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Inter- and Intra-individual Variation in Predator-related Behavioral Plasticity Expressed by Female Green Swordtails (*Xiphophorus hellerii*)

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INTER- AND INTRA-INDIVIDUAL VARIATION IN PREDATOR-RELATED BEHAVIORAL PLASTICITY EXPRESSED BY FEMALE GREEN SWORDTAILS (*XIPHOPHORUS HELLERII*)

by

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INTER- AND INTRA-INDIVIDUAL VARIATION IN PREDATOR-RELATED BEHAVIORAL PLASTICITY EXPRESSED BY FEMALE GREEN SWORDTAILS (*XIPHOPHORUS HELLERII*)

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Phenotypic plasticity is the ability of one genotype to express multiple phenotypes under variable environments. Behavioral plasticity is a type of phenotypic plasticity in which individuals adjust behavior in response to changes in environment. Often, behavioral plasticity is studied at the level of the population, rather than at the level of the individual. Further, few studies have considered the effect of individual traits, such as size and age, on the expression of behavioral plasticity, or, how individual plasticity may be correlated across different contexts. In this study, we used female green swordtails (*Xiphophorus hellerii*) to test the effects of body size at testing and age at maturation on the expression of predator-related behavioral plasticity in two social contexts: (1) intrasexual (two females) and (2) intersexual (two females and a male). We also tested the extent to which plasticity is correlated across contexts within individuals, to elucidate whether females that show a high degree of plasticity in one context also show a high degree of plasticity in another context. For two agonistic behaviors, we found differing effects of the interaction of body size at testing and age at maturation on the expression of predator-related plasticity across social contexts. These results suggest that individual traits interact in a complex manner to affect patterns of plasticity across contexts, and, that alleles influencing age at maturation may be associated with alleles that affect plasticity. Across contexts, we found no evidence of correlated plasticity in a given behavior, suggesting that individuals are not consistently plastic across the contexts tested. Within contexts, however, the plasticity expression for several different behaviors was correlated, and was similarly affected by body size at testing and age at maturation. Overall, this study indicates that the type and degree of individual predator-related behavioral plasticity expressed is partially dependent on multiple traits, and varies substantially between social contexts. Few studies have examined the effect of multiple factors on the expression of plasticity, or plasticity in multiple social contexts, and, our results indicate that the causes and consequences of phenotypic plasticity are likely to be complex.

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CHAPTER 1: Inter-individual variation in predator-related behavioral plasticity expressed by female green swordtails (*Xiphophorus hellerii*)

ABSTRACT

Phenotypic plasticity is the ability of a genotype to express different phenotypes, under different environmental conditions. Behavioral plasticity is a type of phenotypic plasticity in which a change in phenotype can rapidly occur. Though often studied at the level of the population, behavioral plasticity studies at the level of the individual could provide insight into how plasticity evolves and is maintained in a population. Further, individual differences in phenotypes (such as body size) may predict differences in the degree of plasticity expressed by individuals within a population. We used female green swordtails (*Xiphophorus hellerii*) to test the effects of body size at testing and age at maturation on the expression of predator-related behavioral plasticity in an intrasexual social context (*i.e.*, two females). We found that all females, regardless of body size at testing or age at maturation, expressed predator-related plasticity in spatial positioning. Further, later-maturing females darted more from the monitor displaying a film stimulus than earlier-maturing females, whether or not the filmed sequence included a predator. Finally, both body size at testing and age at maturation affected the degree of predator-related plasticity in the expression of an agonistic behavior: transverse display. In the presence of a predator, small, early-maturing females decreased the time spent transverse displaying, whereas small, late-maturing females increased the time spent transverse displaying. Large females expressed the opposite pattern. In the presence of a predator, large, early-maturing females increased the time spent transverse displaying, whereas large, late-maturing females decreased the time spent transverse displaying. This study shows that both body size at testing and age at maturation affect plasticity in at least one behavior in female green swordtails. In general, our results suggest that the degree and direction of plasticity expressed by an individual may be affected by complex interactions of its other traits . Thus, simple predictions concerning the effects of environment on plasticity expression may be misleading, and future studies should consider multiple factors when investigating individual differences in behavioral plasticity.

INTRODUCTION

The ability of one genotype to express different phenotypes under variable environmental conditions is referred to as phenotypic plasticity (West-Eberhard 1989; DeWitt *et al*. 1998). The evolution of such flexibility in fitness-related traits can provide individuals with the means to adaptively respond to environmental changes that occur within their lifetime. Behavioral plasticity is a type of phenotypic plasticity that allows organisms to make rapid behavioral responses to changes in their environment (Komers 1997; Pigliucci 2001).

Plasticity in behavior has been studied predominately at the level of the population (Sih and Bell 2008). Such studies have been accomplished by documenting how populations respond to natural or experimental changes in their environment, *e.g.,* timing of breeding in response to climate change (Nussey *et al*. 2007). Less well-studied is how variation in traits at the level of the individual, such as body size and age, affect individual differences in the degree of plasticity expressed, *e.g.,* timing of breeding by young versus old females in response to climate change. This is particularly true outside the context of mate choice (Stamps 2015; Ah-King and Gowaty 2016). A focus on how differences in individual traits may influence the expression of behavioral plasticity merits further attention because, although population-wide phenotypic plasticity may be favored in a fluctuating environment, the optimal response of different individuals within a population may vary (Wolf *et al*. 2008).

