

## Parallel and nonparallel behavioural evolution in response to parasitism and predation in Trinidadian guppies

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### Abstract

Natural enemies such as predators and parasites are known to shape intraspecific variability of behaviour and personality in natural populations, yet several key questions remain: (i) What is the relative importance of predation vs. parasitism in shaping intraspecific variation of behaviour across generations? (ii) What are the contributions of genetic and plastic effects to this behavioural divergence? (iii) And to what extent are responses to predation and parasitism repeatable across independent evolutionary lineages? We addressed these questions using Trinidadian guppies (*Poecilia reticulata*) (i) varying in their exposure to dangerous fish predators and *Gyrodactylus* ectoparasites for (ii) both wild-caught F0 and laboratory-reared F2 individuals and coming from (iii) multiple independent evolutionary lineages (i.e. independent drainages). Several key findings emerged. First, a population's history of predation and parasitism influenced behavioural profiles, but to different extent depending on the behaviour considered (activity, shoaling or boldness). Second, we had evidence for some genetic effects of predation regime on behaviour, with differences in activity of F2 laboratory-reared individuals, but not for parasitism, which had only plastic effects on the boldness of wild-caught F0 individuals. Third, the two lineages showed a mixture of parallel and nonparallel responses to predation/parasitism, with parallel responses being stronger for predation than for parasitism and for activity and boldness than for shoaling. These findings suggest that different sets of behaviours provide different pay-offs in alternative predation/parasitism environments and that parasitism has more transient effects in shaping intraspecific variation of behaviour than does predation.

### Introduction

Natural enemies such as predators and parasites have strong negative fitness effects on prey and hosts and thereby shape evolutionary trajectories of natural populations (Raffel *et al.*, 2008). Most studies of such effects

have focused on predators rather than parasites, perhaps because parasites are less likely to directly cause mortality (Combes, 2001). Yet indirect effects of both forces are also possible, whereby 'fear' of predators reduces fitness (Clinchy *et al.*, 2013) and infection by parasites alters behaviour, reduces fecundity or mating success (Raffel *et al.*, 2008; Preston *et al.*, 2014). Recognizing the existence of such effects, and the fact that parasites tend to be much more abundant than predators, it remains uncertain which class of enemy has a stronger effect on the evolution of natural populations, especially on behavioural traits (Kortet *et al.*, 2010). In

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this study, we consider the evolutionary effects of both predators and parasites on prey/host behaviour.

Predation is already well established as an important driver of the evolution of behaviour. In fish for instance, the ability to escape predators is influenced by traits such as activity, sociability and boldness (e.g. Dugatkin, 1992; Smith & Blumstein, 2010). As a result, fish populations that have evolved in high-predation environments (relative to those in low-predation environments) generally display lower activity, higher shoaling and higher or lower boldness depending on the study (Seghers, 1974; Magurran & Seghers, 1991; Magurran *et al.*, 1992; Bell, 2004; Bell & Sih, 2007; Dingemanse *et al.*, 2007, 2012; Huizinga *et al.*, 2009; Harris *et al.*, 2010; Kortet *et al.*, 2015). Despite these many supportive studies, more work remains to be done. For instance, few of these studies supported their inferences by examining predation effects across multiple independent evolutionary lineages of prey (but see Dingemanse *et al.*, 2007). Moreover, relatively few studies have used common-garden experiments to confirm that the behaviour differences among populations have a genetic (as opposed to plastic) bases (but see Huizinga *et al.*, 2009).

Parasitism as a driving force in the evolution of behaviour and personality traits has been less studied. Parasites have been shown to play a central role in sexual selection (Hamilton & Zuk, 1982; Folstad & Karter, 1992) and to impact host sexual and social behaviours (Knell & Mary Webberley, 2004; Schmid-Hempel, 2011; Barribeau *et al.*, 2015), yet their role in the evolution of consistent interindividual differences in behaviours and personality traits in wild populations remain less well known (Kortet *et al.*, 2010). A growing number of studies point to its likely importance, but empirical evidence remains scarce (Barber & Dingemanse, 2010; Coats *et al.*, 2010; Poulin, 2010, 2013; Koprivnikar *et al.*, 2012; Preston *et al.*, 2014). On the one hand, infection clearly influences host behaviour in a variety of both direct (behavioural manipulation) or indirect (physiological changes) ways (Barber *et al.*, 2000; Barber & Dingemanse, 2010; Poulin, 2013). On the other hand, host behaviour strongly influences the risks of getting infected and the costs of being infected (Hart, 1990, 1994; Ezenwa, 2004). For instance, the level of sociability will influence the risk of infection by contagious parasites, which should then influence selection on social interactions and social networks (e.g. Krause & Godin, 1994; Barber *et al.*, 1998; Pike *et al.*, 2008; Croft *et al.*, 2011). Moreover, high levels of exploration, activity and boldness can increase exposure to parasites, which should then influence selection on these traits (Wilson *et al.*, 1993; Coleman & Wilson, 1998; Natoli *et al.*, 2005; Boyer *et al.*, 2010; McCabe *et al.*, 2015). For all of these reasons, particular combinations of consistent behavioural and personality traits (for instance, low levels of sociability, activity and boldness) might be

selected for in high-parasitism (as opposed to low-parasitism) environments (Barber & Dingemanse, 2010).

Good reasons therefore exist to suspect that the evolution of behavioural traits will be shaped by both predation and parasitism (Barber & Dingemanse, 2010; Kortet *et al.*, 2010). With this motivation, this study was designed around three key questions. (i) What is the relative importance of predation vs. parasitism in shaping behaviour in a study system where both forces differ dramatically among populations? This question is important because very few studies have considered the relative effects of these two potential drivers in the same system (reviewed in Kortet *et al.*, 2010). (ii) To what extent are behavioural differences among predation/parasitism regimes shaped by plastic vs. genetic effects? This question is important because many studies examine only wild-caught individuals (e.g. Magurran & Seghers, 1991) and thus cannot inform the extent to which differences among populations are genetically based. (iii) How consistent are evolutionary responses to predation and parasitism in independent lineages? This question is important because similar responses across multiple lineages to a specific environmental contrast (i.e. parallelism) greatly increases confidence in causal inferences about general effects of that contrast (Endler, 1986; Schluter, 2000; Arendt & Reznick, 2008; Nosil, 2012).

### Study system

Trinidadian guppy (*Poecilia reticulata*) populations experience sharp contrasts in predation (reviews: Endler, 1995; Magurran, 2005) and parasitism (van Oosterhout *et al.*, 2007; Fraser *et al.*, 2010; Gotanda *et al.*, 2013). This geographical variation arises because numerous waterfalls differentially influence the colonization of guppies, predators and parasites (Magurran, 2005). Importantly, different predation and parasitism regimes are replicated across drainages containing guppy populations with independent evolutionary origins and large genetic differences (Suk & Neff, 2009; Willing *et al.*, 2010). This evolutionary replication facilitates formal tests for parallelism (Arendt & Reznick, 2008; Nosil, 2012): that is, whether evolutionary responses to a given predation/parasitism regime are repeatable across independent evolutionary lineages (Reznick *et al.*, 1996b).

The above contrast in predation has been well studied and is typically dichotomized into 'high-predation' sites with dangerous predatory fishes that impose strong guppy mortality vs. 'low-predation' sites with few dangerous predatory fishes that impose only weak guppy mortality (Reznick *et al.*, 1996a; Weese *et al.*, 2010). These predation differences shape the evolution – often in a parallel fashion – of a large suite of behavioural, morphological, physiological and life history traits (reviews: Endler, 1995; Magurran, 2005). As

examples, predators typically drive the evolution of duller coloration (Endler, 1984), faster life history strategies (Reznick & Endler, 1982; Reznick *et al.*, 2001) and enhanced shoaling behaviour (Seghers, 1974; Breden *et al.*, 1987). Yet the same traits also differ markedly among guppy populations within each of these predation regimes (Magurran & Seghers, 1991; Seghers & Magurran, 1995; Millar *et al.*, 2006; Fitzpatrick *et al.*, 2014), which suggests that other selective factors must also be important.

