

THESIS

GENETIC BACKGROUND AND EXPERIENCE AFFECT COURTSHIP BEHAVIOR IN
MALE TRINIDADIAN GUPPIES (*POECILIA RETICULATA*)

Submitted by
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In partial fulfillment of the requirements
For the Degree of Master of Science
Colorado State University
Fort Collins, Colorado
Spring 2023

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ABSTRACT

GENETIC BACKGROUND AND EXPERIENCE AFFECT COURTSHIP BEHAVIOR IN MALE TRINIDADIAN GUPPIES (*POECILIA RETICULATA*)

An animal's behavior may be shaped by its genetics and life experience, but the extent to which each of these factors contributes to determining behavioral phenotypes is an outstanding question in biology. Mating behaviors are of particular interest due to their importance in determining fitness. We sought to investigate the genetic architecture of mating behaviors and their plasticity in response to mating experience. Trinidadian guppies (*Poecilia reticulata*) occur in streams with either high or low predation rates. This genetic background has shaped the evolution of many behavioral phenotypes, including those involved in male courtship strategy. We observed male guppies from high predation, low predation, and intercross populations in their first encounter with a female, then later repeated the encounter to observe how experience affects mating behaviors. We recorded occurrences of three behaviors – sigmoids, forced copulation attempts, and gonopodial swings – to determine how they are affected by sexual experience and genetic background. We found that the frequencies of sigmoids and gonopodial swings vary depending on genetic background and respond plastically to experience. However, we found no evidence that forced copulation attempt frequency depends on either genetics or experience. Our results also cast doubt on the long-standing hypothesis that low-predation guppies consistently evolve increased sigmoid frequency.

ACKNOWLEDGEMENTS

For this thesis I wish to first thank my parents, who supported me throughout my life as I struggled to choose between my many passions, and who helped me believe that I could find success in every endeavor. I am also deeply grateful for the many friends who I met at CSU. I started this program in 2019, and the past three years have been fraught with disease, political upheaval, and economic uncertainty. From morning climbing sessions to late-night zoom parties, I could not have pushed through these trying times without the tight-knit community that my friends and I fostered here. I especially want to call attention to my writing group mates, Julian Cassano and Leena Vilonen, who gave me the constant reassurance and sage advice necessary to keep me from giving up, and who dragged me across the finish line. And, of course, I must express immense gratitude to my brilliant partner Avery Katz for reminding me to smile, being my emotional support (even while stressing during her law school finals) and believing in me when I couldn't believe in myself.

I thank my advisor, Dr. Kim Hoke, who practiced patience and gave sage counsel as I trudged through an endless sea of statistical analysis and questioned my career path every other week. She is a shining example of mentorship, scholarly integrity, and pedagogy. I also recognize the contributions of my committee, who brought their own perspectives and expertise to the formulation of this thesis.

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GENETICS AND PLASTICITY OF MATING BEHAVIOR IN THE MALE TRINIDADIAN GUPPY

Introduction

An outstanding question in evolutionary biology is the extent to which genes and the environment each contribute to important phenotypic traits like reproductive behavior. The effort to understand the evolution and process of reproductive behaviors requires an investigation of the genetics underpinning them. Nearly all behaviors are at least somewhat heritable (Kendler and Greenspan, 2006). Yet, the genetic architecture of key mating behaviors is not well understood (York, 2018). Some complex behaviors are highly polygenic traits, wherein each allele typically has an additive effect on the resulting phenotype (Trexler, 1990). In these traits, interspecific hybrids frequently show intermediate phenotypes compared to their wild-type parental populations (Trexler, 1990). However, the growing consensus from studies in mice, worms, flies, and humans, is that most behaviors are controlled by a few loci of large effect, along with minor contributions of many loci (York, 2018; Flint and Mackay, 2009). Furthermore, on average, courtship behaviors in particular are controlled by loci of even greater effect than other categories of behavioral traits (York, 2018; Arbuthnott, 2009). For traits like these, hybrid populations often present phenotypes matching one parental line due to one or more dominant alleles. Despite recent advances in our knowledge, the extent to which genetic architecture differs among species, populations, and courtship traits, is not well-understood (York, 2018).

Complementing studies of genetic architecture, understanding the evolution and immediate causes of courtship behaviors requires one to consider the role of phenotypic

plasticity. ‘Plasticity’ refers to the ability of a single genotype to express multiple potential phenotypes depending on the organism’s life experience or environment. This phenomenon is ubiquitous in nature and shapes the evolutionary trajectory of populations (Price et al., 2003). Furthermore, plasticity itself is a trait with a genetic basis, and the plasticity of behavior, in particular, can be important in influencing an organism’s fitness (Ghalambor et al., 2010; Rezucha & Reichard, 2014; Godin, 1995; Endler, 1995). This is especially true in the contexts of courtship and antipredator behavior, which can determine reproductive success and survival. A species’ ability to express plastic behaviors is therefore an important factor in determining its capacity to adjust to varying environmental conditions (Polverino et al., 2019) and persist in the ever-changing world of the Anthropocene. However, the plasticity of traits linked to reproductive success has been insufficiently tested (Polverino et al., 2019). Trinidadian guppies (*Poecilia reticulata*) offer an excellent study system to investigate these pressing questions.

