Predation, parasitism and colour in natural guppy populations

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Abstract

Many studies of evolution adopt a unifactorial approach, where a single causal force appearing to account for a majority of the phenotypic variation becomes the focal point of investigation. However, it seems certain that no single force acts to the complete exclusion of other forces, and so multi-factorial studies can make a crucial contribution to our understanding of the evolution of phenotypic variation. In the Trinidadian guppy system, natural selection acting via predation has long been recognized as playing an important role in shaping trait variation. However, other environmental factors also likely contribute, and my thesis explores the potential role of one of them: parasitism. I specifically focus on Gyrodactylus, a monogenean ectoparasite that has important effects on many fish species and coexists with guppies in the wild. I evaluate the extent to which parasitism varies among guppy populations, particularly in relation to predation. I then ask whether parasitism influences phenotypic variation in guppy traits. This work was executed through a large-scale survey in northern Trinidad, where 26 guppy populations of known predation level (high or low) were surveyed across 10 different rivers. Individual guppies were scanned for *Gyrodactylus* and then photographed for image analyses aimed at quantifying body size and aspects of male colour. I found that among-population variation in parasitism levels was consistent between two dry seasons, and that high-predation localities tend to have higher levels of parasitism than do low-predation localities. However, I found few effects of parasitism on guppy traits and no major modifying influence of parasitism on inferences about the role of predation. Although more work certainly needs to be done with respect to parasitism, our results suggest it might be more profitable to concentrate on other potential causal factors that shape guppy trait variation.

Résumé

De nombreuses études sur l'évolution adoptent une approche uni-factorielle qui se concentrent sur une seule force pouvant expliquer la plupart de la variation phénotypique observée. Cependant, il est évident qu'aucune force n'exerce son effet de façon isolée. C'est pourquoi les études multi-factorielles apportent une contribution cruciale à notre compréhension de l'évolution de la variation phénotypique. Dans le système d'étude des guppies de Trinité, la sélection naturelle imposée par les prédateurs a depuis longtemps été considérée comme un facteur important dans l'élaboration de la variation au niveau des traits. Cependant, d'autres facteurs environnementaux contribuent également à cette élaboration, notamment le parasitisme. Je me penche sur le rôle potentiel que peuvent avoir les parasites du genre Gyrodactylus sur les traits des guppies. Ces ectoparasites monogènes exercent de nombreux effets sur plusieurs espèces de poissons et coexistent avec les guppies dans leur environnement naturel. J'évalue le niveau de variation du parasitisme entre les populations de guppies, et plus spécifiquement en relation avec la prédation. J'explore par la suite l'influence potentielle du parasitisme sur la variation phénotypique des traits des guppies. Pour ce faire, une étude de grande échelle a été effectuée sur 10 rivières dans le nord de Trinité, dans laquelle 26 populations sujettes à des niveaux de prédation connus (élevé ou bas) ont été inventoriées. L'état d'infection par les Gyrodactylus a été répertorié pour chaque guppy, puis les poissons ont été photographiés pour analyser les images dans le but de quantifier la taille corporelle ainsi que de nombreux aspects de la coloration chez les mâles. J'ai trouvé que les niveaux de parasitisme entre les populations étaient consistants entre les deux saisons sèches recensées, et qu'il y a une tendance pour les sites de haute prédation à avoir des niveaux de parasitisme plus élevés que les sites de basse prédation. Cependant, j'ai trouvé que la parasitisme avait peu d'effets sur les traits des guppies et aucune influence majeure n'a été trouvée sur les inférences concernant la prédation. Bien qu'il reste encore beaucoup de travail à faire en ce qui a trait au parasitisme, nos résultats suggèrent qu'il pourrait être plus profitable de se concentrer sur d'autres facteurs potentiels pouvant expliquer la variation de traits chez les guppies.

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Preface

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Contributions of authors

I was the principle researcher for the manuscript. Collections of field samples were assisted by Dr. Andrew Hendry, Dr. Gregor Fussmann, Dr. Paul Bentzen of Dalhousie University, Ian Paterson, Felipe Perez Jvostov, Felipe Dargent, Kiyoko Gotanda, Shahin Muttalib, and Lyndsey Baillie. Photoshop analyses were aided by Cameron Mojarrad. Drs. Andrew Hendry and Gregor Fussmann also aided in the design and analysis of both manuscripts.

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General Introduction

Much evolutionary work takes a unifactorial approach, essentially studying one factor to the principle exclusion of others. Examples include predation, dissolved oxygen, competition and light (e.g., Perry et al. 1981; Reznick & Endler 1982; Porter et al. 1984; Evans & Schmidt 1990; Gray & Robinson 2002; Decker et al 2003; Langerhans & Dewitt 2004; Timmerman & Chapman 2004; Reznick & Bryant 2007). This rather constrained approach might strongly limit our understanding of variation that is shaped by a multitude of factors. This could be problematic given that most traits in most systems are presumably influenced by many causal factors. In this thesis, I approach a long-established view of a classic microevolutionary model system (Trinidadian guppies; *Poecilia reticulata*) and attempt to re-evaluate the main driver of natural selection in this system (predation) by assessing its potential interaction with a little-studied factor (parasitism).

In the Trinidadian guppy system, predation has been shown to be a major selective force driving evolution (Haskins et al. 1961; Seghers 1973; Endler 1980; Reznick & Endler 1982a). Morphology, behaviour and life history traits have been studied in depth, alongside male colour variation (Reznick & Endler 1982a; Reznick 1989; Strauss 1990; Reznick et al. 2001;) and other traits (Endler 1995; Magurran 2005). One of the best-documented patterns is that males found in populations considered "low-predation" are more vibrantly colored than their "high-predation" counterparts (Haskins et al. 1961; Endler 1980; 1983; Endler & Houde 1995). Predator assemblage has generally driven this binary categorization, with the presence or absence of strong predatory fishes dictating the "predation regime". The extraordinary variation present in the guppy system has given researchers an excellent opportunity to observe and test how selection drives phenotypic variation in nature. I will extend this work by examining how parasitism might also influence the evolution of Trinidadian guppies.

Parasitism has been shown in many natural systems to influence the direction of selection (McMinn 1990; Zuk 1992; Lozano 1994; Poulin & Vickery 1993; Houde 1997; Lopez 1998; van Oosterhout et al. 2007), and consequently has

become an area of interest for evolutionary ecologists. In guppies, parasitism is common in natural populations (van Oosterhout et al. 2007), and here the particular focus is on *Gyrodactylus*, a monogenean ectoparasite ubiquitous in teleost fish. There are over 400 species classified today, but it has been estimated that there may in fact be over 20 000 (Bakke et al. 2002; Harris et al. 2004). Gyrodactylus spp. are found in both freshwater and marine environments, as well as brackish water, although relatively few species are euryhaline (i.e. can tolerate wide ranges of salinity) (Bakke et al. 2007). Hooks facilitate the attachment of the parasite to the host's body and fins (Fig. 1), and it feeds primarily on the epithelial cells of the fish (Bakke et al. 2007). Gyrodactylus spp. are visible at low magnification, allowing easy observation and manipulation (L. Delaire, pers. obs.). Many species of this ectoparasite are viviparous, meaning that they reproduce directly on the host, giving birth to live young that attach immediately and begin feeding (Bakke et al. 2007; Bakke et al. 2002). Transmission occurs mostly by direct contact between infected individuals, and although this is most likely with other conspecifics, it is also possible with heterospecific individuals coming into contact with each other if the other species is a suitable host for Gyrodactylus (Bakke et al. 2007). Development of infection by Gyrodactylus is mediated by the immune response of the host, although some host species can be so devastated by the parasite burden that infection often leads to a fatal end (Bakke et al. 1992).

Monogenea are reputed to be one of the most host-specific classes of parasites, but within them *Gyrodactylus* have the biggest range of hosts; the majority are species specific (~70%) while some have as many as sixteen hosts (Bakke et al. 2002; King & Cable 2007). Identification of *Gyrodactylus* species can be complicated, as it is based on morphology and morphometry, with respect to marginal hooks and anchors (hamuli) as well as bars in the attachment organ, or opisthaptor (Fig. 1). In many cases a trained morphologist is required to tell these species apart, and using molecular tools such as PCR to compare DNA sequences is useful to ensure the species of *Gyrodactylus* present. For the purpose of this

work, *Gyrodactylus* spp. were not identified individually to species level, due to such labour-intensive and specific procedures.

The devastating impact of *G. salaris* on non-resistant Norwegian salmon that began with its accidental introduction in the late 1970s (Johnsen & Jensen 1986; Bakke et al. 2007) has since sparked much research around the parasite. *Gyrodactylus* spp. have been called the *Drosophila* of the parasite world (Bakke et al. 2007), as they are easy to manipulate and maintain, and have a fast *in situ* generation time, and studies are being conducted in multiple host-*Gyrodactylus* systems. Current *Gyrodactlyus*-related research foci include their detrimental role in the salmon industry, the invasion of exotic species or the investigation of parasitism as a force affecting the evolution of host and parasite species alike.

Gyrodactylus turnbulli and *Gyrodactylus bullatarudis* are the only two species that have been found on guppies caught in Trinidadian streams (Lyles 1990; Harris & Lyles 1992). Infections with either species can be debilitating and even fatal to their poeciliid hosts (Scott & Anderson 1984; Houde 1997). The guppy-*Gyrodactylus* host-parasite system used in this research is an important and engaging system for study in parasitological, ecological, and evolutionary contexts. Parasitological studies involving guppies and *Gyrodactylus* are numerous (Scott 1982; 1984; 1985a; 1985b; Scott & Anderson 1984; Scott & Nokes 1984; Harris 1986; 1988; 1989; van Oosterhout et al. 2003; 2007; van Oosterhout 2007; Martin & Johnsen 2007), in part because the parasites are easy to use in experimental circumstances, but also because investigation involving them has great implications in understanding of the mechanisms behind such phenomena as host resistance and tolerance (Bakke et al. 1992; Cable et al. 2000; Harris et al. 2004; King & Cable 2007).

One potential effect of *Gyrodactylus* is on the colour of male guppies, particularly colours based on carotenoid pigments. Carotenoids gained from the diet are responsible for the orange colouration of male guppies (Endler 1980; Kodric-Brown 1989), which has thus been suggested as an honest indicator of male fitness (Endler 1983; Kodric-Brown & Brown 1984). Hypothesized by the "good genes" model of sexual selection, females should choose these males in

order to obtain the fittest genes to pass on to their offspring (Trivers 1972; Halliday 1983). Such a relationship between dietary carotenoids and showy colouration has also been brought to light in other species, including threespine stickleback and the red jungle fowl (Milinski & Bakke 1990; Zuk et al. 1990) Orange males are expected to be effective foragers, capable of obtaining more carotenoids from their diet than are less conspicuous males from the same population. In guppies, it is suggested that brightly coloured orange males are not only the best foragers, but also the most successful at evading predators (Endler 1980) - as brightly coloured males (particularly with respect to carotenoid colours) are known to be easier to perceive and thus should be generally more susceptible to predation (Endler, 1978; 1983; Godin & McDonough 2003). Carotenoids also play an important role in guppy physiology. They are crucial in immune system functioning (Lozano 1994), which brings to light a trade-off of carotenoid allocation (Folstad & Karter 1992; Alonso-Alvarez et al. 2004), where guppies must defend against pathogens to remain fit, as well as display brightly to increase their attractiveness to mates.