In contrast to predation, the above contrast in parasitism has only recently received considerable attention, specifically in relation to *Gyrodactylus* spp. These ectoparasites feed on mucus and epithelial cells and reproduce directly on the skin of the fish. *Gyrodactylus* parasites are socially transmitted through fish-to-fish contact or when parasites hang in the water film and infect fish foraging at the water surface (Scott, 1985; Lyles 1990; Cable *et al.*, 2002; Cable & Van Oosterhout, 2007). Natural guppy populations differ markedly in their levels of infection by *Gyrodactylus* spp. (Harris and Lyles 1992; Martin & Johnsen, 2007; van Oosterhout *et al.*, 2007; Fraser *et al.*, 2010), and previous studies have shown very consistent patterns of parasite absence vs. presence across years (Fraser *et al.*, 2010; Gotanda *et al.* 2013), suggesting that populations differ in their long-term history of exposure to parasites. Thus, although *Gyrodactylus* intensity varies among years, *Gyrodactylus* presence/absence (i.e. parasite 'regime') appears to be very consistent (Gotanda *et al.*, 2013). *Gyrodactylus* parasites have strong effects on guppy survival, growth and reproduction (Scott & Anderson, 1984; van Oosterhout *et al.*, 2007; Gotanda *et al.*, 2013; Pérez-Jvostov *et al.*, 2012, 2015; Dargent *et al.*, 2013, 2015), and guppies show corresponding evolutionary responses in resistance (Scott & Anderson, 1984; Dargent *et al.*, 2013; Pérez-Jvostov *et al.*, 2015) and MHC diversity (van Oosterhout *et al.*, 2006; Fraser & Neff, 2009). Of particular relevance to our study, *Gyrodactylus* infection influences, and is influenced by, various aspects of behaviour. For instance, *Gyrodactylus*-infected guppies show impaired swimming ability and reduced activity due to fin damages (López, 1999; Cable *et al.*, 2002), reduced shoaling cohesion (Croft *et al.*, 2011; Hockley *et al.*, 2014) and lower boldness (Richards, 2010). In addition, infection risk is influenced by shoaling and other behaviours (e.g. Richards *et al.*, 2010). For all of these reasons, we expect *Gyrodactylus* to influence the development and evolution of guppy behaviours.

## Objectives

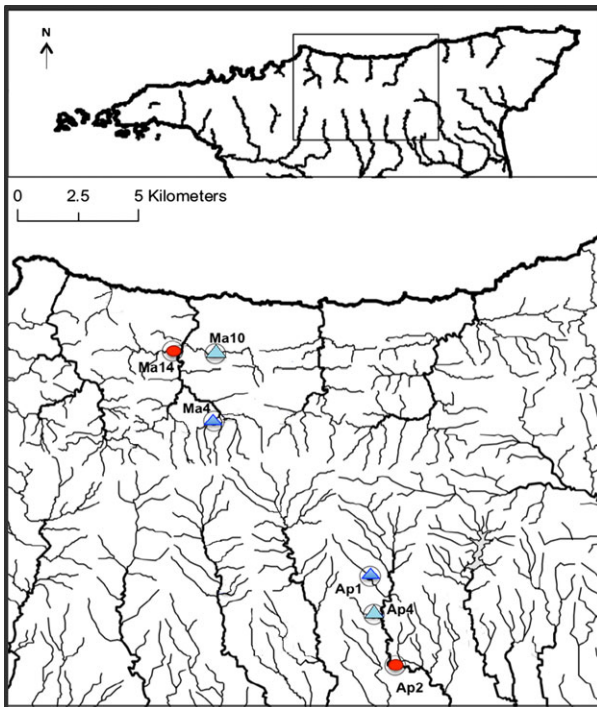
Translating our three key questions (see above) to the guppy system, our specific aims were threefold. First, we quantify (i) the relative importance of predation (high predation vs. low predation) and parasitism

(*Gyrodactylus* presence vs. absence) to behavioural divergence by studying populations from different predation/parasitism regimes. Previous work suggests that predation should have large effects (Magurran & Seghers, 1991; Seghers & Magurran, 1995; Magurran, 2005; Harris *et al.*, 2010), and here, we postulate that parasites could be equally important. Second, we evaluate (ii) the extent to which any documented predation and parasite effects have a genetic vs. plastic basis by comparing results for wild-caught fish (plastic differences present) to those for second-generation fish laboratory reared in common-garden conditions (plastic differences absent). Previous work suggests that population divergence in guppies is shaped by both plasticity and genetic differences (Huizinga *et al.*, 2009; Torres-Dowdall *et al.*, 2012), and we expect similar results for the effects of parasites. Third, we assess (iii) the parallelism of behavioural divergence by studying guppies from the different predation/parasitism regimes in each of two rivers harbouring guppies from distinct evolutionary lineages. Previous work on guppies emphasizes the parallel effects of predators (Reznick *et al.*, 1996b), and we expect that, if parasite effects are also strong, they too will show substantial parallel components. The specific behaviours we study (details in Methods) were chosen to inform not only behavioural divergence (as above), but also to inform current discussions about the evolution of animal 'personalities' and behavioural syndromes (Sih *et al.*, 2004, 2012; Dingemanse & Réale, 2005; Réale *et al.*, 2007; Barber & Dingemanse, 2010; Kortet *et al.*, 2010). That is, we quantify behaviour patterns that, within that larger literature, are considered representative of variation along three personality axes: activity, sociability (shoaling tendency) and boldness (sensu Réale *et al.*, 2007).

## Materials and methods

### Study populations and laboratory rearing

We characterized the behavioural profiles of wild-caught F0 guppies and their laboratory-reared F2 offspring from six populations that evolved under different predation/parasitism regimes: low predation with no *Gyrodactylus* (LP-NG), low predation with *Gyrodactylus* (LP-G) and high predation with *Gyrodactylus* (HP-G) (Fig. 1, Table 1). Natural populations of high-predation guppies that are not exposed to *Gyrodactylus* (HP-NG) have not been found. Indeed, across 23 HP sites examined across previous studies only one has been found to be parasite free (Martin & Johnsen, 2007; Gotanda *et al.*, 2013; Pérez-Jvostov *et al.*, 2012). This suggests that the effect of parasite presence vs. absence mainly occurs in LP sites, and so the evolutionary effects of parasite differences arise mostly when predators are absent in this system. Each predation/parasitism regime was replicated by sampling three populations (one from



**Fig. 1** Location of study sites in the two independent watersheds of Marianne ('Ma'; Northern slope) and Aripo rivers ('Ap'; Southern slope). There are two replicates of each evolutionary regime: High-predation *Gyrodactylus* parasite (HP-G; red circles), Low-predation *Gyrodactylus* (LP-G; light blue triangles) and Low-predation no *Gyrodactylus* (LP-NG; dark blue triangles) (modified from Gotanda *et al.* 2013).

each regime) in each of two rivers (Marianne and Aripo) in the Northern Mountain Range of Trinidad (Fig. 1). These rivers are similar in topography and food availability, but are in two very separate drainage basins, and their guppies represent independent evolutionary lineages that are strongly genetically differentiated (Suk & Neff, 2009; Willing *et al.*, 2010). Our sampling design thus allowed an assessment of parallel evolution of traits in response to similar selection pressures in different evolutionary lineages.

The specific collection sites within each river were chosen based on previous studies establishing the local predation and parasitism regime. Predation regime was assessed based on the presence/absence of dangerous predators (cichlids such as *Crenicichla* sp. and *Aequidens pulcher* in the Aripo River; and eleotrids such as *Eleotris pisonis* and *Gobiomorus dormitor* in the Marianne River) (Reznick *et al.*, 1996b; Magurran, 2005) and on direct measurements of mortality rate (Reznick *et al.*, 1996a; Weese *et al.*, 2010). Parasitism regime was assessed based on the presence/absence of *Gyrodactylus turnbulli* and *Gyrodactylus bullatarudis* parasites on fish skin and fins (Cable & Van Oosterhout, 2007, Gotanda *et al.*, 2013).

