

Parting ways: parasite release in nature leads to sex-specific evolution of defence

F. DARGENT*, G. ROLSHAUSEN†, A. P. HENDRY†, M. E. SCOTT‡ & G. F. FUSSMANN*

*Department of Biology, McGill University, Montreal, QC, Canada

†Redpath Museum, McGill University, Montreal, QC, Canada

‡Institute of Parasitology and Centre for Host-Parasite Interactions, McGill University, Montreal, QC, Canada

Keywords:

enemy release;
experimental evolution;
Gyrodactylus turnbulli;
Poecilia reticulata;
resistance;
sexual dimorphism.

Abstract

We evaluated the extent to which males and females evolve along similar or different trajectories in response to the same environmental shift. Specifically, we used replicate experimental introductions in nature to consider how release from a key parasite (*Gyrodactylus*) generates similar or different defence evolution in male vs. female guppies (*Poecilia reticulata*). After 4–8 generations of evolution, guppies were collected from the ancestral (parasite still present) and derived (parasite now absent) populations and bred for two generations in the laboratory to control for nongenetic effects. These F2 guppies were then individually infected with *Gyrodactylus*, and infection dynamics were monitored on each fish. We found that parasite release in nature led to sex-specific evolutionary responses: males did not show much evolution of resistance, whereas females showed the evolution of increased resistance. Given that male guppies in the ancestral population had greater resistance to *Gyrodactylus* than did females, evolution in the derived populations led to reduction of sexual dimorphism in resistance. We argue that previous selection for high resistance in males constrained (relative to females) further evolution of the trait. We advocate more experiments considering sex-specific evolutionary responses to environmental change.

Introduction

Awareness that populations can evolve rapidly when exposed to novel environments has increased dramatically over the last 15 years (Hendry & Kinnison, 1999; Reznick & Ghalambor, 2001; Merilä & Hendry, 2014). Indeed, ‘contemporary’ (or ‘rapid’) evolution is now a common consideration in basic ecological studies, conservation and management plans, and efforts to improve human well-being (Hendry *et al.*, 2010; Schoener, 2011; Carroll *et al.*, 2014). In most cases, these considerations treat males and females together or analyse only one sex or the other. However, we here show how similar environmental changes can lead to sex-specific responses through which males and females evolve along different trajectories. Such sex-specific

responses to environmental change likely influence overall fitness and population performance.

The extent to which males and females might show different evolutionary responses to the same environmental change is not at all certain (Hendry *et al.*, 2006; Butler *et al.*, 2007). On the one hand, males and females in a given population experience a similar environment and share most of their genetic background – suggesting they should show similar evolutionary responses. On the other hand, males and females can experience a given environment in different ways and do not share their entire genome – suggesting they might show different evolutionary responses. Moreover, the sexes typically exhibit differences in a broad array of behavioural, morphological and physiological traits, and this sexual dimorphism can be extreme (Darwin, 1872; Hedrick & Temeles, 1989; Badyaev, 2002; Delph, 2005). It thus seems plausible that males and females can experience different selective pressures even when they share the same spatial location. Although some studies have examined the sex-specificity of

Correspondence: Felipe Dargent, Department of Biology, McGill University, 1205 Dr. Penfield Av., Montreal, H3A 1B1, QC, Canada.
Tel.: +1 514 835 4235; fax: +1 514 398 5069; e-mail: felipe.dargent@mail.mcgill.ca.

evolutionary responses to environmental differences (Hendry *et al.*, 2006; Butler *et al.*, 2007; Kitano *et al.*, 2012), none has considered sex-specific responses to shifts in parasitism, which is the focus of our study.

Hosts that move into new environments are often exposed to a new parasite community, which can lead to infections by new parasite species (Ostfeld & Keesing, 2000; Kelly *et al.*, 2009; Mastitsky & Veres, 2010; Frankel *et al.*, 2015). Movement to new environments can also lead to the loss of some parasites, such as through sampling effects or the absence of other conditions required by parasites (e.g. definitive/final hosts) (Torchin *et al.*, 2003). These shifts in selection (gains or losses of parasites) should cause evolutionary responses by hosts, as has indeed been observed in many instances (Schmid-Hempel, 2011). For example, populations that are more heavily parasitized often evolve increased resistance (Marshall & Fenner, 1958; Roy & Kirchner, 2000; Boots *et al.*, 2009) – presumably because infection reduces survival (Anderson & May, 1978), fecundity (Herre, 1993) or mating success (Hamilton & Zuk, 1982). By contrast, populations that are less heavily parasitized can evolve reduced resistance (Duncan *et al.*, 2011) – presumably because resistance mechanisms can reduce fitness in the absence of parasites through immunopathology (Graham *et al.*, 2005; Sorci *et al.*, 2013), physiological costs of maintaining immune responses (Lochmiller & Deerenberg, 2000) or antagonistic pleiotropy such as trade-offs with fecundity (Boots & Begon, 1993).

To what extent will males and females evolve similarly or differently to such shifts in parasitism? On the one hand, the sexes can be similarly influenced by parasites. As the genes that influence resistance are often found on autosomes (Murphy *et al.*, 2008), a shift in parasite pressure might lead to similar evolutionary responses in males and females. On the other hand, the sexes often experience different parasite levels, have different costs of infection, and can have different costs of defence (Folstad & Karter, 1992; Zuk & McKean, 1996; Forbes, 2007; Nunn *et al.*, 2009). The result might be sex-specific evolutionary responses to shifts in parasitism. Our goal was to experimentally discriminate between these two plausible alternatives.

Our study

Inferences regarding the evolution of parasite resistance are usually based on field surveys (Jokela *et al.*, 2003; Bonneaud *et al.*, 2011) or laboratory experiments (Schulte *et al.*, 2010; Duncan *et al.*, 2011; Koskella *et al.*, 2012). Although informative, these approaches are limited in several respects. Field surveys are limited because it is difficult to (i) infer causation without experimental manipulations, and (ii) infer rates and directions of evolution when the ancestral state is uncertain. By contrast, laboratory studies are limited because organisms are

removed from the natural context, which could strongly alter evolutionary trajectories. Valuable insights therefore can be gained by combining these two approaches through experimental manipulations in nature, which are rare in general (Reznick & Ghalambor, 2005) and seemingly absent for evolutionary responses to parasite pressure. A very important feature of this methodological hybrid – one in which we were especially interested – is that field manipulations assess evolutionary outcomes in the presence of complicating and covarying factors typical of the natural world. Although the presence of these factors inevitably reduces the precision of inferences regarding mechanisms, it greatly increases the ability to infer what happens when environments change *in nature*.

We implement this field experimental evolution approach by comparing guppies (*Poecilia reticulata*) from a source (ancestral) population where a common and deleterious parasite (*Gyrodactylus* spp) is present to their descendants in four independent parasite-absent experimental field introduction (derived) populations. Ancestral and introduction sites also differed in that the ancestral site had high levels of guppy predators whereas the introduction sites were predator-free (as described in more detail in the Materials and Methods, and Discussion). After 4–8 generations of evolution in these novel environments, we used individual-level laboratory infections to assess resistance to *Gyrodactylus turnbulli*. We previously analysed some data from this experiment, showing that females rapidly and repeatably evolved increased resistance to *Gyrodactylus* after this parasite is removed (Dargent *et al.*, 2013a). This apparently counter-intuitive result reveals the importance of performing manipulations in nature because it shows how the outcomes typical of laboratory experiments can be strongly altered in the complex natural world. In this study, we delve further into the experiment to compare the evolutionary responses of males and females.