Gryrodactylus seem to have several debilitating effects on guppies. Guppy behaviour (such as male courting, female choosiness and foraging) as well as traits such as male colour (specifically orange), have been shown in laboratory studies to be negatively affected by *Gyrodactylus* infection (Kennedy et al. 1987; McMinn 1990; Houde & Torio 1992; Houde 1997; Lopez 1998; van Oosterhout et al. 2003). The fading of orange colour with infection time is particularly interesting as it could represent a trade-off where a male mobilizes carotenoids to defend against infection rather than using them for display. Such effects could also simply be the result of a male's decrease in energy or other negative impact due to being parasitized. Many male traits shown to be affected by *Gyrodactylus* are proven features of female choice (Farr 1980; Endler 1980; Kodric-Brown 1985; Endler & Houde 1995), thus implicating the existence of parasite mediated sexual selection (Kennedy et al. 1987; Moller 1990; Wedekind 1992; Poulin & Vickery 1996).

Parasitism potentially affects many guppy traits even though we rarely know the causal nature of the effect. Predation has been shown to be an important force of natural selection in Trinidadian guppies but is unlikely to explain the full amount of phenotypic variation seen in the traits of these fish. . Here, I add parasitism as a factor affecting guppy evolution and investigate how it may complement, counteract or interact with predation. The goal of this thesis is thus to determine whether parasitism, represented here by infection by the ectoparasite *Gyrodactylus*, plays a role in the evolution of wild Trinidadian guppies, and more specifically, to ascertain any relationship that exists between guppy traits and both predation and parasitism. The first step to this end is a large-scale multi-season field survey of parasitism levels over many populations. Although causation cannot be inferred due to the nature of such a survey, this thesis aims to generate hypotheses that can be built upon in future work.

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PREDATION, PARASITISM AND COLOUR ON NATURAL GUPPY POPULATIONS

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Abstract

Research on phenotypic variation in nature often considers only a single causal factor, such as predation, parasitism, resource levels, or competition. The reality however, is that traits are likely influenced by multiple causal factors that may or may not interact with each other. Our goal is to uncover how phenotypic variation in a classic model system might be influenced by multiple factors. Trinidadian guppies (*Poecilia reticulata*) provide the perfect opportunity as prior studies have shown that variation in traits such as body size and male color are mostly attributed to a single causal factor (predation). Here we observe by way of an extensive stream survey of rivers in northern Trinidad, how phenotypic variation in this situation may be influenced by another casual factor: parasitism. We found that inter-population variation in infection rates of guppies by *Gyrodactylus* parasites was consistent between two dry seasons, and was higher at highpredation sites than at low-predation sites. However, we found only minor influences of infection at the individual or population level on guppy body size and male color. Moreover, the addition of parasite infection levels to statistical models had no influence on the effects of predation levels in those models. These results suggest that parasitism, as it is quantified in this study, is not playing a crucial role in shaping phenotypic variation in these traits. Considerable variation in these guppy traits among populations within a given predation regime however, suggests that additional potential causal factors should be explored.

Introduction

Studies of adaptation are typically conceptualized in terms of unifactorial causality, focusing on single selective forces such as predation (Reznick & Endler 1982; Evans & Schmidt, 1990; Langerhans & Dewitt, 2004, Reznick & Bryant 2007), dissolved oxygen (Decker et al 2003; Timmerman & Chapman 2004; Witte et al. 2008; Mandic et al. 2009; Abaci et al. 2010), competition (Gray & Robinson 2002), or light (Perry et al. 1981; Porter et al. 1984). Although single causal forces can often explain a considerable amount of the phenotypic variation among natural populations, there is no reason to suspect that a single selective factor is overwhelmingly important for any particular trait in any particular

system. What is more likely is that traits are instead under pressure from multiple selective forces (Endler 1986; Schluter 2000).

Potential multifactorial causality has two main consequences for evolutionary inference. First, different causal factors could be closely correlated, and so variation attributable to one factor considered alone could in reality be due to the other factor. As one example, the life history variation in Trinidadian guppies that was originally attributed solely to the direct effect of predator intensity now seems to be at least partly attributable to different resource levels, which covary with predation intensity (Grether et al. 2001; Reznick et al. 2001). Second, effects of one causal factor might interact with, and therefore obscure or intensify, effects of another causal factor. To continue with the guppy example, it has recently been argued that spatial variation in male colour is the result of a complex interaction between spatial variation in predation, canopy cover, and sexual selection (Millar et al. 2006; Karim et al. 2007). For both of these reasons, studies have begun to consider multivariate causality when interpreting patterns of phenotypic variation among natural populations (e.g., Calsbeek & Cox, 2010).

We will here evaluate potential multifactorial causality with respect to predation and parasitism. Parasitism has generally been less studied in an evolutionary diversification context than has predation, but increasing evidence suggests its importance (McMinn 1990; Zuk 1992; Lozano 1994; Poulin & Vickery 1996; Houde 1997; Lopez 1998; van Oosterhout et al. 2007; Eizaguirre et al. 2010). In addition, multivariate causality might be particularly important when it comes to these two selective forces. In particular, predation and parasitism might have similar effects on some traits but different effects on other traits. On the one hand, both forces generally increase mortality rates, and so might similarly lead to the evolution of "fast" life histories (Lee 2008). On the other hand, predators and parasites might select for different levels of some traits, as has been demonstrated for development rates and activity levels (Raffel et al. 2010). Moreover, predation and parasitism levels in nature might sometimes be correlated, either positively or negatively, or might show more idiosyncratic patterns (Decaestecker et al. 2002; MacNeil et al. 2003; Krüger, 2007; Dick et al., 2010; Johnson et al., 2010).

With these possibilities in mind, we revisit a classic case of phenotypic variation (male guppy colour) that has, for the most part, been interpreted in the context of univariate causality (predation) (Endler 1980). We first measure parasitism levels in locations known to have different predation levels, to examine the degree of covariance between these two potential causal forces. We then ask whether parasites influence variation in colour and whether the effect of predation changes when incorporating parasitism levels into analyses.

Trinidadian guppies

Predation is known to be a major selective force shaping phenotypic variation among guppy populations (Haskins et al. 1961; Seghers 1973; Endler 1980; Reznick & Endler 1982; Endler 1995; Houde 1997; Magurran 2005). In particular, the division of guppy populations into those experiencing high predation (strongly piscivorous fishes present) versus low predation (only weakly piscivorous fishes present) explains a substantial portion of the variation among populations in morphology, behaviour, life history, and male colour (Endler 1995; Houde 1997; Magurran 2005). Of particular interest to our study, guppies from low-predation populations are usually larger and more brightly coloured than are those from high-predation populations (Haskins et al. 1961; Endler 1980; 1983; Endler & Houde 1995; Millar et al. 2006; Weese et al. 2010). While predation is thus an important force shaping size and colour variation in this system, it has become increasingly clear that the full story is undoubtedly multifactorial, involving additional selective forces including resource levels (Kodric-Brown, 1989; Grether et al. 2001; Reznick et al. 2001; Millar et al. 2006; Schwartz & Hendry 2007), sexual selection (Endler & Houde 1995; Rodd et al. 2002; Schwartz & Hendry 2007), and parasitism (Martin & Johnsen, 2007). Our contribution will be to take a closer look at the potential role of parasitism, particularly in relation to the accepted role of predation.

Parasitism is common in natural guppy populations (Martin & Johnsen 2007; van Oosterhout et al. 2007), to the extent that up to half of a population can be infected by a single species of *Gyrodactylus*, a monogenean ectoparasite ubiquitous in teleost fish (Lyles 1990; Harris & Lyles 1992). Two species of this parasite, Gyrodactylus turnbulli and Gyrodactylus bullatarudis, have been found on wild-caught Trinidadian guppies (Harris and Lyles 1992). The guppy-Gyrodactylus host-parasite system has been used extensively in parasitological studies (Scott 1982; Scott & Anderson 1984; Scott & Nokes 1984; Scott & Robinson 1984; Scott 1985a; 1985b; Harris 1986; 1988; 1989) as the parasites are visible under a dissecting microscope at low magnification and thus are easily observed and manipulated. Gyrodactylus is a good candidate for investigating the role of parasitism in shaping phenotypic variation in nature owing to its direct life cycle, ease of transmission, and potentially debilitating effects on guppy fitness. For example, Gyrodactylus infection adversely influences several aspects of guppy behaviour, including the rate of male sigmoid display (an important male courting tactic) and the amount of time spent foraging (Kennedy et al. 1987; McMinn 1990; Lopez 1998, 1999; van Oosterhout et al. 2003; Kolluru et al. 2008). Moreover, male colouration, specifically orange, fades in the presence of Gyrodactylus infection (Houde and Torio 1992; Houde 1997). Given that carotenoids are gained through the diet, this could be a direct reflection of a tradeoff in guppy allocation to growth versus parasite defense, where a male mobilizes more carotenoids to defend against infection. Alternatively, it may show the effect of reduced performance due to parasitism decreasing male foraging success.

The first step in considering the causal forces behind phenotypic variation in nature is to generally survey multiple populations that differ in levels of the hypothesized causal factors. To this end, we surveyed parasite prevalence in 26 natural guppy populations of known predation regime in each of two years, seeking to understand covariation between the two factors. We then asked how parasitism levels are related to male guppy traits and whether the effects of predation change when adding parasitism into statistical models. Because this is a field survey, we cannot state with assurance whether the observed patterns result

from genetic variation or phenotypic plasticity. This is doubly so given that body size and aspects of male guppy colour are influenced both by genetic variation (Winge 1927; Haskins et al. 1961; Houde 1992; Reznick & Bryga 1996; Hughes et al. 2005; Tripathi et al. 2009) and phenotypic plasticity (Kodric-Brown 1989; Grether 2000; Ghalambor et al. 2007). Finally, because our study is not manipulative, we cannot directly infer causation. Thus, we view this study as useful for generating causal hypotheses that provide an appropriate context for experimental work.

Methods

Collections and Processing

In February of 2009 and 2010, we collected guppies from 26 sites across 10 rivers in northern Trinidad (Fig. 2; Table 1). Nine of the 26 sites were sampled additionally in September (wet season) of 2009 to allow for a comparison across seasons. Guppies were caught with butterfly nets, briefly placed in a bucket, immediately transferred to individual 18oz Whirl-pak© bags containing river water, and finally placed in an insulated cooler in the shade. The goal of this procedure was to quickly isolate guppies from each other so that individual parasite loads could be determined without worry of the parasites moving between hosts. Approximately thirty males and thirty females were collected per site and transported back to the field station for processing.

Within five hours of collection, the fish were individually anesthetized in buffered 0.2% MS222, and then examined for *Gyrodactylus* under a dissecting microscope at 25x magnification. Gyrodactylids were counted on the entire fish and then the left side of the fish was immediately photographed with a Nikon D80 SRL camera (10.2 megapixels) equipped with a 60mm macro lens and a remote shutter. For the photo, the tail was carefully spread to maximum extent using a fine paint brush. A standard ruler and a colour standard were included in each photograph. Two fluorescent full spectrum lights were used as a light source, with supplemental lighting from a Nikon Speedlight Commander Kit R1C1 flash. All procedures were carried out in accordance with animal use protocols at McGill University.

