Demasculinization of male guppies increases resistance to a common and harmful ectoparasite

FELIPE DARGENT[1,2]*, ADAM R. REDDON[1], WILLIAM T. SWANEY[1,3], GREGOR F. FUSSMANN[1], SIMON M. READER[1], MARILYN E. SCOTT[4] *and* MARK R. FORBES[2]

[1] *Department of Biology, McGill University, 1205 Dr. Penfield Av., Montreal, H3A 1B1, QC, Canada*
[2] *Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, K1S 5B6, ON, Canada*
[3] *School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, UK*
[4] *Institute of Parasitology and Centre for Host-Parasite Interactions, McGill University, 21111 Lakeshore Road, Ste-Anne de Bellevue, H9X 3V9, QC, Canada*

(*Received 11 June 2015; revised 8 August 2015; accepted 1 September 2015; first published online 24 September 2015*)

SUMMARY

Parasites are detrimental to host fitness and therefore should strongly select for host defence mechanisms. Yet, hosts vary considerably in their observed parasite loads. One notable source of inter-individual variation in parasitism is host sex. Such variation could be caused by the immunomodulatory effects of gonadal steroids. Here we assess the influence of gonadal steroids on the ability of guppies (*Poecilia reticulata*) to defend themselves against a common and deleterious parasite (*Gyrodactylus turnbulli*). Adult male guppies underwent 31 days of artificial demasculinization with the androgen receptor-antagonist flutamide, or feminization with a combination of flutamide and the synthetic oestrogen 17 β-estradiol, and their parasite loads were compared over time to untreated males and females. Both demasculinized and feminized male guppies had lower *G. turnbulli* loads than the untreated males and females, but this effect appeared to be mainly the result of demasculinization, with feminization having no additional measurable effect. Furthermore, demasculinized males, feminized males and untreated females all suffered lower *Gyrodactylus*-induced mortality than untreated males. Together, these results suggest that androgens reduce the ability of guppies to control parasite loads, and modulate resistance to and survival from infection. We discuss the relevance of these findings for understanding constraints on the evolution of resistance in guppies and other vertebrates.

Key words: gonadal steroids, feminization, sex-biased parasitism, testosterone, fish, *Gyrodactylus*, *Poecilia reticulata*.

INTRODUCTION

Parasites are pervasive and are known to negatively influence host fitness by reducing reproductive output, growth rate, mating success and survivorship (Price, 1980). In doing so, parasites can be influential drivers of ecological processes and evolutionary patterns (Hamilton, 1982; Hamilton and Zuk, 1982; Lafferty *et al*. 2008; Minchella and Scott, 1991). Parasitism is expected to be a strong source of selection for defensive adaptations that allow hosts to control parasite numbers and mitigate parasite costs. When parasites are present, investment in costly defence mechanisms is expected to be favoured (Schmid-Hempel, 2011). Intriguingly, there is considerable variation amongst individuals within populations in their susceptibility to parasites, suggesting that antiparasite defences are costly and/or trade-off with other fitness enhancing traits, and therefore that maximal defence may not be obtainable or adaptive for all individuals (Lazzaro and Little, 2009; Sheldon and Verhulst, 1996). A striking example of among-individual variation in parasite susceptibility is the common phenomenon of sex-biased parasitism, in which one sex is more frequently infected or carries larger mean parasite loads than the other (Forbes, 2007; Krasnov *et al*. 2012; Nunn *et al*. 2009; Zuk and McKean, 1996). For example, Amo *et al*. (2005), found that wild male wall lizards (*Podarcis murallis*) had higher haemogregarine and ectoparasitic mite infection intensities than did females. Similarly, Krasnov *et al*. (2005) found higher flea abundance in males than females in 6 out of 9 species of desert rodents.

Males and females differ in many ways that may partially account for sex differences in parasite infection rates. For example, males and females often differ in body size and larger individuals typically have more parasites (Guégan *et al*. 1992; Poulin and Rohde, 1997). Males and females may also be exposed to parasites at different rates due to sex differences in space use or social behaviour (Tinsley, 1989). Furthermore, sex differences in time and energy allocation to sexual activities (e.g. courting and fighting) and resource acquisition also could drive sex differences in parasite loads through differences in the amount of resources available for investment in defence (Zuk, 1990).

* Corresponding author. Department of Biology, McGill University, 1205 Dr. Penfield Av., Montreal, H3A 1B1, QC, Canada, Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, K1S 5B6, ON, Canada. E-mail: felipe.dargent@mail.mcgill.ca

doi:10.1017/S0031182015001286

Gonadal steroids play a critical role in sexual differentiation during development, resulting in sex differences in anatomy, physiology and behaviour (Arnold, 2009; Wallen and Baum, 2002), and therefore may have a long-term influence on sex-biased parasitism by organizing phenotypic characteristics during development which in turn affects parasite defence later in life. However, gonadal steroids can also have a more immediate influence on sex-biased parasitism because variation in circulating hormones in adults can mediate sex differences in immune function (Grossman, 1989; Zuk and McKean, 1996). Understanding precisely how circulating gonadal steroids influence defence is a crucial step in understanding individual variation in defence, as well as the potential for and magnitude of sex-biased parasitism, which in turn are necessary for understanding host–parasite dynamics in natural systems. To this end, it is essential to evaluate both the role of gonadal steroids during development and the role that circulating gonadal steroids play in parasite resistance in adults.

Here, we studied guppies (*Poecilia reticulata*) derived from wild populations, and their common and harmful ectoparasites (*Gyrodactylus turnbulli*), to address the importance of circulating gonadal steroids in determining antiparasite defences, i.e. the effect that steroid hormone systems have on adult resistance to parasites. To this end, we manipulated gonadal steroid levels in adult guppies by administering an androgen receptor antagonist (to demasculinize them), or a combination of an androgen receptor antagonist and an artificial oestrogen (to demasculinize and then feminize them), before assessing their resistance to *G. turnbulli*.