We organize our analysis around five questions (Table 1). First, we test for sexual dimorphism in resistance in the ancestral population because the initial state will likely shape evolution in altered environments. Second (for females) and third (for males), we explore the extent to which resistance has evolved across the four replicate experimental introductions. Fourth, we evaluate sexual dimorphism in the derived populations, thus informing how dimorphism has evolved from the ancestral state. Finally, we quantitatively compare the evolutionary trajectories of males and females in the trait space defined by two resistance traits.

Materials and methods

All field procedures were approved by the Trinidad and Tobago Ministry of Fisheries. All laboratory procedures were in accordance with animal care guidelines of McGill University (AUP-5759).

Table 1 Planned comparisons representing specific hypotheses to test for sex-specific evolutionary responses.

Question	Description	Comparison
Q1	Is resistance sexually dimorphic in the source population?	Males vs. females of the Guanapo source
Q2	Did females evolve resistance in the introduced populations?	Introduced females by population vs. Guanapo source females
Q3	Did males evolve resistance in the introduced populations?	Introduced males by population vs. Guanapo source males
Q4	Is resistance sexually dimorphic in the introduced populations?	Males vs. females within introduced populations
Q5	Did the sexes evolve resistance in a similar way?	Change in males vs. change in females from the introduced populations

The set of questions addresses the sex-specificity of evolution under our experimental scenario where we removed parasites and introduced guppies from an ancestral population [Guanapo source (S)] into four tributary streams [Caigual (C), Lower Lalaja (L), Taylor (T) and Upper Lalaja (U)]. The right column lists the planned comparisons of resistance levels between the sexes or populations that need to be conducted to answer the question.

The guppy–*Gyrodactylus* host–parasite system

The guppy is a sexually dimorphic poeciliid fish that has served as an important model for evolutionary studies. In particular, experimental introductions to novel environments have frequently revealed the rapid parallel evolution of behavioural, morphological, and life-history traits (Endler, 1995; Magurran, 2005). Although most work has focused on predation environments, guppies are also known to respond evolutionarily to parasites, specifically the predominantly host-specific monogenean worms *G. turnbulli* and *Gyrodactylus bullatarudis* that are the most prevalent macroparasites of wild guppies (Harris & Lyles, 1992) and dominate the otherwise scarcely diverse parasitic fauna of this fish species (Cable, 2011). These parasites attach to the scales of their host and are transmitted during host-to-host contact (Bakke *et al.*, 2007). *Gyrodactylus* spp. give birth to flukes with fully developed embryos ‘*in-utero*’, which generates parasite epidemics in guppy populations (Scott & Anderson, 1984).

The necessary elements for adaptation by guppies to *Gyrodactylus* have been demonstrated in previous work. First, *Gyrodactylus* are an important selective pressure: (i) they cause high mortality in the field (van Oosterhout *et al.*, 2007) and the laboratory (Cable & van Oosterhout, 2007), (ii) they cause lesions that can serve as entry points for bacterial and fungal secondary infections (Cusack & Cone, 1986; Thoney & Hargis, 1991), (iii) they affect guppy mate choice (Houde & Torio,

1992; Lopez, 1998; Kolluru *et al.*, 2009), and (iv) they reduce the ability of guppies to maintain their position during floods (van Oosterhout *et al.*, 2007). Second, individual guppies differ in their ability to reduce or eliminate gyrodactylid infections (resistance), and this variation has genetic (Fraser & Neff, 2009; Fraser *et al.*, 2010b) and nongenetic (Scott, 1985; Kolluru *et al.*, 2006; Dargent *et al.*, 2013b) components. Consistent with these properties, natural guppy populations differ in their resistance to *Gyrodactylus* infection (Perez-Jvostov *et al.*, 2012; Gotanda *et al.*, 2013) and in the MHC genes that influence this resistance (Fraser & Neff, 2009; Fraser *et al.*, 2010a). Although evidence exists that male and female guppies differ in their resistance to *Gyrodactylus* (Gotanda *et al.*, 2013), it is not known how these differences might influence adaptive divergence between populations and the sexes.

Experimental introductions

In 2008, juvenile guppies were collected with butterfly nets from a site in the Guanapo River (‘Guanapo source’ $-10^{\circ}38'23''N$, $61^{\circ}14'54''W$ and $10^{\circ}39'14''N$, $61^{\circ}15'18''W$) in Trinidad, as part of a large-scale translocation project (Pennisi, 2012; Travis *et al.*, 2014). To create a *Gyrodactylus*- and pathogen-free environment at the introduction sites and avoid the spread of potential infections among collected individuals, all guppies were quarantined and treated for a wide spectrum of pathogens. Treatments included Fungus Eliminator (Jungle Labs, Cibolo, TX, USA), Clout (Sentry AQ Mardel, Omaha, NE, USA), and commercial versions of erythromycin and minocycline (Maracyn and Maracyn Two – Sentry AQ Mardel). The field-collected guppies were maintained separated by sex so that they remained virgin until 3 weeks prior to the introductions when the then mature guppies were mated (i.e. males internally fertilized females) in tanks of five males and five females (mating groups). In March (dry season), these field-collected guppies were introduced into two previously guppy-free upstream tributaries (Lower Lalaja and Upper Lalaja) of the Guanapo River; no major predators of guppies were present in these tributaries. The females of a given ‘mating group’, carrying F1 embryos, were introduced into a different stream than the males from the same ‘mating group’ to maximize effective population size. Of these individuals, 37 and 38 of each sex were introduced into the Lower Lalaja and Upper Lalaja sites, respectively. In 2009, this process was repeated with newly collected Guanapo source guppies that were introduced into two additional guppy-free (and predator-free) upstream tributaries (Taylor and Caigual) of the Guanapo River. On this occasion, 51 and 64 guppies of each sex were introduced into the Taylor and Caigual sites, respectively.

The four tributaries used for the introductions differed from the Guanapo source environment in several

ways. First, the Guanapo source site had a diversity of parasites (including *Gyrodactylus* spp.), whereas the introduction sites were free of guppies and therefore of their host-specific parasites (including *Gyrodactylus* spp.). Furthermore, the antipathogen treatments for the introduced populations were effective in that no presence of *Gyrodactylus* has been reported at these sites which were monthly surveyed for other studies. Second, the Guanapo source site had several important predatory fishes (including the pike cichlid, *Crenicichla frenata*), whereas the introduction sites had only the weakly predatory, gape-limited fish *Rivulus hartii* (Gilliam *et al.*, 1993). Such relaxation in the strength of predation could potentially be an indirect driver of defence evolution mediated through changes in life-history traits (Sheldon & Verhulst, 1996; Dargent *et al.*, 2013a). Nonetheless, it would seem more parsimonious to expect changes in parasitism, rather than in predation, to be the primary cause of the evolution of resistance to parasites, in particular when both selective pressures are relaxed. Third, the Guanapo source site had a more open forest canopy and presumably higher resource availability for guppies than did the introduction sites (Kohler *et al.*, 2012; Handelsman *et al.*, 2013). The different introduction sites were reasonably similar in these properties, except that two (Upper Lalaja and Taylor) had more open canopies, and therefore higher productivity, as a result of experimental canopy thinning (Kohler *et al.*, 2012). More details on the experimental introductions and sites can be found in Kohler *et al.* (2012), Dargent *et al.* (2013a), Handelsman *et al.* (2013), López-Sepulcre *et al.* (2013), Ruell *et al.* (2013), Arendt *et al.* (2014), and Travis *et al.* (2014).