Wild F0 fish from the six populations were collected in November 2012 (Table 1). To confirm the temporal stability of the *Gyrodactylus* presence/absence categorization made for our sites in previous work (Gotanda *et al.* 2013), we assessed *Gyrodactylus* presence on guppies before transporting them to the laboratory, by thoroughly scanning the body of each collected fish under a dissecting microscope ( $\times 10$ ) (e.g. Dargent *et al.*, 2013). Reassuringly, *Gyrodactylus* presence/absence in our sampling year 2012 (Table 1) matched categorizations for the same sites from 2001, 2009 and 2010 (Cable & Van Oosterhout, 2007; Gotanda *et al.*, 2013). This temporal consistency suggests that the guppy populations we studied differ in their long-term history of exposure to *Gyrodactylus*. Thus, although parasite intensity varies among years (Gotanda *et al.*, 2013), all available evidence suggests that *Gyrodactylus* presence/absence (i.e. parasite 'regime') is temporally consistent – at least at our study sites.

All fish were transported to McGill University and housed in 20 L tanks enriched with gravel and plastic plants for 2 months, so as to facilitate acclimation to captivity. On arrival, all fish were treated with Copper-safe (Mardel, USA) for 1 month to remove any *Gyrodactylus* parasites. After this treatment, all of the fish were examined again under the dissecting microscope, which confirmed successful elimination of the parasite. Thus, in this study, direct pathological effects of *Gyrodactylus* infections on behaviour were absent because all fish were de-parasitized before the experiment, but long-lasting physiological or neurological changes due to immune activation could have been present (e.g. Hasselquist & Nilsson, 2012). Throughout this period, the water was filtered, aerated and maintained at 23–25 °C and 7–8 pH, with a 12 : 12 photoperiod. The fish were fed *ad libitum* with brine shrimp *Artemia salina* twice daily.

Laboratory F1 offspring of the wild-caught F0 females were separated by population and raised under the same conditions as described above. Within 24 h of birth, each F1 fry was allocated at random to one of four tanks (i.e. four replicate tanks per population, pooling fish from different broods). For a different study on the maternal effects of parasites, mature F1 offspring in two of the replicate tanks per population were infected with *Gyrodactylus turnbulli*, whereas offspring in the other two tanks were sham-infected. For these infections, each fish was anesthetized with 0.02% Tricaine Methanesulfonate (MS-222, solution buffered to a neutral pH) and two parasites were manually placed onto each fish, following Dargent *et al.* (2013). For the sham infections (control group), the same procedure was followed without the addition of parasites. After 6 days, *Gyrodactylus*-infected F1 fish had accumulated a high number of parasites (mean = 32 parasites), whereas sham-infected F1 fish did not have any parasites. Infected ( $N = 71$ ) and sham-infected ( $N = 64$ ) F1

**Table 1** Characteristics of collection sites. Site numbers and regimes correspond to previous designations and descriptions from Gotanda *et al.* (2013).

Site	UTM Coordinates (Lat, Lon)	Predation	Parasitism	Regime	Total	Number of fish tested F0 generation		Total	Number of fish tested F2 generation	
						Males	Females		Males	Females
Aripo River										
Ap2	694 231, 1 177 709	High Predation	Presence of <i>Gyrodactylus</i>	HP-G	21	7	14	56	19	37
Ap1	693 188, 1 181 605	Low Predation	Presence of <i>Gyrodactylus</i>	LP-G	12	4	8	17	5	12
Ap4	693 328, 1 179 939	Low Predation	No <i>Gyrodactylus</i>	LP-NG	10	6	4	44	20	24
Marianne River										
Ma14	684 934, 1 191 469	High Predation	Presence of <i>Gyrodactylus</i>	HP-G	21	11	10	35	10	25
Ma4	686 692, 1 188 425	Low Predation	Presence of <i>Gyrodactylus</i>	LP-G	29	10	19	58	24	34
Ma10	686 711, 1 191 358	Low Predation	No <i>Gyrodactylus</i>	LP-NG	29	10	19	42	14	28
						Total F0 = 122		Total F2 = 252		

individuals, regardless of their sex, were then housed in groups according to their infection treatment and their population of origin. As these F1 fish gave birth to F2 offspring, those offspring were then transferred to new tanks where they were raised to maturity before testing their behaviour as described below. In total, 135 F1 fish and 252 F2 fish were obtained from the original 122 wild-caught F0 guppies (Table 1).

### Behavioural tests

Each adult F0 and F2 fish was transferred into a transparent cylinder in a five gal test tank (40 cm length × 20.5 cm width × 10 cm water depth). At either end of the test tank were compartments (5 cm length) enclosed by clear Plexiglas partitions with mesh sides permeable to odour cues. One compartment contained a 'stimulus shoal' of four wild-type females (mean body length = 2.53 ± 0.03 cm) from stock populations of mixed origin (mixed F4 broods from multiple drainages), whereas the other compartment did not contain any fish. The side and the identity of the stimulus shoal was changed and randomized regularly to avoid spatial biases (Wright & Krause, 2006). Water was changed and mixed regularly to avoid local accumulation of odour cues. Each tank had gravel on the bottom, and its long axis was divided by marks on the side of the tank into consecutive zones, each 5 cm in length. This experimental design has been shown to effectively reveal variation in shoaling preferences and propensities in guppies (Swaney *et al.*, 2015).

We settled on a 2-min pretrial period to allow for acclimation and for the subject to view its surroundings. This acclimation time was short compared to that typically used for small fish like *Danio rerio* (usually 5 min, see Wright & Krause, 2006). However, we have found that isolation can be a considerable stressor for female guppies (Reader SM, unpublished data) owing to the well-documented sociality of this species (Magurran,

2005). After the acclimation period, the transparent cylinder was gently removed using an overhead string and pulley, and the location of the focal fish was then monitored in real time for 10 min using J-Watcher software (Blumstein *et al.*, 2006) from behind a hide.

'Activity' was recorded using two metrics: the number of times the fish changed zones and the total time spent swimming (i.e. in directed motion and covering a distance > 10% of body length). Both of these metrics are standard in studies of small fishes (e.g. Dingemans *et al.*, 2009). 'Shoaling' was assessed at the same time as activity and was the time spent within 10 cm (approximately four body lengths) of the compartment with the stimulus shoal (Wright & Krause, 2006). Throughout the experiment, the fish showed a strong preference for the compartment containing the stimulus shoal as compared to the empty compartment (Wilcoxon test:  $V = 44\,472$ ,  $P < 0.001$ ), confirming that the test did measure shoaling tendency. Immediately after this joint activity/shoaling test, each fish was captured with a dip net and transferred to a refuge in a new tank. 'Boldness' (behavioural response in a risky situation, *sensu* Réale *et al.*, 2007) was measured in this new tank as the latency for the fish to leave a refuge, with rapid departures assumed to indicate high boldness. The refuge was 10 × 5 × 5 cm and had a 3 cm aperture with a transparent door that was opened remotely by the experimenter after an acclimation time of 2 min. If the fish did not come out of the refuge after 10 min, a maximum score of 600 s was assigned. This boldness metric is a standard one in studies of small fishes (e.g. Harris *et al.*, 2010) and, for Trinidadian guppies, refuge use is a known to be a response to predators (Templeton & Shriner, 2004). After a subject completed the above tests once, it was tested again 20 min later in the same way in the same order, which allowed us to assess the short-term repeatability of behaviours, although not any possible order effects. At the end of all behavioural tests, each fish was

anesthetized as above and body standard length was measured to the nearest millimetre using a ruler.

### Statistical analyses

We first calculated intraindividual repeatabilities for activity, shoaling and boldness across the two trials. Using the two measurements per behaviour per individual, repeatabilities were calculated as the proportion of the total variance explained by individual identity using the rpt.remLMM package in R (Nakagawa & Schielzeth, 2010). To test whether the intraindividual repeatability was different among predation/parasitism regimes, we followed Dingemanse *et al.* (2012). Specifically, we compared (i) mixed models where the intraindividual variance was allowed to differ between regimes (i.e. the random effect of individual was nested within regime) to (ii) reduced models where the intraindividual variance was constrained to have the same value in the different regimes (i.e. random effect of individual only). For this analysis, we standardized the interindividual variance among regimes by rescaling the total variance within regimes to unity (Dingemanse *et al.*, 2012).