Guppy populations have colonized many river systems across the island of Trinidad. These guppies are capable of ascending waterfalls while their predators, such as the pike cichlid (*Crenicichla alta*), are not. This has led to guppies at high elevations existing almost entirely free from predators, while low elevation guppies live under the constant threat of predation. The partial reproductive isolation of these high and low populations has led to the repeated evolution of a variety of behavioral and physiological traits in response to predation. In high predation areas, selective pressure favored guppies with a shorter lifespan and higher reproductive rate . They evolved to spend most of their time and energy on reproduction (Reznick & Endler, 1982). Meanwhile, low-predation populations evolved to be larger, live longer, and spend less time mating than their high-predation ancestors (Reznick & Endler, 1982).

The Trinidadian guppy is a classic example of parallel evolution, in which partially reproductively isolated populations with a common ancestry independently evolve similar phenotypes in response to the same evolutionary pressures (Alexander et al., 2006; Houde, 1997). Their particular evolutionary history and easily measured phenotypes, replicated in the many streams of their native island, make Trinidad a sort of ‘natural laboratory’ for research in various fields, including genetics and evolution. The guppy is also a species well suited to the study of plasticity. They exhibit unusually high behavioral, morphological, and physiological plasticity in traits such as size (Gosline & Rodd, 2008), predator avoidance (Houde, 1997), salinity tolerance (Chervinsky, 1984), and head shape (Torres-Dowdall et al., 2012), to name a few. Most pertinent to this paper, males of this species have been shown to alter their courtship strategies as they gain mating experience, dependent upon their social history (Guevara-Fiore et al., 2012).

One of the distinctive features of male guppies is their exceptionally high rate of courtship. It is estimated that an adult male guppy spends at least half of his time attempting to copulate with females (Magurran and Seghers, 1994). This high reproductive drive coupled with high female fecundity, makes guppies a convenient model organism both in and out of the laboratory. Furthermore, it is relatively easy to quantify such behavior due to their distinctive courtship performances and alternative courtship tactics (Houde, 1997).

Male guppies may choose to employ two different mating tactics, each with associated costs and benefits. The first of these is known as a “sigmoid” display, in which the male attempts to entice the female to allow copulation. In these mating displays, the male flexes his body into an “S” shape and vibrates rapidly (Houde, 1997). In longer sigmoid displays the male may

vibrate more slowly and move his body up and down and will sometimes open the caudal fin to display his colors (Houde, 1997). Female guppies are choosy, so courtship success largely depends on female receptivity (Houde, 1988; Kodric-Brown, 1993).

To circumvent the daunting challenge of successfully courting a female, males may instead forgo sigmoid displays in favor of a forced (or ‘sneaky’) copulation. The male attempts to insert the gonopodium into the female’s genital opening and inseminate the female before she can escape (Houde, 1997). He accomplishes this by lunging with his gonopodium fully extended and twisting his body to access the female’s underside. This is the fastest and least conspicuous strategy, but on average results in fewer successful copulations per attempt compared to the sigmoid display (Godin, 1995; Liley, 1966; Farr, 1980a; Houde, 1988; Kodric-Brown, 1993).

The decision to display to females or employ a sneaky forced copulation approach is influenced by a number of factors, most notably the threat of predation. In high-predation environments a conspicuous sigmoid display may be attractive to a prospective mate, but it also incurs the risk of attracting a predator (Houde, 1997). This means that guppies experience a fitness trade-off between reproductive success and mortality. As a result, high-predation populations have repeatedly been shown to perform fewer sigmoids than their low-predation cousins due to the different selective pressures (Houde, 1997).

The complexity of guppy mating extends beyond the decision to sigmoid or attempt forced copulation. Male guppies also periodically perform a behavior known as a ‘gonopodial swing’, in which the gonopodium is briefly rotated forward from its resting position, usually for less than one second (Rodd & Sokolowski, 1995). Although researchers have not determined the exact function of swings in mating, it has been suggested that they may serve to load the gonopodium with sperm in preparation for copulation (Rodd & Sokolowski, 1995).

Using guppies from high- and low-predation sites in the Quare River, we set out to investigate the genetics and plasticity of courtship behavior. In this study, our first aim was to identify whether the plasticity of mating behavior depends on genetic background. Next, we sought to determine whether genetic line affects courtship behavior. Lastly, we aimed to identify differences in behavior between a guppy's first and second courtship encounters to illuminate behavioral phenotypic plasticity in response to experience.

We expected to find that male guppies change the frequency of mating behaviors after their first encounter with a female, indicating plasticity. We also hypothesized that male guppies alter their behaviors in different directions or to different degrees between encounters, depending on their genetic line. Finally, we hypothesized that the frequency of sigmoids, gonopodial swings, and forced copulation attempts are controlled by few genes of large effect, and therefore predicted that intercross guppies possess phenotypes matching one of their parental lines.

Methods

Guppy collection and breeding

In 2016, wild guppies were captured from two sites in the Quare River drainage in Trinidad, called Quare (QH, high predation, [10.600,-61.100]) and Campo (CM, low predation, [10.683,-61.200]), then transported to Colorado State University. This first wild-type generation consisted of 24 high-predation (QH) families and 28 low-predation (CM) families. In the laboratory, wild-caught females were kept in individual tanks, where offspring were later collected.

Each population was bred separately for two generations to reduce maternal effects from wild guppies. From 6 to 12 weeks of age, guppies were raised in sibling groups separated by sex.