Field collections

Field collections were performed in 2010, 2 years (approximately eight guppy generations) after the introductions into the Upper and Lower Lalaja and 1 year (approximately four guppy generations) after the introductions into the Taylor and Caigual. Guppies were also collected at this time from the Guanapo source population, which continued to be infected with *Gyrodactylus* spp (Gotanda *et al.*, 2013; F. Dargent, pers. obs.). All collections were made with butterfly nets, and the fish were immediately treated with Fungus Eliminator, Clout, Maracyn and Maracyn Two. The guppies were held for about 3 months in Trinidad in population- and sex-specific tanks, before being transported in plastic bottles by airplane to the C. Ghalambor laboratory at Colorado State University. In the laboratory, the guppies were housed in population- and sex-specific tanks following Reznick (1982) with two modifications, (i) aquatic housing systems were used to standardize water quality, and (ii) the tanks were smaller (1.8 L).

To control for plastic and maternal effects, field-collected guppies were bred for two generations under

parasite-absent common garden conditions in the laboratory. When the field-collected guppies reached sexual maturity, each female was randomly mated to a field-collected male from the same site (males were not reused) to produce a first laboratory generation (F1). This F1 generation was then raised as described above before being transferred at 84–140 days of age to our laboratory at McGill University. At McGill, the guppies were mated as described above to produce a second (F2) laboratory generation. All rearing procedures at McGill were the same as those at Colorado State except for a small change of diet. At Colorado State, the guppies were fed brine shrimp and paste made from Tetra-min Tropical Flakes (Tetra, Melle, Germany) [following Reznick (1982)]. At McGill, we replaced the brine shrimp component with the same volume of paste.

Experimental infections

Infection trials for the F2 guppies took place after they were at least 12 weeks old. For each trial, experimental guppies were individually isolated for 7 days prior to infection and for 24 days after initial infection. This isolation took place in 1.8-L containers in an Aquaneering Inc. aquatic housing system (San Diego, CA, USA), with filters to prevent any movement of parasites, food or waste products between tanks. During isolation, each guppy was fed a daily diet of 10 μ L paste of Tetra-min Tropical Flakes. For infection, each individual was first anaesthetized in 0.02% MS222 (Tricaine Methanesulfonate – Argent Chemical Laboratories, Redmond, WA, USA) buffered to a neutral pH with NaCO₃. Each guppy was then weighed to the nearest 0.0001 g, photographed for size [standard length (SL)] measurements in ImageJ, and infected with two *G. turnbulli* (details below). We infected a total of 63 male and 129 female guppies (Table S1). We used a *G. turnbulli* strain that was novel to all the populations. This choice was made for two reasons. First, guppies showed conserved resistance differences among populations when infected with different *Gyrodactylus* strains (Cable & van Oosterhout, 2007; Pérez-Jvostov *et al.*, 2015); second, it avoided the possibility that host–parasite co-evolutionary dynamics, happening exclusively in the source population would influence our results. The benchmark parasites we used were an isogenic strain isolated from – and propagated on – local (Montreal, Canada) pet store guppies.

All guppies were mature and unmated at the time of the experiments. The infections were started by removing scales carrying the parasite from a donor guppy held in a separate container (Scott, 1982), and then placing this scale next to the anaesthetized recipient's caudal fin. This procedure routinely succeeded in transferring two parasites within 5 min. Afterwards, each guppy was allowed to recover and was then returned to its isolation tank. We then monitored parasite popu-

lation growth on each isolated guppy for 24 days or until the guppy died. This monitoring was achieved by – every 2 days – anaesthetizing each guppy and counting its parasites using a dissecting scope at 18× magnification.

Analysis

We first analysed whether the populations or sexes differed in body size, health or mortality. For body size, we used ANOVA with population and sex as factors and SL (distance from the tip of the snout to the end of the caudal peduncle) as the measure of size. As a proxy of fish health at the start of infection, we used the relative condition index (K_n) following Le Cren (1951), where, $K_n = \text{mass}/a(\text{size})^b$ and a and b are the intercept and slope of a least squares regression of log-mass on log-body size across all individuals of a given sex. To assess condition differences, we used an independent ANOVA for each sex with population of origin as a factor. For mortality, we fitted a generalized linear model (GLM) with a binomial response variable (died vs. survived) and a logit link function, with population and sex as predictors. We also tested whether guppies that died differed in their day of death by fitting a GLM with ln-transformed day of death as a response variable and population and sex as predictors. Finally, we performed a chi-square test of independence to assess whether mortality of *Gyrodactylus*-infected individuals differed from that of sham-infected control guppies (females $n = 46$, males $n = 28$).

Measuring resistance

We next analysed two measures of parasite resistance that are used commonly in studies of host–*Gyrodactylus* dynamics (Cable & van Oosterhout, 2007; de Roij *et al.*, 2011; Dargent *et al.*, 2013a). ‘Peak load’ is the maximum number of parasites counted on an individual guppy on any day during the experiment. Analyses based on this measure included individuals that died before the end of the experiment because mortality generally occurred when parasite load had already stabilized or started to decrease. ‘Intrinsic rate of increase’ (r) of the parasite population was estimated as the slope of the regression of ln-transformed *Gyrodactylus* counts between day 0 and day 10 on an individual guppy. Analyses based on this measure included only individuals that survived to at least day 10. Within this framework, lower peak load and lower r represent higher resistance as they indicate a higher capability of the guppies to limit infection.

Resistance (Questions 1–4)

To test whether population and sex influenced parasite peak load and r , we used GLMs. Models included population and sex as factors, size as a covariate and interactions between population and both sex and size. Peak load was modelled with GLMs using a negative

binomial error distribution and the log link function. All other data were modelled with GLMs using the identity link function. Analysis of each model was followed by a series of planned contrasts that specifically informed our questions (Table 1 – ‘Comparison’ column).

Sex-specific evolution (Question 5)

The degree of similarity (i.e. geometric parallelism) in the evolution of resistance between the sexes and populations was assessed by (i) graphical interpretation of divergence in sex trait space and (ii) phenotypic change vector analysis (PCVA) (Adams & Collyer, 2009; Collyer & Adams, 2007) of evolutionary trajectories in multivariate resistance trait space (i.e. orientation of divergence). For the graphical interpretation, relative change in resistance by sex was represented as trait divergence between the source and the introduced populations with change for each sex on a different axis. The source population values were centred to a mean of 0, and the values of the introduced populations were recalculated relative to the new source mean. A 1 : 1 line was used to depict the trajectory of equal amount of change in trait value for both sexes (i.e. similar evolutionary trajectories). For the PCVA, the sex-specific population centroids used in this analysis were multivariate least squares means derived from MANCOVA models with the predictor variable being population contrasts (source vs. each introduced population) and the response variables being r and ln peak load. The respective change vectors (connecting each introduced population to the source) were then compared to each other with respect to their orientation (angle between vectors). Statistical significance for these comparisons was based on permutation of model residuals to obtain $n = 1000$ randomized datasets (Adams & Collyer, 2009; Collyer & Adams, 2007). All statistical procedures were conducted in R 3.0.2 (R Development Core Team 2014).