There is ample evidence that prey species adjust their behavior in response to the presence of a predator—*i.e.*, they exhibit *predator-related plasticity* in behavior. For example, one study found that female Trinidadian guppies (*Poecilia reticulata*) from a high-predation environment (Quaré River) preferred brightly colored males, but reduced this preference in the presence of a predator. Females from a low-predation environment (Paria River) also preferred brightly colored males; however, they did not reduce their response in the presence of a predator (Godin and Briggs 1996). Yet, in a different study, females from the same low-predation environment (Paria River) reduced their preference for a brightly colored male in the presence of a predator, and in some cases switched their preference for a brightly colored male to one that was dull (Gong and Gibson 1996). Such differing results may arise because of differences in individual traits, such as body size or age that may affect the expression of plasticity in mate choice. For example, when female Atlantic mollies (*Poecilia mexicana*) that differed in body size were tested for preference for male size in the presence and absence of a predator, both small and large females preferred large males in the absence of a predator. However, in the presence of the predator, large females reduced their preference for large males. This study suggests that large female Atlantic mollies are behaviorally plastic, but small females are not (Bierbach *et al*. 2011). A better understanding of how different traits affect inter-individual variation in behavioral plasticity could provide insight into the evolutionary trajectory of phenotypic plasticity within a population, as well as how such plasticity can be maintained (Pigliucci 2005; Nussey *et al*. 2007; Dingemanse *et al*. 2010).

Differences in body size among individuals within a population regularly predict differences in their behaviors (Peters 1986). In taxa with determinate growth, an individual's body size (often measured as length), but not necessarily mass, is frequently fixed as an adult, and may affect a variety of behaviors related to fitness (Sebens 1987; Brown *et al*. 1993; Barnett *et al*. 2015). This is the case in bluegill sunfish (*Lepomis macrochirus*), in which mating strategies are contingent upon the size of an individual; small males are consistently 'sneakers' that surreptitiously obtain copulations, whereas large males perform often-elaborate courtship displays (Gross 1991). Plasticity in anti-predator behavior is expressed to different degrees by small and large three-spined sticklebacks (*Gasterosteus aculeatus*). After exposure to a predator, small sticklebacks take less time to emerge from a refuge than large sticklebacks. A possible reason for this difference is that small individuals may have greater metabolic needs than large individuals. If so, small individuals must forage more often than large individuals, despite the predation risk associated with such activity (Krause *et al*. 1998).

Another trait that often affects the expression of behavioral plasticity is age. For example, young female field crickets (*Gryllus lineaticeps*) are plastic in mate preference in response to environments differing in male density, whereas older crickets are not (Atwell and Wagner 2014). The effects of age at maturation *per se* on behavioral plasticity are less well known. Age at maturation and current age may have differing effects on plasticity, because age at maturation is a part of life history strategy (Flatt and Heyland 2011), whereas age is a life stage. Only some individuals share the same life history strategies, including age at maturation, however, many more individuals will progress through the same ages. The effect of age at maturation *per se* on behavioral plasticity compared to age may be different, especially when considering other individual traits associated with age at maturity.

Members of the genus *Xiphophorus* (family Poeciliidae), swordtails and platyfish, are ideally suited to test the effect of individual traits on the expression of behavioral plasticity for several reasons. First, behaviors exhibited by members of this monophyletic group of live-bearing fresh-water fishes have been studied extensively in the laboratory as well as in the field (Rauchenberger *et al*. 1990; Dugatkin 2001; Kazianis and Walter 2002). Second, heritable determination of body size and age at maturation is well studied in this genus, and is controlled by a sex-linked genetic polymorphism at the pituitary locus (P-locus; Kallman and Borkoski 1978; Kallman 1989). Although first discovered in southern platyfish (*X. maculatus)*, it seems that some swordtail species have a P-allele-like system as well (Kallman 1983; Zimmerer and Kallman 1988; Kallman 1989). For example, correlations between size and age at maturity in the green swordtail *X. hellerii* (also known as *X. helleri*; Froese and Pauly 2016) appear to be reflective of a P-allele-like system, and, body size in males appears to be heritable (Kallman 1989; Campton 1992). For species in which the P-locus has been identified, different genotypes for early or later maturation produce fish of varying maturation ages and sizes. At sexual maturation, early-maturers are smaller, whereas late-maturers are larger (Kallman *et al*. 1973; Schreibman *et al*. 1973; Basolo 2008). Finally, while P-alleles are known to only affect age and size at sexual maturation in platyfish, P-alleles have also been identified to be associated with behavior in swordtails (Schreibman *et al*. 1973; Borowsky 1987; Ryan and Causey 1989; Zimmerer and Kallman 1989).

In one species of swordtail, *X. multilineatus* (formerly *X. nigrensis*; Rauchenberger *et al*. 1990), alleles that determine male size and age at maturity are associated with the type of mating behavior exhibited. Genetically large males (metallic blue in color) regularly court females, regardless of social context (*i.e.*, presence of other males). Genetically small males (metallic blue or yellow in color), however, are plastic—they can act as 'sneakers' or they can court, depending on social context. Thus, in this species, there is evidence of behavioral plasticity in males associated with genetically determined body size (Zimmerer and Kallman 1989). Male *X. montezumae* swordtails also exhibit plasticity in mating behavior related to their genetically determined body size; after winning in a male-male competitive interaction, large males were more risk-averse than small males. In addition, contest winners reduced the time spent near females after viewing a high-risk predation condition, but contest losers, usually the small males, increased the time spent near females (Basolo and Nootz *in revision*). Thus, there is further evidence of a genetic link of P-alleles that determine age and size at sexual maturity with predator-related plasticity in male behavior in this genus. This makes members of this genus suitable candidates for investigating how possible genetic links of P-alleles with other alleles that affect behavior can explain differences in inter-individual behavioral plasticity.