At 12 weeks, we randomly paired twenty females from each population with a male from the other population for 24 hours, then separated them. Twenty additional females were paired with a male from a different family in their population. To avoid inbreeding or sexual selection, males and females from different families were randomly selected to produce second-generation families. This breeding process generated wild-type families for both high- and low-predation populations, as well as families with a high-predation mother/low-predation father (CQ) and families with a low-predation mother/high-predation father (QC) (Figure 1). At maturity, we randomly paired intercross offspring from this F1 generation with one of their siblings to maintain between-family genetic variance. In same-population (wild-type) families, we paired unrelated individuals. We repeated this breeding design for two more generations to create F2 and F3 generations. The F3 generations were used in our assays.

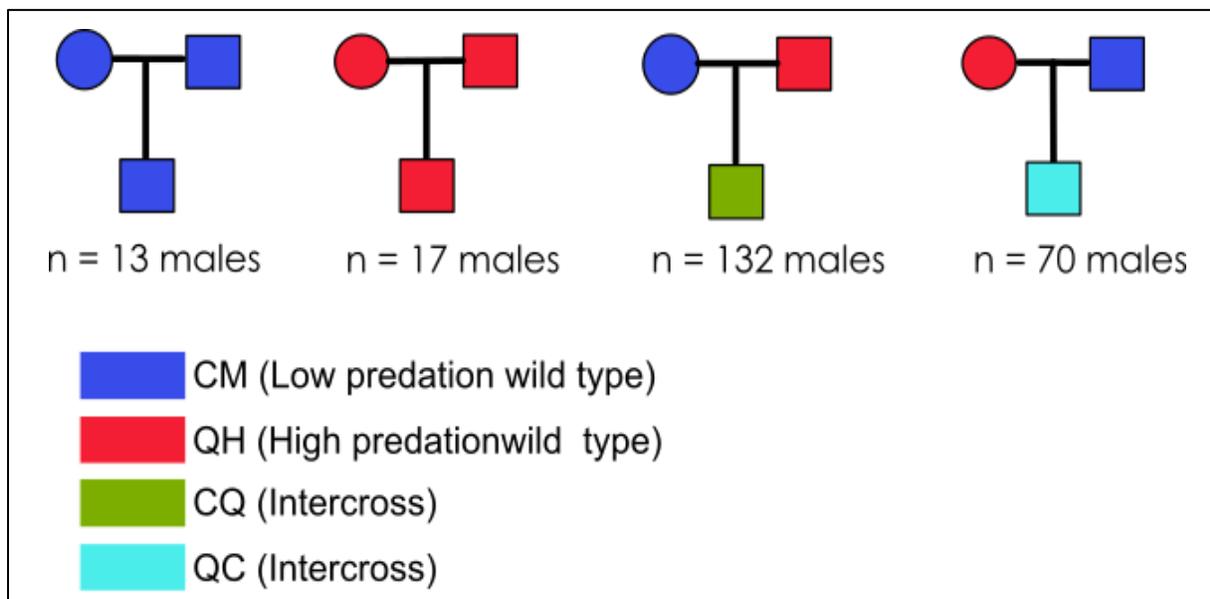


Figure 1. Breeding design for the creation of four genetic lines. Circles represent females, squares represent males. Each genetic line was raised for three generation, and the F3 generation was used in all assays. “N” represents sample size of F3 male guppies used in assays.

Husbandry protocols

Guppies were kept individually in recirculating 1.5-liter acrylic tanks at an average temperature of 25 degrees Celsius and on a 12:12-h light cycle (7:00 am to 7:00 pm). Between 8:00 am and 10:00 am daily, they were alternately fed either Tetramin™ tropical fish flake paste or hatched Artemia cysts. As fish grew, food levels were adjusted weekly based on the age and size of fish (Reznick and Endler, 1982). All experiments and husbandry procedures adhered to the regulations of Colorado State University IACUC #16-6540AA.

Courtship assays and behavior coding

When F3 males reached sexual maturity, we selected virgin males from each genetic line (CM n = 13, CQ n = 132, QC n = 70, QH n = 17) and introduced them to a 2.5-gallon tank with a virgin female from a different family in the same population. Each tank (Dimensions 30.50 x 15.25 x 20.30 cm) contained a gravel bottom and had a 5x2 grid drawn on the front. We allowed the males two minutes to acclimate to their new surroundings before introducing the female, then recorded the first 10 minutes of their encounter with a Casio EX-ZR1100 camera (Casio Computer Company). We left each pair together for one hour to ensure copulation, then separated the two fish. We monitored the females to make sure that they produced offspring, only keeping data from successful encounters. After a 24-hour rest, we repeated the experiment for each male, using a new virgin female. Trials were conducted between 6:00 AM and 9:00 PM.

Three observers recorded all instances of courtship behaviors during the first 10 minutes of each encounter using Jwatcher (<http://www.jwatcher.ucla.edu>). The family and population of each fish were not disclosed to the observers, and each video was observed by only one person.

We recorded the number of sigmoid displays, forced copulation attempts, and gonopodial swings performed during the 10-minute observation period.