Photo analysis

Although parasites were counted in both collection years, colour was analyzed for only the 2009 collections because of the very large effort involved (50 minutes per male (N~760), including both size and colour measurements, and 5 minutes per female (N~830) \Box 700 hours in total). One person (L. Delaire) used Image J (version 1.41) to obtain a series of measurements from the digital photographs, while blind to each fish's site of origin. Five measurements were related to body size: the area, length, and depth of the body, and the area and length of the tail. The other measurements were related to the colour patterns on males. For the first part of this colour analysis ("traditional measurements"), we first categorized individual spots into one of nine colour categories (Millar et al. 2006; Schwartz & Hendry 2010): black, fuzzy black, orange (including red), yellow, blue (including purple), green, bronze-green, violet-blue and silver (these last three colours categories are often considered iridescent). We then recorded the number and area of each coloured spot. Total colour area was then the sum of the areas of all spots in a given colour category.

Repeatability of the above measurements was confirmed by analyzing the same 20 individuals twice and then calculating the correlation coefficient between the two sets of measurements using individual fish as data points. Repeatability was generally high: e.g., body size r = 0.98, carotenoid colour area r = 0.90, structural area r = 0.82, and melanin area r = 0.92. Before statistical analysis (see below), all measures of colour area were corrected for body size by dividing the colour area by the total fish area (sum of area of body and tail) – as in previous work (Nicoletto & Kodric-Brown 1999; Grether, 2000; Pitcher & Evans 2001). When assumptions of normality were violated, log 10 or square root transformations were used, as appropriate. In addition, we found that very few fish had any distinctive "bronze-green" colour. We therefore excluded the few

such measurements from statistical analyses, which improved our ability to meet statistical distribution assumptions.

The above measurements are traditional and have been used in many previous studies (e.g., Endler 1980; Alexander & Breden, 2004, Millar et al. 2006; Pitcher et al. 2007; Martin & Johnsen, 2007; Karim et al. 2007; Weese et al. 2010). These measurements therefore allow comparison to most previous work. We also sought to gain more detailed information about the colour properties of each spot and the overall fish. Thus for the second part of the colour analysis, the February 2009 photographs were analyzed blindly by one person (Cameron Mojarrad) in Photoshop CS4 (version 11.0.1) to obtain measurements of hue, saturation and brightness ("HSB measurements") of the black, orange, yellow and green spots. These specific colour categories were chosen because they were common colours that have considerable contributions from pigments, and are therefore more consistent across different angles of incident light. Although the resulting measurements are still based on expectations for a human visual system, they should nevertheless provide more detailed information that can be used to consider at least some aspects of variation in spectral properties (e.g., Candolin 1999; Karino & Haijima 2001, 2004; Alexander & Breden 2004; Martin & Johnsen 2007). Spectroradiometry measurements of individual colour spots coupled with visual modeling procedures would be even better but are only rarely implemented (Endler, 1991; Grether, 2000; Kemp et al. 2005; Kemp 2006; Kemp et al. 2008) because they are very time consuming and labour-intensive and have only modest repeatability. We therefore elected to use the traditional and Photoshop measurements in an effort to maximize sample sizes while retaining comparability to most previous work.

In Photoshop, hue $(0-360^{\circ})$ refers to the colour's location on a standard colour wheel, where red is approximately 0°, green is approximately 120°, and blue is approximately 240°. Saturation (0-100%), also called chroma, is the intensity or purity of the colour. It represents the amount of grey in proportion to the hue, where grey is 0% and fully saturated is 100%. Brightness (0-100%) refers to the relative lightness or darkness of a colour, black being 0%, and white 100%.
In order to obtain these measurements, each spot (previously selected and outlined using ImageJ) on each male fish was made into its own layer in Photoshop. Three representative points were then chosen on each spot and an average of 101x101 pixels surrounding that spot were used for measurements. The same was done for corresponding colours on the colour standard (black, red, orange, yellow and green) included within the photo of the fish. The values of each colour spot were then standardized by dividing the values for each measure (hue, saturation. and brightness) by the values for the closest corresponding colour in the colour standard. This eliminated any variation in lighting effects across photographs, which should have been minimal anyway owing to our standard lighting set up.

Data preparation

Our analyses focused on predation, parasitism, and guppy traits. Predation level for each collection site was categorized as either "high" (HP) or "low" (LP) based on predator assemblage previously described at each site. This binary categorization is traditional in the literature, and divides sites into those that have predatory fishes with major effects on guppy demographics from those lacking such predators (e.g., Reznick et al. 1996; 1996b; 2001; Rodd et al. 2002; Millar et al. 2006; Martin & Johnsen 2007; van Oosterhout et al. 2007; Schwartz et al. 2010). LP sites on both the northern and southern slopes of the northern mountain range have only the weak guppy predator *Rivulus hartii* (plus freshwater prawns Macrobrachium spp.), whereas HP sites on the two slopes have different sets of major guppy predators (for details see Reznick et al. 1996a; Millar et al. 2006). Although a more quantitative description of predation intensity and type would be better, this blunt HP versus LP division is used because the methods for confirming predation rate are very labour intensive and generally support the above dichotomy anyway (Reznick et al. 1996b; Gordon et al. 2009; Weese et al. 2010), as well as being generally predictive of divergence in guppy traits (Endler 1995; Reznick et al. 1996a; Magurran 2005).

Parasitism was measured in several complementary ways. At the individual guppy level, parasitism was described either by the categorical binary variable

infection presence (infected or not), or by the discrete variable parasite *abundance* (the total number of gyrodactylids per fish). Parasite *abundance* was not used in some of our statistical analyses due to complications resulting from zero-inflated error distributions, as was also the case for Martin & Johnsen (2007). At the level of guppy populations, parasitism was described by the *prevalence* (proportion of infected fish) and the *mean abundance* (average number of gyrodactylids per fish, including uninfected fish).

Statistical analysis

Data analyses were conducted in JMP v8.0.1 and R v2.8.1. The level of significance for all tests was set at $\alpha = 0.05$. Many tests were carried out in this study and in order to avoid problems resulting from multiple testing we used composite analyses as much as possible. In addition, analyses revealed only a few significant effects.

We first examined the consistency of site-specific parasitism levels between years through the correlation (Pearson's r) of parasitism between 2009 and 2010, and between seasons, using pooled data from male and female fish. We then tested for the association between predation category (HP versus LP) and parasitism level through the use of generalized linear mixed models (GLMMs) and general linear models (GLMs). At the individual level, GLMMs tested the level of parasitism in relation to the predictor variables predation level (HP versus LP), year (2009 versus 2010), sex, and site nested within predation level (as a random factor). Parasitism was described here only (as above) with infection presence with a specified binomial distribution. At the population level, GLMs were run on the parasite prevalence and the mean infection abundance (as defined above) with the predictor variables here being predation category and sex. All other statistical tests involving parasitism at the population level (see below) were performed using each of the two above measures of parasitism. Where the results were equivalent, only values for the *prevalence* are reported for simplicity and ease of presentation.

We next examined the factors influencing body size and male guppy colour. All analyses were run at both the individual and population levels. The individuallevel analyses are intended to ask whether trait values differ between infected or uninfected individuals within populations (plastic responses of individuals to parasitism), and whether or not any such differences vary among populations, including in relation to predation level. These differences would then be expected to influence trait divergence among populations in relation to current (plasticity) and historical (evolutionary) levels of parasitism. The population level analyses are then intended to ask whether a given level of parasitism in a population has an influence on trait divergence among populations. This last level of analysis is akin to those that consider environmental correlates of guppy trait variation among natural populations, whether predation or otherwise (e.g., Endler 1978; Grether et al. 2001; Reznick et al. 2001; Millar et al. 2006).

The statistical analyses were based on a set of multivariate and univariate general linear models. Each analysis at both the individual and population level began with a multivariate approach (MANCOVAs). Five separate analyses were run, each considering a different set of guppy traits with a different potential interpretation: (1) the five measures of guppy size, (2) the number of spots of each colour (black, fuzzy black, orange, yellow, blue, green, violet and silver), (3) the average relative size of each spots of each colour, (4) the relative area of each colour category on the fish, and (5) the colour properties (hue, saturation, and brightness) of black, orange, yellow and green. Predictor variables were predation, parasitism (different measures described above for the individual and population levels), sex (for the size analysis only), size (a principal component was generated to represent all five size variables and used only for the traditional measurements of colour), site nested within predation (at the individual level only), and all interactions among non-nested factors. Canonical axes were generated for each significant term in each model, allowing us to see which of the response variables (e.g., which colours) contributed most to the effect of a given predictor variable. Selected univariate analyses (AN(C)OVAs) were used to confirm inferences from the multivariate analyses and these had the same

structure as the MANCOVAs. The only exception was that we could here nest site within predation as a random rather than fixed effect (MANCOVA does not allow nesting of random effects). In order to simplify presentation, non-significant interaction terms were removed and the analyses re-run to obtain a reduced model.

For all of the above tests, analyses were done also excluding parasitism to determine if the inclusion of this variable altered the results with respect to predation. This was done in an effort to see if considering parasitism mattered for interpretations regarding predation regime (see Introduction).

For visual representation of guppy trait data in relation to parasitism and predation, we first selected either a significant effect or an effect that related to an earlier expectation. We then show the results for that trait at both the individual and population levels. At the individual level, we plot the average trait value for infected individuals in each population versus the average trait value for uninfected individuals in the same population. Data points falling on or near the 1:1 line indicate populations with no difference between infected and uninfected individuals for that trait. Points above (or below) the line suggest higher trait values in uninfected (or infected) individuals. We then code the points (populations) by whether they are HP or LP, the comparison of which can indicate whether effects of infection within a population depend on the predation regime of that population. At the population level, we plot the average trait value in the population (including both infected and uninfected individuals) in relation to the measured parasitism level in that population.

Results

Parasitism levels

Parasitism prevalence was highly correlated between years (Fig. 3; r = 0.76, p < 0.0001). Two sites had very low sample sizes in one year (N = 13 in 2009 for Yarra 2 and N = 24 for Quare 2 in 2010; See Table 1), and so estimates of parasitism levels in those years might be imprecise. The correlation after removing these two sites was higher (r = 0.85, p < 0.0001). We can also compare

the two sets of dry season samples to a subset of nine corresponding sites also sampled in the wet season. For this subset, all correlations between the two seasons were positive (February 2009 versus February 2010: r = 0.98, p < 0.0001) but no significant association was detected between wet and dry seasons (February 2009 versus September 2009: r = 0.41, p = 0.269; September 2009 versus February 2010: r = 0.49, p = 0.186).

At the population level, parasitism was higher at HP sites than at LP sites, with respect to both *prevalence* and *mean abundance* (Table 2; Fig. 4). At the individual level (for the response variable *infection presence*), we found a significant main effect of predation (parasitism was higher in HP sites). For the response variable *abundance*, we found an interaction between predation and year (parasitism differences between HP and LP sites were greater in 2010), as well as a significant main effect of predation (parasitism was higher in HP sites) (see Table 2; Fig. 4).