MATERIALS AND METHODS

The study system

The guppy is a sexually-dimorphic poeciliid fish native to the island of Trinidad and Northern South America (Magurran, 2005). *Gyrodactylus turnbulli* is a highly prevalent (Harris and Lyles, 1992) and deleterious ectoparasite of wild guppies (Gotanda *et al*. 2013; van Oosterhout *et al*. 2007*a*). These monogenean flatworms transmit through host-to-host contact, and attach to their host's epithelium where they feed and give birth to flukes with fully developed embryos 'in-utero' (Bakke *et al*. 2007). Therefore, *Gyrodactylus* infections are prone to exponential population increase on individual hosts and epidemic dynamics within guppy populations (Scott and Anderson, 1984), which leads to high guppy mortality in the laboratory (Dargent *et al*. 2013*a*; van Oosterhout *et al*. 2007*b*) and the wild (van Oosterhout *et al*. 2007*a*).

The guppy-*Gyrodactylus* host–parasite system is a convenient model to assess the role of gonadal steroids in the defence against parasites. First, the regulatory effect of androgens on guppy behaviour and colouration may play a critical role in the expression of secondary sexual characters and mating success (Bayley *et al*. 2002, 2003). Second, correlations between carotenoid colouration, mate preference and defence against parasites have long been recognized in guppies (Houde and Torio, 1992; Kennedy *et al*. 1987; Kolluru *et al*. 2006), while the ecological and evolutionary drivers of guppy parasite defence have been the focus of much recent research (Dargent *et al*. 2013*a*, *b*; Fitzpatrick *et al*. 2014; Gotanda *et al*. 2013; Perez-Jvostov *et al*. 2012, 2015; Tadiri *et al*. 2013). Missing from this increasingly well-understood model system is the degree to which circulating gonadal steroids influence defence against *Gyrodactylus* parasites in the guppy.

Guppies used in this research were laboratory-reared from fish collected in Trinidad. In Experiment 1, we used F2 descendants from a Trinidadian population collected in 2013 after having been experimentally translocated in 2009 (Travis *et al*. 2014) from a high-predation site in the Guanapo river where *Gyrodactylus* spp. was present to a tributary stream (Lower Lalaja) where predation was low and *Gyrodactylus* was absent. In Experiment 2, we used descendants of wild-caught fish collected between 2010 and 2011 from the Aripo and Quare rivers from sites where predation is high and *Gyrodactylus* spp. is present. These guppies were housed together as a mixed origin population.

Hormone treatments

Two weeks prior to the start of the hormone treatments, sexually mature guppies were isolated into 1·8 L chambers in an aquatic housing system (Aquaneering Inc., San Diego, USA). The fish were physically isolated, but retained visual contact with their neighbours throughout the experiments. The laboratory was maintained at 23 ± 1 °C with a 13 h:11 h (L:D) photoperiod. We used carbon-filtered municipal water that was conditioned with Prime (Seachem Laboratories, Madison, USA), freshwater Biozyme (Mardel, Oklahoma City, USA), and left to stand for 2 days and warm up before being added to the housing systems. The housing system passed water through a filter pad, a biological filter, a set of carbon filters and a UV sterilization device. Subjects were fed with TetraMin Tropical Flakes (Tetra, Melle, Germany) ground into powder and reconstituted with water to form a thick paste that was delivered using Hamilton microlitre syringes (Hamilton Laboratory Products, Reno, USA). Prior to the start of the hormone treatments, subjects were fed *ad libitum* and their chambers remained connected to the recirculating system, thus each chamber had a complete water change approximately every 8 min.

https://doi.org/10.1017/S0031182015001286 Published online by Cambridge University Press

We gathered data on individual body size (measured as standard length: SL) and mass at two time points: on the first day we began administering the hormone treatments, and 21 days later, the day we began experimental parasite infections (Supplementary Tables S1, S2). To measure SL and mass we anesthetized each guppy in 0·02% Tricaine Methanesulfonate (MS222; Argent Chemical Laboratories, Redmond, USA) buffered to a pH of 7 with $NaCO_3$. Guppies were then weighed to the nearest 0·01 g and photographed on the left side with a Nikon D90 camera (Nikon, Mississauga, Canada). Each image included a ruler for scale.

At the start of the hormone treatments, male guppies (mean ± S.E.M. mass = 0·08 g ± 0·002) were randomly assigned to control, demasculinization or feminization treatments, while females (mean ± S.E.M. mass = 0·13 g ± 0·006) remained untreated. Acetone was used as a solvent to combine the pharmacological agents with ground flake food. We saturated the food with acetone mixed with the hormone treatment and then allowed the acetone to evaporate in a fume hood for 24 hours. Untreated control male and female guppies received food that had been saturated with acetone alone without any pharmacological treatment, guppies in the demasculinization treatment received food that had been dosed with 4·29 mg of the androgen receptor antagonist flutamide (Sigma–Aldrich, Oakville, Canada) per gram of dry food, and guppies in the feminization treatment received food that had been dosed with 4·29 mg of flutamide and 0·04 mg of the synthetic oestrogen 17 β-estradiol (Sigma–Aldrich, Oakville, Canada) per gram of dry food. Each guppy received 5 μL day^{-1} of paste prepared with their respective treatments (in a 7:8 food:water ratio), which is equivalent to 10·40 μg day^{-1} guppy^{-1} of flutamide and 0·10 μg day^{-1} guppy^{-1} of 17 β-estradiol. Guppies ingested all of the food provided to them. The flutamide dosage was based on previous dose-response studies in guppies showing effective inhibition of male-specific traits (Bayley *et al*. 2003; Kinnberg and Toft, 2003), without the increased mortality seen at higher doses (Baatrup and Junge, 2001). The dose of 17 β-estradiol per g body weight was based on dose-response work in goldfish demonstrating robust inhibition of male-specific traits, but no associated weight loss (Bjerselius *et al*. 2001). All hormone treatments lasted for 31 days (i.e. 21 days of treatment without parasite infections and 10 days of treatment after *Gyrodactylus* infection). We performed 2 consecutive experiments. Experiment 1 had 2 treatments: feminization of males and untreated males. Experiment 2 had the same treatments as Experiment 1 in addition to demasculinization of males and untreated females. These experiments were identical in all regards with the exception of the additional treatments (see below) and the use of different wild-derived guppy populations.

