



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



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ARTICLE INFO

Article history:

Received 15 October 2014

Received in revised form 19 January 2015

Accepted 27 January 2015

Available online 11 March 2015

Keywords:

Gyrodactylus

Poecilia reticulata

Local adaptation

Host–parasite coevolution

Evolutionary lineage

Ecological history

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.

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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; Saarinen 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, 1995; 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 maladaptation 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″, E-61°18′25.861″; LP, N10°44′51.85″, E-61°17′30.615″) on the northern slope and the Aripo River (HP, N10°39′25.832″, E-61°13′39.395″; LP, N10°41′15.496″, E-61°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) and then immediately scanned for *Gyrodactylus*, using a dissecting microscope. Infected fish were isolated in individual containers to prevent the spread of infection.

All fish, regardless of whether or not they were initially infected, were treated with N-cyclopropyl-1,3,5-triazine-2,4,6-triamine (cyromazine; Lice And Anchor Worm Treatment, Ecological Laboratories Inc., U.S.A.) which effectively eliminates *Gyrodactylus* (Pérez-Jvostov et al., 2012). When no *Gyrodactylus* were seen on a fish over three consecutive days of visual inspection (as above), the fish was considered parasite-free. Elastomer dyes (Northwest Marine Technology Inc., U.S.A.) were then injected to give each fish a distinct intra-dermic mark, a procedure used effectively in many previous guppy studies (Bassar et al., 2010; Weese et al., 2010; Pérez-Jvostov et al., 2012). The elastomer marks were no longer than 2 mm and no marked fish showed signs of reduced mobility or altered behaviour. Guppies were then held in population- and sex-specific aquaria. No fry were observed in the

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), measured 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