No correlation was evident between activity and boldness (Spearman rank correlation F0:  $\rho = 0.12$ ,  $P = 0.17$ ; F2:  $\rho = 0.015$ ,  $P = 0.81$ ) nor between shoaling and boldness (F0:  $\rho = 0.040$ ,  $P = 0.66$ ; F2:  $\rho = 0.064$ ,  $P = 0.31$ ), whereas activity and shoaling were negatively correlated (F0:  $\rho = -0.41$ ,  $P < 0.001$ ; F2:  $\rho = -0.21$ ,  $P < 0.001$ ). To account for this correlation, which was at least partly due to their quantification from the same assay (see above), we ran subsequent analyses on the residuals of activity (corrected for shoaling tendency) and the residuals of shoaling (corrected for activity) as response variables (these residuals were not correlated:  $\rho = -0.041$ ,  $t = -1.12$ ,  $P = 0.26$ ) (Cohen *et al.*, 2002). All results refer to these residual measures.

To analyse the factors influencing activity (residuals), shoaling (residuals) and boldness, we tested each behaviour separately in each generation (F0 and F2) in Linear Mixed Models (lme function, R, version 3.0.3). The models included a random effect of fish identity (to account for nonindependence of repeated measures on the same individual), a fixed effect of regime (LP-NG, LP-G, HP-G), a fixed effect of river (Marianne or Aripo), a fixed effect of sex (male or female) and all second order interactions. The infection status of F1 parents and its interactions were included as fixed factors in the F2 models. This last factor, which was not known for the F0 fish, was why F0 and F2 fish were not analysed in the same model. Infection status had no significant interaction effects and thus was not considered further in this study. Best models were selected using the AICc criterion (Zuur *et al.* 2009) (details in Supporting information). *Post hoc* tests were conducted in separate rivers/regimes/sexes using the same mixed

models when an interaction was found significant. The relative magnitude of predation and parasitism effects on behaviours was then assessed by calculating the effect sizes of regime separately for each contrast (HP-G vs. LP-G for predation effect, LP-G vs. LP-NG for parasitism effect). Here, we used Cohen's *d* coefficients and their associated 95% confidence intervals (Cohen, 1988; Nakagawa & Cuthill, 2007).

To assess whether fish from different evolutionary lineages (i.e. rivers) had parallel or nonparallel response to predation/parasitism regime, we compared the percentage of total variance explained by the regime vs. the regime-by-river interaction (e.g. Kaeuffer *et al.*, 2012). Indeed, the effect of regime reveals the extent of parallelism (i.e. similar responses to predation/parasitism by the two lineages), whereas the regime-by-river interaction informs the extent of nonparallelism (i.e. different responses to predation/parasitism by the two independent lineages) (e.g. Kaeuffer *et al.*, 2012). To compare the variance explained by parallel vs. nonparallel responses to regime, we compared generalized models including only an effect of regime and river, with models including an effect of regime, river and an effect of the regime-by-river interaction. A power analysis (pwr.f2.test function in R) was conducted to evaluate our ability to detect a significant interaction between regime and river given our sample sizes. This power analysis shows that our sample sizes (F0:  $N = 122$ , F2:  $N = 252$ ) were sufficient to detect a potentially significant interaction of medium effect size. That is, the necessary sample size to detect the effect of an interaction in a linear model with medium effect size of 0.15 with a power of 80% and a significance level of 0.05 was  $N = 92$  (Cohen, 1988). We are therefore confident in our statistical power and hence our ability to detect parallel vs. nonparallel effects.

### Ethical statement

All procedures were carried out in accordance with the Canadian Council on Animal Care and ASAB guidelines and were approved by the Animal Care Committee of McGill University (Protocol #7133 and #5759). Field sampling was approved by the Ministry of Agriculture, Land and Marine Resources of the Republic of Trinidad and Tobago and fish import was approved by the Canadian government (Permit Q-2012-00021-2). At the conclusion of the study, fish were placed into breeding populations at McGill University.

## Results

### Behavioural consistency

The level of individual consistency across trials was  $r = 0.44 \pm 0.044$  [95% CI = (0.34, 0.51), ( $P < 0.001$ ) for activity],  $r = 0.43 \pm 0.044$  [95% CI = (0.34, 0.51),

$P < 0.001$ ] for shoaling, and  $r = 0.48 \pm 0.04$  [95% CI = (0.40, 0.56),  $P < 0.001$ ] for boldness. These repeatability levels did not significantly differ between regimes for any behaviour: activity (F0: Likelihood Ratio =  $4.0 \times 10^{-8}$ ,  $P = 0.99$ ; F2: LR =  $3.81 \times 10^{-8}$ ,  $P = 0.98$ ), shoaling (F0: LR =  $1.10 \times 10^{-7}$ ,  $P = 0.97$ ; F2: LR =  $1.96 \times 10^{-7}$ ,  $P = 0.99$ ) and boldness (F0: LR = 2.06,  $P = 0.15$ ; F2: LR = 1.28,  $P = 0.25$ ).

### Activity

Time spent swimming was positively correlated with the number of zone changes (F0:  $t_{240} = 12.78$ ,  $P < 0.001$ ; F2:  $t_{496} = 11.24$ ,  $P < 0.001$ ), and population differences were the same for either metric (results not shown). We thus henceforth present results for zone changes only. In F0 wild-caught fish, the best model included only the fixed effects of sex, river and regime (Table 2, see Table S1 for all models). Males were marginally more active than females, Aripo fish were less active than were Marianne fish and responses to regime were similar and parallel in both rivers (Fig. 2) (i.e. no regime-by-river interaction, and no significant amount of variance explained by the interaction, Table 3). These parallel responses took the form of HP-G F0 fish being less active than LP F0 fish (both LP-G and LP-NG; Fig. 2). Thus, F0 activity was lower in HP environments, whereas *Gyrondactylus* regime had no effect. In laboratory-reared F2 fish, the above parallel effect of regime largely persisted (Table 3), whereas the effect of river did not: that is, the best model included an effect of parental status and regime alone (Table 2). Specifically, HP-G F2 fish were less active than were LP-G F2 fish

(Fig. 2) (LP-NG fish were somewhat intermediate). Effect size comparisons revealed that predation had a stronger effect than parasitism in both F0 and F2 generations (Table 3, Fig. 5).

### Shoaling

In F0 wild-caught fish, the best model for shoaling included effects of river and a regime-by-sex interaction, with no significant effect of regime alone (Table 2, Fig. 3). Sex-specific tests show that males ( $\chi^2 = 11.02$ ,  $P = 0.0040$ ) but not females ( $\chi^2 = 1.01$ ,  $P = 0.60$ ) showed effects of regime, with HP-G males shoaling more than LP-NG males (Fig. S1). Regime explained a low amount of F0 shoaling variance (Table 3).