During the 31 days of hormone treatment, the guppies remained in their 1·8 L chambers, which we disconnected from the aquatic recirculating system, chemically isolating the fish to ensure that no hormone treatment passed between the chambers. Visual contact between neighbours was retained throughout the experiment and therefore the fish were not socially isolated at any time. To maintain water quality during the treatment period, we changed 75% of the water in each chamber every 4 days and replaced the chamber with an entirely fresh one every 12 days. Water quality was monitored throughout the experiments by performing visual checks for water clarity and residue presence and by weekly tests, in randomly selected chambers, of alkalinity, pH, nitrite, nitrate, hardness and ammonia. Water quality was within normal range throughout the experiment and we did not detect any sign of water quality degradation at any time, or of negative effects of water quality on the hosts or parasites.

Experimental infections

Twenty-one days after the start of the hormone treatments, all fish were individually anaesthetized in 0·02% MS222 and infected with 2 *G. turnbulli* each. We infected each guppy by removing a small piece of fin tissue or a scale carrying *G. turnbulli* from a euthanized infected donor guppy and placing it next to the recipient guppy's caudal fin until we saw, under a Nikon SMZ800 dissecting stereoscope (Nikon Instruments, Melville, USA), that 2 *G. turnbulli* had attached to the experimental fish. After infection, each guppy was allowed to recover from anaesthesia in its home chamber. We monitored *G. turnbulli* numbers on each live subject on days 6, 8 and 10 post infection, by anaesthetizing the fish and counting the parasites using the dissecting stereoscope at 18× magnification. We used *G. turnbulli* from our laboratory population, which was initially obtained in 2009 from domestic guppies purchased from a commercial supplier in Montreal, QC, Canada. This *G. turnbulli* population has been maintained on domestic-origin host guppies, and therefore has not had any period of co-evolution with the wild-origin guppy populations used in this study.

Analysis

To assess whether hormone treatment and guppy body size (SL) had an effect on *G. turnbulli* load on each count day, we fitted a generalized linear model (GLM) with a negative binomial distribution and a log-link function and used Tukey HSD for pairwise *post hoc* comparisons. To assess the general effect of hormone treatment on the growth trajectory of *G. turnbulli* we fitted a repeated

https://doi.org/10.1017/S0031182015001286 Published online by Cambridge University Press

measures GLM with a negative binomial distribution for Experiment 1. We were unable to perform this analysis for Experiment 2 because of the high parasite-induced mortality in the untreated control group. The repeated measures GLM was conducted in SPSS 22 (IBM, New York, USA), all remaining analyses were conducted using the R Language and Environment for Statistical Computing v 3.1.0 (R Development Core Team, 2014). α was set at $P < 0{\cdot}05$. Data are archived in the Dryad repository (doi:10.5061/dryad.k8fg7).

Experiment 1

To assess whether guppy resistance was influenced by the action of circulating gonadal steroids we compared 15 untreated males to 14 males that had been treated simultaneously with flutamide (an androgen receptor antagonist) and 17 β-estradiol (a synthetic oestrogen) (Supplementary Table S3). Guppy body size and mass did not significantly differ between treatments (feminization *vs* untreated) at the start of the experiment (SL: $F_{1,27} = 0{\cdot}91$; $P = 0{\cdot}35$; mass: $F_{1,27} = 0{\cdot}23$; $P = 0{\cdot}63$), nor at the start of infection (i.e. 21 days after the start of hormone treatment; SL: $F_{1,26} = 0{\cdot}14$; $P = 0{\cdot}71$; mass: $F_{1,27} = 0{\cdot}01$; $P = 0{\cdot}91$). Subjects were laboratory-reared F2 descendants from a Trinidadian population experimentally translocated in 2009 (Travis *et al*. 2014).

Experiment 2

To disentangle the relative roles of androgens and oestrogens, we ran a second experiment, repeating both treatments in Experiment 1 along with 2 additional treatments: male demasculinization and untreated females, resulting in 4 total treatment groups (Supplementary Table S4). Males under demasculinization were treated with flutamide only, allowing us to investigate male parasite resistance when androgen receptor signalling is blocked. Different gonadal steroids can have contrasting effects on immune function: androgens generally have immunosuppressive effects, while oestrogens often promote disease resistance, although effects can vary (Klein, 2000, 2004). We also tested untreated females to check for sex-biased parasite resistance in untreated guppies, as this is known to vary between populations (Dargent, 2015; Gotanda *et al*. 2013; Stephenson *et al*. 2015).

As is typical for guppies, the females were larger than the males, both at the beginning of the experiment (mean ± S.E.M. SL: males = 15·46 ± 0·16, females = 17·97 ± 0·3; $F_{3,65} = 21{\cdot}28$; $P < 0{\cdot}001$) and at the time of infection (mean ± S.E.M. SL: males = 15·56 ± 0·14, females = 18·57 ± 0·28; $F_{3,68} = 36{\cdot}99$; $P < 0{\cdot}001$). There was no significant difference in SL among the 3 male treatments at either time point (start of treatments: $F_{2,47} = 1{\cdot}1$, $P = 0{\cdot}34$; infection: $F_{2,50} = 0{\cdot}72$, $P = 0{\cdot}49$). A similar pattern was observed for body mass. Female guppies were heavier than males when they started receiving the hormone treatments (mean ± S.E.M. mass: males = 0·09 ± 0·003, females = 0·13 ± 0·006 g; $F_{3,65} = 14{\cdot}73$; $P < 0{\cdot}001$) and on the first day of infection (mean ± S.E.M. mass: males = 0·08 ± 0·003, females = 0·14 ± 0·006 g; $F_{3,68} = 31{\cdot}17$; $P < 0{\cdot}001$), but mass did not differ between male treatments at the start of the experiment ($F_{2,47} = 0{\cdot}38$; $P = 0{\cdot}68$) nor on the day of infection ($F_{2,50} = 0{\cdot}24$; $P = 0{\cdot}79$). Males did not differ in SL between Experiments 1 and 2 (initial SL: $F_{1,77} = 0{\cdot}42$; $P = 0{\cdot}52$; infection day SL: $F_{1,79} = 0{\cdot}004$; $P = 0{\cdot}95$) but males in Experiment 1 were lighter than those in Experiment 2 (initial mass: $F_{1,77} = 6{\cdot}21$; $P = 0{\cdot}01$; infection day mass: $F_{1,80} = 4{\cdot}53$; $P = 0{\cdot}04$; Supplementary Tables S1, S2).