Results

General effects

Body size (SL) of the experimental guppies did not differ among populations, except that Lower Lalaja guppies were larger than Upper Lalaja guppies (Tables S1 and S2, Figure S1). Female guppies were larger than male guppies, and this sexual dimorphism was similar among populations (nonsignificant interaction between population and sex – Table S2). Guppy health, measured as relative condition index (K_n), did not differ among populations (males: $F_{4,58} = 1.15$, $P = 0.34$; females: $F_{4,124} = 1.92$, $P = 0.11$). *Gyrodactylus*-induced mortality was higher in both sexes relative to sham-infected guppies (63.5% vs. 10.7% in males: $\chi^2 = 19.6$, d.f. = 1, $P < 0.001$; and 82.2% vs. 5.4% in females: $\chi^2 = 88$, d.f. = 1, $P < 0.001$), and infection with *Gyrodactylus* induced higher mortality in female than male

guppies (Table S3). Although the maximal model also included population (results not shown), its effects were not significant and so population was removed from the final model. Among the guppies that died, day of death was not explained by either population, sex or their interaction (Table S4). A survival analysis using Cox proportional hazards led to the same conclusion (Table S5). Note that similarity among populations in overall health and parasite-induced mortality aided our subsequent ability to uncover differences in parasite load because differences in parasite peak load were not then due to a greater accumulation of parasites on individuals that were in poor health at the start of infection or that survived for longer periods of time.

Resistance (Questions 1–4)

Population, sex and their interaction all had strong effects on *Gyrodactylus* peak load and intrinsic rate of increase (r), as did body size on peak load (Table 2). We therefore performed planned comparisons that specifically answered our initial questions about similar or sex-specific evolution of resistance (Table 1). These comparisons were based on simplified models that excluded body size because (i) body size effects were inconsistent (Tables 2, S1 and S6, Figure S1), and (ii) model simplification through stepwise deletion tended to drop size from all models (except for female peak load). Nonetheless, including body size as a covariate generally did not qualitatively change the results (Table S7).

Answer 1 – On sexual dimorphism in the source population: guppies from the Guanapo source population were sexually dimorphic in resistance – males had

higher resistance (lower peak load and lower r) than females (Table 3). Answer 2 – On female evolution: females from three of the four introduced populations had higher resistance (lower peak loads and lower r) than females from the Guanapo source population (Table 3), indicating the evolution of increased resistance in the absence of parasite pressure (as earlier reported by Dargent *et al.* (2013a)). Answer 3 – On male evolution: in contrast to the higher resistance that had evolved in females from three of the four introduced populations (Dargent *et al.*, 2013a), male resistance to parasites did not differ between the introduced populations and the Guanapo source population for r , and only differed in one case for peak load (Table 3). The exception was that Taylor males had a higher peak load (i.e. decreased resistance) than Guanapo source males. Together, these results indicate lack of evolution of resistance in male guppies in the absence of parasites. Answer 4 – On sexual dimorphism in the introduced populations: of the three populations in which females evolved increased resistance, only one (Lower Lalaja) showed a difference in peak load between males and females (Table 3), and none of the three populations showed a difference between males and females in r (Table 3). In the fourth introduced population (Cai-gual), neither females nor males evolved and sexual dimorphism was maintained in both r and peak load. Overall, sexual dimorphism in resistance decreased in the introduced populations as females evolved to become more similar to the ancestrally more-resistant males (Fig. 1a), which did not themselves show any (except Taylor for peak load) post-introduction evolution of resistance.

Sex-specific evolution or the lack thereof (Question 5)

To more formally assess the degree of similarity in the evolution of resistance between the sexes and populations, we performed two analyses. The first analysis considered trait divergence between the source and introduction populations in male (y -axis) vs. female (x -axis) trait space. This analysis most directly evaluates the relative amount of change in males vs. females within populations (Fig. 2), which thus informs the extent to which the sexes evolved in a similar or independent manner. In this analysis, three of the four introduced populations showed resistance trait changes that did not overlap with the 1 : 1 line, thus confirming sex-independent evolution.

The second analysis considers evolutionary trajectories in resistance trait space (Fig. 1), which assesses both similarity, or not, between the sexes (as above) and between the populations within sexes. In this analysis, visual inspection showed that females from the four introduced populations shared the same orientation of phenotypic change vectors, indicating a clear pattern of parallel evolution among female populations, towards

Table 2 Generalized linear model for *Gyrodactylus* peak load and intrinsic rate of increase (r) on *Poecilia reticulata*.

Response	Independent variables				
		d.f.	Mean sq	F	P -value
Peak load	Population	4	87 392	5.38	< 0.001
	Sex	1	107 815	6.64	0.011
	Size	1	294 972	18.15	< 0.001
	Population : Sex	4	59 945	3.69	0.007
	Population : Size	4	31 981	1.97	0.101
	Residuals	177	16 249		
r	Population	4	0.015	4.36	0.002
	Sex	1	0.13	37.22	< 0.001
	Size	1	0.002	0.66	0.418
	Population : Sex	4	0.009	2.69	0.033
	Population : Size	4	< 0.001	0.17	0.954
	Residuals	159	0.003		

Generalized linear model results for peak load (integer variable with a negative binomial distribution) and intrinsic rate of increase (continuous variable with a normal distribution) of *Gyrodactylus turnbulli* on *Poecilia reticulata* with host population and sex as factors and guppy standard length as a covariate.

Table 3 Planned contrasts of *Gyrodactylus* peak load and intrinsic rate of increase (r) by guppy population and sex.

Question	Comparison	Peak load†	r ‡
Q1	S♀ vs. S♂	-3.84***	-4.42***
Q2	S♀ vs. C♀	-1.11	-1.43
	S♀ vs. L♀	-3.8***	-3.83***
	S♀ vs. T♀	-2.75**	-3.19**
	S♀ vs. U♀	-3.95***	-3.36**
Q3	S♂ vs. C♂	0.22	-1.32
	S♂ vs. L♂	-1.91	-0.86
	S♂ vs. T♂	2.98**	0.65
	S♂ vs. U♂	-0.25	-0.22
Q4	C♀ vs. C♂	-2.42*	-4.93***
	L♀ vs. L♂	-3.78***	-2.27§
	T♀ vs. T♂	1.81	-0.83
	U♀ vs. U♂	-1.08	-1.88

Planned comparisons with generalized linear models for peak load (integer variable with a negative binomial distribution) and intrinsic rate of increase (r – continuous variable with a normal distribution) of *Gyrodactylus turnbulli* on *Poecilia reticulata*. Comparisons test whether peak load or r : [Q1] was sexually dimorphic in the ancestral population; [Q2] evolved in the introduced females; [Q3] evolved in the introduced males; and [Q4] was sexually dimorphic in the introduced populations.

Abbreviations for population names: Guanapo source (S) population, and Caigual (C), Lower Lalaja (L), Taylor (T), and Upper Lalaja (U) introduced populations.

† z -values reported for peak load and

‡ t -values for r .

