, is distinctively larger than *Gyrodactylus* under a stereo-microscope (Schelkle *et al*. 2011).

Source and maintenance of experimental animals

All experiments were conducted in Tobago in a makeshift indoor laboratory (24–31 °C) or in outside ponds (22–28 °C) using wild caught fish and parasites. Guppies and *R. hartii* were collected using seine nets in June in 3 consecutive years (2006–2008) from the Naranjo River, Trinidad (Grid Ref: UTM 20P – 692498·44 E, 118257·53 N). The site is an upland, low-predation site where *R. hartii* is the main guppy predator. Guppies from this population experience natural mixed species infections of *G. bullatarudis* and *G. turnbulli*. Guppies and *R. hartii* were maintained separately in aerated tanks with twice-weekly water changes, and fed daily with *Artemia* and/or Aquarian® fish flakes. All experimental fish were unlikely to have been parasite naïve.

Guppies for use in the experiments were 'cleaned' of natural gyrodactylid infections by removing any worms present on individually anaesthetized fish (0·02% MS222) using watchmakers' forceps under a stereo-microscope (Schelkle *et al.* 2009). Single worms from infected guppies from the same site were then transferred to the cleaned fish to culture parasites. Culture fish were housed individually in 0·6 L pots, fed daily and the water changed every other day. When a culture fish had more than 10 worms, additional uninfected fish were added to the culture and at least one parasite was removed for identification. Light microscope flat mounts of the hamuli were prepared according to Harris *et al.* (1999). Once the parasite species had been confirmed, the mono-species cultures were maintained through the weekly addition of uninfected fish and/or removal of heavily infected or previously infected hosts. These cultures provided parasites for the experimental infections. New strains of each species were established from the wild every year. *Rivulus hartii* used in the experimental transfers were also screened under anaesthetic approximately 1 week after capture for the presence of gyrodactylids but none was found. All *R. hartii* and guppies used for the experiments were of a similar size range: 17–24 and 16–21 mm standard length, respectively. Experiments 1, 2 and 4 were conducted in 2006. Experiment 3 was split between 2007 and 2008 because of difficulties in establishing the parasite monocultures. Replicates using both species were performed each year.

Experiment 1: Attachment and survival of parasites on predator

A single worm, *G. bullatarudis* or *G. turnbulli*, attached to a fragment of guppy fin tissue was positioned next to an anaesthetized *R. hartii* using watchmakers' forceps (this methodology has been widely used for gyrodactylid infections of guppies (van Oosterhout *et al.* 2003) and other teleosts (Bakke *et al.* 2007)). The time until attachment was recorded. Worms that had not attached within 10 min were discarded and the fish allowed to recover for at least 3 h (individually maintained in a 0·6 L pot) before a second (or maximum third) attempt was made with the same gyrodactylid species. After attachment, *R. hartii* were kept under light anaesthesia for 5 min. If the infection persisted, the fish was revived and the infection checked every hour thereafter until the worm was lost.

Experiment 2: Parasite transfer

Rivulus hartii and guppies were infected with one *G. bullatarudis* or one *G. turnbulli* as described above. One infected and one uninfected fish were placed together in a 10 L tank. There were four treatments: *G. bullatarudis* infected guppy/uninfected *R. hartii* ($n = 21$); *G. bullatarudis* infected *R. hartii*/uninfected guppy ($n = 7$); *G. turnbulli* infected guppy/uninfected *R. hartii* ($n = 19$); *G. turnbulli* infected *R. hartii*/uninfected guppy ($n = 10$). Trials lasted 24 h; both donor and potential recipient fish were then screened for parasites under anaesthetic.