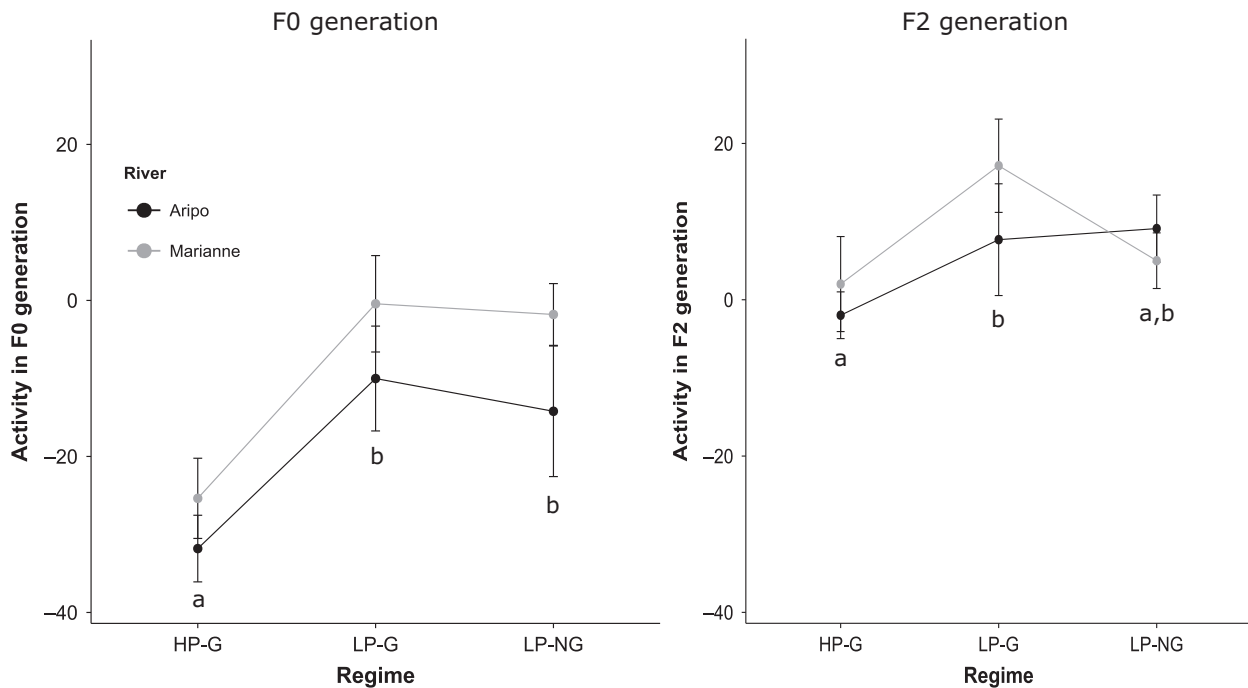
In laboratory-reared F2 fish, different patterns emerged. First, a river-by-regime interaction was evident (Table 2), indicating a nonparallel responses of the two lineages to regime in the F2 laboratory-reared generation (Table 3). This nonparallelism was principally driven by between-river differences for the HP-G regime: Aripo HP-G fish shoaled more than did Marianne HP-G fish, whereas no difference was found for other regimes (Fig. 3). Effect size comparisons indicated similar effect sizes of parasitism and predation on shoaling for both F0 and F2 fish (Table 3, Fig. 5).

### Boldness

In F0 wild-caught fish, the best model for boldness included sex (males were bolder; i.e. they left the refuge sooner), river and regime (Table 2) and, hence,

**Table 2** Best mixed models explaining activity, shoaling and boldness levels in F0 ( $N = 122$ ) and F2 guppies ( $N = 252$ ) from different predation/parasitism regimes in two guppy lineages (two independent rivers). Individual identity was included as a random effect, and the best models were chosen using the AICc criterion (see Supporting information S1 for details). Triple interactions were not retained in any final model. ‘–’ indicates that the factor or interaction was not retained in the final model.

	Activity			Shoaling			Boldness		
	$\chi^2$	d.f.	$P$	$\chi^2$	d.f.	$P$	$\chi^2$	d.f.	$P$
F0 generation									
Sex	2.77	1,117	0.097	7.85	1115	0.005	4.33	1,118	0.037
River	6.18	1,117	0.013	2.48	1115	0.11	3.47	1,118	0.062
Regime	19.97	2,117	< 0.001	1.71	2115	0.47	6.60	2,118	0.037
River*Regime	–	–	–	–	–	–	–	–	–
Sex*Regime	–	–	–	9.39	2115	0.0091	–	–	–
F2 generation									
Parent infection	5.00	1,248	0.025	11.63	1244	< 0.001	–	–	–
Sex	–	–	–	–	–	–	0.0012	1,244	0.97
River	–	–	–	23.06	1244	< 0.001	31.66	1,244	< 0.001
Regime	11.18	2,248	0.0037	8.74	2244	0.013	13.96	2,244	< 0.001
River*Regime	–	–	–	3.68	2244	< 0.001	–	–	–
Sex*Regime	–	–	–	–	–	–	6.95	2,244	0.031
Sex*River	–	–	–	3.68	1244	0.055	11.64	1,244	< 0.001



**Fig. 2** Mean ( $\pm$  SE) activity level (residuals of number of zone changes) in F0 and F2 guppies (males and females combined) from populations differing in predation/parasitism regime (HP-G: High-predation Gyrodactylus parasites, LP-G: Low-predation Gyrodactylus, LP-NG: Low-predation no Gyrodactylus) in two replicate rivers (Marianne River in grey and Aripo River in black). A parallel effect of regime was found in F0 and F2 generation (i.e. similar effects of regime between rivers see Table 2). Different letters represent significant differences between regimes in *post hoc* tests.

fish from the both rivers and sexes showed reasonably parallel responses to regime (Table 3). Specifically, parasitism was associated with low boldness in that LP-G fish were less bold than were LP-NG fish (Fig. 4). Accordingly, effect size comparison shows that parasitism had a stronger effect on F0 boldness than predation (Table 3, Fig. 5).

In laboratory-reared F2 fish, the best model included sex-by-river and sex-by-regime interactions, as well as main effects of regime and river (Table 2). Overall, Aripo F2 fish were bolder than Marianne F2 fish (Fig. 4), and HP-G F2 fish were less bold than were LP-NG F2 fish (Fig. 4). Subsequent sex-specific tests revealed that these differences were mainly driven by females given that (i) males from different rivers ( $\chi^2_2 = 0.010$ ,  $P = 0.99$ ) and regimes ( $\chi^2_2 = 2.41$ ,  $P = 0.29$ ) did not differ in boldness, (ii) Aripo females were bolder than were Marianne females ( $\chi^2_1 = 31.61$ ,  $P < 0.001$ ), and (iii) HP-G females were less bold than were females from other regimes (Fig. S2). Effect size comparisons show that parasitism and predation had similar effect sizes on F2 boldness (Table 3, Fig. 5).

## Discussion

Our key findings, stated as generally as possible, were as follows. First, a population's history of co-evolution

with predators and parasites influenced their behaviour, with predation having generally a more consistent and stronger effect than parasitism. Second, genetic effects on behavioural divergence (i.e. present in F2 laboratory-reared individuals) were mainly evident in relation to predation regime, whereas plastic effects (i.e. present in wild-caught but not F2 individuals) predominated in relation to parasitism. Third, the two independent evolutionary lineages of guppies (from different drainages) showed a mixture of parallel (activity and boldness) and nonparallel (shoaling) responses to predation/parasitism regime. We further discuss each of these key findings in the sections below.

## Predation and parasitism

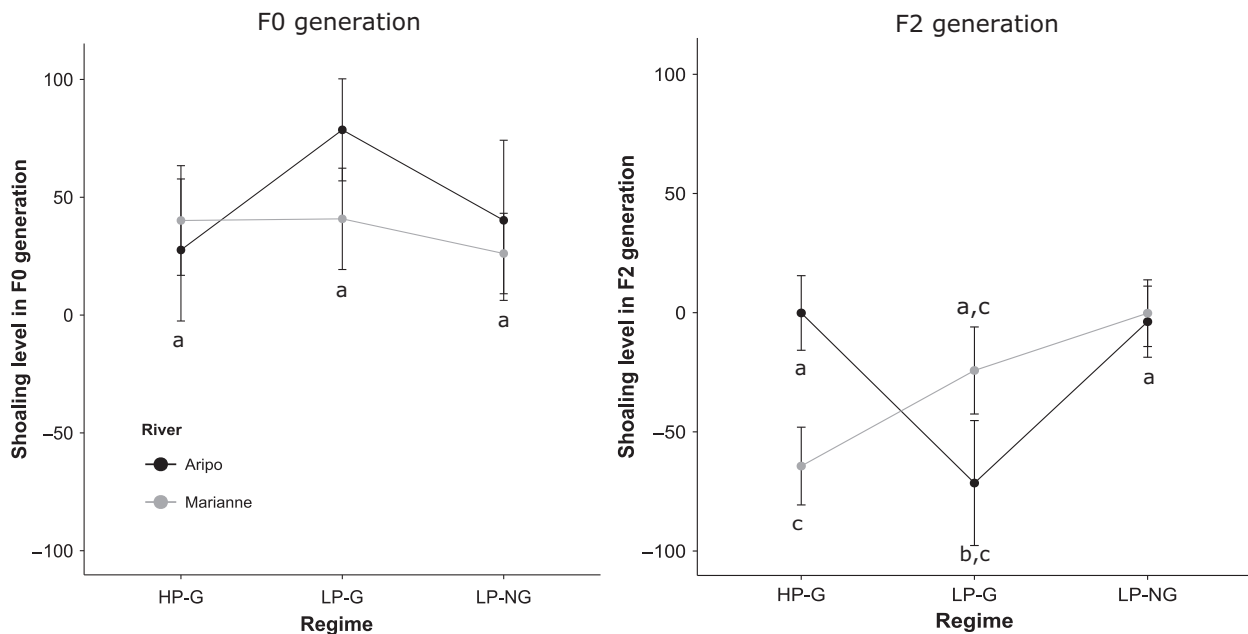
Predators and parasites were both expected to have strong effects because behaviours are key traits determining the risk of both predation and parasitism. Considering predation first, we found strong and generally consistent effects of predation regime on some guppy behaviours. For instance, HP fish were less active (Fig. 2) and shoaled more (at least in males, Fig. S1) than LP guppies in most cases, differences consistent with previous work on other guppy populations (Magurran, 2005). These differences are thought to arise because lower activity increases crypsis and