Post infection mortality was high in Experiment 2 and so we used a Cox proportional hazards model to determine whether hormone treatment and body size (SL) influenced guppy survival up to 13 days post infection (i.e. 3 days after we had finished treating the guppies with hormones). Standard length and its interaction with hormone treatment had no significant effects on survival and thus were dropped from the model by AIC stepwise model selection.

RESULTS

Experiment 1

Guppies that underwent feminization via combined treatment with flutamide and 17 β-estradiol had significantly lower *G. turnbulli* loads than untreated guppies throughout the infection period (repeated measures GLM effect of treatment: $F_{1,77} = 4{\cdot}94$; $P < 0{\cdot}029$), and specifically on both days 8 and 10 of infection (Table 1; Fig. 1; Supplementary Figure S1). We did not detect any significant effect of SL or of its interaction with the hormone treatment on parasite load (Table 1). Mortality following infection was low. Only 3 individuals had died (10%) by day 10 of infection, all of which were in the untreated group (Supplementary Table S3). *Gyrodactylus turnbulli* populations on individual guppies continued to grow through the duration of the experiment (Supplementary Figure S1). We observed no obvious pathological effects of treatment with flutamide and 17 β-estradiol in concert (feminization) and this treatment significantly increased resistance to *G. turnbulli* on all guppies.

Experiment 2

Hormone treatment had a significant effect on parasite load at both day 8 and day 10 (Table 2; Fig. 2; Supplementary Figure S2). As in Experiment 1, males that underwent feminization also had lower

https://doi.org/10.1017/S0031182015001286 Published online by Cambridge University Press

Table 1. *Gyrodactylus turnbulli* parasite load is significantly higher in untreated male guppies compared with males under feminization on day 8 and 10 of infection (Experiment 1)

	Day 6[a]	Day 8[b]	Day 10[c]
Hormone treatment	3	5·49*	5·01*
SL	2·73	2·44	1·18
Treatment: SL	0·83	0·35	0·12

Generalized linear model results for *G. turnbulli* load (integer variable with a negative binomial distribution) on guppies for days 6, 8 and 10 of infection, with treatment (feminization *vs* untreated males) as factor and guppy standard length (SL) as a covariate. *F*-values reported (* = $P < 0{\cdot}05$).
[a] $n = 29$.
[b] $n = 28$.
[c] $n = 26$.

Table 2. *Gyrodactylus turnbulli* parasite load varies with sex and hormone treatment on day 8 and 10 of infection (Experiment 2)

	Day 6[a]	Day 8[b]	Day 10[c]
Hormone treatment	2·5	3·26*	11·25***
SL	0·81	0·26	0·29
Treatment: SL	0·49	0·24	2·68

Generalized linear model results for *G. turnbulli* load (integer variable with a negative binomial distribution) on guppies for days 6, 8 and 10 of infection, with treatment (untreated males, untreated females, males under demasculinization, and males under feminization) as factor and guppy standard length (SL) as a covariate. *F*-values reported (* = $P < 0{\cdot}05$, *** = $P < 0{\cdot}001$).
[a] $n = 72$.
[b] $n = 62$.
[c] $n = 40$.

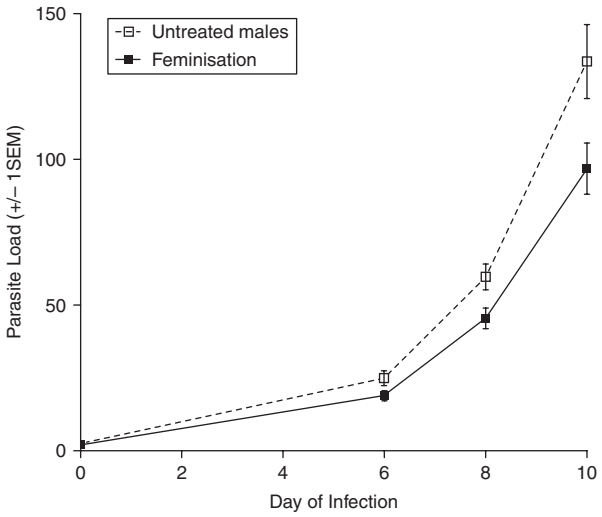

Fig. 1. Mean ± S.E.M.*Gyrodactylus turnbulli* parasite load per host for untreated male guppies (dashed line) or males treated with flutamide and 17 β-estradiol (feminization – solid line) by day of infection (Experiment 1).