Guppy traits

We examined many guppy traits in a larger number of analyses, each with multiple predictor variables. These results are summarized by trait type below and, to avoid unnecessary complication, we stick to general conclusions. The details appear in the tables. The main overall effects that emerged from the following analyses are that predation sometimes had considerable effects on guppy traits (particularly body size and orange colouration), but that parasitism rarely did. (We will note some interesting exceptions.) Not surprisingly then, the exclusion of parasitism terms from statistical models had essentially no influence on the predation terms in those same models.

<u>Body size</u>: In individual-level and population–level multivariate analyses, females were larger than males and LP fish were larger than HP fish, whereas no main effect of parasitism was evident (see Table 3; Appendix Table A1). For additional factors that could be examined in the individual-level analyses, we found a significant effect of site nested with predation regime, a significant twoway interaction between sex and predation (the difference in size between

predation regimes was greater for females), and a significant three-way interaction between sex and predation and parasitism (the difference between infected and uninfected fish was greater for HP fish than for LP fish to a degree that was greater for males than females) (Table 3). Exclusion of parasitism from the models had no effect on the significance of sex, predation, or their interaction (Table 3). Overall, these effects were generally the same in the univariate analyses of each size measure by itself (Appendix Table B1), with the results for body area shown in Fig. 5.

<u>Spot number</u>: In population-level analyses, no effects were significant (Table 4). In individual-level analyses, we found a significant effect of site nested with predation regime (canonical variates suggest that HP males had more silver, green, and blue spots, but fewer fuzzy black spots), and body size (large males had more spots than small males) (Table 4; Appendix Table A2). Parasitism did not have a significant effect, and removal of the parasitism term did not change the above effects (Table 4). Univariate analyses of individual colour categories generally corroborated these effects. In particular, larger fish had more colour spots, and LP fish had more fuzzy black spots and fewer green spots (Appendix Table B2). The only effect of parasitism in these univariate analyses was that parasitized fish had more fuzzy black spots at individual level (main effect) and more parasitized populations had more fuzzy black spots for LP sites but not HP sites at the population level (interaction) (Appendix Table B2; Fig. 6).

<u>Spot size</u>: In population-level analyses, populations with larger fish had relatively larger spots (particularly fuzzy black, violet, yellow, green and silver), no effect of predation was evident, and more heavily parasitized populations had larger silver spots and smaller green and fuzzy black spots (Table 5; Appendix Table A3). In individual-level analyses, no effects were significant (Table 5). Removal of parasitism levels from the models had no influence on significance of the other terms. Univariate analyses of individual colour categories showed that larger fish had relatively larger spots, that LP males had larger orange spots than HP males (population level), and that populations with more parasites had larger silver spots (population level) and smaller fuzzy black spots (individual level;

Appendix Table B3), with data for fuzzy black shown in Fig. 7. Some interaction terms were also significant (Appendix Table B3).

<u>Colour area</u>: In population-level analyses, no effects were significant (Table 6). In individual-level analyses, colour area differed among sites nested within predation regime and was greater for LP fish than for HP fish (particularly with respect to orange area), with no effect of parasitism (Table 6 and Appendix Table A4). Accordingly, removal of parasitism from the model had no effect on significance of the other factors (Table 6). Univariate analyses were non-significant for individual colours, although the effect for orange area was close and very suggestive on visual inspection of the data (Fig. 8). We therefore also ran a post hoc analysis on the biologically relevant category of "carotenoid colours" (yellow plus orange plus red), which showed that LP males have marginally more carotenoid colour than do HP males (F = 4.254, p = 0.0499), paralleling the trend suggested in the individual-level multivariate analysis.

<u>Photoshop measurements</u>: In population-level analyses, no effects were significant (Table 7). In individual-level analyses, we found a significant effect of site nested within predation regime and predation regime (LP males had yellow spots that were more orange [hue] and more saturated, and orange spots that were brighter and more saturated) (Appendix Table A5). Removal of parasitism had no effect on the significance of other terms in the model (Table 7). Univariate analyses found few significant effects on the brightness, hue, or saturation of colour spots (Appendix Table B5). However, many effects were in the same general direction as that indicated in the multivariate analysis, with the results for orange saturation shown in Fig. 9.

Discussion

We explored the potential for multiple causal factors to influence phenotypic variation among populations in a classic evolutionary system: Trinidadian guppies. We specifically considered whether the previous conclusions about effects of predation regime (high or low) on body size and male colour (Endler, 1978; Endler, 1980; Millar et al. 2006; Kemp et al. 2008) were modified by a

simultaneous evaluation of parasitism. In order for our measures of parasitism to provide a meaningful comparison to predation regime, we first needed to confirm that differences in parasitism between populations were reasonably consistent through time.

We found that variation in parasitism levels across populations was very highly correlated between two dry season samples (Fig. 3). Differences in parasitism thus have the potential to make a reasonable contribution to phenotypic variation among guppy populations. Interestingly, parasitism levels in these dry season samples were not well correlated with those in the intervening wet season. The likely reason is that heavy and frequent rainfall during the wet season can create difficult conditions for guppies (Weese et al. 2011), particularly those infected with Gyrodactylus (van Oosterhout et al. 2007). Our data thus suggest that repeatable environmental differences between sites (dry seasons) lead to similar patterns of parasitism levels as under stable conditions, even if those levels are perturbed by intervening extreme environmental conditions (flooding during the wet season). Studies of other host-parasite systems have similarly revealed that repeatable environmental variation among populations drives at least somewhat repeatable patterns of parasitism (e.g., Duffy et al. 2010; Eizaguirre et al. 2010; MacColl & Chapman 2010; Poulin et al. 2011). Of course, different conditions during the intervening wet season dictate that adaptation to parasitism at any given site might be compromised by seasonal variation in parasitism levels. Indeed, temporal variation in parasite dynamics can have important consequences for host adaptation (Decaestecker et al. 2007). These results further highlight the importance of considering both spatial and temporal variation in parasitism (Duffy et al. 2010, Eizaguirre & Lenz 2010; Stireman et al. 2002).

We also found that parasitism levels differed on average between high predation (HP) and low predation (LP) guppy populations, being higher in the former. This result is consistent with the single-year survey of Martin and Johnsen (2007). It seemingly conflicts, however, with the studies of Cable and van Oosterhout (2006) reporting higher parasite levels in an upland (LP) site than a lowland (HP) site in the Aripo River. Interestingly, however, we found

inconsistent results concerning parasite mean abundance and prevalence for HP and LP sites within the Aripo River (Fig. 4), indicating that this river appears to be an exception to the general trend (as do Marianne and Quare). Returning to this general trend, higher parasitism levels in HP than LP populations could simply relate to geography, where dispersal of LP guppies downstream into HP sites (but only rarely in the reverse) would continually remove infected guppies from LP sites and add them to HP sites (van Oosterhout et al. 2007). In addition, HP populations shoal more in defense against predators (Magurran, 1990; 2005), which could increase the opportunity for, and ease of, parasite transmission. Of course, these are merely hypotheses given that HP and LP environments and HP and LP guppies differ in many ways (Endler 1995; Reznick et al. 2001) that could influence parasitism levels.

Effects of parasitism

Our results suggest that *Gyrodactylus* is not a particularly important factor driving natural variation in guppy body size and male colour. We draw this conclusion because infected and uninfected individuals generally do not differ consistently in body size or colour within populations, and the level of parasitism was only rarely related to the average size or colour of guppies across populations (apparent exceptions are noted below). This conclusion is consistent with Martin and Johnsen (2007), who did not find any association in Trinidadian guppy populations between *Gyrodactylus* infection levels and orange color. Both of these field surveys, however, appear in conflict with laboratory studies reporting that *Gyrodactylus* infection influences some aspects of guppy colour. For instance, the intensity of orange color on male guppies fades following infection by *Gyrodactylus* (Houde & Torio 1990; Houde 1997).

We can see several potential reasons why parasitism levels are not associated with guppy traits in field surveys even though they can be in laboratory studies. First, the static nature of point-in-time field samples does not take into account an individual guppy's infection history, which laboratory studies show is important in the responses of guppies to *Gyrodactylus* (Scott & Anderson 1984; Scott

1985b; 1987). That is, a guppy that was not infected in our survey could well have been infected just previously. Alternatively, a guppy that was infected in our survey could have been uninfected until only recently. Thus, it seems possible that effects of *Gyrodactylus* on guppy traits could be better assessed by tracking individuals through time to relate infection history to trait development. This will be difficult in natural populations but could be attempted through mark-recapture studies, which are feasible for guppies (e.g., van Oosterhout et al. 2007; Weese et al. 2010).

Another possibility is that laboratory studies often control for variation in factors that obscure associations between infection status and guppy traits in natural populations. For instance, variation in resource acquisition can influence both infection by *Gyrodactylus* (Kolluru et al. 2006; 2008) and body size and male colour (Grether et al. 1999; 2001). Also, infection by other parasites may well overwhelm any signal of infection by *Gyrodactylus*, particularly given that many other parasites are known in natural guppy populations. In addition, guppy age can influence parasite infection (Poulin & Vickery 1993; Thomas et al. 2009) and also body size and colour (Miller & Brooks 2005). So even if *Gyrodactylus* infection is important for guppy traits when removing other influences in the laboratory, this effect might be washed out by other factors varying in the more complicated natural milieu.

Although our general conclusion is that *Gyrodactylus* is not an important factor driving variation in guppy traits, we did record a few potential exceptions that might be worth exploring in future studies. At the individual level, the number of fuzzy black spots was lower in populations with higher levels of parasitism (Fig. 6), and fuzzy black spots were also larger on infected individuals (Fig. 7). Previous studies have shown that fuzzy black spots change in size during high-energy activities, such as mating, dominant and aggressive behaviours (Price et al. 2008; L. Delaire personal obs.). It is also known that parasitized guppies show a reduced rate of such behaviours (Kennedy et al. 1987; McMinn 1990). Thus, energy devoted to fight infection might detract from the ability to intraspecific signals, here the size of fuzzy black spots. At the population level,

populations with higher infection rates had fewer black spots. We have no concrete explanation for this variation but it is tempting to speculate that the above constraints on spot size changes might mean that guppies evolved reduced reliance on this signal in sites with high parasitism. We also found that populations with higher infection rates had more silver spots, but these spots are not very numerous and we have no obvious explanation for why they might vary in this manner.

Predation and parasitism

A primary motivation of our study was to determine whether a consideration of parasites would modify previous interpretations regarding the role of predators in driving variation in guppy traits. Such a consideration is important given our demonstration that predation and parasitism levels are correlated in nature, meaning that variation originally attributed to predation could well be influenced by coincident variation in parasitism. At the same time, the correlation between these two potential causal factors was not perfect (Fig. 3), providing the opportunity to disentangle their effects.

We first confirmed that predation does indeed correlate with some aspects of guppy trait variation. For instance, guppies were larger in LP sites than in HP sites (Fig. 5). This pattern has been observed in previous work, and may be due to a combination of plastic, demographic, evolutionary responses to higher mortality rates and low competition in HP sites (Endler 1978; Reznick & Endler 1982; Rodd & Reznick 1997; Grether et al. 2001b). In addition to body size differences, LP guppies tended in some analyses to have more numerous and larger black spots (Figs. 6 and 7), more orange area on the body (Fig. 8), and more saturated orange spots (Fig. 9). These results also generally fit with previous work in confirming that predation intensity does have an influence on guppy colour variation, ostensibly because conspicuousness increases predation risk (Endler, 1980; Kemp et al. 2008; Millar et al. 2006).