We considered the focal male to exhibit a sigmoid when he curved his body into an “S” shape, positioned himself in front of or beside the female, and rapidly vibrated his body with lateral muscle contractions (Liley, 1966; Baerends et al., 1955). A forced copulation attempt (also known as a gonopodial thrust) is an alternative mating tactic, in which the male will instead try to inseminate the female before she can escape by approaching her from behind and lunging with a fully extended gonopodium.). We chose to observe all forced copulation attempts, not only successful forced copulations, because our study concerns courtship strategy decision-making rather than the effectiveness of each mating tactic. Gonopodial swings were characterized by a brief extension of the gonopodium to approximately a 90° angle relative to the body, without a forced copulation attempt. We selected gonopodial swings as an indicator of overall interest in a female, as they have been found to covary with a number of other courtship behaviors and female receptivity (Guevara-Fiore et al., 2010; Fischer et al., 2016; Polverino et al., 2019).

Analysis

We used a generalized linear mixed model approach to investigate the extent to which genetic line and sexual experience contribute to determining the frequency of sigmoids, forced copulation attempts, and gonopodial swings. Considering our high number of zeroes for all three response variables and the overdispersion in non-zero data, and by using metrics of model fit compared to Poisson and normal distributions, we decided that a zero-inflated negative binomial distribution was most appropriate. Specifically, we selected our distribution based on Q-Q plots

and the Akaike Information Criterion. We created all models using the glmmTMB (Brooks et al., 2017) package in Rstudio (R Core Team v. 4.1.2).

We ran the model for each behavior using genetic line and experience as our fixed effects, with an interaction between the two. We included fish ID nested within family as a random effect. At the outset, we included body length as a covariate but found that it obscured population differences because of disparities in each genetic line’s mean length. We also tested a model with the time of day of each assay as a covariate but found an extremely low effect size. We therefore omitted both time and body length from the final model. AIC rankings of all models are shown in Table 1.

Table 1. Generalized linear mixed models with zero-inflated negative binomial distribution for each behavior, ranked by AIC. “Y” represents the behavior as a response variable. “Experience” is a factor with two levels for the first and second encounters. “Line” is the genetic line (CM, QH, CQ, QC). “ID” is the individual guppy, nested within the family of origin. “Length” is body length. “Time” is the time of day when the assay was performed. The CM population is not included in the Sigmoid models.

Behavior	Model formula	AIC	Rank
Sigmoids	Y ~ Experience + Line + (1 Family/ID)	814.4	1
	Y ~ Experience * Line + (1 Family/ID)	816.2	3
	Y ~ Experience * Line + Length + (1 Family/ID)	818.1	4
	Y ~ Experience * Line + Length + Time + (1 Family/ID)	814.9	2
Forced Copulation Attempts	Y ~ Experience + Line + (1 Family/ID)	1222.6	2
	Y ~ Experience * Line + (1 Family/ID)	1227.1	3
	Y ~ Experience * Line + Length + (1 Family/ID)	1217.7	1
	Y ~ Experience * Line + Length + Time + (1 Family/ID)	1229.7	4
Gonopodial Swings	Y ~ Experience + Line + (1 Family/ID)	2604.2	2
	Y ~ Experience * Line + (1 Family/ID)	2608.5	3
	Y ~ Experience * Line + Length + (1 Family/ID)	2609.3	4
	Y ~ Experience * Line + Length + Time + (1 Family/ID)	2599.0	1

We were unable to include fish from the Low Predation (Campo, CM) genetic line in our sigmoid model because they performed no sigmoids during any trial, so the model was unable to converge. To solve this, we removed CM from the linear model. To test whether the four lines were different from each other, we used a Kruskal-Wallis rank-sum test (R Core Team, 2021).

We followed this with a Conover-Iman test of multiple comparisons using rank sums (Dinno, 2017) for pairwise comparisons among lines.

Results

Most males had little to no apparent interest in the female during our observation periods and spent much of their time distracted by their reflection in the tank glass. We also observed very low mean counts for all three behaviors. We found that the low-predation wild-type line CM performed no forced copulation attempts in the first courtship encounter, and no sigmoids in either encounter. The estimated marginal mean number of forced copulations and sigmoids in a 10-minute encounter never exceeded 1.5 for any genetic line, and gonopodium swings never exceeded 10. Despite the low number of mating behaviors, statistical analysis did reveal differences between genetic lines and between encounters.

Table 2. Mixed effects model Chi-square test of sigmoids, forced copulation attempts, and gonopodium swings as a function of genetic line, experience, and a two-way interaction. We made a reduced model for each behavior by removing the interaction term after finding no statistical evidence for interaction. Green indicates $p < 0.05$. Yellow indicates $p < 0.1$.

	Genetic Line	Experience	Genetic Line*Experience
Sigmoids	$X^2_3 = 0.57, p = 0.75$	$X^2_1 = 4.26, p = 0.04$	$X^2_3 = 2.05, p = 0.36$
Reduced Model	$X^2_3 = 0.49, p = 0.78$	$X^2_1 = 5.86, p = 0.02$	--
Forced Copulation Attempts	$X^2_3 = 3.59, p = 0.31$	$X^2_1 = 1.57 e^{-5}, p = 0.99$	$X^2_3 = 3.65, p = 0.30$
Reduced Model	$X^2_3 = 5.52, p = 0.14$	$X^2_1 = 0.63, p = 0.43$	--
Gonopodial Swings	$X^2_3 = 7.10, p = 0.07$	$X^2_1 = 1.86, p = 0.17$	$X^2_3 = 1.71, p = 0.63$
Reduced Model	$X^2_3 = 9.21, p = 0.03$	$X^2_1 = 5.13, p = 0.02$	--