§Nonsignificant after FDR correction.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

higher resistance than the Guanapo source population and towards the level of resistance in the ancestral male position (Fig. 1a). Consistent with the visual inspection, the angles of female trajectories in trait space did not differ among the introduced populations in the PCVA (Table 4A). Furthermore, variation around the mean female phenotypic trait spaces only overlapped between the Caigual introduced population and the Guanapo source population, consistent with the lack of resistance evolution in these females (Fig. 1b). Males showed a very different pattern, with visual inspection implying divergent evolution among populations (i.e. different orientations of phenotypic change vectors). However, only two of six paired comparisons of male phenotypic vector orientations among the four introduced populations were significant (Table 4A), presumably owing to high variation in resistance traits among males (Fig. 1c). Visual inspection (and the first analysis above) indicated differences between males and females in the orientation of phenotypic trait vectors, although this was statistically significant for guppies from the Taylor introduced population only (Table 4B) – for the same reason (high variation in males). Thus, the vector analysis had sufficient power to detect divergence in evolution when substantial evolution had occurred but not when little evolution had occurred.

Discussion

In the ancestral population (Guanapo source), resistance to infection by *Gyrodactylus* parasites was greater for male than for female guppies (Question 1 in Table 1). After release from parasite pressure through replicate experimental introductions, the evolution of resistance proceeded in a sex-specific manner (Question 5). In particular, males did not show much (if any) evolution of increased or decreased resistance (Question 3), whereas females generally evolved increased resistance (Question 2). The end result was an evolutionary reduction of sexual dimorphism in resistance to parasite infection (Question 4). Our main focus will be on potential causal explanations for the observed sex-specific evolution, but we must first consider the possibility that the observed sexual dimorphism – and hence sex-specific evolution – could be a correlated effect of other differences between the sexes.

Male and female guppies differ in a number of ways that might influence parasite loads. First, female guppies might owe their higher *Gyrodactylus* numbers in nature (Gotanda *et al.*, 2013) to being more gregarious than males (Richards *et al.*, 2010), which should promote parasite transmission. Second, lower *Gyrodactylus* loads on males in nature might result from greater (relative to females) infection-induced mortality caused by predation or flooding (van Oosterhout *et al.*, 2007), which would truncate the accumulation of parasites by truncating the life of heavily infected hosts. Third, studies in other systems have suggested that ecological differences between the sexes, such as different diets or microhabitat use, could lead to sexual dimorphism in parasite loads (Reimchen & Nosil, 2001). While the above effects could have altered the selective environment of the field populations, none of them are relevant to our laboratory assays because (i) the fish were isolated and so parasite transmission was not possible, (ii) predators and flooding were absent, and (iii) the sexes had similar diets and habitats. Finally, differences in body size can influence parasite loads (Cable & van Oosterhout, 2007), but the larger size of females did not in our study explain their higher parasite loads (and therefore lower resistance) (Table 2, Tables S6 and S7). These observations suggest that sexual dimorphism in resistance is not simply a correlated effect of (at least some) other differences between the sexes.

The key question that thus emerges is as follows: why should resistance evolve differently for males than for females when both are similarly and totally released from exposure to a common and deleterious parasite? One possibility is that parasite removal, although experienced by both sexes, might have led to different strengths of relaxed selection for males vs. females. This argument starts from the realization that higher resistance of males than females in the ancestral population implies males have historically been under stronger

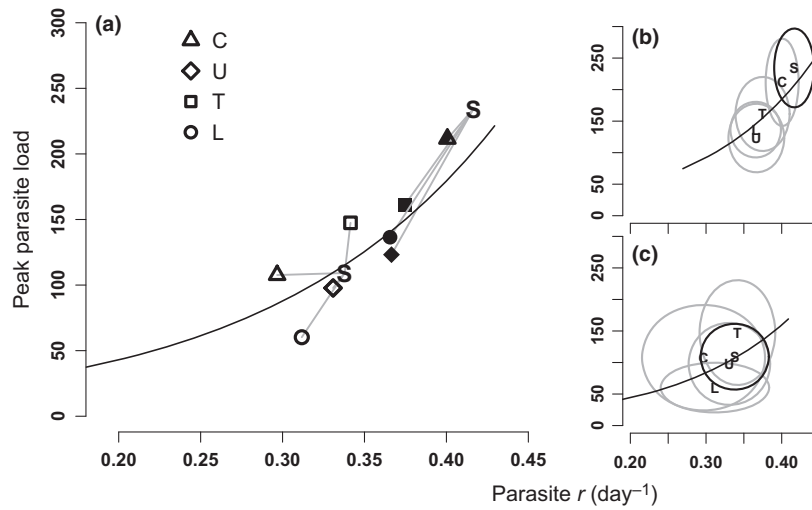


Fig. 1 Evolutionary divergence for males and females in resistance trait space. (a) For each of the four introduced populations (C, L, T and U), the evolutionary trajectory within the sexes (males: empty symbols; females: filled symbols) is depicted as the line connecting the source population centroid (S) to the introduced population centroids. Panels b (females) and c (males) show the same data for each sex separately, with letters depicting population centroids and ellipses representing the 75% data spread. Curved trajectories in each of the three panels depict the negative binomial generalized linear model fit to the raw data to further illustrate the evolutionary trajectories overall (a), for females (b) and for males (c).

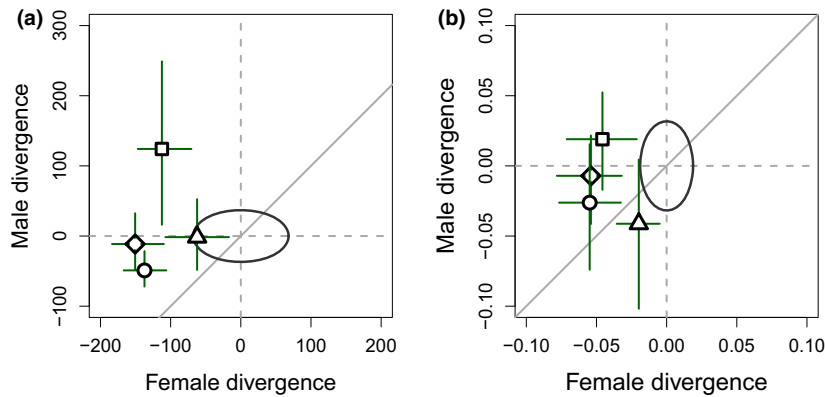


Fig. 2 Symmetry plots of the evolution of resistance by sex. Evolution of parasite resistance for females (x -axis) and males (y -axis) in the four introduced populations (C, L, T and U; symbols as in Fig. 1) based (a) on the mean number of *Gyrodactylus* at peak load, and (b) on the mean intrinsic rate of *Gyrodactylus* increase from the start of infection to day 10 of infection. In each case, population means are shown relative to the Guanapo source population mean (the origin in each graph); for example, intrinsic rates of increase of parasites on Taylor female guppies were on average 0.046 day^{-1} lower than those on Guanapo Source females but 0.019 day^{-1} higher when the respective male populations were compared with one another. Points depict population means with 95% CIs, and ellipses depict 95% CIs for the source mean (CIs based on $n = 1000$ bootstrap samples). The 1 : 1 line represents the absence of sex-specific evolution, that is, where both males and females would have experienced identical changes. Nonoverlap of the introduced population CIs with the 1 : 1 line suggests that the sexes in a given population evolve resistance in a sex-specific manner.

selection than have females. This idea meshes with observations that (i) males invest much more of their time and energy into sexual activities – as opposed to feeding – than do females (Magurran & Seghers, 1994; Griffiths, 1996) and thus should be less able to increase resource acquisition to combat infection, (ii) males are more likely to be preyed upon than females (Weese

et al., 2011) and infection-induced morbidity could make them even more susceptible, (iii) infected males are more likely than females to be displaced downstream during flooding events (van Oosterhout et al., 2007), and (iv) infected males (but presumably not females) have reduced mating opportunities (Houde & Torio, 1992). However, the idea does *not* mesh with

Table 4 Vector angles of resistance evolutionary trajectories.