We used female green swordtails, *Xiphophorus hellerii* (subfamily Poeciliinae), to investigate the effect of body size at testing and age at sexual maturity on predator-related behavioral plasticity. These sexually dimorphic fish are livebearers found in Central America (Belize, Guatemala, Honduras) and Mexico (Heckel 1848). According to Kallman (1989), while male green swordtails exhibit variation in age and size at maturation, female green swordtails mature at a young age and small size (similar to early-maturing males). The Basolo Laboratory, however, has evidence suggesting that females from multiple wild populations express growth and maturation trajectories consistent with a P-allele system operating in both sexes (unpublished data). The population used for our study is one of these. In fact, this population reflects a P-allele system like that found in the southern platyfish (*X. maculatus*) (Schreibman *et al*. 1973; Kallman and Borkoski 1978; Kallman 1989). That is, males and females show similar maturation trajectories, and, females continue to grow after maturation, while males do not (Kallman *et al*. 1973; Kallman 1989; Royle *et al*. 2006; Basolo 2008). Thus, females with a P-genotype for early maturation will mature at a relatively small size, yet may ultimately become large due to post-maturation growth (see Figure 1.1 reproduced from Basolo 2008). If there is a genetic link of P-like-alleles with alleles that affect behavioral expression in green swordtails (as found in some other swordtails within the genus, *e.g.,* Zimmerer and Kallman 1989) these associations may underlie inter-individual differences in behavioral plasticity. The existence of such a genetic link can be elucidated by evaluating differences in predator-related behavioral plasticity exhibited by females that are similar in body size, but are dissimilar in age at maturation.

Beyond the context of mate-choice, females are infrequently used as test subjects in behavioral studies (Gowaty 1997; Ah-King and Gowaty 2016). Few studies have investigated plasticity in behaviors exhibited during female-female interactions in general (for examples see Forsgren *et al*. 2004; Gavasa *et al*. 2012). To our knowledge, no studies have addressed behavioral plasticity in female-female social behaviors in green swordtails. We tested whether in an intrasexual social context (two females), female green swordtail body size at testing is associated with the degree of predator-related behavioral plasticity. In the wild, male and female green swordtails that co-occur with piscivorous fish are larger on average than those from sites in which piscivorous fish are absent (Basolo and Wagner 2004). And, in a laboratory study of a congener, the southern platyfish (*X. maculatus*), platyfish size increased in populations in which there was a piscivorous fish predator, and decreased in populations in which a predator was not present (Basolo 2008). These body size differences may be, in part, a product of predator gape width limitations (Basolo and Wagner 2004; Basolo 2008). Based on these two studies, we assumed that small individuals incur a greater risk of predation than large individuals. As such, we predicted that small females would express a greater degree of predator-related plasticity than large females.

We further considered that inter-individual differences in behavioral plasticity may be driven by genetic links of alleles for age and size at sexual maturation with alleles that affect behavioral expression, given that associations of P-alleles with behavior have previously been shown in other *Xiphophorus* species. If females that matured small and relatively early (early/large) that have grown to be similar in body size to females that matured later (late/large), show similar degrees of plasticity, this would suggest that current size, not P-like-alleles (*i.e.*, age at maturation), affects plasticity. Conversely, if females dissimilar in body size at testing, but similar in ages at maturation behave similarly, this would suggest that P-like-alleles (*i.e.*, age at maturation), not current size, affect plasticity.

METHODS

Source of Test Fish

Green swordtails, *Xiphophorus hellerii*, were collected from Little Barton Creek near Unitedville, Belize, at approximately mile marker 59 on the Western Highway (N 17° 12' 28''/W 88° 56' 36") in March 2013 and 2014. These Generation 0 (G0) male and female fish were transported to the University of Nebraska—Lincoln and held individually in 20.8 L glass tanks with a handful of the freshwater plant Java moss, an under-gravel filter, and a loose gravel bottom (depth of gravel=1.5 cm). G0 females that were brought back to the laboratory, but did not have offspring, were mated in the laboratory to G0 males collected from the same location. Twenty-five G0 females produced between one and five broods with 1 to 89 Generation 1 (G1) offspring per brood. When broods were born, up to 14 newborn G1 fish were collected within 10 hours of birth.

G1 offspring were held individually in a 6.6 L plastic “home” tank (29.9 x 19.7 x 20.3 cm) with a loose gravel bottom (depth of gravel=1 cm), 10-15 small snails, and a handful of Java moss to help maintain the quality of the environment in the home tanks. All fish were housed in a room in which available light (12L:12D) and temperature (26-28°C) were controlled. G1 offspring were physically, but not visually isolated from one another throughout rearing.

To standardize experience during rearing of G1 offspring, each individual’s home tank was rotated weekly within (same height) and across (different heights) shelving rows, and between shelving units, in order to standardize any effects due to variation in light, temperature, and disturbance levels across the room. To further standardize conditions, we fed all individuals at the same hour, four times daily with Tetra brand TetraMin® tropical fish flakes at 0900 h and 1400 h, and hatched Brine Shrimp Direct live brine shrimp nauplii at 1200 h and 1600 h.

