Testing for local host–parasite adaptation: an experiment with Gyrodactylus ectoparasites and guppy hosts

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ABSTRACT

Hosts and parasites are in a perpetual co-evolutionary “arms race”. Due to their short generation time and large reproductive output, parasites are commonly believed to be ahead in this race, although increasing evidence exists that parasites are not always ahead in the arms race – in part owing to evolutionary lineage and recent ecological history. We assess local adaptation of hosts and parasites, and determine whether adaptation was influenced by ecological or evolutionary history, using full reciprocal cross-infections of four Gyrodactylus ectoparasite populations and their four guppy (Poecilia reticulata) host populations in Trinidad. To consider effects of evolutionary lineage and recent ecology, these four populations were collected from two different river drainages (Marianne and Aripo) and two different predation environments (high and low). The highest infection levels were obtained when parasites from the Aripo lineage infected guppies from the Marianne lineage, indicating a higher infectivity, virulence and/or reproductive success of the Aripo parasites. Aripo lineage guppies were also better able to limit Gyrodactylus population growth than guppies from the Marianne River, indicating their strong “resistance” to Gyrodactylus regardless of the source of the parasite. Predation environment had no detectable influence on host–parasite population dynamics of sympatric or allopatric combinations. The much stronger effect of evolutionary lineage (i.e., river) than recent ecological history (i.e., predation) emphasises its importance in driving co-evolutionary dynamics, and should be explored further in future studies on local host–parasite adaptation.

Keywords:
Gyrodactylus
Poecilia reticulata
Local adaptation
Host–parasite coevolution
Evolutionary lineage
Ecological history

1. Introduction

Adaptation in host–parasite systems is a dynamic “arms race” in which adaptive peaks for the host and the parasite continuously shift in response to evolution of the opposing party (Ebert, 1994; Kaltz and Shykoff, 1998; Gandon and Michalakis, 2002; Kawecki and Ebert, 2004). Parasites are generally considered to be ahead in this arms race due to their shorter generation times which should increase their evolutionary speed (see Lively, 1999; Gandon and Michalakis, 2002; Greischar and Koskella, 2007; Hoeksema and Forde, 2008), and because hosts are usually exposed to many parasite species which makes adaptation to any one species more difficult (Kawecki and Ebert, 2004). Consistent with this, many studies have found that parasites show stronger signals of local adaptation to their hosts than hosts do to their parasites (reviewed in: Greischar and Koskella, 2007; Hoeksema and Forde, 2008) as evidenced by higher infection levels for a given parasite population on sympatric hosts than on allopatric hosts (Ebert, 1994; Saairen and Taskinen, 2005). However, other studies have not found evidence of local parasite adaptation, or have found apparent local maladaptation of parasites: e.g., infection levels are higher on allopatric than sympatric hosts (Lemoine et al., 2012; Roth et al., 2012; Konijnendijk et al., 2013; Sternberg et al., 2013).

One set of potential reasons for these varied results is methodological. First, many studies have measured parasite fitness (e.g., infection levels) without also measuring host fitness (e.g., survival or growth) which means that local adaptation cannot be considered independently for both host and parasite. Second, many studies have been conducted in the laboratory whereas very different results might be obtained in the natural environment (Brockhurst and Koskella, 2013). Third, many studies have not performed full reciprocal cross-infection experiments which makes it

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difficult to separate the confounding influences of virulence and resistance co-evolution (Greischar and Koskella, 2007).

Another set of potential reasons for varied results in local host-parasite adaptation studies is untested interactions with other factors related to evolutionary history or ecological context (Thompson, 1994, 1999; Morgan et al., 2005). Evolutionarily, different host lineages and their co-evolved parasites could have had different histories of selection, genetic bottlenecks, drift and founder events which might have strongly shaped co-evolutionary trajectories. Ecologically, recent ecological history whereby different host–parasite populations have experienced different biotic or abiotic conditions could have imposed selection that directly or indirectly influenced co-evolutionary trajectories (Thompson, 1999). As one example, environments with high predation-induced host mortality are likely to select both for parasites that reproduce more quickly (and thus might be more virulent), and for hosts that invest less in parasite defence (Lively, 1999; Gandon and Michalakis, 2002). Of course, the inverse might occur if parasitism increases susceptibility to other sources of mortality (Choo et al., 2003).