Gonopodial Swings

Because analyzing the full model provided no evidence for an interaction between genetic line and experience ($X^2_3 = 1.71, p = 0.63$), we moved on to examine the main effects by dropping the interaction, creating a more parsimonious model (Table 1, Table 2). Models lacking the interaction term provided moderately strong evidence that genetic line affected gonopodial swings (reduced model $X^2_3 = 9.21, p = 0.03$). We found that the two intercross lines performed

more gonopodial swings than the low-predation CM guppies (Table 2, Figure 2). However, we did not find evidence for a difference between CM and the high-predation QH wild-type guppies. Our reduced model also supplied evidence that courtship experience affected the number of gonopodial swings ($X^2_1 = 5.13$, $p = 0.02$; Table 2, Figure 2).

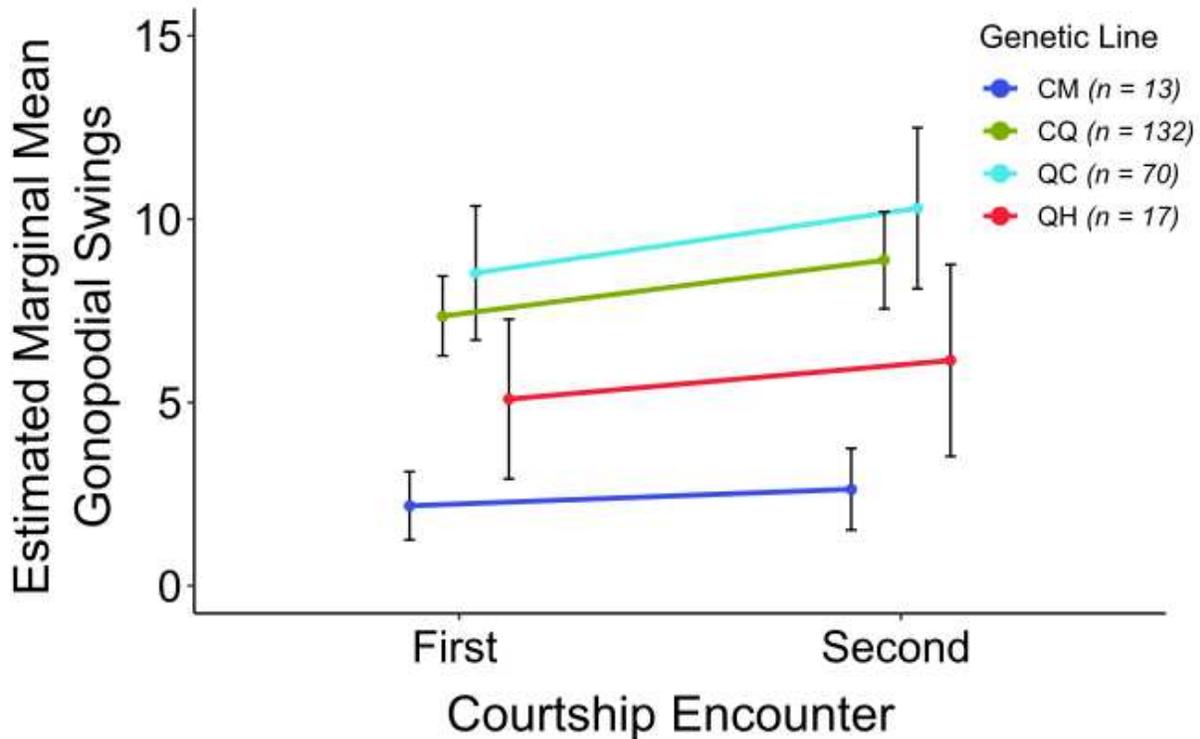


Figure 2. Genetic line and courtship experience affect the number of gonopodial swings performed. Points represent the estimated marginal mean number of gonopodial swings in a 10-minute courtship encounter with error bars showing standard error. Lines show the change between the first and second encounters. CM (Campo) and QH (Quare) are wild-type guppies from low-predation and high-predation sites, respectively. The CQ (Campo-Quare) line was bred from a CM mother and QH father, while the QC (Quare-Campo) line was bred from a QH mother and CM father.

Forced Copulation Attempts

We found no evidence that genetic line ($X^2_3 = 3.59$, $p = 0.31$), courtship experience ($X^2_1 = 1.57 \times 10^{-5}$, $p = 0.99$), or their interaction ($X^2_3 = 3.65$, $p = 0.30$) affect the number of forced copulation attempts performed in a 10-minute courtship period (Table 2, Figure 3). This held true

even after dropping the interaction term (genetic line $X^2_3 = 5.52$, $p = 0.1$; experience $X^2_1 = 0.63$, $p = 0.43$).