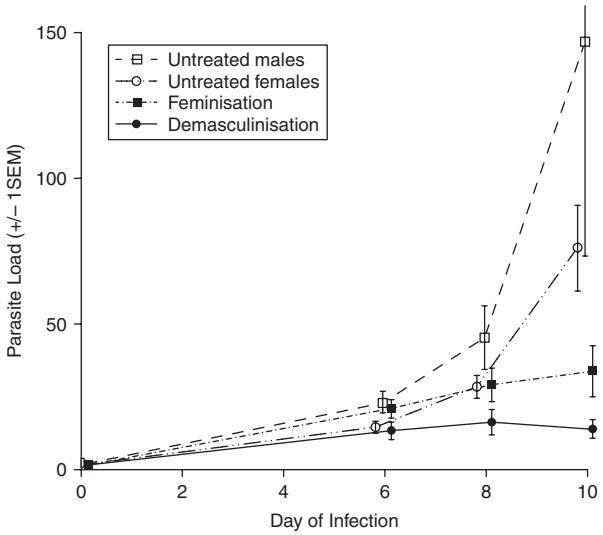

Fig. 2. Mean ± S.E.M. *Gyrodactylus turnbulli* load per host in male guppies treated with flutamide (demasculinization), with flutamide and 17 β-estradiol (feminization), and in untreated males and females, compared across days after infection (Experiment 2). Points are slightly offset on the *x*-axis to reduce overlap.

G. turnbulli loads on both days 8 and 10 of infection compared with untreated males (Tables 2, 3; Fig. 2), although this difference was only statistically significant on day 10. Males that underwent the demasculization treatment had significantly lower *G. turnbulli* loads compared with untreated males on days 8 and 10 of infection (Tables 2, 3; Fig. 2). Parasite loads were not significantly different between those males that underwent demasculinization and those that underwent feminization at any time point, and both had lower loads than untreated females on day 10 (Tables 2, 3; Fig. 2). With few exceptions, *G. turnbulli* populations on individual guppies continued to grow for the duration of the experiment while their hosts remained alive, but growth trajectories differed between treatments (Supplementary Figure S2). We observed no significant effects of SL or any interaction effects between body size and treatment on parasite load (Table 2). Contrary to previous studies on wild guppy populations (Gotanda *et al*. 2013), we found no evidence that guppies from our Aripo/Quare mixed-origin laboratory-bred population were sexually dimorphic in *G. turnbulli* resistance (Table 3). In contrast to Experiment 1, guppy mortality after infection with *G. turnbulli* was high in the mixed Aripo/Quare population: 67% of all fish had died by the 13th day of infection (56% by the 10th day). This mortality was significantly higher in the untreated males than in either group of treated males (demasculinization or feminization) or the untreated females (Table 4; Supplementary Figure S2).

https://doi.org/10.1017/S0031182015001286 Published online by Cambridge University Press

Table 3. *Post hoc* pairwise comparisons of *Gyrodactylus turnbulli* load by treatment (Experiment 2)

Treatment pair	Day 8 diff.	adj. P	Day 10 diff.	adj. P
UF–UM	−19·25	0·24	−80·15	0·1
FeM–UM	−18·36	0·27	−127·83	<0·01
DeM–UM	−33·05	0·01	−150·15	<0·001
FeM–UF	0·88	0·99	−47·68	0·05
DeM–UF	−13·81	0·44	−70	<0·01
DeM–FeM	−14·69	0·37	−22·32	0·60

Tukey HSD *post hoc* pairwise comparison among treatments for guppies in Experiment 2. A negative difference indicates that the second group in a treatment pair had a higher parasite load than the first treatment. UM, untreated males; UF, untreated females; DeM, males under demasculinization; and FeM, males under feminization.

Table 4. Guppy survival by treatment post infection with *Gyrodactylus turnbulli*

Coefficient	Estimate	SEM	*Z*-value	$P(>\|z\|)$
Untreated females	−2	0·47	−4·3	<0·001
Feminization males	−1·16	0·37	−3·1	0·002
Demasculinization males	−1·48	0·4	−3·67	<0·001

Cox proportional hazards results for survival until day 13 after infection, 'day of death' as a response variable, and 'treatment' as explanatory variable. Values are for individuals of a given treatment compared with untreated males.

DISCUSSION

We conducted two independent experiments with different populations of wild-origin guppies and found that gonadal steroids affect the ability of male guppies to control infection by the ectoparasite *G. turnbulli*. *Gyrodactylus turnbulli* populations on individual hosts increased over the experiment, but treatment with the androgen receptor antagonist flutamide (resulting in 'demasculinized' males) or a combination of flutamide and the oestrogen 17 β-estradiol (resulting in 'feminized' males) resulted in reduced *G. turnbulli* loads compared with untreated males or females. These differences were not explained by differences in body size. Furthermore, males under both feminization and demasculinization treatments showed significantly greater survival compared with untreated males following infection in our second experiment. Variation in *G. turnbulli* population growth within treatments and between experiments is likely to be influenced by the autocorrelative nature of *Gyrodactylus* population growth (Ramírez *et al*. 2012), yet the effects of gonadal steroid manipulation generated significantly different parasite loads between treatments in both experiments. Taken as a whole, these results suggest that androgens have a detrimental effect on guppy resistance to parasitism.

To our knowledge, only one previous study has experimentally assessed the role of gonadal steroids on *Gyrodactylus* resistance. Buchmann (1997) evaluated the effect of testosterone on female trout's (*Oncorhynchus mykiss*) resistance to *Gyrodactylus derjavini* and concluded that testosterone injections led to higher parasite loads. However, the results of the Buchmann (1997) study could not distinguish between a detrimental effect of testosterone on host defence and the alternative hypothesis that testosterone has a direct positive effect on *Gyrodactylus* reproduction. Our results suggest that a detrimental effect of androgens on the host is more likely than a direct effect of testosterone on *Gyrodactylus* reproduction. Our experimental fish received flutamide, which binds to androgen receptors, broadly inhibiting the host physiological responses to multiple androgens in teleost fishes (including both testosterone and 11-ketotestosterone; de Waal *et al*. 2008; Jolly *et al*. 2006) without altering the circulating levels of these hormones (Jensen *et al*. 2004).

Oestrogens also may have immunomodulatory effects in teleost fishes (e.g. Cuesta *et al*. 2007; Watanuki *et al*. 2002), but the degree to which they enhance or reduce host immunity seems to be highly system- and species-specific (Chaves-Pozo *et al*. 2012). When we consider the role of oestrogens on defence against *Gyrodactylus*, 2 lines of evidence suggest that it did not have a major effect in the guppy. First, male guppies treated with flutamide and 17 β-estradiol did not differ in resistance from male guppies treated with flutamide alone, suggesting that 17 β-estradiol did not have a substantial additional effect on defence. Second, untreated female guppies were not more resistant than males that underwent demasculinization and, in fact, they had higher parasite burdens on day 10 of infection.