Despite this general pattern, we were struck by the overall inconsistency of predation regime effects. For instance, predation was important in only some

analyses (Tables 4-7; Appendices A and B) and considerable overlap in colour was evident between predation categories. That is, a number of LP guppy populations had very small individuals with few and small black spots, low orange area, and low orange saturation (Figs. 4-8). Indeed, within-watershed LP versus HP differences varied in direction and magnitude among watersheds. Similar complications and nuances to the predation story have been revealed in other recent studies (Millar et al. 2006; Karim et al. 2007; Weese et al. 2010; Kemp et al. 2008). Overall, then, recent work suggests that, although predation regime is certainly important, the causes of guppy colour variation in nature are undoubtedly multifactorial.

Our focus here was whether or not parasitism should be a part of this emerging multifactorial story. As noted above, we found that the effects of *Gyrodactylus* parasitism on colour variation were relatively minor, and would not be expected to have a serious modifying influence on interpretations regarding predation. Fitting with this idea, the inclusion or exclusion of parasitism from statistical models had no influence on the apparent effects of predation. This tells us that when building up a multi-factorial view of the drivers of colour variation, we perhaps need to be less concerned with parasites, *Gyrodactylus* at least, than with other factors such as resource levels and canopy cover (Grether et al. 1999; Millar et al. 2006; Schwartz & Hendry, 2010) and sexual selection (Houde & Endler 1990; Rodd et al. 2002; Schwartz & Hendry 2007).

Conclusions

Our main findings were that variation in levels of *Gyrodactylus* parasitism on guppies (1) are consistent across dry seasons, (2) are higher in high-predation guppy populations than in low-predation guppy populations, (3) have only minor influences on guppy trait variation, and (4) do not modify the inferred effects of predation. These results suggest that parasitism, at least as it was quantified in our study, is not that important to guppy trait variation; the static nature of our survey of only a subset of guppy traits was not effective at detecting the effects of this parasite. Parasitism is a dynamic process with effects that are most obvious when

tracking individual hosts through time. In addition, other environmental factors, including other parasites, might swamp or otherwise obscure any effects of *Gyrodactylus* in natural guppy populations. Also, other guppy traits, such as life history and behavior, might be more strongly influenced by parasitism. Overall it seems that much variation in guppy traits remains to be explained both within and among populations, suggesting that multiple causal factors are important in this system.

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Tables

Table 1: Sites sampled in Fe	ebruary 2009 and 2010.
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	Site		UTM	Predation	Sample	Sample	Gvrodactvlus
	5110		Coordinates	Regime	Size	Size	e ji o date t ji das
			(x, y)	(H-high,	2009	2010	
				L-low)			
1	Arima 1	Am1	686581	Н	68	51	Yes
			1183042				
2	Arima 2	Am2	687219	L	60	50	No
2	A minute 2	A	1181021		65	15	Vee
3	Arima 5	Ams	080815	п	65	45	res
Δ	Aripo 1	An1	693188	т	68	49	Ves
7	7 uipo i	n pi	1181605	L	00	т <i>)</i>	103
5	Aripo 2	Ap2	694231	Н	60	50	Yes
	1	•	1177709				
6	Aripo 4	Ap4	693328	L	54	50	No
			1179939				
7	Damier 1	Da1	682413	Н	66	50	Yes
0		DA	1193774	Ŧ	50	47	N
8	Damier 2	Da2	682467	L	58	47	No
0	El Cedro 1	Cel	689627	т	60	51	Ves
/	Li Ccuio i	cei	1179248	L	0)	51	105
10	El Cedro 2	Ce2	689674	н	68	52	Yes
			1178592				
11	Guanapo 1	Gu1	690856	Н	62	52	Yes
	-		1178253				
12	Guanapo 2	Gu2	689337	L	76	51	No
			1184456	-	•	-	
13	Marianne 3	Ma3	687359	L	20	50	No/Yes (2000/2010)
14	Marianna 4	Mod	1188/02	т	69	40	(2009/2010) V as
14	Marianne 4	IV1a4	1188425	L	00	49	108
15	Marianne 8	Ma8	685300	L	68	51	Yes
10		1.140	119193	-	00	01	100
16	Marianne 10	Ma10	686711	L	73	50	No
			1191358				
17	Marianne 14	Ma14	684934	Н	62	50	Yes
			1191469	_			
18	Marianne 16	Mal6	685561	L	69	44	No
10	Domio 11	Do 11	1188185	т	66	51	Vaa
19	Parla 11	Pall	1100784	L	00	51	ies
20	Paria 7	Pa7	689639	L	72	48	Yes
20	Turru /	147	1188582	Ľ	12	10	105
21	Quare 1	Qu1	697548	Н	69	53	Yes
			1179275				
22	Quare 2	Qu2	697183	L	43	24	Yes/No
			1180514				(2009/2010)
23	Turure 2	Tu2	700200	Н	66	45	Yes
24	Tummo 2	Tu2	1178350	т	75	10	NT-
24	Turure 5	103	118130	L	15	48	INO
25	Yarra 1	Ya1	683415	L	60	44	Yes
	1	1	1187541	-			100
26	Yarra 2	Ya2	680306	Н	13	44	Yes
			1193940				

Table 2: Results of GLMMs and GLMs examining parasitism at the individual (N = 3514) and population (N=26) levels. Parasitism is measured as *infection presence* (the presence or absence of infection) and *abundance* (number of gyrodactylids per fish, including uninfected fish) at the individual level, and by *prevalence* (the proportion of the population that is infected) and *mean abundance* (the mean number of gyrodactylids per guppy per population, including uninfected fish) at the population level. Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

Individual Level	Infection Presence		Abu	ındance	
Model	Infection Presence = Predation+Year+Sex+S(r)+Interactions		Abundance = Predation+	-Year+Sex+S(r)+Interactions	
Factor	Z P		Z	Р	
Predation	-10.25	<u><0.0001</u>	9.191	<u><0.0001</u>	
Year	1.245	0.213	1.800	0.0719	
Sex	-0.468 0.640		-0.6610	0.508	
Predation*Year	_	_	-3.490	<u>0.0005</u>	
Population Level	Pr	evalence	Mean Abundance		
Model	Prevalence = Predation+Year+Interaction		Mean Abundance = Predation+Year+Interaction		
	F _{2,49} P		F _{2,49}	Р	
Model	12.47	<u>0.0001</u>	7.896	<u><0.0011</u>	
Year	0.0819	0.7759	0.2558	0.6153	
Predation	24.84	<u><0.0001</u>	15.54	<u>0.0003</u>	

Table 3: Results of MANCOVAs examining size variation among sexes and levels of predation and parasitism at the individual and population levels. Parasitism is measured as *infection presence* (individual level) and *prevalence* (population level). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

	Individual	level		Population	level	
				F	d.f.	Р
Factor	F	d.f.	Р			
Whole Model	38.35	155, 7680	<u><0.0001</u>	4.904	10, 90	<u><0.0001</u>
Wilk's λ	0.0586			0.4759		
Sex	336.7	5, 1551	<u><0.0001</u>	46.71	5,45	<u><0.0001</u>
Predation	68.88	5, 1551	<u><0.0001</u>	5.711	5, 45	<u>0.0004</u>
Parasitism	0.8615	5, 1551	0.5063	0.8615	5, 45	0.3885
Sex*Pred	3.838	5, 1551	<u>0.0019</u>	-	-	-
Sex*Para	0.8207	5, 1551	0.5348	-	-	-
Pred*Para	1.006	5, 1551	0.4129	-	-	-
Sex*Pred*Para	2.278	5, 1551	<u>0.0447</u>	-	-	-
Site[Pred]	29.22	120, 7627.6	<u><0.0001</u>			
B) Excluding Parasitism	Individual	level		Population	level	
Factor	F	d.f.	Р	F	d.f.	Р
Whole Model	44.14	135, 7694.4	0.0000	21.94	10, 90	
Wilk's λ	0.0591			0.0846		<u><0.0001</u>
Sex	530.1	5, 1559	<u>0.0000</u>	47.91	5, 45	<u><0.0001</u>
Predation	120.1	5, 1559	<u><0.0001</u>	8.107	5, 45	<u><0.0001</u>
Sex*Pred	5.106	5, 1559	<u>0.0001</u>	-	-	-
Site[Pred]	29.81	120, 7666.9	<u>0.0000</u>			

Table 4: Results of MANCOVAs examining variation in number of male coloured spots among levels of predation and parasitism at the individual and population levels. Parasitism is measured as *infection presence* (individual level) and *prevalence* (population level). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

A) Including	· ·· · ·	,, ,		Population level				
Parasitism	Individua	l level						
Factor	F	d.f.	Р	F	d.f.	Р		
Whole Model	4.171	216, 5579.1	<u><0.0001</u>	1.089	24, 44.12	0.3926		
Wilk's λ	0.316			0.2592				
Predation	4.095	8,723	<u><0.0001</u>	1.376	8, 15	0.2826		
Parasitism	0.674	8,723	0.7148	0.8335	8, 15	0.5875		
Size (PC1)	7.547	8,723	<u><0.0001</u>	1.192	8, 15	0.3655		
Site[Pred]	4.126	192, 5523.9	<u><0.0001</u>					
B) Excluding	x 1· · 1	11 1			1 1			
B) Excluding Parasitism	Individual	l level		Population	level			
B) Excluding Parasitism Factor	Individual F	d.f.	P	Population F	<i>level</i> d.f.	Р		
B) Excluding Parasitism Factor Whole Model	Individual F 4.309	d.f. 208, 5570.3	Р <u><0.0001</u>	Population F 1.268	level d.f. 16, 32	Р		
 B) Excluding Parasitism Factor Whole Model Wilk's λ 	Individual F 4.309 0.3183	d.f. 208, 5570.3	Р <u><0.0001</u>	Population F 1.268 0.3746	level d.f. 16, 32	P 0.2752		
B) Excluding Parasitism Factor Whole Model <i>Wilk's λ</i> Predation	Individual F 4.309 0.3183 3.947	d.f. 208, 5570.3 8, 724	P < <u><0.0001</u> < <u><0.0001</u>	Population F 1.268 0.3746 0.1650	level d.f. 16, 32 8, 16	P 0.2752 0.1650		
B) Excluding Parasitism Factor Whole Model <i>Wilk's λ</i> Predation PC1	Individual F 4.309 0.3183 3.947 7.574	d.f. 208, 5570.3 8, 724 8, 724	P <0.0001 <0.0001 <0.0001	Population F 1.268 0.3746 0.1650 0.2996	level d.f. 16, 32 8, 16 8, 16	P 0.2752 0.1650 0.2996		
B) Excluding Parasitism Factor Whole Model <i>Wilk's λ</i> Predation PC1 Pr*PC1	Individual F 4.309 0.3183 3.947 7.574 -	d.f. 208, 5570.3 8, 724 8, 724 -	P <u><0.0001</u> <u><0.0001</u> <u><0.0001</u> -	Population F 1.268 0.3746 0.1650 0.2996 -	level d.f. 16, 32 8, 16 8, 16 -	P 0.2752 0.1650 0.2996 -		

Table 5: Results of MANCOVAs examining variation in average size of male coloured spots among levels of predation and parasitism at the individual and population levels. Parasitism is measured as *infection presence* (individual level) and *prevalence* (population level). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