Comparison		Angle (°)	P-value
(A) Between populations			
Males	Caigual vs. Taylor	164.5	0.122
	Lower Lalaja vs. Caigual	13.6	0.376
	Lower Lalaja vs. Taylor	178.1	0.007
	Lower Lalaja vs. Upper Lalaja	1.3	0.945
	Upper Lalaja vs. Caigual	14.9	0.652
	Upper Lalaja vs. Taylor	179.4	0.007
Females	Caigual vs. Taylor	3.1	0.908
	Lower Lalaja vs. Caigual	1.6	0.92
	Lower Lalaja vs. Taylor	4.7	0.712
	Lower Lalaja vs. Upper Lalaja	4.1	0.648
	Upper Lalaja vs. Caigual	5.7	0.726
	Upper Lalaja vs. Taylor	8.8	0.463
Population		Angle (°)	P-value
(B) Within populations (males vs. females)			
Caigual		1.8	0.953
Lower Lalaja		17	0.136
Taylor		165.2	0.045
Upper Lalaja		14.9	0.303

Quantification of evolutionary trajectories in resistance trait space based on measures of orientation (vector angles in degrees) derived from phenotypic change vector analysis. (A) Comparisons of female and male trajectories within each of the introductions relative to the source populations. (B) Comparisons between populations within the sexes. Observed significance levels are based on empirically generated ($n = 1000$) residual permutations (Adams & Collyer, 2009; Collyer & Adams, 2007).

results from our experiment. Specifically, theory (Boots *et al.*, 2009) predicts that, under the above conditions, males released from parasitism should have evolved decreased resistance (assuming resistance is costly) and females should have evolved in the same direction but less rapidly because they begin with a lower level of resistance. By contrast, we found that males did not evolve increased or decreased resistance at all (except Taylor males for peak load) and females evolved increased resistance (Fig. 1).

Another possible explanation for our results is that the shift in parasitism was accompanied by a shift in other selective forces that shaped resistance evolution. Indeed, our experimental populations also experienced a decrease in predation and changes in productivity relative to the ancestral populations (Kohler *et al.*, 2012; López-Sepulcre *et al.*, 2013; Travis *et al.*, 2014). Predation, in particular, is known to exert a very large influence on guppy evolution (Endler, 1995; Magurran, 2005), and we previously argued that the evolution of increased resistance in females following parasite removal might be a pleiotropic by-product of evolution in response to release from predation (Dargent *et al.*, 2013a). Although we cannot exclude the possibility that the shift in predation exerted a stronger selective

pressure on resistance than did the shift in parasitism, predation effects cannot explain the new finding reported here that resistance did *not* evolve in males. The reason is that predation pressure is stronger on males than on females in the ancestral environment (Weese *et al.*, 2011), and so release from predation should have a stronger effect on males yet their resistance did not evolve as much as did that of females.

In the absence of a clear *selective* reason for sex-specific evolution of resistance, we are drawn to the idea of stronger evolutionary constraints on males than on females. First, if historical selection for resistance was stronger on males than on females (as argued above), males might retain lower available genetic variance for resistance, leading to a weaker response even under an equal shift in selection (Bell, 2007). Supporting this idea, studies of other systems have shown that evolutionary responses to shifts in predation can be limited owing to a lack of variation in defence traits (Lahti *et al.*, 2009), and a similar situation could perhaps occur in response to shifts in parasitism. Second, the costs of increasing resistance might not be linear, making progress towards ever higher resistance progressively more costly and likely to occur at a decelerating rate. In our study, this constraint would be greater for males than for females given the former's initially higher resistance (i.e. males are closer to the absolute limit of having no parasites). In either case, it is conceivable that males could be experiencing selection for increased resistance (perhaps for the same reason as females) but they might not increase further in resistance, or they might do so more slowly. Thus, we suggest that sexual dimorphism in the source population (males more resistant than females) might be constraining further increases in resistance more strongly for males than for females; that is, initial sexual dimorphism in resistance can constrain the evolution of the sexes along the same trajectory in response to a similar shift in selection.

Conclusions

Our study merged a field experiment in relaxed selection (*sensu* Lahti *et al.*, 2009) with the consideration of sex-specific evolutionary responses (Hendry *et al.*, 2006; Butler *et al.*, 2007; Kitano *et al.*, 2012). Our most striking result was that males and females showed considerable differences in their evolution of resistance in response to parasite removal. This outcome could arise because the same environmental shift has different consequences for selection on males vs. females. However, we here suggest that the same result can emerge owing to sex-specific constraints on evolution. As one type of constraint, the sex that has ancestrally experienced stronger selection might harbour less genetic variation in the trait. As another, the sex with higher trait values might have more difficulty evolving ever higher trait values if the costs or benefits of increased trait expression are

nonlinear. Further experiments could explore the effects of increased selection (i.e. adding parasites) as opposed to relaxed selection, and could assess the degree to which predation and other sources of multifarious selection affect the evolution of resistance.

Studies of sex-biased parasitism have long recognized that physiological as well as behavioural differences between males and females cause divergent parasite loads (Zuk & McKean, 1996), yet they continue to ignore the potential effects of sex-specific evolution on those traits that govern infection. Our results suggest that sex-biased parasitism is a highly dynamic character, which could clarify why it has been so hard to elucidate general patterns and mechanisms (e.g. Nunn *et al.*, 2009). In a broader context, studies on the evolutionary consequences of environmental change would benefit from increasing attention to sexual differences and their potential consequences. We further suggest that our findings are relevant to understanding sexual conflict. For instance, differences between the sexes in optimal investment in resistance could lead to intralocus sexual conflict whereby each sex constrains the other's adaptive evolution of defence (Chapman *et al.*, 2003). Yet the loss of sexual dimorphism that we found could alleviate sexual antagonism in resistance by aligning the sexes investment in this trait.

Acknowledgments

We thank D. Reznick, C. Ghalambor, E. Ruell, D. Fraser and the FIBR team for supplying us with guppies derived from the FIBR sites in Trinidad, for their support, and for their advice. We thank S. Portalier, E. Pedersen, A. Reddon and D. Adams for advice on data analysis, A. López-Sepulcre, L. Jacquin, J. Raeymaekers and two anonymous reviewers for comments on the manuscript, and C. Tاديري, N. Okere and A. Howard for laboratory work. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada through a Special Research Opportunity grant (G.F.F., M.E.S., and A.P.H. – # 356373-07), a Vanier CGS to F.D., and a NSERC-RTI to A.P.H. Research at the Institute of Parasitology is supported by an FQRNT Regroupement. Research at the Ghalambor lab was supported by a National Science Foundation's Faculty Early Career grant to C.K.G. (DEB-0846175). The guppy introductions were funded by a United States National Science Foundation – Frontiers in Integrative Biological Research grant to D. Reznick P.I. (EF-0623632). A.P.H. also thanks Darwin's pub the *George and Dragon* in Downe, England, for the inspiration necessary to facilitate his final comments.