The Trinidadian guppy is frequently used in evolutionary studies due to its capacity for rapid and repeatable adaptation to different ecological environments (see reviews: Endler, 1985; Houde, 1997; Magurran, 2005; Dargent et al., 2013). The ecological force that has received the most attention is predation intensity, with guppy populations commonly classified as either high predation (HP), with many dangerous predatory fishes that have major effects on guppy survival, or low predation (LP), with fewer and less dangerous predatory fishes that have only minor effects on guppy survival (Reznick et al., 1996a; Gordon et al., 2009; Weese et al., 2010). In response to these different mortality regimes, HP and LP guppies have evolved a number of behavioural, life history and morphological differences (see reviews: Endler, 1995; Houde, 1997; Magurran, 2005). As one example, HP guppies show earlier maturation and increased reproductive investment, with more frequent reproductive events and many but smaller embryos (Reznick, 1982; Reznick and Endler, 1982). Moreover, this evolution occurs rapidly following experimental introductions in nature (Reznick and Bryga, 1987; Reznick et al., 1990, 1997; Gordon et al., 2009) and is repeatable across watersheds colonised by very divergent guppy lineages and with different predator faunas (Reznick and Bryga, 1996; Reznick et al., 1996b).

Guppies are commonly infected by the monogenean worm Gyrodactylus, a genus of ubiquitous host-specific ectoparasites on fishes (Harris and Lyles, 1992; Kearn, 1994; Harris et al., 2004). Gyrodactylus are viviparous and reproduce directly on the host, exhibiting hyperviviparity: a mature female has in its uterus a fully developed embryo that in turn has a developing embryo within its uterus (Kearn, 1994). Transmission between hosts occurs through contact when the parasite ‘jumps’ to a new host. These characteristics result in a rapid increase in parasite numbers on an individual host and epidemic spread of infection through fish populations (Scott and Anderson, 1984). Infections by Gyrodactylus can cause high guppy mortality in the laboratory (Scott and Anderson, 1984; van Oosterhout et al., 2003; Cable and van Oosterhout, 2007a,b) and in nature (van Oosterhout et al., 2007). Not surprisingly, then, some evidence exists that guppy populations have evolved in response to Gyrodactylus, particularly through variation in the immune response (van Oosterhout et al., 2003) and at loci of the Major Histocompatibility Complex (MHC) (Fraser and Neff, 2009; Fraser et al., 2010).

In a previous study (Pérez-Jvostov et al., 2012), we used experimental infections in semi-natural mesocosms to test whether adaptation to different predation environments (HP versus LP) influenced Gyrodactylus–guppy interactions. We found strong and repeatable differences in Gyrodactylus infection dynamics between host–parasite assemblages taken from different field locations, but we found that the differences were not related to predation regime. However, because each guppy population was infected only with its own local parasite population, we were unable to disentangle the confounding effects between highly resistant hosts and highly virulent parasites, and those from low-resistance hosts and low-virulence parasites, which restricted any potential inferences on local adaptation.

The objective of this study was to assess local adaptation of hosts and parasites, and to determine whether adaptation was influenced by ecological or evolutionary history, using the well-studied ectoparasite Gyrodactylus infecting the Trinidadian guppy (Poecilia reticulata). Our design allowed us to circumvent methodological limitations (Hoeksema and Forde, 2008) by (i) generating separate measures of parasite and host fitness, (ii) conducting experiments in reasonably natural (mesocosm) environments, and (iii) conducting a full reciprocal cross-infection experiment with four Gyrodactylus–guppy populations to disentangle local adaptation from effects of host–parasite co-evolution. We specifically tested whether parasites or hosts showed evidence of local adaptation (higher performance of parasites with sympatric than with allopatric hosts, or higher performance of hosts with sympatric than with allopatric parasites), and whether any local mal-adaptation was related to drainage of origin (evolutionary lineage) or predation regime (ecological differences).