Further, every four weeks, each G1 offspring was temporarily removed from its home tank for 2-4 min in order to clean the tank. This involved emptying the tank (including all snails and Java moss), rinsing the gravel, and refilling the tank with clean water. The snails, Java moss, and G1 fish were then returned to the home tank, and the tank was placed back on the shelving unit.

To track the stages of sexual maturation, G1 offspring were measured when they reached 77 +/- 7 days old, and measured every 17.5 +/- 5.5 days thereafter until they reached sexual maturity. Four months after the date G1 females were identified as sexually mature (*i.e.*, a gravidity spot present indicative of the presence of yolked eggs per Basolo and Melie unpublished data), they were considered ready to be used in test trials.

Individual Traits of Test Fish Relevant to Analyses

From the pool of mature G1 females, focal individuals were categorized as either small or large, and non-focal females were categorized as intermediate-sized. Size classes attributed to the test fish were relative to the mean body sizes of the pool of G1 females at testing (standard length ranged from 37.15 to 60.14 mm; body mass ranged from 1.06 to 5.68 g). Our measure of standard length was defined as the length of the body from the tip of the rostrum to the midway point of the caudal vein. In our laboratory pool of test fish, as found in field populations (Basolo and Wagner 2004), standard length (mm) and body mass (g) are highly correlated (Pearson's correlation coefficient r= 0.984, n= 71, p< 0.001). Because of this correlation, our proxy for size class is based on standard length alone. Size classes were created by comparing the mean and range of female standard lengths within the pool of test fish, and by then choosing representative focal females from both ends of the range.

Body sizes at testing for each size class were as follows: small focal females (standard length ranged from 37.15 to 45.46 mm; body mass ranged from 1.06 to 2.47 g; n= 20), large focal females (standard length ranged from 49.00 to 60.14 mm; body mass ranged from 2.71 to 5.68 g; n= 30), and intermediate non-focal females (standard length ranged from 41.05 to 53.75 mm; body mass ranged from 1.55 to 3.52 g; n= 77). Intermediate non-focal females were chosen based on their body size relative to the focal individual, and were representative of the middle of the range of standard lengths within the pool of test fish at testing. On average, non-focal females differed in standard length from the focal female by 7.50 mm (standard length range of difference was 2.28 to 17.92 mm; body mass range of difference was 0.21 to 4.08 g). Intermediate non-focal females were used as non-focal individuals between 1-7 times, with a mode of 4 tests per non-focal female.

Test fish within both size classes represented a wide range of ages at sexual maturation (in days). Because individuals were grouped into size classes based on the standard length measured six days before the trials began, age and size at testing was not necessarily close to age and size at maturation. This means that some individuals that had been relatively small, early-maturers were ultimately grouped within the large size class, as a result of continued post-maturation growth. Focal females of the small size class had matured between 112 and 229 days old, and focal females of the large size class had matured between 98 and 279 days old.

Some individuals tested were half-or full-siblings (sibs) of one another. A total of 23 families were represented in this study. Of the focal females tested, at least one small and one large individual were represented from 10 of the 23 families. Individuals within the small size class came from 18 different families, and individuals within the large size class came from 16 different families. Non-focal intermediate-sized females came from

24 different families. Of these 24 non-focal female families, 20 families were also represented by focal individuals, although neither half- nor full-sibs were tested with one another.

Pre-trial Acclimation of Test Fish

To acquaint test subjects to the environmental conditions experienced during testing, focal and non-focal fish experienced a series of pre-trial acclimation periods. Five days prior to the first test day, fish were individually introduced to a conspecific “socializer” female of a smaller size in a tank (60 x 30 x 30 cm) with a bottom white board partitioned into a 3 x 6 grid of equally-sized squares (9 x 9 cm), and covered with a thin layer of gravel (depth=3 mm) secured with silicon aquarium sealant (American Sealant, Inc.). The tank was partitioned into two sections of equal size (30 x 30 x 30 cm) by a clear, permeable plastic divider placed in the middle. Thus fish were physically, but not visually or chemically isolated from one another for 20 min.

On the fourth evening prior to when trials began, an empty foraging ring, identical to the foraging ring of the testing tank, was placed in their home tanks overnight. Finally, three days prior to trial commencement, focal and non-focal fish were individually placed in a tank (60 x 30 x 30 cm) with the 3 x 6 gridded gravel board bottom and two sides (one 60 x 30 cm and one 30 x 30 cm) covered with blue felt. On the other 30 x 30 cm side of the tank was a monitor (Truetech 2-in-1 17” LCD TV + DVD Model No. PVS21175S1) that played a 34-minute film sequence of a predation event by a large Jack Dempsey cichlid predator, *Rocio octofasciata* (known at various times by nine other species names; Froese and Pauly 2016), on a male conspecific (*X. hellerii*). Monitor side was randomly determined for each individual.

Experimental Design

A testing framework was developed in which each focal female was tested twice per week over a four-week period (with three to four days in between tests). Within each test week, a female was tested within one context in two different treatments. The treatments for this study were two predator environments: (1) predator-absent and (2) predator-present. Context order was randomized separately for each focal female prior to the start of a set of trials. Within each context, treatment order was also randomized.