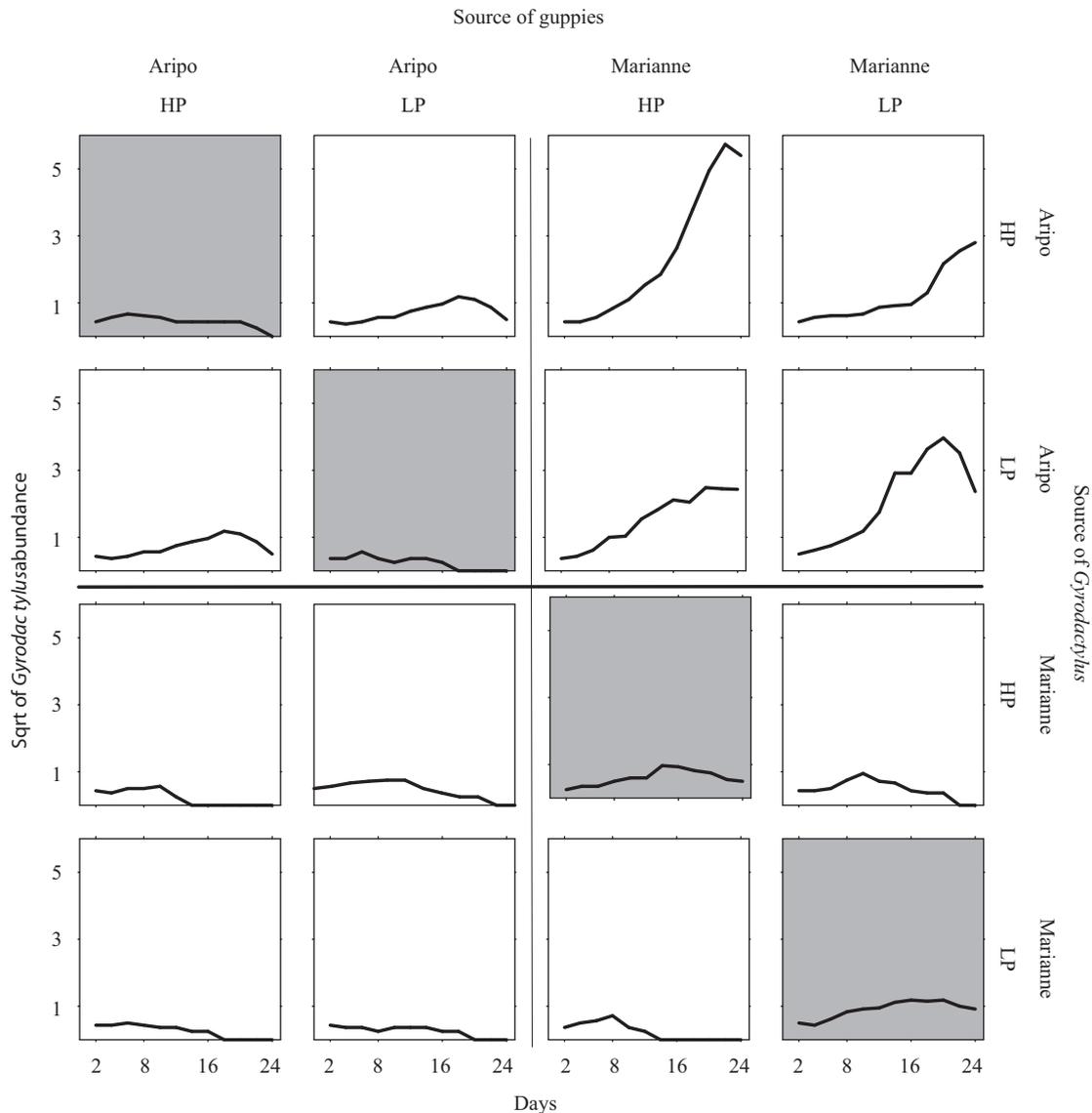


Fig. 1. A schematic of the experimental design coupled with results on *Gyrodactylus* population dynamics when infecting each guppy population in semi-natural mesocosms. Horizontal labels indicate the field source (Aripo or Marianne Rivers, Trinidad) of guppies (*Poecilia reticulata*), vertical labels indicate the field source of the parasites used in each mesocosm. White squares represent allopatric combinations (guppies and *Gyrodactylus* from different field locations) and gray squares represent sympatric combinations (guppies and *Gyrodactylus* from the same field locations). HP, high predation; LP, low predation; Sqrt, square root.

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 *P* values were obtained using a Satterthwaite approximation for degrees of freedom with the package *lmerTest*, 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 host–parasite combination (sympatric versus allopatric – see Fig. 1), predation regime (HP versus LP) of *Gyrodactylus*, and drainage (Marianne versus Aripo Rivers) source of *Gyrodactylus*. Simplified alternative models did not have lower Akaike Information Criterion (AIC) values (not shown), thus we only present results for the full model including all interactions. In this analysis, local *Gyrodactylus* adaptation would be inferred if parasite performance was higher on sympatric than allopatric hosts, taking into account the predation regime and drainage of origin of *Gyrodactylus*.

As a second step in evaluating *Gyrodactylus* performance, the 16 host–parasite combinations were categorised according to the “degree of similarity” between hosts and parasites. We generated a new fixed factor with four levels representing hosts and parasites from (i) the same drainage and same predation regime (“sympatric” as described above), (ii) the same drainage but different predation regimes, (iii) different drainages but the same predation

regime, or (iv) different drainages and different predation regimes. We then fitted a general linear model with the response variable being mean intensity of infection (log transformed) and the explanatory variables being the degree of similarity, predation regime (high versus low) of *Gyrodactylus*, and drainage (Marianne versus Aripo Rivers) source of *Gyrodactylus*. In this analysis we purposely ranked the degree of similarity based on drainage source rather than predation, based on the assumption that host genetic makeup would be more important than the predation environment, but the opposite could also have been explored. Local *Gyrodactylus* adaptation would be inferred relative to ecological difference (is *Gyrodactylus* performance higher on guppies from the same predation regime?) and phylogenetic distance (is *Gyrodactylus* performance higher on guppies from the same drainage source?).