2. Materials and methods

2.1. Fish collection and treatment

Immature guppies were collected from an HP population and an LP population within each of two rivers in the northern mountain range of Trinidad: the Marianne River (HP, N10°46'30.525", E61°18'25.861"; LP, N10°44'51.85", E61°17'30.615") on the northern slope and the Aripo River (HP, N10°39'25.832", E61°13'39.395"; LP, N10°41'15.496", E61°14'4.455") on the southern slope. These two rivers represent different guppy lineages (and probably separate colonisation events) as genetic distances between them are very large (see Suk and Neff, 2009; Willing et al., 2010). The Gyrodactylus populations in these different drainages are probably also distinct (given their host specificity for guppies), but this has not yet been confirmed.

At each site, the fish were collected with butterfly nets and immediately placed in individual 8 oz. whirl-pak bags (Spectrum Nasco, U.S.A.) to prevent movement of parasites among fish. After transfer to our laboratory in Trinidad, all fish were anaesthetised with MS-222 (Finquel MS222 from Fisher Canada; 1:8000 dilution and buffered to a neutral pH using NaHCO3) to prevent the spread of infection.

All fish, regardless of whether or not they were initially infected, were treated with N-cyclopentyl-1,3,5-triazine-2,4,6-triamine (cyromazine; Lice And Anchor Worm Treatment (E-61), E61®) on the southern slope. These two rivers represent different guppy lineages (and probably separate colonisation events) as genetic distances between them are very large (see Suk and Neff, 2009; Willing et al., 2010). The Gyrodactylus populations in these different drainages are probably also distinct (given their host specificity for guppies), but this has not yet been confirmed.

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recovery aquaria, confirming that females had been virgin prior to
the experiment.

2.2. Mesocosms

The mesocosms were 0.5 m wide by 3 m long by 0.2 m deep, and received continuous flowing water from a tributary adjacent
to the Arima River without guppies, thus also preventing any
potential introduction of Gyrodactylus into the mesocosms. This
natural flow allowed colonisation of the mesocosms by algae and
invertebrates, including natural foods for guppies, but excluded
any non-experimental guppies. These specific mesocosms have
been used in a number guppy studies and are a good mimic of
natural conditions (for technical specifications see Palkovacs
et al., 2009; Bassar et al., 2010; Pérez-Jvostov et al., 2012).

2.3. Experimental design

Our experiment used a fully reciprocal cross-infection design
for the four host–parasite populations (Fig. 1). Each of the four
guppy populations was tested with each of the four Gyrodactylus
populations. This design led to four sympatric pairs (hosts and
parasites from the same locations) and 12 allopatric pairs (hosts
and parasites from different locations). Due to a limited number
of mesocosms (16 channels), we were unable to perform replicates
for the particular guppy–Gyrodactylus combinations.

2.4. Experimental protocol

Four weeks after parasite removal and marking (see
Section 2.1), each fish was weighed (to the nearest 0.1 mg), mea-
sured for standard length (to the nearest 1 mm), and scanned for
Gyrodactylus. No parasites were found, confirming that parasite
treatment had eliminated Gyrodactylus from all experimental fish.
Guppies were then separated into 16 experimental groups (four for
each population) each with eight females and eight males. The 16
groups were then introduced into 16 mesocosms – one group per
mesocosm.

Gyrodactylus for the experiment came from an infected “donor”
fish collected immediately prior to the experiment from each of the

![Fig. 1](image-url)
four natural populations. To initiate a *Gyrodactylus* epidemic, we first transferred two to four parasites from the caudal fin of each of the four infected “donor” fish onto a male guppy selected from each of the four populations from the above-described recovery tanks. This transfer was done using a dissecting microscope by individually moving *Gyrodactylus* from a donor fish onto a naïve male. The experimentally infected males were kept overnight in individual 1 L containers and parasite establishment was confirmed the following day by inspection using a microscope. One infected male guppy was then introduced into each mesocosm to generate every possible combination of hosts and parasite sources.

*Gyrodactylus* epidemics in each mesocosm were monitored every second day over a period of 23 days. All fish were captured individually, anaesthetised (see Section 2.1), identified and inspected using a dissecting microscope to count parasites. After each inspection, the fish were released back into their mesocosm.