For this Chapter, females were tested in an intrasexual social context, in which a focal female was paired with an intermediate-sized non-focal (NF) female. For Chapter 2, females were tested in an intersexual social context, in which a focal female was paired with an intermediate-sized NF female and a NF male. In Chapter 2, the results for the two social contexts are compared. Finally, for a separate study, the females were tested in two non-social contexts: general activity and foraging (Coit and Basolo *in prep*). Of the four contexts described above, only the intrasexual social context is addressed in this chapter.

Each trial was 30-min in duration. Trials were divided into three 10-min periods: acclimation (P0), pre-exposure (P1), exposure (P2). For the predator-absent treatment, one of 13 film stimuli was randomly designated and displayed on a monitor at one end of the test tank during a trial. In the predator-absent treatment, P1 and P2 were identical to the acclimation (P0) period because in these periods, a film stimulus showed an environment devoid of predators for the entire 30 min. For the predator-present treatment, one of 12 film stimuli was randomly designated and displayed on a monitor at one end of the test tank during a trial. In the predator-present treatment, the third 10-min period (P2) differed from the predator-absent treatment P2 in that the film stimulus showed a large, field-collected fish, a Jack Dempsey cichlid (*Rocio octofasciata*), swimming across the monitor, in and out of view, in varying depths-of-field. Jack Dempsey cichlids are piscivorous predators that co-occur with green swordtails in the wild (Basolo and Wagner 2004). In the laboratory, *R. octofasciata* consume *X. hellerii* (DiSciullo personal observation). The film stimuli used in this study were created for previous experiments with green swordtails (Melie and Basolo *in revision*). Monitor side was randomly designated for each trial.

Testing Chamber Set-up

Trials were conducted in one of four designated testing chambers in the Basolo Laboratory. A testing chamber included a 60 x 30 x 30 cm test tank with a monitor (DELL UltraSharp 2005FPW 51.05-cm Wide Aspect Flat Panel LCD Monitor) randomly placed at one of the two ends of the test tank (30 x 30 cm) to display a film stimulus. The glass at the other end of the tank (30 x 30 cm), opposite the monitor, was covered on the outer side with blue felt. One of the long sides of the tank (30 x 60 cm) was randomly designated the back of the tank, and, was covered (on the outer side) with blue felt. The other long side of the tank (30 x 60 cm) was designated the front.

We created a visually distinct 3 x 6 grid of 18, 9 x 9 cm squares on a white plastic panel by siliconing (American Sealants, Inc.) a thin layer of gravel across the panel, except at the borders of the 18 squares. The grid panel was situated on the bottom of the tank during experimental trials, allowing us to precisely track the position of test subjects throughout a trial. The six 9 x 9 cm squares closest to the monitor displaying a stimulus were designated Zone 1, the six 9 x 9 cm squares in the middle of the tank were designated Zone 2, and the six 9 x 9 cm squares farthest from the monitor displaying a stimulus were designated Zone 3 (Figure 1.2). Two plastic plant refuges (Imagine Gold Ambulia Green, 18 cm in length) were suspended in the middle of the tank, at the border of Zones 1 and 2, and, at the border of Zones 2 and 3. A clear plastic foraging ring was suspended across the tank from front to back, and equidistant to the ends of the tank.

Two cameras were positioned in the chamber: one 33.8 cm above the tank (top-facing; DCR-Sony SR47 Handycam or Sony DCR-SR68 Handycam; see Figure 1.2) and one in front of the tank, 130.8 cm away from the tank (front-facing; DCR-Sony SR47 Handycam, Sony DCR-SR68 Handycam or Panasonic 5100HS WV-PS03). Filmed sequences of trials from these cameras were recorded onto Fuji Pro VHS tapes or Sony Memory Stick PRO Duo 4GB memory cards. In addition, trials were viewed remotely via two monitors located outside of the test chamber. A light fixture suspended above the tank was equipped with two 40 Vita light™ broad-spectrum lamps. To reduce glare on the surface of the tank water, the light fixture was fitted with a sheet of vellum.

Conducting and Scoring Trials

Fish were not fed for a two-hr period prior to testing. Prior to the start of a trial, the testing chamber was fully prepared, including starting and pausing the film stimulus, such that the monitor displayed a predator-absent environment (P0) when test subjects were introduced into a test tank. For each trial, the focal individual was placed in the tank, then the non-focal female, and finally a red TetraMin flake was placed on the surface of the water in the middle of the test tank, to ensure that females were not hungry. Then, the film stimulus was resumed and the cameras started recording the fish in the tank.

At the end of the 30-min test period, all test subjects were returned to their home tanks. If the trial series was not yet complete, the next trial was run three to four days after the previous trial. Because trials in the intersexual social context may have preceded trials in the intrasexual social context for some focal females, we took steps to ensure that females would not be pregnant. This was done by modifying the sexually developed anal fin (gonopodium) of every non-focal male tested in the intersexual social context (see Chapter 2). Once a focal fish had completed all trial types, she was removed from the study and monitored for five weeks to assess health and reproductive status (no females were impregnated during the trial series).

Following each trial, the tank was drained immediately and the tank and its components were rinsed with an 80% denatured ethyl alcohol solution to eliminate any trace of the previous occupants. Trials were conducted between 0900 and 1700 h.