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;

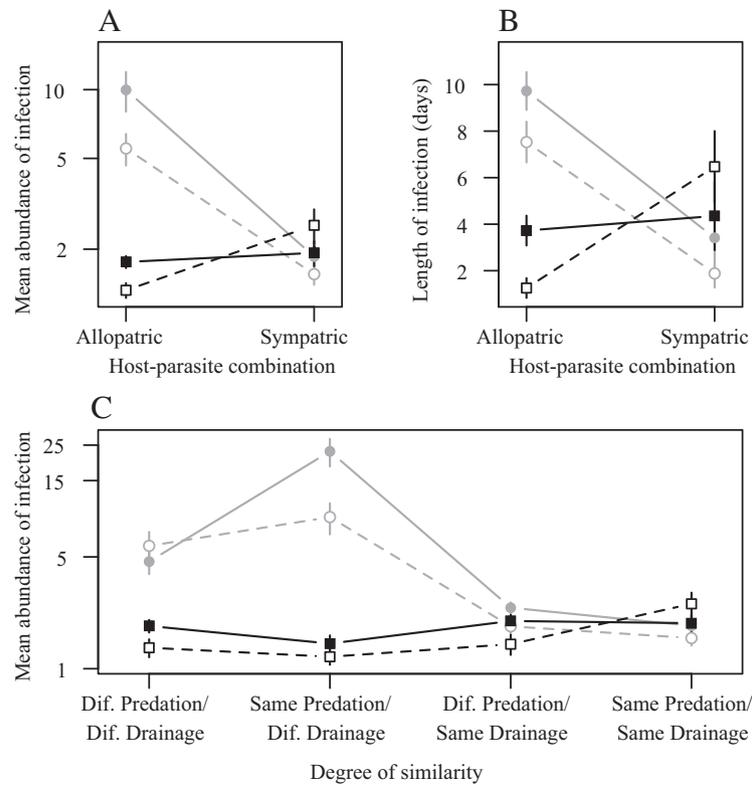


Fig. 2. Least square means for *Gyrodactylus* performance. (A) Mean number of parasites/fish/day when infecting the sympatric host versus all allopatric hosts, (B) mean duration of infection on sympatric versus all allopatric hosts, (C) mean number of parasites/fish/day according to the degree of similarity between the parasite strain and the guppy strain in the mesocosms. Marianne River, Trinidad high predation *Gyrodactylus* (filled black squares), Marianne River low predation *Gyrodactylus* (empty black squares), Aripo River, Trinidad high predation *Gyrodactylus* (filled gray circles) and Aripo River low predation *Gyrodactylus* (empty gray circles). Error bars represent S.E. Dif., different.

Table 1

Statistical analysis for *Gyrodactylus* performance on sympatric versus allopatric guppy populations. Analyses were performed using a linear mixed effects model. *P* values and denominator degrees of freedom (d.f.) were obtained using a Satterthwaite approximation for degrees of freedom.

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

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

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 on 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