Female guppies are larger than males and sexual dimorphism in body size is a common explanation for sex-biased parasitism in vertebrates. The larger

https://doi.org/10.1017/S0031182015001286 Published online by Cambridge University Press

sex is expected to have higher parasite loads since larger individuals have a wider area exposed to parasite attacks or a larger resource base for the parasite population to grow (Zuk and McKean, 1996). However, we did not detect a difference in parasite loads between untreated males and females, nor did body size correlate with variation in resistance in either experiment. This finding might appear surprising, given that field surveys (Gotanda *et al*. 2013) and laboratory experiments (Dargent, 2015) report sex differences in *Gyrodactylus* loads in certain guppy populations, and in at least one instance such a sex difference has been linked to size dimorphism (Cable and van Oosterhout, 2007). However, sex-biased parasitism in guppies is not consistently male biased and appears to be influenced by predation regime. For example, Gotanda *et al*. (2013) reported higher *Gyrodactylus* spp. loads on females compared with males in natural streams where the risk of predation was high, but the reverse pattern at sites where the risk of predation was low, suggesting that body size differences are not a comprehensive explanation for sex-biased parasite loads in guppies. Furthermore, experimentally translocated wild guppies have been shown to rapidly evolve resistance to *Gyrodactylus* in a sex-specific manner, leading to the loss of sexual dimorphism in resistance (Dargent, 2015). Thus, it is possible that the population we used for Experiment 2 is one that shows minimal sexual dimorphism in resistance to *Gyrodactylus*, although we did observe higher mortality in untreated males than females. It is possible that the high mortality in the untreated male group, which considerably reduced our sample size, precluded our ability to detect an otherwise significant dimorphism in parasite loads. Nevertheless, our results clearly show that regardless of any initial sexual dimorphism in defence, interfering with androgen signalling augments resistance to *G. turnbulli* in male guppies.

The significantly higher mortality of untreated males compared with untreated females suggests that androgens may reduce guppy tolerance to *Gyrodactylus* (i.e. the ability of hosts to reduce the negative impacts of a given parasite load; Raberg *et al*. 2007). This line of reasoning is supported by the lower mortality of males that underwent both demasculinization and feminization compared with the untreated males. We did observe a difference in untreated male mortality between Experiments 1 and 2, possibly the result of population differences in subjects' susceptibility to *Gyrodactylus* induced mortality. Given that laboratory conditions were identical between the two experiments, the most likely cause for particular differences in mortality and parasite loads between Experiments 1 and 2 is the fish population of origin. However, regardless of population origin, guppies that underwent hormone treatments (demasculinization or feminization) experienced lower mortality during infection and carried lower parasite loads than untreated males in both experiments.

The suppressive effect of the androgen system on guppy defence against *G. turnbulli* suggests a trade-off between resistance to these ectoparasites and other fitness-related traits of male guppies. On the one hand, female guppies typically prefer to mate with males that show brighter carotenoid colouration (Houde and Endler, 1990) and more active courtship (Kodric-Brown and Nicoletto, 2001), traits which have positive correlations with circulating androgens (Baatrup and Junge, 2001; Bayley *et al*. 2003), and thus higher levels of circulating androgens would seem to increase male fitness. On the other hand, infections by *Gyrodactylus* are known to decrease male carotenoid colouration and display rate, and consequently decrease female preference for males with higher *Gyrodactylus* loads (Houde and Torio, 1992; Kennedy *et al*. 1987). Furthermore, *Gyrodactylus* infection may compromise predator evasion, for example, via increased morbidity and decreased swimming performance (Cable *et al*. 2002). Thus, *Gyrodactylus* can decrease male guppy host fitness through the direct effect of increased mortality and through the indirect effect of decreased mating opportunities, which may counterbalance the fitness enhancing properties of their androgen hormones. A further possibility is that an increase in circulating androgens could promote carotenoid accumulation, which in turn counterbalances the immunosuppressive effects of androgens (e.g. Blas *et al*. 2006; McGraw and Ardia, 2007). However, this possibility is unlikely here, given that males with intact androgen levels had higher parasite burdens than those under the feminization and demasculinization treatments.

In conclusion, a reduced response of androgen receptors to circulating androgens was found to lead to decreased parasite burdens and parasite-induced mortality. Future work should determine if androgens have a direct immunosuppressive effect or, alternatively, if androgen dependent changes in sexual traits and reproductive investment indirectly affect investment in immunity. Our findings are consistent with the idea that androgens modulate immune function, but run contrary to the view that size determines parasite loads, and therefore help further the understanding of inter-individual variation in parasitism. The developmental and current (circulating) effects of gonadal steroids on the immune system and resistance to infection, as well as their indirect effects on secondary sexual traits that affect fitness, are underappreciated in studies addressing the ecology and evolution of vertebrate defence against parasites. Our results on a model host–parasite system strongly suggest that gonadal steroids should be considered in concert with morphological or behavioural differences

https://doi.org/10.1017/S0031182015001286 Published online by Cambridge University Press

when accounting for variation among individuals and between the sexes.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0031182015001286.

ACKNOWLEDGEMENTS

We thank HJ Pak, K White, D Uthayakumar and G Daggupati for laboratory assistance, A Morrill and S Portalier for figure coding advice, and A Hendry for use of his aquatic housing systems (NSERC-RTI #148297). We thank D Reznick, C Ghalambor, E Ruell, D Fraser, and the FIBR team for supplying us with guppies used in Experiment 1.