At the end of the experiment, the weight (to the nearest 0.1 mg) and length (to the nearest 1 mm) were recorded for all fish. All females were euthanised with MS-222 and then dissected to count their embryos. Reproductive allocation was calculated as the percentage of gained weight devoted to embryo weight.

All procedures in the experiments were in accordance with ethical practices and approved by the McGill University, Canada, Animal Use Committee (Protocol No. 5759).

2.5. Statistical analysis

Our analyses focus on two aspects of local host–parasite adaptation: (i) *Gyrodactylus* performance on different guppy populations, and (ii) guppy performance when exposed to different *Gyrodactylus* populations. *Gyrodactylus* performance was evaluated in two separate types of model, and guppy performance was evaluated in a third type of model. All analyses were performed in R version 2.14.1 (R Core Development Team 2011), and all statistical tests were two-tailed with levels of significance set at $P < 0.05$.

2.5.1. Models of *Gyrodactylus* performance

As a first step in evaluating *Gyrodactylus* performance, two linear mixed effects (LME) models were constructed with different response variables: (i) mean abundance of infection (average number of parasites observed on all guppies throughout the experiment), and (ii) duration of infection (number of consecutive days each guppy was infected throughout the experiment). Mean abundance of infection was log-transformed to meet the assumptions of normality and homoscedasticity of residuals. In both models, the random factor was guppy population and the fixed factors were (i) the degree of similarity, (ii) number of embryos. In these models, the random factor was *Gyrodactylus* population and the fixed factors were host–parasite combination (sympatric versus allopatric – see Fig. 1), predation regime (high versus low) of the guppy population, and drainage source (Marianne versus Aripo Rivers) source of the guppy population.

2.5.2. Models of guppy performance

To evaluate guppy performance, three LME models were constructed with different response variables: (i) change in female body mass (final weight – initial weight), (ii) reproductive allocation (proportion of body mass devoted to embryonic mass), and (iii) number of embryos. In these models, the random factor was *Gyrodactylus* population and the fixed factors were host–parasite combination (sympatric versus allopatric – see Fig. 1), predation regime (high versus low) of the guppy population, and drainage source (Marianne versus Aripo Rivers) of the guppy population. Initial female mass was also added as a covariate. Based on AIC comparisons of alternative models (not shown), we present a reduced model that excluded the three-way interactions, second order interactions with host–parasite combination (sympatric versus allopatric) and the initial mass covariate.

3. Results

3.1. *Gyrodactylus* performance

*Gyrodactylus* infections established and spread through the experimental guppy population in all of the guppy–*Gyrodactylus* combinations (Fig. 1). Nonetheless, parasite performance on allopatric pairs varied greatly whereas parasite performance on sympatric hosts, measured as mean abundance, was similar across all *Gyrodactylus* populations (Fig. 2A). We first describe results based on the two response variables for sympatric–allopatric comparisons and then results based on host–parasite “degree of similarity”.

Sympatric–allopatric analyses based on mean intensity of infection showed that *Gyrodactylus* from the Aripo River were maladapted to their sympatric hosts in that they achieved higher intensities on allopatric Marianne River guppies (Fig. 2A; Table 1). This pattern held, regardless of the predation regime of the hosts or parasites, suggesting that maladaptation is best explained at the drainage source level. This was best exemplified in the Marianne River LP *Gyrodactylus*, which was the only parasite population showing a higher intensity on its sympatric host than on allopatric hosts – even though the infection intensity of Marianne River HP *Gyrodactylus* was similar in sympatric and allopatric comparisons (Figs. 2A, B and 3A, B).

Sympatric–allopatric analyses based on the duration of infection on individual fish yielded results similar to those described above for the mean intensity of infection. In particular, Aripo River *Gyrodactylus* (both LP and HP) were maladapted in that infections were 6–8 days shorter on sympatric than allopatric hosts; Marianne River LP *Gyrodactylus* were locally adapted in that infections were up to 6 days longer on sympatric than allopatric hosts;
and Marianne River HP Gyrodactylus maintained similar infection durations on sympatric and allopatric guppies (Fig. 2B; Table 1). ‘Degree of similarity’ analyses showed that Aripo River Gyrodactylus performance was the highest on allopatric hosts that shared the same predation regime, especially for HP Gyrodactylus (Fig. 2C; Table 2). By contrast, Marianne River Gyrodactylus performed similarly on all allopatric hosts, regardless of the degree of similarity in the environment (HP versus LP) and phylogenetics (drainage source).