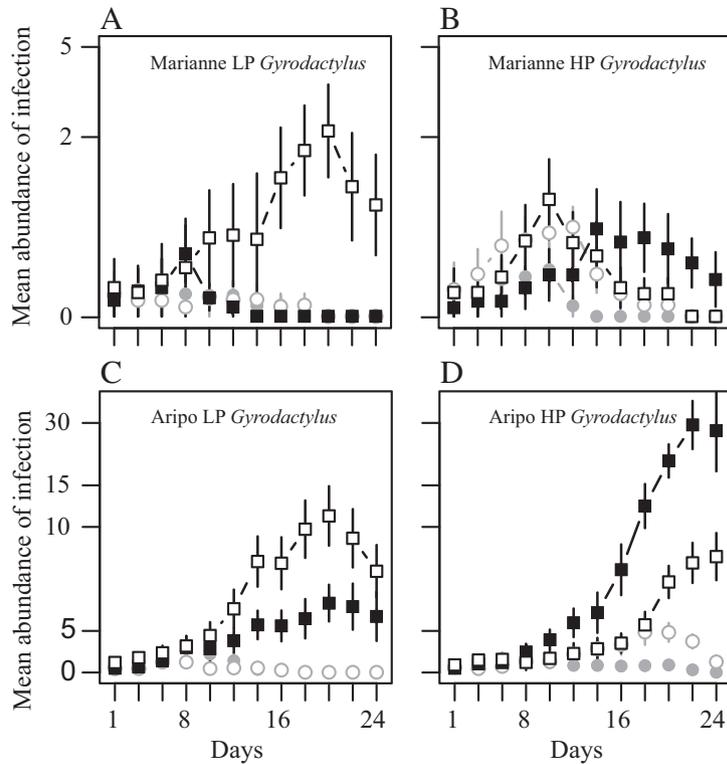


Fig. 3. Population dynamics of (A) Marianne River, Trinidad low predation *Gyrodactylus*, (B) Marianne River high predation *Gyrodactylus*, (C) Aripo River, Trinidad low predation *Gyrodactylus*, and (D) Aripo River high predation *Gyrodactylus* when infecting mesocosm guppies from four field populations: 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.

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.

| Variables | Mean intensity of infection | |
|--|-----------------------------|------------------|
| | F (d.f.) | P |
| <i>Gyrodactylus</i> drainage of origin (Marianne versus Aripo River, Trinidad) | 137.167 (1, 256) | <0.001 |
| <i>Gyrodactylus</i> predation of origin | 15.473 (1, 256) | <0.001 |
| Degree of similarity | 24.672 (3, 256) | <0.001 |
| <i>Gyrodactylus</i> drainage of origin × <i>Gyrodactylus</i> predation regime of origin | 2.045 (1, 256) | 0.153 |
| <i>Gyrodactylus</i> drainage of origin × Degree of similarity | 57.439 (3, 256) | <0.001 |
| <i>Gyrodactylus</i> predation regime of origin × Degree of similarity | 4.289 (3, 256) | 0.005 |
| <i>Gyrodactylus</i> drainage of origin × <i>Gyrodactylus</i> predation regime of origin × Degree of similarity | 4.723 (3, 256) | 0.0031 |

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

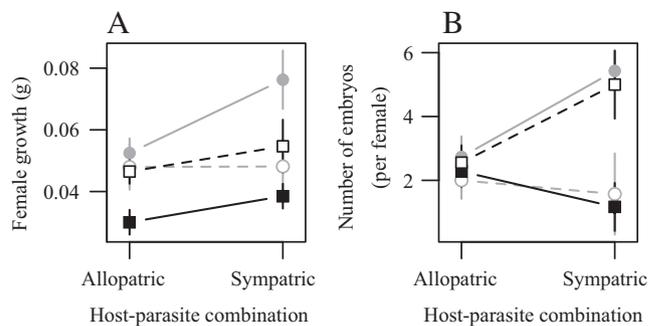


Fig. 4. Least square means for guppy performance when infected with sympatric versus allopatric hosts. (A) Female guppy growth over 23 days, (B) number of embryos per female. Symbols represent guppy populations: Marianne River, Trinidad high predation (filled squares), Marianne River low predation (empty squares), Aripo River, Trinidad high predation (filled circles) and Aripo River low predation (empty circles). Error bars represent S.E.