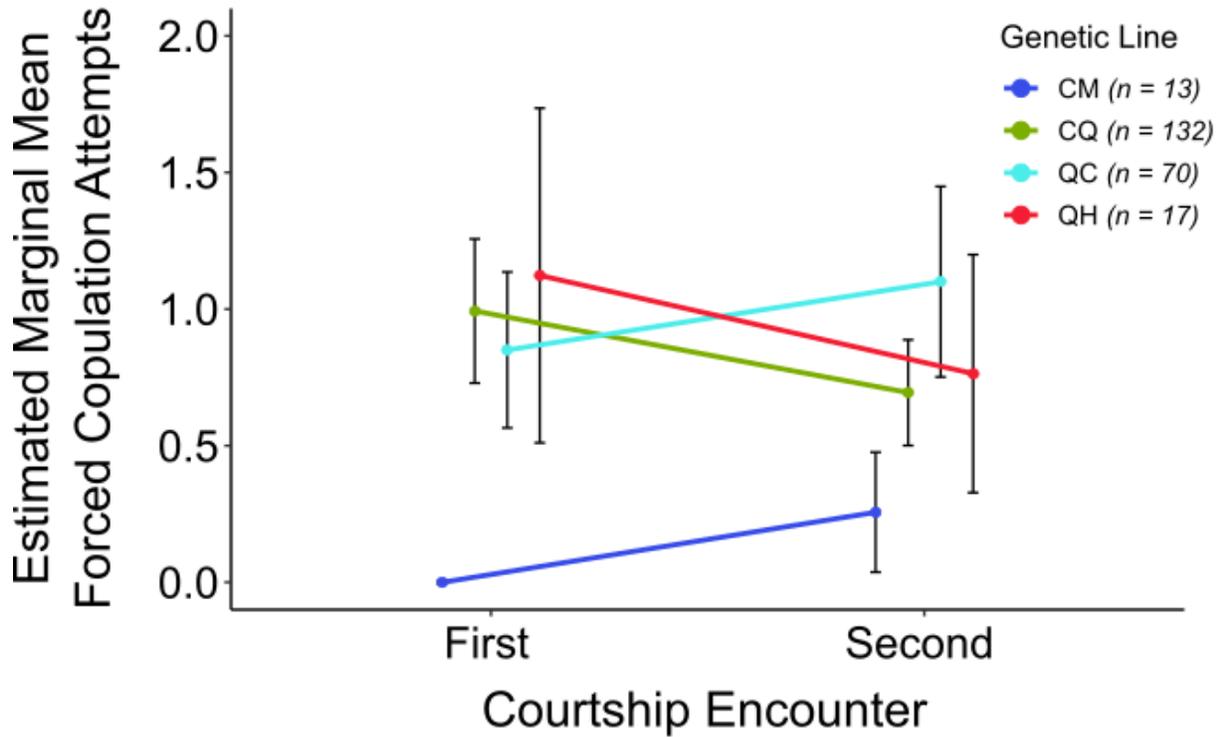


Figure 3. Genetic line and courtship experience do not affect the number of forced copulation attempts performed. Points represent the estimated marginal mean number of forced copulations attempted in a 10-minute courtship encounter with error bars showing standard error. Lines show the change between the first and second encounters. Genetic line acronyms same as Figure 2.

Sigmoids

We were unable to include CM guppies in our linear mixed model because they performed no sigmoids in either encounter (Figure 4). In the remaining three genetic lines, we found that courtship experience affected the number of sigmoids performed in a 10-minute courtship encounter (full model $X^2_1 = 4.26$, $p = 0.04$; reduced model $X^2_1 = 5.86$, $p = 0.02$) (Table 2). Our estimated marginal means contrasts indicated that guppies from QH, CQ, and QC performed 0.57 fewer sigmoids ($Z = 2.06$, $p = 0.04$) on average in the second encounter compared to the first. A Kruskal-Wallis rank-sum test found only weak evidence of population

differences ($X^2_3 = 7.77$, $p = 0.051$). To determine which genetic lines accounted for the marginal evidence of population-level differences, we used a Conover-Iman test of multiple comparisons using rank sums. Using this method, we found that CM was lower than all three other lines, which were not different from each other (Table 3). Our linear model did not find that the effect of experience differed between genetic lines (full model $X^2_3 = 2.05$, $p = 0.36$).