References

Adams, D.C. & Collyer, M.L. 2009. A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution* **63**: 1143–1154.

- Anderson, R.M. & May, R.M. 1978. Regulation and stability of host-parasite population interactions: I. Regulatory processes. *J. Anim. Ecol.* **47**: 219–247.
- Arendt, J.D., Reznick, D.N. & López-Sepulcre, A. 2014. Repliated origin of female-biased adult sex ratio in introduced populations of the Trinidadian guppy (*Poecilia reticulata*). *Evolution* **68**: 2343–2356.
- Badyaev, A.V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol.* **17**: 369–378.
- Bakke, T.A., Cable, J. & Harris, P.D. (2007) The biology of gyrodactylid monogeneans: the “Russian-doll killers”. In: *Advances in Parasitology*, Vol. 64 (J.R. Baker, R. Miller & D. Rollinson, eds), pp. 161–378. Elsevier Academic Press Inc, San Diego.
- Bell, G. 2007. *Selection: The Mechanism of Evolution*, 2nd edn. Oxford University Press, Oxford.
- Bonneaud, C., Balenger, S.L., Russell, A.F., Zhang, J.W., Hill, G.E. & Edwards, S.V. 2011. Rapid evolution of disease resistance is accompanied by functional changes in gene expression in a wild bird. *Proc. Natl. Acad. Sci. USA* **108**: 7866–7871.
- Boots, M. & Begon, M. 1993. Trade-offs with resistance to a granulosis-virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct. Ecol.* **7**: 528–534.
- Boots, M., Best, A., Miller, M.R. & White, A. 2009. The role of ecological feedbacks in the evolution of host defence: what does theory tell us? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**: 27–36.
- Butler, M.A., Sawyer, S.A. & Losos, J.B. 2007. Sexual dimorphism and adaptive radiation in Anolis lizards. *Nature* **447**: 202–205.
- Cable, J. (2011) Poeciliid parasites. In: *Ecology and Evolution of Poeciliid Fishes* (J.P. Evans, A. Pilastro & I. Schlupp, eds), 409 pp. University of Chicago Press, Chicago.
- Cable, J. & van Oosterhout, C. 2007. 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.
- Carroll, S.P., Jørgensen, P.S., Kinnison, M.T., Bergstrom, C.T., Denison, R.F., Gluckman, P. *et al.* 2014. Applying evolutionary biology to address global challenges. *Science* **346**.
- Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends Ecol. Evol.* **18**: 41–47.
- Collyer, M.L. & Adams, D.C. 2007. Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology* **88**: 683–692.
- Cusack, R. & Cone, D.K. 1986. A review of parasites as vectors of viral and bacterial diseases of fish. *J. Fish Dis.* **9**: 169–171.
- Dargent, F., Scott, M.E., Hendry, A.P. & Fussmann, G.F. 2013a. Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proc. R. Soc. Lond. B Biol. Sci.* **280**: 1–9.
- Dargent, F., Torres-Dowdall, J., Scott, M.E., Ramnarine, I. & Fussmann, G.F. 2013b. Can mixed-species groups reduce individual parasite load? A field test with two closely related poeciliid fishes *Poecilia reticulata* and *Poecilia picta*. *PLoS One* **8**: e56789.
- Darwin, C. 1872. *The Descent of Man, and Selection in Relation to Sex*. D. Appleton and Company, Broadway.
- Delph, L.F. 2005. Processes that constrain and facilitate the evolution of sexual dimorphism. *Am. Nat.* **166**: S1–S4.

- Duncan, A.B., Fellous, S. & Kaltz, O. 2011. Reverse evolution: selection against costly resistance in disease-free microcosm populations of *Paramecium caudatum*. *Evolution* **65**: 3462–3474.
- Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**: 22–29.
- Folstad, I. & Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**: 603–622.
- Forbes, M.R. 2007. On sex differences in optimal immunity. *Trends Ecol. Evol.* **22**: 111–113.
- Frankel, V.M., Hendry, A.P., Rolshausen, G. & Torchin, M.E. 2015. Host preference of an introduced 'generalist' parasite for a non-native host. *Int. J. Parasitol.* **45**: 703–709.
- Fraser, B.A. & Neff, B.D. 2009. MHC class IIB additive and non-additive effects on fitness measures in the guppy *Poecilia reticulata*. *J. Fish Biol.* **75**: 2299–2312.
- Fraser, B.A., Ramnarine, I.W. & Neff, B.D. 2010a. Selection at the MHC class IIB locus across guppy (*Poecilia reticulata*) populations. *Heredity* **104**: 155–167.
- Fraser, B.A., Ramnarine, I.W. & Neff, B.D. 2010b. Temporal variation at the MHC class IIB in wild populations of the guppy (*Poecilia reticulata*). *Evolution* **64**: 2086–2096.
- Gilliam, J.F., Fraser, D.F. & Alkins-Koo, M. 1993. Structure of a tropical stream fish community: a role for biotic interactions. *Ecology* **74**: 1856–1870.
- Gotanda, K.M., Delaire, L.C., Raeymaekers, J.A.M., Pérez-Jvostov, F., Dargent, F., Bentzen, P. *et al.* 2013. Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia* **172**: 155–166.
- Graham, A.L., Allen, J.E. & Read, A.F. 2005. Evolutionary causes and consequences of immunopathology. *Annu. Rev. Ecol. Syst.* **36**: 373–397.
- Griffiths, S.W. 1996. Sex differences in the trade-off between feeding and mating in the guppy. *J. Fish Biol.* **48**: 891–898.
- Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds - a role for parasites. *Science* **218**: 384–387.
- Handelsman, C.A., Broder, E.D., Dalton, C.M., Ruell, E.W., Myrick, C.A., Reznick, D.N. *et al.* 2013. Predator-induced phenotypic plasticity in metabolism and rate of growth: rapid adaptation to a novel environment. *Integr. Comp. Biol.* **53**: 975–988.
- 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.
- Hedrick, A.V. & Temeles, E.J. 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends Ecol. Evol.* **4**: 136–138.
- Hendry, A.P. & Kinnison, M.T. 1999. The pace of modern life: measuring rates of contemporary microevolution. *Evolution* **53**: 1637–1653.
- Hendry, A.P., Kelly, M.L., Kinnison, M.T. & Reznick, D.N. 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *J. Evol. Biol.* **19**: 741–754.
- Hendry, A.P., Lohmann, L.G., Conti, E., Cracraft, J., Crandall, K.A., Faith, D.P. *et al.* 2010. Evolutionary biology in biodiversity science, conservation, and policy: a call to action. *Evolution* **64**: 1517–1528.
- Herre, E.A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**: 1442–1445.
- Houde, A.E. & Torio, A.J. 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behav. Ecol.* **3**: 346–351.
- Jokela, J., Lively, C.M., Dybdahl, M.F. & Fox, J.A. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. *Biol. J. Linn. Soc.* **79**: 165–181.
- Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R. & Tompkins, D.M. 2009. Parasite spillback: a neglected concept in invasion ecology? *Ecology* **90**: 2047–2056.
- Kitano, J., Mori, S. & Peichel, C.L. 2012. Reduction of sexual dimorphism in stream-resident forms of three-spined stickleback *Gasterosteus aculeatus*. *J. Fish Biol.* **80**: 131–146.
- Kohler, T.J., Heatherly, T.N., El-Sabaawi, R.W., Zandonà, E., Marshall, M.C., Flecker, A.S. *et al.* 2012. Flow, nutrients, and light availability influence Neotropical epilithon biomass and stoichiometry. *Freshw. Sci.* **31**: 1019–1034.
- Kolluru, G.R., Grether, G.F., South, S.H., Dunlop, E., Cardinali, A., Liu, L. *et al.* 2006. The effects of carotenoid and food availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*). *Biol. J. Linn. Soc.* **89**: 301–309.
- Kolluru, G.R., Grether, G.F., Dunlop, E. & South, S.H. 2009. Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). *Behav. Ecol.* **20**: 131–137.
- Koskella, B., Lin, D.M., Buckling, A. & Thompson, J.N. 2012. The costs of evolving resistance in heterogeneous parasite environments. *Proc. R. Soc. Lond. B Biol. Sci.* **279**: 1896–1903.
- Lahti, D.C., Johnson, N.A., Ajite, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T. *et al.* 2009. Relaxed selection in the wild. *Trends Ecol. Evol.* **24**: 487–496.
- Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *J. Anim. Ecol.* **20**: 201–219.
- Lochmiller, R.L. & Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**: 87–98.
- Lopez, S. 1998. Acquired resistance affects male sexual display and female choice in guppies. *Proc. R. Soc. Lond. B Biol. Sci.* **265**: 717–723.
- López-Sepulcre, A., Gordon, S.P., Paterson, I.G., Bentzen, P. & Reznick, D.N. 2013. Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proc. R. Soc. Lond. B Biol. Sci.* **280**.
- Magurran, A.E. 2005. *Evolutionary Ecology: The Trinidadian Guppy*. Oxford University Press, New York.
- Magurran, A.E. & Seghers, B.H. 1994. Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. Lond. B Biol. Sci.* **255**: 31–36.
- Marshall, I.D. & Fenner, F. 1958. Studies in the epidemiology of infectious myxomatosis of rabbits: V. Changes in the innate resistance of Australian wild rabbits exposed to myxomatosis. *J. Hyg.* **56**: 288–302.
- Mastitsky, S. & Veres, J. 2010. Field evidence for a parasite spillback caused by exotic mollusc *Dreissena polymorpha* in an invaded lake. *Parasitol. Res.* **106**: 667–675.
- Merilä, J. & Hendry, A.P. 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol. Appl.* **7**: 1–14.
- Murphy, K.P., Travers, P., Walport, M. & Janeway, C. 2008. *Janeway's Immunobiology*. Garland Science, New York.