Behaviors (including anti-predator and social agonistic behaviors) were scored from the filmed trials that were uploaded to a computer (see Table 1.1). Behaviors exhibited during the pre-exposure (P1) and exposure (P2) periods were scored and compared within and across treatments. The social behaviors that we scored had previously been described as expressed by members of the genus *Xiphophorus*. In these previous studies, behaviors were exhibited by males toward other males and females, and, by females toward males (Clark *et al*. 1954; Franck 1964; Beaugrand *et al*. 1984; Basolo 1995). In this study, we found that many of these same behaviors were exhibited by focal females toward non-focal females. A freeware program, Solomon Coder (version beta 16.06.26; Péter 2016) was used to track frequency (number of times a behavior was exhibited, *i.e.*, count) and duration (the time spent exhibiting the behavior from start to finish, sec) of the behaviors scored.

ANALYSES

Effect of Treatment and Individual Traits on Behavioral Plasticity

We used linear mixed models with a Gaussian distribution to assess the effects of predator treatment and the covariates on the response variables. Data were analyzed in R (version 3.2.2; R Core Team 2015). We used the *lmer* function for linear mixed modeling in package *lme4* (version 1.1-12; Bates *et al.* 2015).

Because we were interested in the possible effect of each predictor on a number of different behaviors, we used the same global model for each response variable. The global model included: the fixed effect of treatment (predator-absent or predator-present environment); focal individual body size at testing (standard length in mm) and age at maturation (number of days old when determined to be sexually mature) as covariates; and all possible two- and three-way interactions. Focal fish ID and family to which the fish belonged were included as random effects to account for the repeated measures design and the lack of independence of fish from the same family. Fish ID was nested within family ID. The response variable was defined as the difference in behavior expressed (*i.e.*, the number of times or length of time a behavior was exhibited) during the exposure period and the pre-exposure period. Our measure of plasticity was the change in that response from the predator-present treatment to the predator-absent treatment.

In this study, if there was a significant effect of treatment (*i.e.*, predator environment), or a significant effect of the interaction of treatment with body size at testing and/or age at maturation on the response variable, then predator-related plasticity was expressed.

To determine the effect of each predictor on the response variables, we used the *drop1* function to run chi-squared likelihood ratio tests and obtain p-values by comparing models with and without the predictors of interest. To validate our models, we checked all fixed/random effects and covariates for collinearity, and checked all residuals of the models for violations of assumptions of normality and homoscedasticity.

Alpha Correction for Multiple Tests

To account for multiple comparisons, we applied the Benjamini-Hochberg (B-H) procedure to control the false discovery rate across the multiple complex models applied to data from the same dataset (Benjamini and Hochberg 1995). We set the false discovery rate at Q = 0.05 and adjusted critical p-values (P) to q-values (Q), by considering the effects of all predictors across the 10 models simultaneously. Each of the 70 significance values were then ranked and adjusted per the B-H procedure.

RESULTS

Effect of Predator on Plasticity in Anti-Predator Behaviors

There were no effects of the three- or any two-way interactions on predator-related plasticity in anti-predator behaviors after controlling the false discovery rate (FDR). As a result, these interactions were dropped from the models for all anti-predator-related behavioral response variables (all $Q \geq 0.182$).

Time spent in Zone 1. There were no significant effects of body size at testing or age at maturation on female time spent in Zone 1 (the third of the tank closest to the monitor displaying a stimulus) after controlling the FDR (Table 1.2). Treatment significantly affected time spent in Zone 1 (Table 1.2); females spent less time in Zone 1 during the exposure period in the predator-present treatment, compared to the predator-absent treatment (Figure 1.3). As such, females expressed predator-related behavioral plasticity in spatial positioning.

Dart away from stimulus. There were no significant effects of treatment or body size at testing on female darts from the stimulus after controlling the FDR (Table 1.3). Age at maturation significantly affected female darts from the stimulus; females that matured relatively later darted more from the stimulus, regardless of treatment or body size at testing (Figure 1.4).

Remain motionless facing stimulus. Neither the treatment nor the covariates of interest (body size at testing and age at maturation) affected female time spent facing the monitor displaying the stimulus after controlling the FDR (all $Q \geq 0.764$).

Effect of Predator on Plasticity in Social Behaviors

Transverse display toward female. There was a significant effect of the three-way interaction between treatment, body size at testing, and age at maturation on female transverse displays after controlling the FDR (Table 1.4). This indicates that there is predator-related plasticity in female transverse displays, and, that body size at testing and age at maturation affect female transverse displays differently between treatments (Figure 1.5). As age at maturation increased, small females increased time spent transverse displaying (Figure 1.6). Large females, however, decreased time spent transverse displaying as age at maturation increased (Figure 1.6). While females of both size classes expressed predator-related plasticity related to age at maturation, small females expressed a greater degree of predator-related plasticity than large females, with the direction of plasticity depending on female age at maturation.

Other social behaviors. After controlling the FDR, there were no significant effects of the three-way interactions (all Q ≥ 0.070), of any two-way interactions (all Q ≥ 0.376), or of any predictors (treatment, body size at testing, age at maturation; all Q ≥ 0.36) on the other social behaviors tested (*i.e., displace from female, charge at female, dart away from female, bite female, simultaneous circling*).

Effect of Predator on Plasticity in General Activity

Movement. After controlling the FDR, there were no significant effects of the three-way interaction, of any two-way interactions, or of any predictors (treatment, body size at testing, age at maturation) on movement throughout the trial (all Q ≥ 0.398).