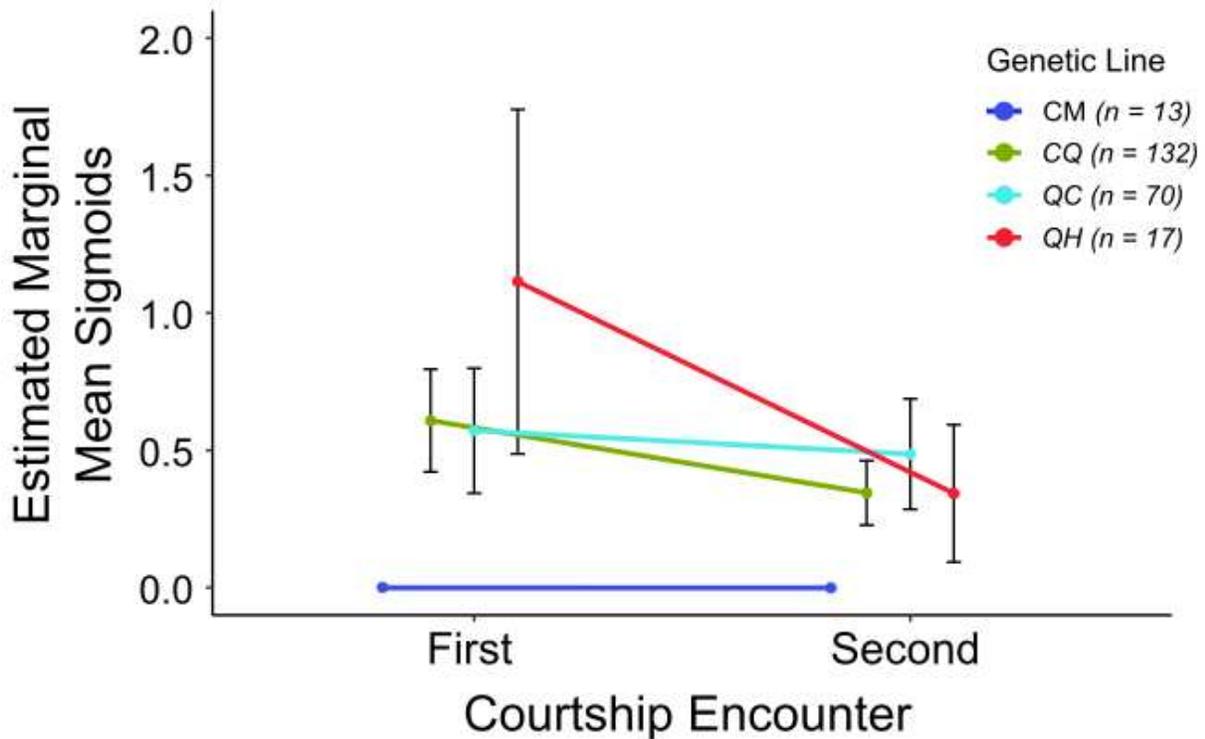


Figure 4. Courtship experience affects the number of sigmoids performed. Points represent the estimated marginal mean number of sigmoids in a 10-minute courtship encounter with error bars showing standard error. Lines show the change between the first and second encounters. Genetic line acronyms same as in Figure 2.

Table 3. Conover-Iman test of multiple comparisons using rank sums, comparing mean sigmoids between each genetic line. “Difference” represents the column mean minus the row mean. Green highlights indicate $p < 0.05$.

	CM	CQ	QC
CQ <i>Difference</i>	-2.54	--	--
<i>p-value</i>	0.006	--	--
QC <i>Difference</i>	-2.51	-0.14	--
<i>p-value</i>	0.0062	0.445	--
QH <i>Difference</i>	-2.62	-0.87	-0.75
<i>p-value</i>	0.005	0.194	0.227

Discussion

Population-level differences in genetically controlled traits have been observed in many guppy behaviors (Houde, 1997; Endler, 1980). We hypothesized that plasticity in courtship behavior is genetically controlled as well. We predicted that male guppies alter their behaviors in different directions or to different degrees between encounters depending on their genetic line. Although we cannot rule out the possibility that a larger sample size could have allowed us to detect a difference between lines in the magnitude or direction of experience effects, with the data available our statistical analysis ultimately provided no evidence of genetic differences in plasticity. It is also possible that the male guppies’ distraction with their reflection masked such effects.

Numerous studies have established mating behavior differences between low- and high-predation guppies (Houde, 1997). Low-predation guppies employ different courtship strategies than their high-predation counterparts (Magurran and Seghers, 1994). They usually show less courtship activity overall but perform more sigmoids because they do not experience the predation-related drawbacks of such flashy displays (Godin, 1995). We therefore predicted that in concordance with existing literature, our CM guppies would perform the most sigmoids, but show lower counts of forced copulation attempts and gonopodium swings. As expected, they did

perform the fewest gonopodium swings. However, we found no differences in forced copulations between the genetic lines. Most surprisingly, we saw that the low predation CM guppies not only did not perform more sigmoids than the other lines, but in fact performed the fewest. The Kruskal-Wallis rank-sum test showed marginal statistical evidence of an overall difference between genetic lines ($p = 0.051$), and the Conover-Iman test showed that CM was lower than the other three lines ($p < 0.05$). However, we must interpret this difference cautiously due to our limited sample size, low overall sigmoid counts, and inability to include CM in the linear models. Our findings challenge existing guppy literature and call into question the assumption that low predation pressure causes the evolution of greater sigmoid frequency.

We recommend further investigation to determine whether our results for these wild populations are truly representative of the behavior of the guppies in this river. If so, it would indicate that guppies in the Quare river are different from others in Trinidad, challenging the standing assumption that parallel evolution in these three behaviors is consistent across the island. This would raise questions about what unique factors in the Quare river system or its ancestral population of guppies resulted in a different evolutionary trajectory.

Plasticity in response to experience has been observed in many taxa (Bhat et al., 2015; Hofmann, 2003) and in many behavioral traits, including those involved in mating (Godin, 1995). We expected male guppies would change the frequency of mating behaviors after their first encounter with a female. Prior studies in guppies have found that sigmoids are more effective per attempt compared to forced copulations (Godin, 1995; Liley, 1966; Farr, 1980b; Houde, 1988; Kodric-Brown, 1993). We therefore predicted that males would have greater

success with sigmoids in their first encounter, and therefore attempt fewer forced copulations and more sigmoids in the second encounter.