**Table 3** A. Predation and parasitism effect sizes were estimated by the Cohen's  $d$  coefficients and their 95% CI [see Fig. 5 (Cohen, 1988)]. HP-G and LP-G populations were compared to estimate the predation effect and LP-G and LP-NG populations to estimate the parasitism effect. NS: nonsignificant; neg.: negative association between factor and behaviour (e.g. predation depresses activity); pos.: positive association between factor and behaviour. Non overlapping CIs indicate significantly different effect sizes between predation and parasitism regimes. B. Parallelism of regime effects across rivers was assessed by comparing the variance explained by the effect of regime (parallelism) vs. the effect of regime-by-river interaction (nonparallelism) (see methods). Percentage of variance explained is based on sums of squares (SS): SS effect/SS total (Kaeuffer *et al.*, 2012). A significant nonparallel response means that regime had different effects between the two rivers (Kaeuffer *et al.*, 2012) (see Methods).

A. Predation vs. parasitism regime effects		B. Parallel response to regime			General conclusion		
Predation effect size	Parasitism effect size	Predation vs. parasitism	Parallelism (regime effect)	Nonparallelism (regime-by-river interaction)			
<b>Activity</b>							
F0	-0.95 (-1.41, -0.49) $t_{81} = -3.98$ $P < 0.001^*$	0.06 (-0.38, 0.51) $t_{78} = -0.26$ $P = 0.79$	Predation (neg) > Parasitism (NS)	$F_{2,115} = 12.7$ $P < 0.001^*$ Var 17.6%	$F_{2,115} = 0.12$ $P = 0.80$ Var 0.17%	$F_{1,117} = 0.12$ $P = 0.89$	Parallel divergence across predation regimes in both F0 and F2
F2	-0.47 (-0.80, -0.14) $t_{148} = -2.81$ $P = 0.0056^*$	0.25 (-0.08, 0.57) $t_{159} = 1.50$ $P = 0.13$	Predation (neg) > Parasitism (NS)	$F_{2,245} = 4.41$ $P = 0.013^*$ Var 3.4%	$F_{2,245} = 0.92$ $P = 0.39$ Var 0.72%	$F_{1,248} = 0.93$ $P = 0.39$	Genetic-based negative effect of predation on activity
<b>Shoaling</b>							
F0	-0.16 (-0.60, 0.28) $t_{81} = -0.72$ $P = 0.47$	0.22 (-0.23, 0.67) $t_{78} = 0.98$ $P = 0.33$	Predation (NS) = Parasitism (NS)	$F_{2,115} = 0.47$ $P = 0.62$ Var 0.80%	$F_{2,115} = 0.49$ $P = 0.61$ Var 0.84%	$F_{1,117} = 0.49$ $P = 0.61$	No parallel divergence across regimes in F0 nor in F2
F2	0.11 (-0.22, 0.44) $t_{148} = 0.68$ $P = 0.49$	-0.33 (-0.66, -0.010) $t_{159} = -2.04$ $P = 0.043^*$	Predation (NS) = Parasitism (neg)	$F_{2,245} = 2.30$ $P = 0.10$ Var 1.80%	$F_{2,245} = 4.55$ $P = 0.012^*$ Var 3.49%	$F_{1,248} = 4.54$ $P = 0.011^*$	No consistent effect of regime on shoaling
<b>Boldness</b>							
F0	0.37 (-0.07, 0.81) $t_{81} = 1.70$ $P = 0.093$	-0.58 (-1.04, -0.13) $t_{78} = -2.61$ $P = 0.011^*$	Predation (NS) < Parasitism (neg)	$F_{2,115} = 3.37$ $P = 0.037^*$ Var 5.32%	$F_{2,115} = 0.82$ $P = 0.44$ Var 3.50%	$F_{1,117} = 0.82$ $P = 0.44$	Parallel divergence across parasitism regimes in F0 but not in F2
F2	-0.17 (-0.50, 0.16) $t_{148} = 1.01$ $P = 0.31$	-0.15 (-0.47, 0.17) $t_{159} = -0.91$ $P = 0.36$	Predation (NS) = Parasitism (NS)	$F_{2,245} = 2.63$ $P = 0.074$ Var 1.9%	$F_{2,245} = 1.03$ $P = 0.35$ Var 0.76%	$F_{1,248} = 1.03$ $P = 0.35$	Plastic negative effect of parasitism on boldness

\* indicate significant effects ( $P < 0.05$ )



**Fig. 3** Mean ( $\pm$  SE) shoaling level (residuals of time spent near conspecifics in seconds) in F0 and F2 guppies (males and females combined) from populations differing in predation and parasitism regime (HP-G: High-predation *Gyrodactylus* parasites, LP-G: Low-predation *Gyrodactylus* parasites, LP-NG: Low-predation no *Gyrodactylus*) in two replicate rivers (Marianne River in grey and Aripo River in black). No effect of regime was found in F0 generation, and a nonparallel effect of regime was found in the F2 generation (i.e. different effects of regime between rivers, see Table 2). Different letters represent significant differences between regimes and rivers in *post hoc* tests.

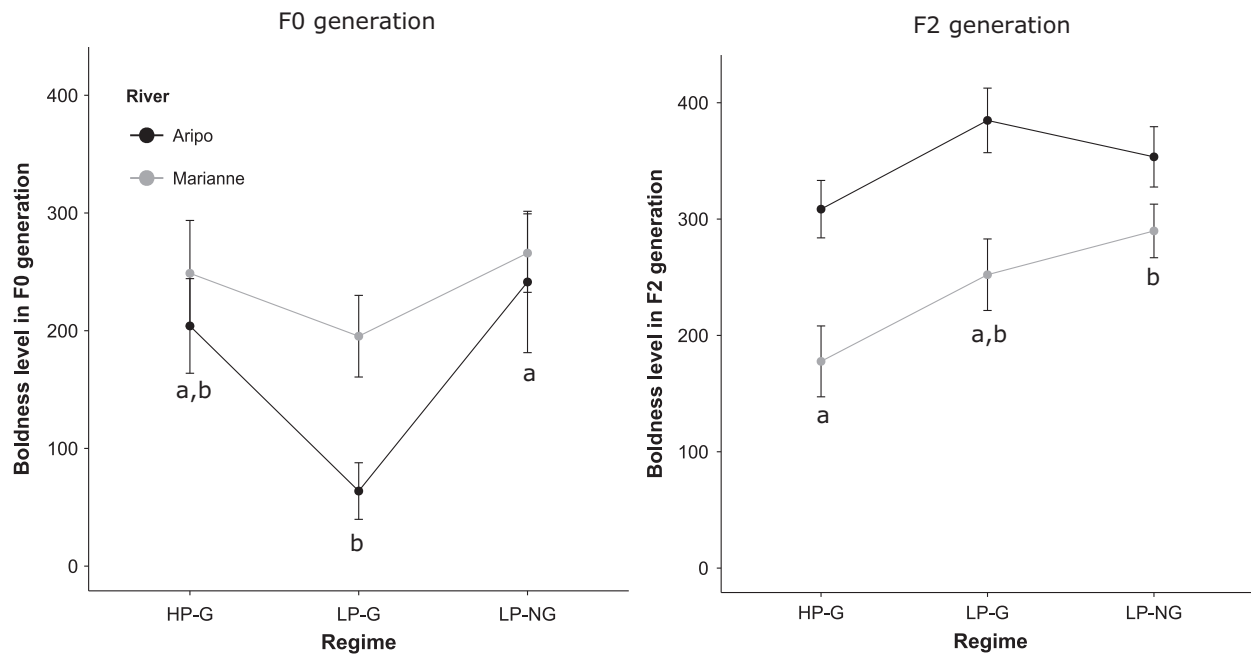
greater shoaling dilutes risk (Magurran, 2005), both of which should decrease predation. Overall, then, our findings support previous assertions that predation is an important driver of the evolution of behavioural variation in nature.