3.2. Guppy performance

Female guppy growth was higher when fish were exposed to sympatric than allopatric parasites for three of the four guppy populations (Fig. 4A; Table 3). Overall, Aripo River HP females showed the highest growth rate, particularly when infected with their sympatric parasite, whereas Marianne River HP females had the lowest growth rate regardless of parasite origin. The number of embryos per female was similar among populations when females were

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Table 1

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>Mean abundance of infection</th>
<th>Duration of infection</th>
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<tbody>
<tr>
<td></td>
<td>F (d.f.)</td>
<td>P</td>
</tr>
<tr>
<td>Gyrodactylus drainage of origin</td>
<td>49.065 (1, 263.20) &lt;0.001</td>
<td>11.75 (1, 262.67) &lt;0.001</td>
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<tr>
<td>Gyrodactylus predation regime of origin</td>
<td>1.358 (1, 263.20) 0.244</td>
<td>3.451 (1, 262.67) 0.064</td>
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<td>Host–parasite combination</td>
<td>15.086 (1, 260.96) &lt;0.001</td>
<td>5.008 (1, 260.99) 0.026</td>
</tr>
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<td>Gyrodactylus drainage of origin × Gyrodactylus predation regime of origin</td>
<td>4.063 (1, 263.20) 0.044</td>
<td>1.980 (1, 262.67) 0.160</td>
</tr>
<tr>
<td>Gyrodactylus drainage of origin × Host–parasite combination</td>
<td>4.945 (1, 263.16) 0.027</td>
<td>12.702 (1, 219.90) &lt;0.001</td>
</tr>
<tr>
<td>Gyrodactylus predation regime of origin × Host–parasite combination</td>
<td>7.925 (1, 263.16) 0.005</td>
<td>0.708 (1, 219.90) 0.400</td>
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<tr>
<td>Gyrodactylus drainage of origin × Gyrodactylus predation regime of origin × Host–parasite combination</td>
<td>3.06 (1, 263.16) 0.081</td>
<td>2.615 (1, 219.90) 0.107</td>
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<td>Random effects</td>
<td>Variance S.D.</td>
<td>Variance S.D.</td>
</tr>
<tr>
<td>Guppy population (intercept)</td>
<td>0.1708 0.4133</td>
<td>0.6564 0.8102</td>
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<tr>
<td>Residual</td>
<td>0.4144 0.6435</td>
<td>5.9542 2.4401</td>
</tr>
</tbody>
</table>

d.f., degrees of freedom. Significant P values are presented in bold.
exposed to allopatric *Gyrodactylus* (Fig. 4B; Table 3). However, when infected with sympatric *Gyrodactylus*, Aripo River LP and Marianne River HP guppies produced fewer embryos, whereas sympatric infection of Aripo River HP and Marianne River LP guppies resulted in a higher number of embryos. The analysis of reproductive allocation did not reveal any significant effects of predation regime, drainage source or sympatric/allopatric association (Table 3).

4. Discussion

Many previous studies of host–parasite interactions have not been designed in a way that allows clear insights into local adaptation and co-evolution (Hoeksema and Forde, 2008). In an effort to reduce a number of these limitations, we tracked separate measures of parasite and host fitness in a fully reciprocal cross-infection design conducted in stream mesocosms using four