Table 3

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.

| Fixed effects | Female growth | | Number of embryos | | Reproductive allocation | |
|--|-------------------|--------------|-------------------|--------------|-------------------------|----------|
| | <i>F</i> (d.f.) | <i>P</i> | <i>F</i> (d.f.) | <i>P</i> | <i>F</i> (d.f.) | <i>P</i> |
| Guppy drainage of origin | 7.546 (1, 97.56) | 0.003 | 0.033 (1, 87.99) | 0.855 | 3.123 (1, 87.95) | 0.080 |
| Guppy predation of origin | 0.214 (1, 97.56) | 0.644 | 0.291 (1, 87.99) | 0.590 | 1.052 (1, 87.95) | 0.307 |
| Host–parasite combination | 4.043 (1, 97.56) | 0.047 | 2.574 (1, 87.99) | 0.111 | 0.380 (1, 87.95) | 0.539 |
| Guppy drainage of origin × Guppy predation of origin | 10.349 (1, 97.56) | 0.001 | 8.183 (1, 87.99) | 0.005 | 1.573 (1, 87.95) | 0.213 |
| Random effects | Variance | S.D. | Variance | S.D. | Variance | S.D. |
| <i>Gyrodactylus</i> population | 0.001 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 |
| Residual | 0.005 | 0.022 | 5.865 | 2.422 | 0.032 | 0.179 |

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 maladaptation 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., 1999; Koskela et al., 2000; Lemoine et al., 2012; Roth et al., 2012; Konijnendijk et al., 2013; Sternberg et al., 2013). Our study reduced the methodological limitations of many previous studies, and our data indicate that *Gyrodactylus* does not generally show strong local adaptation to their sympatric guppy 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 maladaptation, 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 (Schelkle 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 Storer, 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 (Willing 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 drainages (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

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-Jvostov 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-Jvostov 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 emphasising 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 (Kaeuffer 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.

Acknowledgments

We would like to thank David Reznick and the NSF FIBR Program (From Genes to Ecosystems) for facilitating use of the experimental channels, valuable suggestions and overall support. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of a Special Research Opportunity Grant to G. Fussmann, A. Hendry, P. Bentzen and M. Scott, NSERC Discovery grant to M. Scott, and a PhD scholarship from the Consejo Nacional de Ciencia y Tecnología (Mexico) to F. Pérez-Jvostov. Research at the Institute of Parasitology, McGill University, is supported by a regroupement stratégique from Fonds Québécois de la recherche sur la nature et les technologies, Canada.

References

- Alizon, S., Hurford, A., Mideo, N., van Baalen, M., 2008. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.* 22, 245–259.
- Altizer, S.M., 2001. Migratory behaviour and host–parasite co-evolution in natural populations of monarch butterflies infected with a protozoan parasite. *Evol. Ecol. Res.* 3, 611–632.
- Anderson, R.M., May, R.M., 1982. Coevolution of hosts and parasites. *Parasitology* 85, 411–426.
- Bassar, R.D., Marshall, M.C., Lopez-Sepulcre, A., Zandona, E., Travis, J., Pringle, C.M., Flecker, A.S., Thomas, S.A., Fraser, D.F., Reznick, D.N., 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *Proc. Natl. Acad. Sci.* 107, 3616–3621.
- Brockhurst, M.A., Koskella, B., 2013. Experimental coevolution of species interactions. *Trends Ecol. Evol.* 28, 367–375. <http://dx.doi.org/10.1016/j.tree.2013.02.009>.
- Brown, G.E., Ferrari, M.C.O., Elvidge, C.K., Ramnarine, I.W., Chivers, D.P., 2013. Phenotypically plastic neophobia: a response to variable predation risk. *Proc. R. Soc. B.* 280, 20122712.
- Cable, J., van Oosterhout, C., 2007a. The impact of parasites on the life history evolution of guppies (*Poecilia reticulata*): the effects of host size on parasite virulence. *Int. J. Parasitol.* 37, 1449–1458.
- Cable, J., van Oosterhout, C., 2007b. The role of innate and acquired resistance in two natural populations of guppies (*Poecilia reticulata*) infected with the ectoparasite *Gyrodactylus turnbulli*. *Biol. J. Linn. Soc.* 90, 647–655.
- Choo, K., Williams, P.D., Day, T., 2003. Host mortality, predation and the evolution of parasite virulence. *Ecol. Lett.* 6, 310–315. <http://dx.doi.org/10.1046/j.1461-0248.2003.00425.x>.
- Dargent, F., Scott, M.E., Hendry, A.P., Fussmann, G.F., 2013. Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proc. R. Soc. B.* 280. <http://dx.doi.org/10.1098/rspb.2013.2371>.
- Dybdahl, M.F., Storfer, A., 2003. Parasite local adaptation: Red Queen versus Suicide King. *Trends Ecol. Evol.* 18 (3), 523–530.
- Ebert, D., 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science* 265, 1084–1086.
- Endler, J.A., 1986. *Natural Selection in the Wild*. Princeton Univ. Press, Princeton, NJ, USA.
- Endler, J.A., 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* 10, 22–29.
- Evans, J.P., Gasparini, C., Pilastro, A., 2007. Female guppies shorten brood retention in response to predator cues. *Behav. Ecol. Sociobiol.* 61, 719–727. <http://dx.doi.org/10.1007/s00265-006-0302-1>.