Behavioural divergence was also influenced by parasite regime, but the effects were generally weaker and less consistent than for predation. For instance, guppies that had evolved with *Gyrodactylus* (LP-G) shoaled less (but only in Aripo F2 individuals) and were less bold (but only in F0 individuals) than were guppies that had evolved without *Gyrodactylus* (LP-NG). The existence of at least some effects on shoaling is consistent with the idea that grouping behaviour influences the transmission of socially transmitted ectoparasites, such as *Gyrodactylus*, and should therefore diverge between populations that differ in the risk of parasitism (Richards *et al.*, 2010; Johnson *et al.*, 2011). Likewise, the existence of at least some effects on boldness is consistent with arguments that this trait influences exposure to parasites and should therefore diverge between populations with different risks or costs of parasitism (e.g. Wilson *et al.*, 1993; Coleman & Wilson, 1998; Boyer *et al.*, 2010). Despite these effects, however, the effects of parasites tended to be less consistent than the effects of predators.

The greater importance of predation than parasitism to behavioural divergence in guppies converges with similar arguments recently made for other guppy traits,

especially male colour (Martin & Johnsen, 2007; Gotanda *et al.*, 2013). In addition to the explanation that predators are simply stronger agents of mortality than are parasites (Combes, 2001; Introduction), several other facts might explain this difference. First, existing studies of guppies have focused on only a single parasite (*Gyrodactylus*), whereas guppies can be infected by a diverse suite of parasites (e.g. Kennedy *et al.*, 1987). Perhaps other parasites (or entire parasite communities) would have stronger effects (Rigaud *et al.*, 2010). And yet, plenty of prior evidence exists to suspect that *Gyrodactylus* is an important agent of mortality and morbidity (Scott & Anderson, 1984; Cable & Van Oosterhout, 2007; Pérez-Jvostov *et al.*, 2012) to which guppies evolve resistance (Scott & Anderson, 1984; Cable & Van Oosterhout, 2007; Dargent *et al.*, 2013; Pérez-Jvostov *et al.*, 2015). It is however possible that other behaviours not measured here are more important for parasite avoidance, such as exploration, or the ability to discriminate and avoid infected conspecifics from healthy conspecifics (e.g. Hart, 1992; Houde, 1997).

Second, we could only assess the effects of parasitism (*Gyrodactylus* presence vs. absence) in the absence of high predation, whereas predation and parasitism could have strong interactive effects, such as when parasitism increases susceptibility to predation (e.g. Hudson *et al.*, 1992; Hatcher *et al.*, 2006). Thus, the effects of parasites might be more important in the presence of dangerous predators (synergistic: Kortet *et al.*, 2010). Antagonistic



**Fig. 4** Mean ( $\pm$  SE) boldness level (maximum time minus the latency to come out of a shelter) in guppies (males and females combined) from populations differing in predation and parasitism regime (HP-G: High-predation Gyrodactylus parasites, LP-G: Low-predation Gyrodactylus, LP-NG: Low-predation no Gyrodactylus) in two independent rivers (Marianne River in grey and Aripo River in black). High values thus indicate a rapid latency to leave the shelter and are assumed to represent high boldness. A parallel effect of regime was found in the F0 generation (i.e. similar effects of regime between rivers see Table 2). Different letters represent significant differences between regimes in *post hoc* tests.

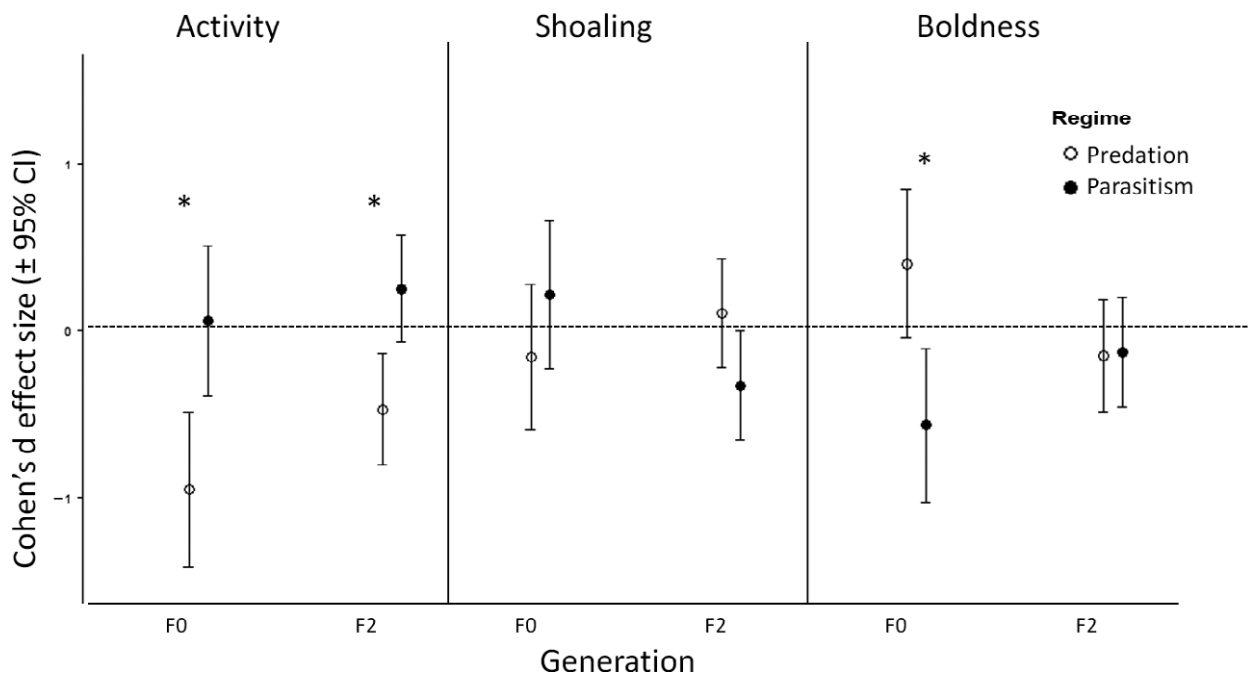
effects are also possible (Raffel *et al.*, 2008; Stephenson *et al.*, 2015); for instance, grouping behaviour (shoaling) could be beneficial in the presence of predators but detrimental in the presence of contagious parasites (Coté & Poulin 1995). If we could find a set of parasite-free high-predation guppy populations, we would be able to test these ideas in a fully crossed predation-by-parasite design. However, such populations are very rare (Martin & Johnsen, 2007; Gotanda *et al.*, 2013), probably because any site accessible to dangerous predators is also accessible to parasites. At the same time, note that predators with effects on guppies (Rodd and Reznick 1997; Millar *et al.*, 2006; McKellar and Hendry 2011) are present even in low-predation sites, they are just weaker agents of mortality than the predators in high-predation sites (Magurran, 2005). Thereby, although our study outlined more consistent effects of predation compared to parasitism on the investigated behaviours, further work is now needed on a larger number of behavioural traits and in alternative environments to test the generality of our conclusions.