**Table 2**

Statistical analysis for *Gyrodactylus* performance on guppy populations, according to their degree of similarity (same drainage and same predation environment, same drainage and different predation environment, different drainage and same predation environment, different drainage and different predation environment). Analyses were performed using a general linear model.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean intensity of infection</th>
<th>F (d.f.)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gyrodactylus</em> drainage of origin (Marianne versus Aripo River, Trinidad)</td>
<td>137.167 (1, 256)</td>
<td>&lt;0.001</td>
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<tr>
<td><em>Gyrodactylus</em> predation of origin</td>
<td>15.473 (1, 256)</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Degree of similarity</td>
<td>24.672 (3, 256)</td>
<td>&lt;0.001</td>
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<tr>
<td><em>Gyrodactylus</em> drainage of origin × <em>Gyrodactylus</em> predation regime of origin</td>
<td>2.045 (1, 256)</td>
<td>0.153</td>
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<tr>
<td><em>Gyrodactylus</em> drainage of origin × Degree of similarity</td>
<td>57.439 (3, 256)</td>
<td>&lt;0.001</td>
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<tr>
<td><em>Gyrodactylus</em> predation regime of origin × Degree of similarity</td>
<td>4.289 (3, 256)</td>
<td>0.005</td>
<td></td>
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<tr>
<td><em>Gyrodactylus</em> drainage of origin × <em>Gyrodactylus</em> predation regime of origin × Degree of similarity</td>
<td>4.723 (3, 256)</td>
<td>0.0031</td>
<td></td>
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</tbody>
</table>

d.f., degrees of freedom. Significant P values are presented in bold.

exposed to allopatric *Gyrodactylus* (Fig. 4B; Table 3). However, when infected with sympatric *Gyrodactylus*, Aripo River LP and Marianne River HP guppies produced fewer embryos, whereas sympatric infection of Aripo River HP and Marianne River LP guppies resulted in a higher number of embryos. The analysis of reproductive allocation did not reveal any significant effects of predation regime, drainage source or sympatric/allopatric association (Table 3).

![Fig. 3](image-url) Population dynamics of (A) Marianne River, Trinidad low predation *Gyrodactylus*, (B) Marianne River high predation *Gyrodactylus*, (C) Aripo River, Trinidad low predation *Gyrodactylus*, and (D) Aripo River high predation *Gyrodactylus* when infecting mesocosm guppies from four field populations: Marianne high predation (filled black squares), Marianne low predation (empty black squares), Aripo high predation (filled gray circles) and Aripo low predation (empty gray circles). Each figure represents the mean number of parasites on individual fish. Error bars represent S.E.

![Fig. 4](image-url) Least square means for guppy performance when infected with sympatric versus allopatric hosts. (A) Female guppy growth over 23 days, (B) number of embryos per female. Symbols represent guppy populations: Marianne River, Trinidad high predation (filled squares), Marianne River low predation (empty squares), Aripo River, Trinidad high predation (filled circles) and Aripo River low predation (empty circles). Error bars represent S.E.
populations that differed in recent ecological history and evolutionary history. Our first key finding was that parasites were not – contrary to typical expectations – locally adapted to their hosts. This raises questions about the conditions under which parasites or hosts are more likely to lead the evolutionary “arms race”. Our second key finding was that patterns of local parasite mal-adaptation were strongly influenced by evolutionary lineage (drainage source and therefore host, and perhaps parasite, lineage) but were not influenced by recent ecological history (predation regime). In the following sections we expand on the potential explanations for local Gyrodactylus maladaptation, as well as the relative importance of ecological history and evolutionary lineage as drivers of host–parasite co-evolution.

Although parasites are generally expected to have an evolutionary advantage over hosts (Ebert, 1994; Saarinen and Taskinen, 2005) due to their short generation time and potentially high host specificity, parasites have not always shown signatures of local adaptation to their sympatric hosts (Kaltz et al., 1999; Oppliger et al., 2005) due to their short generation time and potentially high host specificity, parasites have not always shown signatures of local adaptation to their sympatric hosts. As an example, Aripo River Gyrodactylus achieved the highest and the longest infection intensities when exposed to guppies from the Marianne River drainage, yet relatively low infection levels when infecting guppies from their shared drainage – regardless of their predation environment (Figs. 2A, B and 3).