A) Including Parasitism	Individual	level		Population	level	
	F	d.f.	Р	F	d.f.	Р
Factor						
Whole Model	1.002	112, 53.69	0.5083	4.503	24,	<u><0.0001</u>
Wilk's λ	0.0004			0.0239	41.205	
Predation	2.779	8,6	0.1149	2.016	8,14	0.1201
Parasitism	2.276	8,6	0.1660	3.477	8, 14	<u>0.0202</u>
Size (PC1)	1.221	8,6	0.4154	12.70	8, 14	<u><0.0001</u>
Site[Pred]	0.9701	88, 48.8	0.5574			
B) Excluding	Individual	level		Population	level	
B) Excluding Parasitism	Individual	level		Population	level	
B) Excluding Parasitism Factor	Individual F	<i>level</i> d.f.	Р	Population F	level d.f.	Р
B) Excluding Parasitism Factor Whole Model	Individual F 0.8965	<i>level</i> d.f. 104, 59.18	P 0.6902	<i>Population</i> F 5.142	<i>level</i> d.f. 16, 30	P <0.0001
 B) Excluding Parasitism Factor Whole Model Wilk's λ 	Individual F 0.8965 0.0015	<i>level</i> d.f. 104, 59.18	P 0.6902	Population F 5.142 0.0714	<i>level</i> d.f. 16, 30	P <u><0.0001</u>
 B) Excluding Parasitism Factor Whole Model Wilk's λ Predation 	Individual F 0.8965 0.0015 2.676	<i>level</i> d.f. 104, 59.18 8, 7	P 0.6902 0.1061	Population F 5.142 0.0714 2.128	level d.f. 16, 30 8, 15	P <u><0.0001</u> 0.0986
 B) Excluding Parasitism Factor Whole Model Wilk's λ Predation PC1 	Individual F 0.8965 0.0015 2.676 0.6975	level d.f. 104, 59.18 8, 7 8, 7	P 0.6902 0.1061 0.6891	Population F 5.142 0.0714 2.128 12.87	level d.f. 16, 30 8, 15 8, 15	P <u><0.0001</u> 0.0986 <u><0.0001</u>
 B) Excluding Parasitism Factor Whole Model Wilk's λ Predation PC1 Pr*PC1 	Individual F 0.8965 0.0015 2.676 0.6975 -	level d.f. 104, 59.18 8, 7 8, 7	P 0.6902 0.1061 0.6891	Population F 5.142 0.0714 2.128 12.87 -	level d.f. 16, 30 8, 15 8, 15	P <u><0.0001</u> 0.0986 <u><0.0001</u> -

Table 6: Results of MANCOVAs examining variation in male coloured area among levels of predation and parasitism at the individual and population levels. Parasitism is measured as *infection presence* (individual level) and *prevalence* (population level). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

A) Including	Individual loval			Population level			
Parasitism	Inaiviaua	l level					
Factor	F	d.f.	Р	F	d.f.	Р	
		200 5570			16.00		
Whole Model	6.833	208, 5578	<u><0.0001</u>	1.128	16, 32	0.3726	
Wilk's λ	0.1753			0.4089			
Predation	13.4	8,725	<u><0.0001</u>	0.766	8, 16	0.6370	
Parasitism	0.4029	8,725	0.9191	1.391	8,16	0.2728	
Site[Pred]	6.901	192, 5539.2	<u><0.0001</u>				
B) Excluding	¥ 1 1				1 1		
Parasitism	Individual	l level		Population			
	F	df	D	F	df	P	
Factor	1	u.1.	I	1	u.1.	1	
Whole Model	7.098	200, 5567.3	<u><0.0001</u>	0.4426	8, 17		
Wilk's λ	0.1761					0.5099	
Predation	13.55	8,726	<u><0.0001</u>	0.9406	8,17	0.5099	
Site[Pred]	6.974	192, 5546.8	<u><0.0001</u>				

Table 7: Results of the MANCOVAs examining the hue, saturation and brightness of black, orange, yellow and green coloured spots among levels of predation and parasitism at the individual and population levels. Parasitism is measured as *infection presence* (individual level) and *prevalence* (population level). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

A) Including							
Parasitism	Individual	level		Population level			
Factor	F	d.f.	Р	F	d.f.	Р	
Whole Model	2.558	312,	<u><0.0001</u>	1.180	24, 24	0.3439	
Wilk's λ	0.1175	3686.4		0.2103			
Predation	2.917	12, 334	<u>0.0007</u>	1.599	12, 12	0.2140	
Parasitism	0.7673	12, 334	0.6842	0.7290	12, 12	0.7037	
Pr*Pa	-	-	-	-	-	-	
Site[Pred]	2.456	204,	<u><0.0001</u>				
		3379.7					
B) Excluding	x 1· · 1	1 1			1 1		
Parasitism	Individual	level		Population	Population level		
Factor	F	d.f.	Р	F	d.f.	Р	
Whole Model	2.632	300,	<0.0001	1.896	12, 13	0.1334	
Wilk's λ	0.1207	3671.4					
Predation	2.881	12, 335	<u>0.0008</u>	1.896	12, 13	0.1334	
Site[Pred]	2.590	288,	<u><0.0001</u>				
		3642.8					

Figures



Figure 1: Morphology of Gyrodactylus spp. Photo courtesy of Marilyn Scott.



Figure 2: Collection sites in northern Trinidad. Blue triangles indicate lowpredation sites and red circles indicate high-predation sites. Site abbreviations and UTM coordinates are shown in Table 1.



Figure 3: Correlation of prevalence (proportion of infected fish per population) by collection year. Blue represents low predation (LP) sites, and red represents high predation (HP) sites (r = 0.76, p < 0.0001). With outliers removed due to sample sizes (Quare 2 LP and Yarra 2 HP, represented by a blue cross and a hollow red square, respectively, on the figure.) r = 0.85, p < 0.0001. Dashed line is the one-to-one line.



Figure 4: Parasitism levels by river and predation level (insets): A) Prevalence: proportion of infected fish per population; B) Mean infection abundance: mean number of parasites per fish per population; by river and predation regime at the population level. A) HP sites have more infected fish than LP sites (p < 0.0001). B) HP sites have more Gyrodactylids than LP sites (p = 0.003). HP populations are represented by red circles, LP populations by blue triangles. Box plots in insets represent 25% and 75% quantiles with the middle line in the boxes identifing the median sample value.



Figure 5: The body area of guppies in relation to predation (blue triangles = low predation; red circles = high predation) and parasitism. A) and C): Correlation of average body sizes of infected guppies vs. uninfected guppies per site; each point represents a site with at least one infected guppy. Values for populations without infected individuals are shown in the column at left. Data show that infection had no consistent influence on the size of guppies within populations. B) and D) Guppy size as a function of parasite prevalence (zero prevalence = uninfected sites) Guppies from low-predation populations were generally larger than those from high-predation populations but parasitism levels had no obvious effect.



Figure 6: The number of fuzzy black spots on male guppies in relation to predation (blue triangles = low predation; red circles = high predation) and parasitism. Panel (A) shows that infected individuals generally had more black spots than did uninfected individuals (values for populations without infected individuals are shown in the column at left) and that high-predation populations generally had males with fewer black spots. Panel (B) suggests again the effect of predation at the population level (although not here statistically significant), while showing no obvious effect of parasite prevalence.


Figure 7: The average size of fuzzy black spots on male guppies in relation to predation (blue triangles = low predation; red circles = high predation) and parasitism. Panel (A) suggests that infected individuals generally had smaller black spots than did uninfected individuals, although this was not statistically significant (values for populations without infected individuals are shown in the column at left) and that no effect of predation was evident. Panel (B) suggests an effect of predation but this was not statistically significant.



Figure 8: The area of orange colour on male guppies in relation to predation (blue triangles = low predation; red circles = high predation) and parasitism. Panel (A) shows that infection had no consistent influence on the area of orange on guppies within populations (values for populations without infected individuals are shown in the column at left) but suggests that low-predation fish had more orange than high-predation fish. Panel (B) shows that orange area is not influenced by parasite prevalence. The effect of predation regime at the individual level (Panel A) is no longer significant at the population level (sites included where no guppies were infected).



Figure 9: The saturation (from Photoshop) of orange spots (degrees, from $0-360^{\circ}$) on male guppies in relation to predation (blue triangles = low predation; red circles = high predation) and parasitism. Panel (A) shows that infection had no consistent influence on orange saturation (values for populations without infected individuals are shown in the column at left) and suggests that males from low-predation populations had greater orange saturation, although this effect was only significant in multivariate analyses. Panel (B) shows the lack of influence of parasitism levels on orange saturation but again suggests an effect of predation level – although this was not significant.

General Conclusions

Parasitism can have a significant influence on the evolution of many organisms (Borgia & Collis 1989; Combes 1998; Zuk 1992; Chadwick & Little 2005; Eizaguirre & Lenz 2010). They often regulate population dynamics beginning at the host level by altering host population dynamics, natural mortality rates and levels of reproductive success (Tompkins & Begon 1999; Chadwick & Little 2005; Telfer et al. 2005). Behavioural, morphological or reproductive effects on hosts can result in subsequent changes to life history characteristics, general fitness and of course, survival (Minchella & Scott 1991; Moore 2002). By affecting such crucial components of host lifecycle, parasites are ideally placed to act as a selective pressure driving evolution. Trinidadian guppies might also experience such effects, and *Gyrodactylus*, among many other parasites, has recently piqued the interest of evolutionary biologists.

In the Trinidadian guppy system, a very important factor influencing variation in guppy traits is predation, particularly the contrast between highpredation and low-predation localities. However, a substantial fraction of the variation in traits remains unexplained, suggesting the importance of additional causal factors. The goal of my thesis was to expand current knowledge of this system by examining the potential causal role of parasitism, including in relation to predation.

I found that variation among guppy populations in levels of parasitism by *Gyrodactylus* spp. are consistent across dry seasons, and are higher in highpredation sites than in low-predation sites. This suggests the potential for parasitism to be an important factor shaping guppy trait variation in nature. However, parasitism had only minor effects of guppy traits both within and between populations, and its consideration did not alter interpretations regarding the role of predation. Setting aside this generality, I did find some potential associations that could be focus of future work. For instance, the number of fuzzy black spots was lower in populations where parasitism levels were higher. The biological significance of these isolated findings is unclear. Overall, it is possible that the general lack of detected effects of parasitism is due to the static nature of

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our survey or additional factors that vary in nature and obscure the effects of parasites. Or perhaps such effects would be detectable for other traits not measured here.

This survey provides a framework from which to further explore factors contributing to phenotypic variation in the Trinidadian guppy system. It is important that future work look at parasitism levels temporally, with a more complete overview of parasite fauna, while also incorporating the study of additional potential casual factors. I hope that this thesis will inspire further research, and contribute to a greater understanding of the evolution of complex systems.

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APPENDIX A

Eigenvectors and Pearson correlation coefficients derived from MANCOVAs

Table A1: Eigenvectors and Pearson correlation coefficients derived from the predator regime, sex, and size terms of the

 MANCOVA for guppy size. Variables exhibiting an absolute value of the correlation coefficient ≥ 0.4 are in bold text.