- Fitzpatrick, S.W., Torres-Dowdall, J., Reznick, D.N., Ghalambor, C.K., Funk, W.C., 2013. Parallelism isn't perfect: could disease and flooding drive a life history anomaly in Trinidadian guppies? *Am. Nat.* 183, 290–300.
- Fraser, B., Neff, B.D., 2009. Parasite mediated homogenizing selection at the MHC in guppies. *Genetica* 138, 273–278.
- Fraser, B., Rannaraine, I.W., Neff, B.D., 2010. Temporal variation at the MHC class IIB in wild populations of the guppy (*Poecilia reticulata*). *Evolution* 64, 2086–2096.
- Gandon, S., Michalakis, Y., 2002. Local adaptation, evolutionary potential and host–parasite coevolution: interactions between migration, population size and generation time. *J. Evol. Biol.* 15, 451–462.
- Gordon, S.P., Reznick, N.D., Kinnison, M.T., Bryant, M.J., Weese, D.J., Rasanen, K., Millar, N.P., Hendry, A.P., 2009. Adaptive changes in life history and survival following a new guppy introduction. *Am. Nat.* 174, 34–45.
- Gosline, A.K., Rodd, F.H., 2007. Predator-induced plasticity in guppy (*Poecilia reticulata*) life history traits. *Aquat. Ecol.* 42, 693–699. <http://dx.doi.org/10.1007/s10452-007-9138-7>.
- Gotanda, K.M., Delaire, L.C., Raeymaekers, J.A.M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M.E., Fussmann, G.F., Hendry, A.P., 2013. Adding parasites to the guppy–predation story: insights from field surveys. *Oecologia* 172, 155–166. <http://dx.doi.org/10.1007/s00442-012-2485-7>.
- Greischar, M.A., Koskella, B., 2007. A synthesis of experimental work on parasite local adaptation. *Ecol. Lett.* 10, 418–434. <http://dx.doi.org/10.1111/j.1461-0248.2007.01028.x>.
- Harris, P.D., Lyles, A.M., 1992. Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) in Trinidad. *J. Parasitol.* 78, 912–914.
- Harris, P.D., Shinn, A.P., Cable, J., Bakke, T.A., 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Syst. Parasitol.* 59 (1), 1–27.
- Hoeksema, J.D., Forde, S.E., 2008. A meta-analysis of factors affecting local adaptation between interacting species. *Am. Nat.* 171, 275–290. <http://dx.doi.org/10.1086/527496>.
- Houde, A.E., 1997. Sex, Color, and Mate Choice in Guppies. Princeton University Press, Princeton, NJ, USA.
- Johnson, M.B., Lafferty, K.D., van Oosterhout, C., Cable, J.C., 2011. Parasite transmission in social interacting hosts: monogenean epidemics in guppies. *PLoS One* 6, e22634. <http://dx.doi.org/10.1371/journal.pone.0022634>.
- Kaeuffer, R., Peichel, C.L., Bolnick, D.I., Hendry, A.P., 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution* 66, 402–418. <http://dx.doi.org/10.1111/j.1558-5646.2011.01440.x>.
- Kaltz, O., Shykoff, J., 1998. Local adaptation in host–parasite systems. *Heredity* 81, 361–370. <http://dx.doi.org/10.1046/j.1365-2540.1998.00435.x>.
- Kaltz, O., Gandon, S., Michalakis, Y., Shykoff, J.A., 1999. Local maladaptation of the plant pathogen *Microbotryum violaceum* to its host *Silene latifolia*: evidence from a cross-inoculation experiment. *Evolution* 53, 395–407.
- Kawecki, T.D., Ebert, D., 2004. Conceptual issues in local adaptation. *Ecol. Lett.* 7, 1225–1241.