Experiment 3: Parasite transfer in semi-natural conditions

Twenty-four replicate plastic ponds (dia. 122 cm) with a bamboo frame cover and lined with a net bag (to prevent escape of *R. hartii*) were filled to a depth of 12 cm with dechlorinated water. A similar experimental set-up has been used previously (van Oosterhout *et al.* 2007). Six male and 6 female uninfected guppies were placed into each pond, along with an uninfected *R. hartii*. An additional 17 *R. hartii* were infected with either *G. bullatarudis* ($n = 12$) or *G. turnbulli* ($n = 12$) using the methods described for Experiment 1 (mean no. worms per fish ± S.E.: 2·82 ± 0·54). A single infected *R. hartii* was then added to each of the 24 ponds. At 6, 24 and 48 h, all fish in each pond were caught and screened for parasites before being returned to the ponds.

Experiment 4: Parasite survival on predator out of water

Individual *R. hartii* were infected as before with a single worm of either *G. bullatarudis* ($n = 21$) or *G. turnbulli* ($n = 25$). After 5 min the infection was confirmed and the fish revived and allowed to swim in fresh water for 1 min. Infected fish were placed individually into covered buckets (depth 60 cm, total volume 48 L) containing saturated leaf litter (collected from local rivers) to a depth of 14 cm. Leaf litter often covers the riparian zone of guppy rivers (personal observation) and is therefore likely to be the substrate that *R. hartii* most commonly migrates across when moving between guppy habitats. After 5 min fish were screened for parasite presence. In further trials individual hosts were left for up to 6 h in the leaf litter, and screened for parasites every hour.

Ethics statement

All animal work was approved by Cardiff University ethical committee and covered by UK Home Office regulations (PPL 30/2357).

Table 2. Number of attempts required for *Gyrodactylus bullatarudis* and *G. turnbulli* to attach to *Rivulus hartii*, and time taken to attach during the first attempt. In a few cases (once for *G. bullatarudis* and five times for *G. turnbulli*), parasites failed to attach and these individuals were discarded after the third attempt

		No. infection attempts			Time to infection (s)	
Parasite	*N*	1st	2nd	3rd	Mean time first attempt	Range
G. bullatarudis	64	57	6	0	51·8	1–458
G. turnbulli	70	58	7	1	117·8	3–307

Statistical analyses

Data were analysed using Minitab 12.1. Chi-squared tests and Fisher Exact tests were used to test for differences between *G. bullatarudis* and *G. turnbulli* in parasite attachment and survival. Inter-specific differences in parasite transfer under semi-natural conditions were analysed using a binomial mass function, using the rate of transfer of *G. bullatarudis* to calculate the probability of observing no transfers of *G. turnbulli* in a given number of trials. A Kruskal–Wallis was used to analyse differences in survival and attachment times. The time gyrodactylids spent attached to *R. hartii*, as well as the time required to transfer the worm to an uninfected *R. hartii* host, was analysed using a regression with life data analysis. The model used a Weibull distribution to describe the gyrodactylids' mortality rate (or attachment rate). In this, we assumed that failure to remain attached is a function of time. In the model, gyrodactylid species was the predictor, and a Newton–Raphson algorithm was used to calculate maximum likelihood of the parameters.

RESULTS

Field data

Wild caught *P. reticulata* and *R. hartii* in the Caroni (Caura River) and South Eastern Drainages (Mayaro River) from Trinidad were naturally infected with *Gyrodactylus* spp. (Table 1). Both *G. bullatarudis* and *G. turnbulli* were commonly found on guppies, with prevalences ranging from 0 to 97·5%. Three individuals of *Gyrodactylus* spp. were recovered from *R. hartii* but it was not possible to identify them to species level. Guppies caught at the same sites as the infected *R. hartii* exhibited highly variable parasite burdens (9·9 and 97·5% prevalence at the Upper Caura and Mayaro, respectively) (Table 1).