 Pearson
 Pearson

 Pearson
 Pearson

Response Variable	Eigenvector	Pearson		Pearson		Pearson		Pearson
		Correlation		Correlation		Correlation		Correlation
		Coefficient		Coefficient		Coefficient		Coefficient
<u>Individual level</u>	Predation		Sex		Sex*Pred		Sex*Pred*Pa	
L Body	-0.023907	-0.0301	-0.0373682	-0.6341	-0.0481194	-0.0681	-0.0295564	-0.5490
L Tail	-0.016985	-0.0208	0.00334305	-0.3634	-0.002725	-0.0279	-0.0006817	-0.3189
BD	0.16006701	0.3165	0.13417366	-0.3253	0.10849837	0.2799	0.16175877	-0.2261
A Body	-0.0019894	0.0786	-0.0011666	-0.5363	0.0026886	0.0987	-0.0025946	-0.4616
A Tail	0.00194984	0.1766	0.00156289	-0.3467	0.00039819	0.1184	0.00124468	-0.2908
Population level								
Mean L Body	0.25424252	0.6316	-0.244911	-0.7740				
Mean L Tail	-0.2828047	0.5061	0.38632091	-0.3912				
Mean BD	1.1018767	0.8456	0.19147584	-0.5964				
Mean A Body	-0.0502615	0.6829	-0.0114154	-0.7625				
Mean A Tail	0.00105057	0.6710	0.04020148	-0.4305				

Table A2: Eigenvectors and Pearson correlation coefficients derived from the predator regime and size terms of the MANCOVA for number of male coloured spots. Variables exhibiting an absolute value of the correlation coefficient ≥ 0.4 are in bold text.

Response Variable	Eigenvector	Pearson	Eigenvector	Pearson
		Correlation		Correlation
		Coefficient		Coefficient
<u>Individual level</u>	Predation		Size (PC1)	
Black	0.00546479	0.1055	0.00420018	0.1767
Fuzzy Black	-0.015679	-0.4196	0.00040431	0.2397
Orange	-0.0081258	-0.1877	0.01168691	0.4424
Yellow	-0.005369	-0.1578	0.01389614	0.4319
Blue	0.01718218	0.4263	0.0140093	0.4636
Green	0.01796364	0.4564	0.00783614	0.2981
Violet	0.02297823	0.3920	0.02514461	0.5501
Silver	0.03420083	0.4807	0.03251339	0.4762

Table A4: Eigenvectors and Pearson correlation coefficients derived from the predator regime term of the MANCOVA for male coloured area. Variables exhibiting an absolute value of the correlation coefficient ≥ 0.4 are in bold text.

Response Variable	Eigenvector	Pearson Correlation Coefficient
<u>Individual level</u>	Predation	
Black Area Corrected	0.34810378	0.2978
Fuzzy Black Area Corrected	0.1954486	0.2560
Orange Area Corrected	1.19468527	0.8844
Yellow Area Corrected	0.43432269	0.3528
Blue Area Corrected	-0.7418944	-0.0208
Green Area Corrected	-0.4649715	-0.1888
Violet Area Corrected	-0.319629	-0.0567
Silver Area Corrected	-1.2158075	-0.2092

Table A5: Eigenvectors and Pearson correlation coefficients derived from the predator regime term of the MANOVA for the hue, saturation and brightness of black, orange, yellow and green. Variables exhibiting an absolute value of the correlation coefficient \geq 0.4 are in bold text.

Response Variable	Eigenvector	Pearson Correlation Coefficient
Individual level	Predation	
B_Hcorr	0.05168832	0.0036
B_Scorr	0.01370592	0.0557
B_Bcorr	0.01109051	-0.0501
O_Hcorr	-0.0043633	0.2922
O_Scorr	0.00256508	0.3781
O_Bcorr	0.00109107	0.3037
Y_Hcorr	-0.0316587	-0.6508
Y_Scorr	0.06640038	0.4261
Y_Bcorr	0.02474287	0.0890
G_Hcorr	-0.0517515	-0.0713
G_Scorr	-0.011295	0.1571
G_Bcorr	-0.0045578	0.1157

APPENDIX B

Univariate Analyses

Table B1: Fixed effects of univariate analyses on guppy size at the individual (Indi.) and population (Pop.) levels. Fixed effects included Sex (male or female), **Pr**edation level (high or low) and **Pa**rasitism (individual level: *infection presence*; population level: *prevalence*). At the individual level, Site nested within Predation was included as a random factor (not shown). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

Level	Level Effect		rea of	Body	Le	ength o	of Body		Body De	pth		Area of	Tail	L	ength o	f Tail		PC1	
(N =	= 1587)	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р
Indi.	S	1558	411.8	<u><0.0001</u>	1557	512.8	<0.0001	1558	196.7	<u><0.0001</u>	1557	108.2	<0.0001	1561	100.8	<0.0001	1557	254.9	<u><0.0001</u>
	Pr	26.09	4.910	<u>0.0356</u>	25.59	3.251	0.0832	25.98	9.621	<u>0.0046</u>	25.43	3.865	0.0603	24.29	1.523	0.2289	26	4.418	<u>0.0455</u>
	Pa	1580	0.0075	0.9310	1578	0.2061	1 0.6499	1580	0.0561	0.8128	1576	0.1538	0.695	1580	0.0027	0.9587	1578	0.0025	0.9599
	S*Pr	1558	11.87	<u>0.0006</u>	1558	7.424	<u>0.0065</u>	1558	10.46	<u>0.0012</u>	1558	7.236	<u>0.0072</u>	-	-	-	1558	8.959	<u>0.0028</u>
	S*Pa	1558	1.367	0.2425	1558	1.250	0.2638	1558	0.5035	0.4781	1558	0.9688	0.3251	-	-	-	1558	1.029	0.3105
	Pr*Pa	1580	2.563	0.1096	1578	3.647	0.0563	1580	2.8205	0.0933	1576	1.740	0.1873	-	-	-	1578	2.606	0.1067
	S*Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B1	continu	ed:
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Level	vel Effect Mean Area of Bod (N = 26) DF F P		of Body	Mean .	Length	of Body	Me	an Bod	ly Depth	Mear	n Area o	of Tail	Mean	Lengtl	h of Tail		Mean P	PCI	
(N	= 26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
Pop.	Model	3,48	21.30	<0.0001	3, 48	56.72	<0.0001	3, 48	15.47	<u><0.0001</u>	3, 48	6.376	<0.0001	3, 48	4.584	<u>0.0067</u>	3, 48	12.48	<u><0.0001</u>
	S		50.34	<u><0.0001</u>		51.98	<u><0.0001</u>		24.32	<u><0.0001</u>		8.746	<u>0.0048</u>		6.679	<u>0.0129</u>		24.68	<u><0.0001</u>
	Pr		11.25	<u>0.0016</u>		9.120	<u>0.004</u>		18.90	<u><0.0001</u>		9.398	<u>0.0036</u>		5.599	<u>0.0221</u>		11.18	<u>0.0016</u>
	Pa		0.914	0.3438		1.275	0.2645		0.6205	0.4348		0.9012	0.3472		3.124	0.0835		1.413	0.2403

Table B2: Fixed effects of univariate analyses on the number of coloured spots at the individual (Indi.) and population (Pop.) levels. Fixed effects included predation level (high or low) and parasitism (individual level: *infection presence*; population level: *prevalence*). At the individual level Site nested within Predation was included as a random factor (not shown). Non-significant interaction terms were removed from the model (not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$. Marginally insignificant effects, or significant effects in an insignificant model, are in italics.

Level	Effect	Та	tal Spot.	\$	В	lack Spo	ots	Fuzzy	Black	Spots	C	Drange Sp	pots	Ye	ellow Sp	ots	E	lue Spo	ots
(N	[= 760)	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р
Indi.	Pr	23.72	0.0004	0.9847	27.58	0.1215	0.7301	27.11	4.478	<u>0.0437</u>	24.95	0.3075 0).5842	25.19	0.5383	0.4699	26.9	1.495	0.2320
	Pa	741.2	1.515	0.2188	621.1	0.1388	0.7096	535.5	4.522	<u>0.0339</u>	755.4	0.7373 ().3908	748.9	0.0130	0.9091	744.2	0.3078	3 0.5792
	PC1	392.9	36.84	<u><0.0001</u>	171.5	2.132	0.1461	128.8	3.100	0.0807	640.3	7.132 <	<0.0078	434.2	9.110	<u><0.0027</u>	404.1	8.696	<u><0.0034</u>
	Effect	Gr	een Spot	ts	V	iolet Spa	ots	Si	lver Sp	ots									
		Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	-								
	Pr	27.6	4.170	0.0508	26.26	0.8415	0.3673	26.84	1.320	0.2608	-								
	Pa	691.4	0.1993	0.6554	712.1	0.0415	0.8387	750.2	0.0168	0.8970									
	PC1	244.7	13.00	<u><0.0004</u>	280	17.83	<u><0.0001</u>	455.2	2 10.38	<u><0.0014</u>									

Laual	Effect	Mean N	Number o	f Total	Mean .	Number o	f Black	Mean	Number	of Fuzzy	Me	an Numb	er of	Mean	Number	of Yellow	Mear	ı Number	of Blue
Levei	Ljjeci		Spots			Spots		1	Black Sp	ots	0	range Sp	oots		Spots			Spots	
(N	(=26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
Pop	Model	3, 22	0.9540	0.4318	3, 22	0.2567	0.8557	6, 19	2.705	<u>0.0452</u>	3, 22	1.355	0.2825	3, 22	0.7071	0.5580	3, 22	0.1141	0.9509
	Pr	1	1.330	0.2611	1	0.0182	0.8939	1	7.455	0.7806	1	3.411	0.0783	1	1.105	0.3046	1	0.0974	0.7579
	Pa	1	0.8182	0.3755	1	0.2216	0.6425	1	0.775	0.3897	1	1.446	0.2419	1	0.2436	0.6265	1	0.1026	0.7518
	PC1	1	0.2312	0.6354	1	0.4487	0.5099	1	3.277	0.0861	1	1.897	0.1822	1	0.0948	0.7610	1	0.9581	0.9581
	Pr*Pa	-	-	-	-	-	-	1	5.357	<u>0.0320</u>	-	-	-	-	-	-	-	-	-
	Pr*PC1	-	-	-	-	-	-	1	3.573	0.0741	-	-	-	-	-	-	-	-	-
	Pa*PC1	-	-	-	-	-	-	1	0.6883	0.4171	-	-	-	-	-	-	-	-	-
	F (C)	Mean N	lumber of	f Green	Mean	Number o	f Violet	Mean	Number	of Silver									
	Еђест		Spots			Spots			Spots										
		DF	F	Р	DF	F	Р	DF	F	Р	-								
	Model	3, 22	4.345	<u>0.0151</u>	3, 22	0.3404	0.7963	2, 33	0.4820	0.6982	-								
	Pr	1	4,5611	<u>0.0441</u>	1	0.0189	0.0189	1	0.0189	0.8918									
	Pa	1	0.2711	0.6078	1	1.100	0.3056	1	1.100	0.3056									
	PC1	1	10.87	<u>0.0033</u>	1	0.0063	0.9374	1	0.0063	0.9374									

Table B2 continued:

^a Distribution not normal

Table B3: Fixed effects of univariate analyses on the average size of guppy spots at the individual (Indi.) and population (Pop.) levels. Fixed effects included predation level (high or low) and parasitism (individual level: *infection presence*; population level: *prevalence*). At the individual level Site nested within Predation was included as a random factor (not shown). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