### Plastic and genetic effects

Patterns of behavioural divergence frequently differed between F0 wild-caught guppies and F2 laboratory-

reared guppies; yet several population differences were observed even in F2 fish. This is consistent with previous work showing that both plastic (personal exposure to predators and parasites) and genetic responses (evolutionary responses to divergent selection) shape behavioural profiles through adaptive and nonadaptive plasticity in guppies (Huizinga *et al.*, 2009; Torres-Dowdall *et al.*, 2012). Interestingly, several additional nuances emerged.

For instance, the genetic contribution to behavioural divergence was greater for predation regime (i.e. differences in F0 fish often persisted in F2 fish) than for parasite regime (i.e. effects generally evident only in F0 fish). This genetic contribution to trait divergence between predation regimes is a typical outcome when studying guppy traits and is consistent with the expectation that predators induce strong selection that leads to important genetically based trait divergence (Reznick *et al.*, 1996b; Torres-Dowdall *et al.*, 2012). Thus, the weaker genetically based divergence in relation to parasite regime could be taken as support for the idea that parasites often have milder and more transient effects that might impose weaker selection (Combes, 2001). In addition, the consistency of parasitism (intensity and virulence) within and between generations might be lower than the consistency of predation, which should favour plastic responses to parasitism rather than



**Fig. 5** Effect sizes of predation (white dots) and parasitism (black dots) on activity, shoaling and boldness in F0 and F2 generations. HP-G and LP-G populations were compared to estimate the predation effect and LP-G and LP-NG populations to estimate the parasitism effect. Effect sizes were calculated using Cohen's *d* coefficients and their 95% confidence intervals CIs (Cohen, 1988; Nakagawa & Cuthill, 2007). A negative effect size indicates a decreasing effect of predation/parasitism on behaviour. Nonoverlapping CIs indicate significantly different effect sizes between parasitism and predation regime and are indicated by an asterisk.

genetic-based divergence (Raffel *et al.*, 2008; Schmid-Hempel, 2011).

### Parallelism and nonparallelism

Some behaviours showed strong parallel responses to predation/parasitism (i.e. the main effect of regime was strong, whereas the river-by-regime interaction was weak), which aids inferences about specific causal agents of selection. For instance, activity showed strong parallel *phenotypic* and *genetic* divergence (both F0 and F2 generations) associated with predation, whereas boldness showed strong parallel *phenotypic* divergence (F0 generation only) associated with parasitism. These results are broadly consistent with work showing that the HP-LP contrast generates roughly similar trait divergence across multiple independent guppy lineages, even though the specific predators differ markedly among drainages (e.g. Endler, 1995; Reznick *et al.*, 1996b). Our results are also consistent with previous studies arguing that *Gyrodactylus* parasites have some effects on guppy behaviour, especially shoaling and boldness (e.g. Richards, 2010; Croft *et al.*, 2011). Given that all fish were treated for parasites well before the behavioural assays, our results indicate at least some persistent behavioural effects of previous parasite exposure (for

instance through immune activation) although the underlying mechanisms remain to be explored.

At the same time, many variations could not be explained by parallel responses to predation/parasitism regime. First, the main effect of river was sometimes very strong: for instance, across predation/parasitism regimes, Marianne F0 guppies were more active than were Aripo F0 guppies, and Aripo F2 guppies were bolder than were Marianne F2 guppies. Second, the effects of regime sometimes differed between rivers (i.e. regime-by-river interaction). For instance, F2 HP-G guppies shoaled more than did LP-G guppies, but only in the Aripo River. These results indicate that local factors other than predation and parasite regime play a major role in shaping behavioural divergence. One such factor could be resource availability, which differs among sites within and between rivers (Grether *et al.*, 2001; Reznick *et al.*, 2001; McKellar *et al.*, 2009) and is known to shape social behaviours and shoaling in fishes (e.g. Hensor *et al.*, 2003). Another potential factor relates to the different suites of predators found on the north (Marianne) vs. south (Aripo) slopes of the Northern Mountain Range (Endler, 1984; Reznick *et al.*, 1996b; Magurran, 2005; Millar *et al.*, 2006). That is, different predators are expected to select for different behaviours in the fishes on which they prey

(Templeton & Shriner, 2004). Exploring these potential explanations will require additional studies of more sites and lineages.

Another interesting aspect of nonparallelism was that behavioural responses to predation/parasitism often differed between the sexes (i.e. sex-by-regime interaction). For instance, only F0 males differed in shoaling between regimes, and F0 females were shyer than males. This last result is consistent with previous work showing that females are generally shyer than males and show stronger risk-taking responses to predation (Magurran *et al.*, 1992; Magurran & Seghers, 1994; Harris *et al.*, 2010). Such sex-specific behavioural responses might be due to the sex-specific payoff functions associated with risk (Laland & Reader, 1999; Reader, 2015) and the fact that males are more willing than females to incur the risk associated with seeking additional mating opportunities (Magurran & Seghers, 1994). More attention should be paid to the differential evolutionary responses of males and females to the same environmental differences, as previously suggested for other guppy traits (Hendry *et al.*, 2006; Dargent *et al.*, 2016).

### Personalities

We conducted repeated tests to measure individual consistency in behaviour. The different behaviours were somewhat repeatable across trials and were within the range of repeatability values usually found in fishes (Bell *et al.* 2009). Similar results have been found in other studies of guppies (e.g. Harris *et al.*, 2010; Smith & Blumstein, 2010; Brown *et al.*, 2014), suggesting they fit the accepted concept of personalities (Réale *et al.*, 2007). In this context, our results add to the literature suggesting that predation, and to a lesser extent parasitism, shape the evolution of animal personalities (Barber & Dingemans, 2010; Kortet *et al.*, 2010). Interestingly, we detected no consistent difference across regimes in the level of behavioural repeatability (consistency), suggesting that predation and parasitism do not affect the level of behavioural consistency. Despite these inferences, some limits remain. In particular, repeatabilities were not especially strong and our experimental design likely generated a bias in their favour. In particular, our 20 min interval between trials is a short time lag, which could artificially increase repeatability of certain behaviours. Further studies are thus needed to compare personalities tested in additional ways in a greater number of replicate populations with different evolutionary origins.

### Conclusions and implications

Our study reveals that both predation and parasitism shape behavioural traits, but in quite different ways.

Effects of predation tend to be strong, genetically based and parallel across independent host lineages experiencing different predators. Effects of parasitism tend to be plasticity-based and less parallel across host lineages. As noted above, these patterns are in accordance with the expected difference between predators and parasites in the strength and temporal consistency of selection (Combes, 2001; Raffel *et al.*, 2008; Schmid-Hempel, 2011). Yet much remains to be learned. For instance, predator regimes were characterized based on an entire community, whereas parasitism regime was characterized based on a single species. We now need to consider the effects of individual predators and of parasite communities. In addition, the specific mechanism by which parasite exposure leads to persistent behavioural effects after parasites are removed needs to be elucidated. Finally, predators and parasites might have decoupling effects on behavioural correlations, which could affect the evolution of behavioural syndromes and hence the evolution of multivariate phenotypes (Sih *et al.*, 2004; Bell & Sih, 2007; Coats *et al.*, 2010; Poulin, 2013). We look forward to exploring these questions in future work.

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## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article: **Figure S1**. Mean ( $\pm$  SE) shoaling level (residuals of time spent near conspecifics) of male (left) and female (right) F0 guppies from populations differing in predation and parasitism regime (HP-G: High Predation-



Gyrodactylus parasites, LP-G: Low Predation-Gyrodactylus parasites, LP-NG: Low Predation- No Gyrodactylus) in two replicate rivers (Marianne in grey and Aripo in black).

**Figure S2.** Mean ( $\pm$  SE) boldness level (maximum time minus latency to leave a refuge) of male (left) and female (right) F2 guppies from populations differing in predation and parasitism regime (HP-G: High Predation-Gyrodactylus parasites, LP-G: Low Predation-

Gyrodactylus parasites, LP-NG: Low Predation- No Gyrodactylus) in two replicate rivers (Marianne River in grey and Aripo River in black).

**Table S1** All best models ( $\Delta$ AICc < 2) explaining activity, shoaling and boldness in F0 and F2 individuals.

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