- Kearn, G.C., 1994. Evolutionary expansion of the monogenea. *Int. J. Parasitol.* 24, 1227–1271.
- Konijnendijk, N., Raeymaekers, J.A.M., Vandeuren, S., Jacquemin, L., Volckaert, F.A.M., 2013. Testing for local adaptation in the *Gasterosteus–Gyrodactylus* host–parasite system. *Evol. Ecol. Res.* 3, 489–502.
- Koskela, T., Salonen, V., Mutikainen, P., 2000. Local adaptation of a holoparasitic plant, *Cuscuta europaea*: variation among populations. *J. Evol. Biol.* 13, 749–755.
- Lemoine, M., Doligez, B., Richner, H., 2012. On the equivalence of host local adaptation and parasite maladaptation: an experimental test. *Am. Nat.* 179, 270–281. <http://dx.doi.org/10.1086/663699>.
- Lively, C.M., 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. *Am. Nat.* 153, S34–S47.
- Magurran, A.E., 2005. Evolutionary Ecology the Trinidadian Guppy. Oxford Univ. Press, Oxford, UK.
- Morgan, A.D., Gandon, S., Buckling, A., 2005. The effect of migration on local adaptation in a coevolving host–parasite system. *Nature* 437, 253–256. <http://dx.doi.org/10.1038/nature03913>.
- Oppliger, A., Vernet, R., Baez, M., 1999. Parasite local maladaptation in the Canary lizard *Gallotia galloti* (Reptilia: Lacertigae) parasitized by haemogregarian blood parasite. *J. Evol. Biol.* 12, 951–955.
- Packer, C., Holt, R.D., Hudson, P.J., Lafferty, K.D., Dobson, A.P., 2003. Keeping the herds healthy and alert: implications of predator control for infectious disease. *Ecol. Lett.* 6, 797–802.
- Palkovacs, E.P., Marshall, M.C., Lamphere, B.A., Lynch, B.R., Weese, D.J., Fraser, D.F., Reznick, D.N., Pringle, C.M., Kinnison, M.T., 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. Trans. R. Soc. B* 364, 1617–1628.
- Pérez-Jvostov, F., Hendry, A.P., Fussmann, G.F., Scott, M.E., 2012. Are host–parasite interactions influenced by adaptation to predators? A test with guppies and *Gyrodactylus* in experimental stream channels. *Oecologia* 170, 77–88. <http://dx.doi.org/10.1007/s00442-012-2289-9>.
- Reznick, D.N., 1982. The impact of predation on life history evolution in Trinidadian guppies: genetic basis of observed life history patterns. *Evolution* 36, 1236–1250.
- Reznick, D.N., Endler, J.A., 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36, 160–177.
- Reznick, D.N., Bryga, H.A., 1987. Life-history evolution in guppies (*Poecilia reticulata*): 1. Phenotypic and genetic changes in an introduction experiment. *Evolution* 41, 1370–1385.
- Reznick, D.N., Bryga, H.A., Endler, J.A., 1990. Experimentally induced life-history evolution in a natural population. *Nature* 346, 357–359.
- Reznick, D.N., Bryga, H.A., 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). V. Genetic basis of parallelism in life histories. *Am. Nat.*, 339–359.
- Reznick, D.N., Butler IV, M.J., Rodd, F.H., Ross, P., 1996a. Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution* 50, 1651–1660.
- Reznick, D.N., Rodd, F.H., Cardenas, M., 1996b. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. *Am. Nat.* 147, 319–338.