Experiment 1: Attachment and survival of parasites on R. hartii

Gyrodactylus bullatarudis was more successful at infecting *R. hartii* during all infection attempts ($\chi^2 \geqslant 8{\cdot}21$, D.F. = 1, $P = 0{\cdot}004$) and took less time to infect (mean ± S.E. = 51·8 ± 10·2 s) than *G. turnbulli* (117·8 ± 15·2 s) during the first infection attempt (Kruskal–Wallis test, $H = 8{\cdot}34$, D.F. = 1, $P = 0{\cdot}004$) (Table 2; Fig. 1). *Gyrodactylus bullatarudis* also survived significantly longer on *R. hartii* (mean ± S.E. = 135·8 ± 21·5 min, max. 6 h) than *G. turnbulli* (mean ± S.E. = 61·5 ± 13·2 min, max. 5 h) (regression with life data: Log-likelihood = −75·97, $z = -2{\cdot}81$, $P = 0{\cdot}005$) (Fig. 1). Only a single parasite (*G. bullatarudis*) gave birth on a *Rivulus*, 1–2 h after its transfer, and by 3 h post-infection both mother and daughter parasite had been lost.

Experiment 2: Parasite transfer

Transmission of gyrodactylids from an infected guppy to an uninfected *R. hartii* was significantly higher for *G. bullatarudis* (5 out of 21 trials) than *G. turnbulli* (0 out of 19 trials; Fisher's Exact Test, $P = 0{\cdot}048$). There was no difference in transmission of *G. bullatarudis* (5 out of 7 trials) and *G. turnbulli* (5 out of 10 trials) from *R. hartii* to guppies (Fisher's Exact Test, $P > 0{\cdot}05$).

Experiment 3: Parasite transfer in semi-natural conditions

Transmission of *G. bullatarudis* from *R. hartii* to guppies occurred in 3 out of 12 trials (4 guppies total) compared with no transmission for *G. turnbulli* (binomial probability: $P = 0{\cdot}0317$). No *R. hartii* was infected with *G. turnbulli* at the 24 h time point, whereas 4 *R. hartii* maintained their *G. bullatarudis* infection for at least 24 h, with one individual still infected after 48 h.

Experiment 4: Parasite survival on R. hartii *out of water*

Significantly more *G. bullatarudis* survived for 5 min on isolated *R. hartii* in leaf litter than *G. turnbulli* (18 out of 21 *G. bullatarudis* and 12 and of 25 *G. turnbulli*; $\chi^2 = 7{\cdot}619$, D.F. = 1, $P = 0{\cdot}006$). The mean (± S.E.) survival time of *G. bullatarudis* (87·8 ± 26·0 min) was significantly longer than that of *G. turnbulli* (24·1 ± 8·9 min) (regression with life data: Log-likelihood = −52·17, $z = -2{\cdot}54$, $P = 0{\cdot}011$; Fig. 1).