One possible reason for this apparent lack of local adaptation by Gyrodactylus – or even maladaptation in the case of Aripo River parasites – is a strong divergence in resistance between Marianne River and Aripo River guppies. Such differences, coupled with strong divergence in Gyrodactylus virulence, could result in an apparent lack of strong local parasite adaptation, or even mal-adaptation, when highly virulent parasites interact with low resistance hosts. Some evidence for this possibility exists in our study as Aripo River guppies were least affected by their sympatric parasites, with highest growth rates (and sometimes more embryos) when faced with sympatric, relative to allopatric, parasites, while Aripo River Gyrodactylus performed best when infecting the less resistant Marianne River guppies (Fig. 4A, B). Yet another possible explanation is that guppies are leading the “arms race” and have become locally adapted to their sympatric parasites. Previous work has suggested that guppies from the Paria River, with common MHC alleles, have lower Gyrodactylus infection levels in the laboratory (Fraser and Neff, 2009), with similar observations in the wild (Fraser et al., 2010), suggesting that, indeed, guppies could be locally adapted to their sympatric Gyrodactylus.

Why might guppies be leading the “arms race”? First, it should be noted that, although Gyrodactylus have much shorter generation times than guppies, guppies have relatively short generation times (approximately four per year) compared with hosts in other frequently studied host–parasite associations. Furthermore, it is conceivable that the reproductive system of Gyrodactylus (i.e., hyperviviparity and parthenogenesis), although allowing for very fast population growth, can also result in a reduction of genetic diversity in the population. Indeed, a recent laboratory study reported that sexual reproduction accounted for only 3.7% to 10.9% of population diversity, suggesting that the vast majority of the individuals are clones (Scheikle et al., 2012). Such a reduction in Gyrodactylus genetic diversity could allow outbred guppy populations to quickly adapt to the most common parasite genotype and thus put them at a lesser evolutionary disadvantage than other hosts to their parasites. Second, Gyrodactylus can cause high guppy mortality in the field and, especially, in the laboratory (Scott and Anderson, 1984; van Oosterhout et al., 2003; Cable and van Oosterhout, 2007a,b). It may be conceivable that very high virulence in Gyrodactylus might be maladaptive if it increases host mortality in such way that the basic Gyrodactylus reproductive rate is reduced (Dybdahl and Storfer, 2003; Alizon et al., 2008). In this way, lower virulence in Gyrodactylus could evolve in response of the lower resistance of their guppy host (Altizer, 2001; Sternberg et al., 2013) – although the opposite effect has also been reported (Hoeksema and Forde, 2008). Some evidence for this exists in our study in that the least resistant hosts (Marianne River HP and LP) also had the parasites with the lowest performance. Of course, cause and effect could be reversed here in that Marianne River Gyrodactylus do not have to evolve high infectivity because their hosts show such low resistance. Regardless of the specific reasons, these results should be taken as another challenge to the established paradigm that parasites generally are ahead in the “arms race” with hosts (Hoeksema and Forde, 2008).

Beyond this basic result, considerable variance was present between our measures of both parasite fitness (number of parasites and duration of infection) and host fitness (growth and reproduction). We now explore the causes of this variation by considering different evolutionary histories (different guppy, and likely Gyrodactylus, lineages) and differences in recent ecological histories (HP versus LP).

How evolutionary history has shaped host–parasite interactions remains to be determined. One possibility is that patterns of guppy genetic variation differ dramatically between the two lineages, which could then cause different evolutionary trajectories even in the case of similar selection. For instance, recent studies have shown considerable variation between northern and southern slope guppies in the genetic basis for adaptive traits, potentially due to founder effects (Willig et al., 2010). Another possibility is that genetic differentiation between HP and LP populations in a given drainage is relatively low – thus making Gyrodactylus adaptation to particular genotypes easier. Indeed, low microsatellite genetic differentiation between predation environments has been shown for both Marianne River and Aripo River drainage (Suk and Neff, 2009), but is still unknown for genes of the immune system (e.g., MHC). Irrespective of the particular mechanisms by which evolutionary history could affect the evolution of host resistance and parasite infectivity, our study indicates that these effects

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<td>Statistical analyses of guppy performance when infected with sympatric versus allopatric Gyrodactylus. Analyses were performed using linear mixed effects models. P values and denominator degrees of freedom (d.f.) were obtained using a Satterthwaite approximation for degrees of freedom. Significant P values are presented in bold.</td>
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have major implications on co-evolutionary dynamics. Taking evolutionary history into account is thus important in studies of host–parasite dynamics.