- Reznick, D.N., Shaw, F.H., Rodd, F.H., Shaw, R., 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia Reticulata*). *Science* 275, 1934–1937.
- Richards, E.L., van Oosterhout, C., Cable, J., 2010. Sex-specific differences in shoaling affect parasite transmission in guppies. *PLoS One* 5, e13285. <http://dx.doi.org/10.1371/journal.pone.0013285>.
- Rodd, F.H., Reznick, D.N., 1991. Life history evolution in guppies: III. The impact of prawn predation on guppy life histories. *Oikos* 62, 13. <http://dx.doi.org/10.2307/3545440>.
- Rodd, F.H., Reznick, D.N., 1997. Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology* 78, 405–418.
- Roth, O., Keller, I., Landis, S.H., Salzburger, W., Reusch, T.B.H., 2012. Hosts are ahead in a marine host–parasite coevolutionary arms race: Innate immune system adaptation in pipefish *Syngnathus typhie* against *Vibrio phylotypes*. *Evolution* 66, 2528–2539. <http://dx.doi.org/10.5061/dryad.4h1q3qb2>.
- Saariinen, M., Taskinen, J., 2005. Local adaptation in a crustacean parasite–molluscan host interaction: a field experiment. *Evol. Ecol. Res.* 7, 1191–1199.
- Schekle, B., Faria, P., Johnson, M.B., van Oosterhout, C., Cable, J., 2012. Mixed infections and hybridisation in monogenean parasites. *PLoS One* 7 (7), e39506.
- Schluter, D., 2000. The Ecology of Adaptive Radiation. Oxford Univ. Press, Oxford, UK.
- Scott, M.E., Anderson, R.M., 1984. The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology* 89, 159–194.
- Sternberg, E.D., Li, H., Wang, R., Gowler, C., de Roode, J.C., 2013. Patterns of host–parasite adaptation in three populations of monarch butterflies infected with a naturally occurring protozoan disease: virulence, resistance, and tolerance. *Am. Nat.* 182, E235–E248. <http://dx.doi.org/10.1086/673442>.
- Suk, H.Y., Neff, B.D., 2009. Microsatellite genetic differentiation among populations of the Trinidadian guppy. *Heredity* 102, 425–434. <http://dx.doi.org/10.1038/hdy.2009.7>.
- Thompson, J.N., 1994. Specific hypotheses on the geographic mosaic of coevolution. *Am. Nat.* 153, S1–S14.
- Thompson, J.N., 1999. The evolution of species interactions. *Science* 284, 2116–2118.
- Torres-Dowdall, J., Handelsman, C.A., 2012. Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 66, 3432–3443. <http://dx.doi.org/10.5061/dryad.84gf5>.
- van Oosterhout, C., Harris, P.D., Cable, J., 2003. Marked variation in parasite resistance between two wild populations of the Trinidadian guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Biol. J. Linn. Soc.* 79, 645–651.
- van Oosterhout, C., Mohammed, R.S., Hansen, H., Archard, G.A., McMullan, M., Weese, D.J., Cable, J., 2007. Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int. J. Parasitol.* 37, 805–812.
- Weese, D.J., Gordon, S.P., Hendry, A.P., Kinnison, M.T., 2010. Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution* 64, 1802–1815.
- Willing, E.M., Bentzen, P., van Oosterhout, C., Hoffmann, M., Cable, J., Breden, F., Weigel, D., Dreyer, C., 2010. Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. *Mol. Ecol.* 19, 968–984. <http://dx.doi.org/10.1111/j.1365-294X.2010.04528.x>.