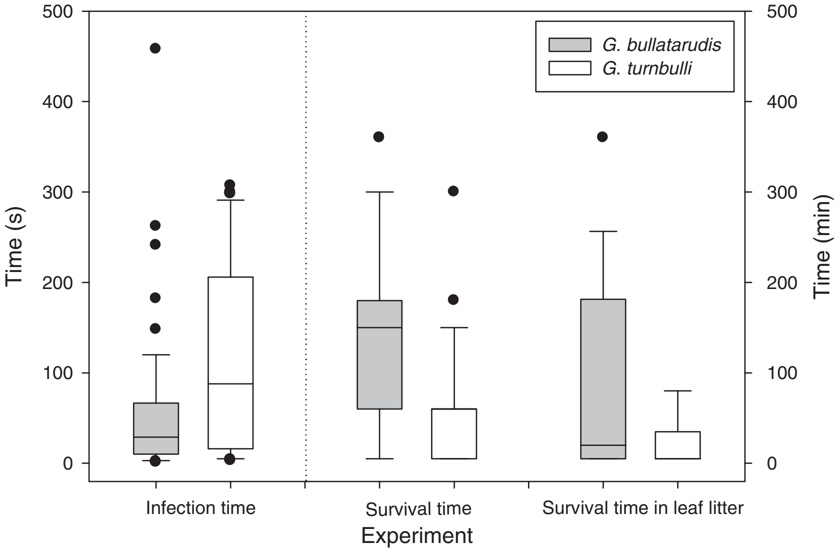

Fig. 1. Infection time (in seconds, 'Time (s)'), survival time and survival time in leaf litter (in min, 'Time (min)') of *Gyrodactylus bullatarudis* (grey bars) and *G. turnbulli* (open bars) on the paratenic host, *Rivulus hartii*. The dots represent outliers, the bars, the lower and upper limits and the box represents the first and third quartile value with the median. Note: in the text, means ± S.E. are presented.

DISCUSSION

Only *G. bullatarudis* transferred from infected guppies, *P. reticulata* (the prey) to uninfected killifish, *R. hartii* (the predator). Under experimental conditions, *G. bullatarudis* also infected killifish more quickly and survived for longer than *G. turnbulli*. When fish were held in heterospecific pairs, both parasite species showed a similar transmission rate from infected killifish to uninfected guppies. Although sample sizes were low, it was clear that both parasites showed a preference to return to their optimal host. When infected killifish were released into semi-natural ponds containing uninfected guppies, only *G. bullatarudis* transferred to the guppies, possibly indicating that *G. turnbulli* transmission is impaired after infecting the predator. Out of water, *G. bullatarudis* remained attached for longer than *G. turnbulli* (mean 88 min compared with 24 min), which suggests it is more likely to survive in the wild when killifish migrate overland (Reznick, 1995). How far *R. hartii* can migrate overland, and how long it survives out of water, have not been explicitly tested. Descriptions of population differentiation between drainages on Trinidad argue against this fish undergoing long-distance migrations (Jowers *et al*. 2008; Walter *et al*. 2011), but there is evidence that they reach headwaters by bypassing waterfalls, and use pools separate from the main river channel (Gilliam and Fraser, 2001). This suggests that the survival times of *Gyrodactylus* spp. on *R. hartii* out of water observed here are sufficient for this to be a useful dispersal mechanism for the parasites.

Gyrodactylus bullatarudis can cause mass guppy mortality and may be more virulent than *G. turnbulli*, but it is also more prone to extinction, at least in laboratory cultures (Richards and Chubb, 1996; Richards and Chubb, 1998; Cable and van Oosterhout, 2007; reviewed by Cable, 2011). Nevertheless, its reduced host specificity and increased ability to survive on *R. hartii* in terrestrial habitats may explain why *G. bullatarudis* is more common than *G. turnbulli* in many isolated upland sites (see Cable, 2011). This suggestion of more versatile use of host species by *G. bullatarudis* is analogous to Diamond's (1974) 'supertramp' species. It could allow *G. bullatarudis* to be an early colonizer of new habitats and monopolize this habitat (cf. Monopolization Hypothesis; De Meester *et al*. 2002). Further work is needed to investigate interspecific competition in these gyrodactylids, particularly with reference to the establishment of new populations.

Theory suggests that the reduced reliance of *G. bullatarudis* on their primary host for transmission is likely to influence virulence (Galvani, 2003). In wild populations, the costs of parasite virulence are presumably less severe for *G. bullatarudis* if it can exploit killifish as a paratenic host and vector into naïve guppy populations. *Rivulus hartii* is more common upstream, above waterfalls than in downstream habitats (Seghers, 1978), and its migration is influenced by water level and its own threat of predation (Gilliam and Fraser, 2001). Given that a single individual of any *Gyrodactylus* species introduced into a naïve host population can be sufficient to initiate an epidemic of these viviparous, hermaphrodite pathogens (Cable and Harris, 2002), the small behavioural difference we observe between these parasite species may have profound consequences on their evolutionary success and distribution. Understanding what drives the evolution of different transmission strategies is important as it will inform our understanding of both predator–prey and host–parasite interactions in a systems biology approach.

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