- Nunn, C.L., Lindenfors, P., Pursall, E.R. & Rolff, J. 2009. On sexual dimorphism in immune function. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**: 61–69.
- van Oosterhout, C., Mohammed, R.S., Hansen, H., Archard, G.A., McMullan, M., Weese, D.J. et al. 2007. Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int. J. Parasitol.* **37**: 805–812.
- Ostfeld, R.S. & Keesing, F. 2000. Biodiversity and disease risk: the case of Lyme disease. *Conserv. Biol.* **14**: 722–728.
- Pennisi, E. 2012. The great guppy experiment. *Science* **337**: 904–908.
- Perez-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.
- Pérez-Jvostov, F., Hendry, A.P., Fussmann, G.F. & Scott, M.E. 2015. Testing for local host–parasite adaptation: an experiment with *Gyrodactylus ectoparasites* and guppy hosts. *Int. J. Parasitol.* **45**: 409–417.
- Reimchen, T.E. & Nosil, P. 2001. Ecological causes of sex-biased parasitism in threespine stickleback. *Biol. J. Linn. Soc.* **73**: 51–63.
- R Development Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reznick, D. 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. & Ghalambor, C. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* **112–113**: 183–198.
- Reznick, D.N. & Ghalambor, C.K. 2005. Selection in nature: experimental manipulations of natural populations. *Integr. Comp. Biol.* **45**: 456–462.
- Richards, E.L., van Oosterhout, C. & Cable, J. 2010. Sex-specific differences in shoaling affect parasite transmission in guppies. *PLoS One* **5**: e13285.
- de Roij, J., Harris, P.D. & MacColl, A.D.C. 2011. Divergent resistance to a monogenean flatworm among three-spined stickleback populations. *Funct. Ecol.* **25**: 217–226.
- Roy, B.A. & Kirchner, J.W. 2000. Evolutionary dynamics of pathogen resistance and tolerance. *Evolution* **54**: 51–63.
- Ruell, E.W., Handelsman, C.A., Hawkins, C.L., Sofaer, H.R., Ghalambor, C.K. & Angeloni, L. 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. *Proc. R. Soc. Lond. B Biol. Sci.* **280**.
- Schmid-Hempel, P. 2011. *Evolutionary Parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics*. Oxford University Press, Oxford, New York.
- Schoener, T.W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* **331**: 426–429.
- Schulte, R.D., Makus, C., Hasert, B., Michiels, N.K. & Schulenburg, H. 2010. Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proc. Natl. Acad. Sci. USA* **107**: 7359–7364.
- Scott, M.E. 1982. Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* **85**: 217–236.
- Scott, M.E. 1985. Dynamics of challenge infections of *Gyrodactylus bullatarudis* Turnbull (Monogenea) on guppies, *Poecilia reticulata* (Peters). *J. Fish Dis.* **8**: 495–503.
- 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.
- Sheldon, B.C. & Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**: 317–321.
- Sorci, G., Cornet, S. & Faivre, B. 2013. Immune evasion, immunopathology and the regulation of the immune system. *Pathogens* **2**: 71–91.
- Thoney, D.A. & Hargis, W.J. Jr 1991. Monogenea (platyhelminthes) as hazards for fish in confinement. *Annu. Rev. Fish Dis.* **1**: 133–153.
- Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J. & Kuris, A.M. 2003. Introduced species and their missing parasites. *Nature* **421**: 628–630.
- Travis, J., Reznick, D., Bassar, R.D., López-Sepulcre, A., Ferrere, R. & Coulson, T. (2014) Chapter one – do eco-evo feedbacks help us understand nature? Answers from studies of the trinidadian guppy. In: *Advances in Ecological Research*, Vol. 50 (J.R. Moya-Laraño J. Rowntree & G. Woodward, eds), pp. 1–40. Academic Press, London.
- Weese, D.J., Schwartz, A.K., Bentzen, P., Hendry, A.P. & Kinison, M.T. 2011. Eco-evolutionary effects on population recovery following catastrophic disturbance. *Evol. Appl.* **4**: 354–366.
- Zuk, M. & McKean, K.A. 1996. Sex differences in parasite infections: patterns and processes. *Int. J. Parasitol.* **26**: 1009–1023.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Experimental population characteristics and summary results.

Table S2 ANOVA for guppy standard length by population and sex.

Table S3 GLM for death during experiment by sex.

Table S4 GLM for day of death by population and sex.

Table S5 Survival analysis using Cox proportional hazards.

Table S6 GLMs for *Gyrodactylus turnbulli* peak load and intrinsic rate of increase (r) on *Poecilia reticulata* by sex.

Table S7 Planned contrasts of *Gyrodactylus* peak load on *Poecilia reticulata* with size as a covariate.

Figure S1 Guppy size (mm) at start of infection by population and sex.

Data deposited at Dryad: doi: 10.5061/dryad.867ts

Received 1 October 2014; revised 2 September 2015; accepted 6 September 2015