DISCUSSION

Individual females expressed predator-related plasticity in time spent in Zone 1 (third of the tank closest to the monitor playing the stimulus) (Table 1.2). Females darting from the stimulus did not show predator-related plasticity, but age at maturation affected the expression of darts from the stimulus (Table 1.3). Body size at testing and age at maturation affected the expression of predator-related plasticity in transverse displaying toward the female (Table 1.4).

Females, regardless of body size at testing or age at maturation, expressed predator-related plasticity in the time spent in Zone 1 (Table 1.2, Figure 1.3). Focal females reduced the time spent in Zone 1 when the predator was present in the exposure period compared with the pre-exposure period, but showed little difference in the time spent in Zone 1 across periods when the predator was absent. It is widely known that organisms across a wide range of taxa regularly assess the risk of predation and modulate behaviors in response to predator environment (reviewed in Lima and Dill 1990). The finding of this study provides further evidence of this well-documented response, and is supported by the results of a similar study conducted in our laboratory, using male green swordtails (Melie and Basolo *in revision*). Females recognized the predator as a threat from the visual cue alone, and adjusted their behavior accordingly. Here, this behavioral response manifested as females moving away from the perceived threat (*i.e.,* the monitor displaying the film stimulus), thereby increasing the distance between focal females and the predator and by doing so, likely reducing the perceived risk of predation. The expression of plasticity in this anti-predator behavior was similar across all individuals within the population, regardless of body size at testing or age at maturation.

Females that were older at maturity darted more from the stimulus than females that were younger at maturity, regardless of treatment or body size at testing (Table 1.3, Figure 1.4). This result suggests that later-maturing individuals may be more risk-sensitive to non-biological stimuli (such as the monitor displaying a film stimulus) than earlier-maturing females. Previous work has shown that female green swordtails respond to a monitor playing a film of a biological stimulus in a manner highly similar to that of how they respond to a live stimulus (Trainor and Basolo 2000). Yet in this study, response to the predator environment stimuli did not follow an expected pattern, because later-maturing females frequently darted from the monitor whether the film displayed a biological stimulus (*i.e.*, a predator) or not. This could be explained by the fact that there is a trade-off between age at maturation and other fitness-related traits (Flatt and Heyland 2011). Maturing later can be risky given that an individual may succumb to predation or other sources of mortality prior to reproducing. But, this high-risk life history strategy may result in the high-reward of more abundant and larger, thus highly advantaged, offspring (Abrahams 1993; Reznick *et al*. 1993; Belk and Tuckfield 2010; O'Dea *et al*. 2015). Late-maturing females may thus be more risk-sensitive in behavior overall than are early-maturing females in order to ensure survival to reproduction. This sensitivity may manifest in females as a tendency to increase responsiveness to non-biological stimuli (*i.e.*, monitor displaying the stimulus) over time. This may explain why later-maturing females expressed more darting throughout the trial, regardless of treatment (*i.e.*, predator environment) or body size at testing.

This result also suggests that the alleles that determine age at maturation appear to have an associated effect on the expression of this anti-predator behavior. We had predicted that females dissimilar in size but similar in ages at maturation would behave similarly in the trials if the P-like-alleles are associated with behavior alleles. Indeed, later-maturing females, regardless of body size at testing, darted more from the monitor displaying the stimuli than early-maturing females. This finding provides evidence in support of our prediction that P-like-alleles (here, alleles for later maturation) affect behavior expression—in this case that is how skittish or wary an individual is in response to non-biological stimuli (*i.e.*, a monitor displaying a film stimulus). Treatment (*i.e.*, predator environment) did not effect darts from the stimulus, which we had considered an anti-predator behavior. This suggests that the overall tendency of later-maturing females to exhibit risk-sensitive behavior, such as darting from non-biological stimuli, may obfuscate female response specifically to a change in predator environment.

The interaction between body size at testing and age at maturation affected focal female expression of time spent transverse displaying toward the non-focal female (Table 1.4, Figure 1.5). Moreover, the interactive effect of these two covariates on expression of this threatening behavior differed in the presence and absence of the predator. This result indicates that body size at testing and age at maturation affect predator-related plasticity in behavior (Figure 1.6). For females that were small at testing, age at maturation had a major effect on predator-related plasticity; small, early-matured females decreased time spent transverse displaying in the presence of a predator, while small, late-maturing females increased time spent expressing this behavior. Large females showed the opposite pattern of small females.

We had predicted that small females would express a greater degree of behavioral plasticity than large females, as it appears that small females incur a greater risk of predation in wild populations of green swordtails than do large individuals (Basolo and Wagner 2004). Because the effect of size depends on age at maturation, our results are inconsistent with this prediction. For example, small early-maturing females, and large late-maturing females, appear to express less risky behavior (*i.e.*, interacting with the non-focal female through transverse displaying) in the presence of a predator, whereas small late-maturing females, and large early-maturing females, appear to express more risky behavior (*i.e.*, interacting with the non-focal female through transverse displaying) in the presence of a predator. We do not yet know why size and age at maturation interact as they do to affect female transverse displaying. What is clear, however, is that plasticity can be affected by complex interactions between other traits expressed by an individual. As a result, simple predictions about environmental effects on behavior and about differences in plasticity based on single traits (such as size or age at maturation) may often be misleading because of these complexities.