Our results provided strong evidence of plasticity in sigmoid displays, and moderate evidence of plasticity in gonopodium swings, indicating that males do adjust their courtship strategy. However, the patterns we observed did not line up with our predictions. We found no difference in forced copulations between genetic lines, and our guppies performed fewer sigmoids in the second encounter. We cannot determine whether this adjustment is based on effectiveness or some other factor; nor can we say whether any changes in their behavior improve their future mating success. Studies in multiple species have found evidence that an organism's prior courtship experience does affect mating strategy and success. While some experiments point to improved mating success (Saleem et al., 2014; Milonas et al., 2011; Perez-Staples et al., 2010), others disagree (Iglesias-Carrasco et al., 2019). In mosquitofish (*Gambusia holbrooki*), for example, an organism closely related to guppies, mating experience does not improve a male's ability to copulate (Iglesias-Carrasco et al., 2019). Yet, studies in other organisms found courtship plasticity to be effective (Saleem et al., 2014; Milonas et al., 2011; Perez-Staples et al., 2010). Regardless of whether our guppies became more successful in their second mating encounter, our study supports existing literature showing that guppy mating behavior responds plastically to experience. While their forced copulation efforts are fixed, they adjust the frequency of sigmoids and gonopodial swings after their first encounter with a female.

We hypothesized that the frequency of sigmoids, gonopodial swings, and forced copulation attempts are controlled by a few genes of large effect. Therefore, we predicted, intercross guppies would possess phenotypes matching one of their ancestral populations. Based

on our models, we can state that intercross guppies showed no difference in forced copulation attempts compared to CM and QH. However, they were higher than CM and matched QH in gonopodium swings. The Kruskal-Wallis and Conover-Iman tests showed that the same was true for sigmoids. Our results provide suggestive evidence that gonopodium swings and sigmoids may be controlled genes that are dominant in the QH population. Yet, further research will be necessary to confidently determine the number and location of genes that determine the frequency of these traits.

In general, guppies from all four genetic lines performed abnormally few courtship behaviors compared to existing literature (Fischer et al., 2016; Godin, 1995; Farr, 1980; Farr, 1975a). This is an especially surprising observation in our low predation guppies, which were expected to show greater sigmoid activity than the high-predation population (Magurran and Seghers, 1994; Houde, 1997). One possible explanation for the low courtship activity we observed is that the overhead lights used in the assays were too bright. Guppy courtship behavior is known to vary with ambient light intensity (Chapman et al., 2009; Godin, 1995; Endler, 1987; Reynolds, 1993; Reynolds et al., 1993). In some studies, high light intensity caused males to court less and use less conspicuous behaviors (Archard et al., 2009; Endler, 1987), while others have found the opposite effect (Chapman et al., 2009). Although the effect of light intensity is debated, if our guppies were in fact performing fewer behaviors due to lighting this may have obscured any effect of genetic line or experience. Future laboratory studies would do well to consider the natural lighting conditions of their guppies' ancestral wild populations.

The standard period of observation varies greatly among guppy courtship studies. Some studies report that male guppies took as long as 30 minutes to display full courtship behavior

(Barbosa et al., 2013). It is important to consider that we left males together with each female for a full 24 hours to ensure successful copulation, but we do not know at what point copulation occurred. Only 10 (2.4%) of our 421 recordings contained an insemination, but all fish mated within 24 hours as determined by later offspring production; so, 97.6% of mating pairs only successfully copulated after the first 10 minutes. Our guppies may have significantly changed their behavior later in their encounters, outside the scope of our observation. It is possible that behavioral differences among populations or experiential effects are hidden due to courtship latency. For example, CM guppies may have performed more behaviors than the other genetic lines, as hypothesized, but only started courting later. Future studies should consider giving their guppies greater acclimation time or recording a longer courtship period.

One logical follow-up to this experiment would be to validate our results with fish from previously studied rivers using the same methods but a larger sample size for the wild-type populations. If such an experiment found different results from those usually observed in guppies from that river, it would suggest that the methods used in our experiment interfered with the guppies' normal mating behavior. Such a result would be informative in the design of future studies in the guppy system.

It is also worth considering that male guppies may change their behaviors differently after further courtship experiences. Our fish did not change their forced copulation attempts in their second encounter with a female, but they may simply need more experience to alter their behavior. Conversely, while we observed a change in sigmoid behavior and gonopodium swings, these behaviors may have returned to normal or even increased further in subsequent assays. Future studies of mating behavior plasticity would benefit from observing three or more sexual encounters.

Lastly, it is still unclear whether male guppies change their mating behaviors differently depending on which mating tactics were most effective in prior encounters, and whether their courtship strategy is more effective in later encounters. This question may be addressed in future studies by observing which courtship behaviors immediately precede successful copulation in the first and second encounters.

Conclusions

By comparing the behavior of male guppies in their first and second encounters with a female, we found that they exhibit plasticity in the frequency of sigmoids and gonopodial swings, but not forced copulations. Forced copulation attempt frequency appears to be fixed, but sigmoid and gonopodial swing frequencies may be adjusted after gaining courtship experience. We did not find evidence of genetic variance underlying the plasticity of mating behavior. However, our results revealed surprising behavioral differences between genetic lines. Contrary to previous studies, we found that low-predation guppies performed fewer sigmoids than their ancestral high-predation population. This casts doubt on the long-standing hypothesis that sigmoids consistently evolve to increase in the absence of predation pressure. We also found that intercross guppies matched the gonopodial swing and sigmoid phenotypes of the QH genetic line, suggesting that these behaviors may be controlled by loci that are dominant in the high-predation population.

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