FINANCIAL SUPPORT

We thank the Quebec Centre for Biodiversity Science (FD); the Natural Sciences and Engineering Research Council of Canada (NSERC) (GFF – #356373-07; SMR – #418342-2012 and #429385-2012; ARR), Richard H. Tomlinson fund (ARR) and the Canada Foundation for Innovation (SMR – #29433). Research at the Ghalambor laboratory was supported by a NSF Faculty Early Career (DEB-0846175). The guppy introductions were funded by a United States NSF Frontiers in Integrative Biological Research grant to D Reznick P.I. (EF-0623632).

REFERENCES

Amo, L., López, P. and Martín, J. (2005). Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. *Parasitology Research* **96**, 378–381.

Arnold, A. P. (2009). The organizational–activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Hormones and Behavior* **55**, 570–578. doi: http://dx.doi.org/10.1016/j.yhbeh.2009.03.011.

Baatrup, E. and Junge, M. (2001). Antiandrogenic pesticides disrupt sexual characteristics in the adult male guppy *Poecilia reticulata*. *Environmental Health Perspectives* **109**, 1063–1070.

Bakke, T. A., Cable, J. and Harris, P. D. (2007). The biology of gyrodactylid monogeneans: The 'Russian-doll killers'. *Advances in Parasitology*, **64**, 161–376.

Bayley, M., Junge, M. and Baatrup, E. (2002). Exposure of juvenile guppies to three antiandrogens causes demasculinization and a reduced sperm count in adult males. *Aquatic Toxicology* **56**, 227–239.

Bayley, M., Larsen, P. F., Baekgaard, H. and Baatrup, E. (2003). The effects of vinclozolin, an anti-androgenic fungicide, on male guppy secondary sex characters and reproductive success. *Biology of Reproduction* **69**, 1951–1956.

Bjerselius, R., Lundstedt-Enkel, K., Olsén, H., Mayer, I. and Dimberg, K. (2001). Male goldfish reproductive behaviour and physiology are severely affected by exogenous exposure to 17 β-estradiol. *Aquatic Toxicology* **53**, 139–152.

Blas, J., Pérez-Rodríguez, L., Bortolotti, G. R., Viñuela, J. and Marchant, T. A. (2006). Testosterone increases bioavailability of carotenoids: Insights into the honesty of sexual signaling. *Proceedings of the National Academy of Sciences* **103**, 18633–18637.

Buchmann, K. (1997). Population increase of *Gyrodactylus derjavini* on rainbow trout induced by testosterone treatment of the host. *Diseases of Aquatic Organisms* **30**, 145–150.

Cable, J., Scott, E. C., Tinsley, R. C. and Harris, P. D. (2002). Behavior favoring transmission in the viviparous Monogenean *Gyrodactylus turnbulli*. *Journal of Parasitology* **88**, 183–184.

Cable, J. and 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. *International Journal for Parasitology* **37**, 1449–1458.

Chaves-Pozo, E., García-Ayala, A. and Cabas, I. (2012). Sex steroids modulate fish immune response. In *Sex Steroids* (ed. Kahn, S. M.), pp. 199–220. InTech. http://www.intechopen.com/books/sex-steroids

Cuesta, A., Vargas-Chacoff, L., García-López, A., Arjona, F. J., Martínez-Rodríguez, G., Meseguer, J., Mancera, J. M. and Esteban, M. A. (2007). Effect of sex-steroid hormones, testosterone and estradiol, on humoral immune parameters of gilthead seabream. *Fish & Shellfish Immunology* **23**, 693–700.

Dargent, F. (2015). The wild side: Assessing evolutionary ecology of defence against parasites in nature. Ph.D. Thesis. pp. 223. McGill University, Montreal.

Dargent, F., Scott, M. E., Hendry, A. P. and Fussmann, G. F. (2013*a*). Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proceedings of the Royal Society B: Biological Sciences* **280**, 1–9.

Dargent, F., Torres-Dowdall, J., Scott, M. E., Ramnarine, I. and Fussmann, G. F. (2013*b*). 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.

de Waal, P. P., Wang, D. S., Nijenhuis, W. A., Schulz, R. W. and Bogerd, J. (2008). Functional characterization and expression analysis of the androgen receptor in zebrafish (*Danio rerio*) testis. *Reproduction* **136**, 225–234.

Fitzpatrick, S. W., Torres-Dowdall, J., Reznick, D. N., Ghalambor, C. K. and Funk, W. C. (2014). Parallelism isn't perfect: Could disease and flooding drive a life-history anomaly in Trinidadian guppies? *The American Naturalist* **183**, 290–300.

Forbes, M. R. (2007). On sex differences in optimal immunity. *Trends in Ecology & Evolution* **22**, 111–113.

Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E., Fussmann, G. F. and Hendry, A. P. (2013). Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia* **172**, 155–166.

Grossman, C. (1989). Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *Journal of Steroid Biochemistry* **34**, 241–251.

Guégan, J.-F., Lambert, A., Lévêque, C., Combes, C. and Euzet, L. (1992). Can host body size explain the parasite species richness in tropical freshwater fishes? *Oecologia* **90**, 197–204.

Hamilton, W. (1982). Pathogens as causes of genetic diversity in their host populations. In *Population Biology of Infectious Diseases* (eds. Anderson, R. M., and May, R. M.), pp. 269–296. Springer, Berlin.

Hamilton, W. D. and Zuk, M. (1982). Heritable true fitness and bright birds – a role for parasites. *Science* **218**, 384–387.

Harris, P. D. and Lyles, A. M. (1992). Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) in Trinidad. *Journal of Parasitology* **78**, 912–914.

Houde, A. E. and Endler, J. A. (1990). Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* **248**, 1405–1408.

Houde, A. E. and Torio, A. J. (1992). Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioral Ecology* **3**, 346–351.

Jensen, K. M., Kahl, M. D., Makynen, E. A., Korte, J. J., Leino, R. L., Butterworth, B. C. and Ankley, G. T. (2004). Characterization of responses to the antiandrogen flutamide in a short-term reproduction assay with the fathead minnow. *Aquatic Toxicology* **70**, 99–110.