Level	Effect	E	Black Spo	ts	Fuzz	y Black	Spots	Ог	range Sp	ots	Y	ellow Sp	ots	1	Blue Spo	ts
(1	N = 760)	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р
[ndi	Pr	25.61	2.375	0.1355	29.17	0.0002	0.9897	24.43	3.012	0.0952	36.36	0.7024	0.4075	30.03	0.0742	0.7872
	Pa	745.9	0.1701	0.6801	606.1	5.885	<u>0.0156</u>	743.3	0.0009	0.9762	680.5	0.4238	0.5153	246.1	0.6463	0.4222
	PC1	745.9	49.58	<u><0.0001</u>	169.5	89.85	<u><0.0001</u>	706	31.77	<u><0.0001</u>	613	27.87	<u><0.0001</u>	285.2	6.974	<u>0.0087</u>
	Pr*Pa	-	-	-	-	-	-	-	-	-	687.5	0.0695	0.7922	251.4	0.0814	0.7756
	Pr*Pc1	-	-	-	-	-	-	-	-	-	495.5	8.159	<u>0.0045</u>	183.7	0.4481	0.5041
	Pa*Pc1	-	-	-	-	-	-	-	-	-	692.6	0.1665	0.6833	358.1	6.512	<u>0.0111</u>
	Pr*Pa*Pc1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Effect	Gi	reen Spot	\$	V	iolet Spo	ots	S	ilver Spo	ots						
		Den DF	F	Р	Den DF	F	Р	Den DF	F	Р	-					
	Pr	27.66	0.0289	0.8663	43.15	0.052	0.8196	20.55	0.1717	0.6829	-					
	Pa	370.4	0.3919	0.5317	392.7	0.002	0.9597	66.63	1.201	0.2771						
	PC1	206.1	14.32	<u>0.0002</u>	334	22.80	<u><0.0001</u>	69.01	9.115	<u>0.0036</u>						
	Pr*Pa	-	-	-	392.7	0.8676	6 0.3522	-	-	-						
	Pr*Pc1	-	-	-	334	3.364	0.0675	-	-	-						
	Pa*Pc1	-	-	-	447.3	0.0013	0.9711	-	-	-						
	Pr*Pa*Pc1	-	-	-	447.3	3.985	<u>0.0465</u>	-	-	-						

Level	Effect		Mean Bl	ack	Мес	ın Fuzzy l	Black	Me	ean Orang	ge	1	Mean Yell	0W		Mean Bl	ue
	(N = 26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
Pop	Model	3, 22	2.985	0.0532	3, 22	13.36	<u><0.0001</u>	3, 22	2.860	0.0602	6, 19	5.516	<u>0.0019</u>	3, 22	0.6056	0.6183
	Pr	1	3.361	0.0803	1	0.0194	0.8904	1	7.878	<u>0.0103</u>	1	0.6142	0.4429	1	0.0271	0.8707
	Pa	1	0.327	0.5732	1	0.8194	0.3551	1	0.5944	0.4489	1	0.0058	0.9403	1	0.3449	0.5630
	PC1	1	0.5821	0.4536	1	29.04	<u><0.0001</u>	1	0.4323	0.5177	1	8.210	<u>0.0099</u>	1	1.0367	0.3197
	Pr*Pa	-	-	-	-	-	-	-	-	-	1	3.844	0.0648	-	-	-
	Pr*PC1	-	-	-	-	-	-	-	-	-	1	4.616	<u>0.0305</u>	-	-	-
	Pa*PC1	-	-	-	-	-	-	-	-	-	1	5.462	<u>0.0448</u>	-	-	-
	Pr*Pa*Pc1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Effect	1	Mean Gre	een	i	Mean Vio	let	Λ	Mean Silv	er						
		DF	F	Р	DF	F	Р	DF	F	Р						
	Model	3, 22	4.369	<u>0.0147</u>	3. 22	6.819	<u>0.0020</u>	6, 18	9.399	<0.0001						
	Pr	1	0.8952	0.3543	1	0.6234	0.4382	1	3.406	0.0815						
	Pa	1	0.7096	0.4087	1	0.1794	0.6760	1	30.84	<u>0.0001</u>						
	PC1	1	12.01	<u>0.0022</u>	1	18.39	<u>0.0003</u>	1	1.143	0.2991						
	Pr*Pa	-	-	-	-	-	-	1	6.025	<u>0.0245</u>						
	Pr*PC1	-	-	-	-	-	-	1	6.286	<u>0.0220</u>						
	Pa*PC1	-	-	-	-	-	-	1	0.5635	0.4625						
	Pr*Pa*Pc1	-	-	-	-	-	-	-	-	-						

Table B3 continued:

^a Distribution not normal

Table B4: Fixed effects of univariate analyses on guppy colour at the individual (Indi.) and population (Pop.) levels. Fixed effects included predation level (high or low) and parasitism (individual level: *infection presence*; population level: *prevalence*). At the individual level Site nested within Predation was included as a random factor (not shown). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$. P-values in italics indicate marginal non-significance or a significant value in a non-significant model.

Level	Effect		Orange			Black		Fuz	zy Blaci	k		Yellow ^a			Blue ^a			Green ^a	
(N	= 760)	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р	Den DF	F	Р	Den DF	F	Р	Den DF	F	Р
Indi.	Pr	24.28	3.224	0.0850	24.43	1.056	0.3142	736.3	1.107	7 0.3027	24.56	3.016	0.0950	35.45	0.0859	0.7712	24.52	0.7268 (0.4022
	Pa	745.6	1.222	0.2692	755.9	0.0699	0.7915	25.41	0.5941	0.4411	749.5	0.6329	0.6329	685.3	0.7301	0.3931	716.5	0.0698 (0.7917
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-	685.3	4.376	<u>0.0368</u>	-	-	-
	Effect		Violet ^a			Silver ^a													
		DenDF	F	Р	DenDF	F	Р												
	Pr	24.68	0.1256	0.7261	24.77	0.5947	0.4479	_											
	Pa	731	0.9614	0.3272	743.2	0.9493	0.9493												
	Pr*Pa	-	-	-	-	-	-												

vel Effect		Mean Orange Area_log			Mean Black Area			Mean Fuzzy Black Area			Mean Yellow Area			Mean Blue Area			Mean Green Area		
(N =	= 26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
. N	Aodel	2, 23	1.351	0.2787	2, 23	0.5131	0.6053	2, 23	0.9174	0.4137	2, 23	1.680	0.2083	2, 23	0.4445	0.6465	2, 23	0.3546	0.7052
F	Pr	1	2.692	0.1144	1	0.5105	0.4821	1	0.9443	0.3413	1	2.003	0.1701	1	0.4149	0.5259	1	0.4120	0.5273
F	Pa	1	0.4833	0.4939	1	0.1648	0.6885	1	0.2718	0.6071	1	0.3194	0.5774	1	0.1634	0.6897	1	0.0788	0.7883
P	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Effect	Mean Violet Area		Mean Silver Area_log10															
_		DF	F	Р	DF	F	Р												
N	Aodel	2, 23	0.0027	0.9973	2, 23	2.849	0.0785												
P	Pr	1	0.0001	0.9925	1	0.0371	0.8489												
P	Pa	1	0.0050	0.9441	1	4.5905	0.0843												
	מיי ר																		

^a Distribution not normal

Table B4 continued:

Table B5: Fixed effects of univariate analyses on the hue, saturation and brightness of orange, black, yellow and green guppy spots at the individual (Indi.) and population (Pop.) levels. Fixed effects included predation level (high or low) and parasitism (individual level: *infection presence*; population level: *prevalence*). At the individual level Site nested within Predation was included as a random factor (not shown). Non-significant interactions were removed from the model (values not shown). P-values in bold and underlined indicate significance at $\alpha = 0.05$.

Level	Effect Orange Hue ^a			Bi	lack Hu	e	Ye	llow Hue	a	Green Hue			
(N :	= 760)	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р
Indi.	Pr	24.28	2.625	0.1181	24.36	1.944	0.1758	27.16	0.1283	0.7230	17.68	1.800	0.1967
	Pa	714.1	0.5145	0.4734	653.1	0.7089	0.4001	375.8	0.2423	0.6228	259.7	0.1333	0.7153
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-
Level	Effect	Orang	ge Satur	ation ^a	Black	k Satura	tion	Yello	w Satura	tion	Green Saturation		
(N :	= 760)	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р
Indi.	Pr	24.28	1.485	0.2347	25.11	1.964	0.1733	27.67	0.3062	0.5845	22.29	2.767	0.1102
	Pa	721.8	0.0329	0.8562	699.6	0.0012	0.9725	562.3	0.9996	0.3178	268.3	1.025	0.3122
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-
Level	Effect	Orang	ge Brigh	tness ^a	Black Br	rightnes	s_log10	Yellov	v Brightn	ess ^a	Gree	n Bright	ness
(N :	= 760)	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р	DenDF	F	Р
Indi.	Pr	24.21	0.2196	0.6435	24.83	0.0814	0.7778	30.5	0.6569	0.4239	18.76	0.1384	0.7140
	Pa	676.8	0.9421	0.9421	717.1	5.551	<u>0.0187</u>	459.2	4.585	<u>0.0328</u>	255.5	0.5537	0.4575
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-

Level Effect		Mean Orange Hue			Mean	Black H	lue	Med	ın Yellow	Hue	Mean Green Hue		
(N	= 26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
Pop	Model	2, 23	1.225	0.3121	2, 23	1.565	0.9346	2, 23	0.7607	0.4787	2, 23	0.5478	0.5856
	Pr	1	2.138	0.1572	1	0.0029	0.2235	1	0.0057	0.9405	1	0.3543	0.5575
	Pa	1	0.0003	0.9875	1	0.9573	0.4072	1	1.2491	0.2753	1).3394	0.5658
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-
Level	Effect	Mean O	range S	aturation	Mean Bl	ack Satu	ration	Mean Y	Yellow Sa	turation	Mean	Green Sc	uturation
(N	= 26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
Pop	Model	2, 23	0.8307	0.4484	2, 23	1.337	0.2824	2, 23	0.2548	0.7772	2, 23	2.077	0.1482
	Pr	1	1.072	0.3113	1	1.038	0.3188	1	0.2904	0.5951	1	0.9292	<u>0.0081</u>
	Pa	1	0.1119	0.7410	1	0.6656	0.4230	1	0.4010	0.5328	1	3.464	0.0755
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-
Level	Effect	Mean O	range B	rightness	Mean Bl	ack Brig	htness	Mean Y	Yellow Br	ightness	Mean	Green Bi	rightness
(N	= 26)	DF	F	Р	DF	F	Р	DF	F	Р	DF	F	Р
Pop	Model	2, 23	0.4848	0.6219	2,23	0.6291	0.5420	2, 23	1.160	0.3313	2, 23	0.622 6	0.5453
	Pr	1	0.6275	0.4364	1	0.1461	0.7058	1	0.8548	0.3648	1	0.0867	0.7711
	Pa	1	0.6968	0.4124	1	0.7070	0.4091	1	2.146	0.1565	1	1.231	0.2788
	Pr*Pa	-	-	-	-	-	-	-	-	-	-	-	-

Table B5 continued:

^a Distribution not normal