Theoretical models often predict that increased parasite transmission (and therefore perhaps increased virulence) should evolve under increased host mortality (Anderson and May, 1982; Gandon and Michalakis, 2002). Given that higher parasite reproductive and transmission rates can increase local parasite adaptation (Alizon et al., 2008), Gyrodactylus from HP environments would be expected to perform better (e.g., parasite growth rate, infectivity, prevalence, mean abundance) on their sympatric compared with allopatric hosts (higher performance leads to higher fitness) – although this of course would also select for higher resistance in HP guppies. This expectation has not been demonstrated in our study as we found no relationship between predation regime and local maladaptation by parasites. For instance, of the two parasite populations showing the highest maladaptation (i.e., lowest growth rate on their sympatric host), one was from an HP site and the other was from an LP site.

One possible reason for our inability to detect an effect of recent ecological history is that our experimental conditions were not realistic enough – for instance, we excluded predators from the mesocosms. Predators and predator cues are certainly known to have very strong plastic effects on guppy behaviour and growth (Rodd and Reznick, 1997; Evans et al., 2007; Gosline and Rodd, 2007; Brown et al., 2013), and these guppy traits are known to influence infection (Johnson et al., 2011; Richards et al., 2010). Thus, perhaps we would have found very different results had we exposed guppies to predators or predator cues.

Beyond parasites, we might also expect different predation regimes to influence guppy resistance to parasites. For instance, high mortality rates in HP environments might reduce the benefits of investing in resistance (or tolerance) to parasites (Dargent et al., 2013). Alternatively, heavily infected individuals might experience higher predation risk, which would thus select for increased resistance by the hosts (Packer et al., 2003). However, our study failed to find an association between the predation regime of guppy hosts and their response to parasites. Perhaps the two effects described above (high parasite-independent predation and effects of infection on predation) offset each other, leading to no net effect of predation regime. This possibility might be interesting to investigate in future experiments.

Although the precise mechanisms will have to be further established, our study adds to the growing body of work that suggests that parasites and predation do not seem to have strong interactive effects on guppies (Gotanda et al., 2013; Pérez-Jostov et al., 2012; Dargent et al., 2013). We understand the limitations of our lack of replication for any given guppy–Gyrodactylus combination, yet we are confident of our results given that our sympatric infection dynamics closely resemble those of a previous study where strong drainage source effects were also observed (Pérez-Jostov et al., 2012). Predation is only one ecological factor that might influence host–parasite co-evolution, thus it might seem tenuous to use our results to conclude that ecological context is not important. However, predation is thought to be the strongest ecological context shaping the evolution of guppy traits (Endler, 1995; Houde, 1997; Magurran, 2005) – thus it was a reasonable place to start.

Our observation that parasites were not locally adapted to their hosts may stimulate further work on the conditions under which parasites or hosts are more likely to be leading the evolutionary “arms race”. Our finding that patterns of local host and parasite maladaptation were not influenced by the predation regime but were strongly influenced by the drainage source and therefore host (and perhaps parasite) lineage runs counter to the idea that natural selection owing to ecological differences leads to deterministic patterns of parallel (or convergent) evolution (Endler, 1986; Schluter, 2000), and to evidence from a number of guppy traits for deterministic responses to predation (Reznick and Endler, 1982; Reznick et al., 1990; Rodd and Reznick, 1991; Endler, 1995, but see Torres-Dowdall et al., 2012). However, recent studies are increasingly emphasizing the fact that evolution in similar environments is often not very similar (i.e., non-parallel or non-convergent) which suggests a considerable role for historical contingency (Kauffman et al., 2012; Fitzpatrick et al., 2013). Our study provides direct support for this contingency by showing that patterns of local host–parasite maladaptation are predictable by drainage source (and likely lineage) rather than the (otherwise) most important ecological context for guppies (predation regime). Overall, our study thus provides additional support for the importance of considering non-deterministic aspects of evolution and the causes thereof.

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