Jolly, C., Katsiadaki, I., Le Belle, N., Mayer, I. and Dufour, S. (2006). Development of a stickleback kidney cell culture assay for the screening of androgenic and anti-androgenic endocrine disrupters. *Aquatic Toxicology* **79**, 158–166.

Kennedy, C. E. J., Endler, J. A., Poynton, S. L. and McMinn, H. (1987). Parasite load predicts mate choice in guppies. *Behavioral Ecology and Sociobiology* **21**, 291–295.

Kinnberg, K. and Toft, G. (2003). Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). *Ecotoxicology and Environmental Safety* **54**, 16–24.

Klein, S. L. (2000). Hormones and mating system affect sex and species differences in immune function among vertebrates. *Behavioural Processes* **51**, 149–166.

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology* **26**, 247–264.

Kodric-Brown, A. and Nicoletto, P. (2001). Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behavioral Ecology and Sociobiology* **50**, 346–351.

Kolluru, G. R., Grether, G. F., South, S. H., Dunlop, E., Cardinali, A., Liu, L. and Carapiet, A. (2006). The effects of carotenoid and food

availability on resistance to a naturally occurring parasite (*Gyrodactylus turnbulli*) in guppies (*Poecilia reticulata*). *Biological Journal of the Linnean Society* **89**, 301–309.

Krasnov, B., Morand, S., Hawlena, H., Khokhlova, I. and Shenbrot, G. (2005). Sex-biased parasitism, seasonality and sexual size dimorphism in desert rodents. *Oecologia* **146**, 209–217.

Krasnov, B. R., Bordes, F., Khokhlova, I. S. and Morand, S. (2012). Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. *Mammalia* **76**, 1–13.

Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., De Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T. J., Kuris, A. M., Marcogliese, D. J., Martinez, N. D., Memmott, J., Marquet, P. A., McLaughlin, J. P., Mordecai, E. A., Pascual, M., Poulin, R. and Thieltges, D. W. (2008). Parasites in food webs: the ultimate missing links. *Ecology Letters* **11**, 533–546.

Lazzaro, B. P. and Little, T. J. (2009). Immunity in a variable world. *Philosophical Transactions of the Royal Society B* **364**, 15–26.

Magurran, A. E. (2005). *Evolutionary Ecology: The Trinidadian Guppy*, Oxford University Press, New York.

McGraw, K. J. and Ardia, D. R. (2007). Do carotenoids buffer testosterone-induced immunosuppression? An experimental test in a colourful songbird. *Biology Letters* **3**, 375–378.

Minchella, D. J. and Scott, M. E. (1991). Parasitism: A cryptic determinant of animal community structure. *Trends in Ecology & Evolution* **6**, 250–254.

Nunn, C. L., Lindenfors, P., Pursall, E. R. and Rolff, J. (2009). On sexual dimorphism in immune function. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 61–69.

Perez-Jvostov, F., Hendry, A. P., Fussmann, G. F. and 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. and Scott, M. E. (2015). Testing for local host–parasite adaptation: an experiment with Gyrodactylus ectoparasites and guppy hosts. *International Journal for Parasitology* **45**, 409–417.

Poulin, R. and Rohde, K. (1997). Comparing the richness of metazoan ectoparasite communities of marine fishes: Controlling for host phylogeny. *Oecologia* **110**, 278–283.

Price, P. W. (1980). *Evolutionary Biology of Parasites*, Princeton University Press, New Jersey, USA.

R Development Core Team (2014). R: *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raberg, L., Sim, D. and Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* **318**, 812–814.

Ramírez, R., Harris, P. D. and Bakke, T. A. (2012). An agent-based modelling approach to estimate error in gyrodactylid population growth. *International Journal for Parasitology* **42**, 809–817.

Schmid-Hempel, P. (2011). *Evolutionary Parasitology: the Integrated Study of Infections, Immunology, Ecology, and Genetics*, Oxford University Press, Oxford.

Scott, M. E. and 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. and Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* **11**, 317–321.

Stephenson, J. F., van Oosterhout, C., Mohammed, R. S. and Cable, J. (2015). Parasites of Trinidadian guppies: evidence for sex- and age-specific trait-mediated indirect effects of predators. *Ecology* **96**, 489–498.

Tadiri, C. P., Dargent, F. and Scott, M. E. (2013). Relative host body condition and food availability influence epidemic dynamics: a *Poecilia reticulata-Gyrodactylus turnbulli* host-parasite model. *Parasitology* **140**, 343–351.

Tinsley, R. C. (1989). The effects of host sex on transmission success. *Parasitology Today* **5**, 190–195.

Travis, J., Reznick, D., Bassar, R. D., López-Sepulcre, A., Ferriere, R. and Coulson, T. (2014). Do eco-evo feedbacks help us understand nature? Answers from studies of the Trinidadian guppy. In *Advances in Ecological Research*, Volume **50** (eds. Moya-Laraño, J. R., Rowntree, J. and Woodward, G.), pp. 1–40. Academic Press, London.

van Oosterhout, C., Mohammed, R. S., Hansen, H., Archard, G. A., McMullan, M., Weese, D. J. and Cable, J. (2007*a*). Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *International Journal for Parasitology* **37**, 805–812.

van Oosterhout, C., Smith, A. M., Haenfling, B., Ramnarine, I. W., Mohammed, R. S. and Cable, J. (2007*b*). The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. *Conservation Biology* **21**, 1573–1583.

Wallen, K. and Baum, M. J. (2002). Masculinization and defeminization in altricial and precocial mammals: comparative aspects of steroid hormone action. *Hormones, Brain and Behavior* **4**, 385–423.

Watanuki, H., Yamaguchi, T. and Sakai, M. (2002). Suppression in function of phagocytic cells in common carp *Cyprinus carpio* L. injected with estradiol, progesterone or 11-ketotestosterone. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **132**, 407–413.

Zuk, M. (1990). Reproductive strategies and disease susceptibility: an evolutionary viewpoint. *Parasitology Today* **6**, 231–233.

Zuk, M. and McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology* **26**, 1009–1023.