In all, just one (transverse display) of the ten behaviors that we analyzed provides evidence that individual traits affect the degree of predator-related plasticity expressed in dissimilar ways. Further, age at maturation, but not treatment, also affected the expression of another behavior (dart from stimulus). Findings for darts from stimulus suggest that there may be an association between the alleles for size and age at maturation with alleles that affect behavior, because females of dissimilar size at testing, but similar ages at maturation behaved similarly. This study shows that the effects of individual traits on the expression of behavioral plasticity by individuals are integral to consider when investigating differences in behavioral plasticity within a population. Complex interactions of individual traits and changes in environment may direct the degree to which behavioral plasticity is expressed, as we found for plasticity in transverse displaying. Studies continuing to investigate plasticity at the level of the individual may provide greater insight into the maintenance and direction of evolution in plasticity within a population (Dingemanse and Wolf 2013). Further analyses evaluating the extent to which individuals are consistent in the degree of plasticity expressed, both within and across contexts, will lead to a better understanding of the effect of individual traits on behavioral plasticity.

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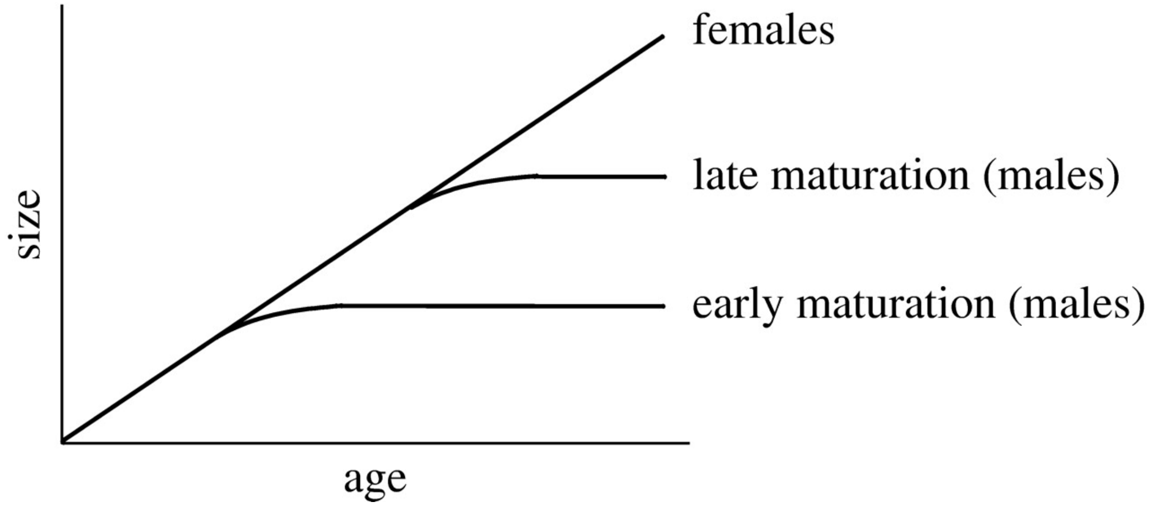

Figure 1.1 Antagonistic pleiotropy results in trade-offs between age and size at sexual maturation in platyfish, with alternate P-alleles affecting life histories differently (Reproduced from Basolo 2008).

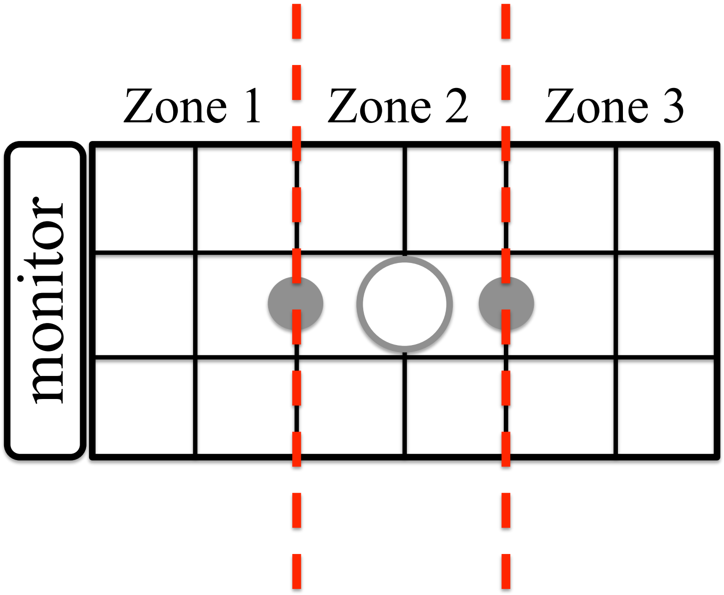

Figure 1.2 Top view of test tank with Zones demarcated.

Table 1.1 Behavioral responses measured in the intrasexual social context.

Behavior name	**Type of behavior**	**Measurement method**	**Description of behavior**
Time spent in Zone 1	Anti-predator	Duration (sec)	Amount of time spent in close proximity to the monitor, regardless of predator environment
Dart away from stimulus	Anti-predator	Count	Quick swim (*i.e.*, dart) from stimulus (*i.e.*, monitor displaying the stimulus) whether or not predator was on screen
Remain motionless facing stimulus	Anti-predator	Duration (sec)	Fish is unmoving at the bottom, top, or middle of the water column, body is oriented toward the monitor displaying the stimulus, regardless of predator environment
Transverse display toward female	Agonistic	Duration (sec)	Body shaped in an "S" or "C" curve, or, held straight perpendicularly to NFF
Displace from female	Agonistic	Count	Move or displace body/part of body in response to lunge or bite from NFF
Charge at female	Agonistic	Count	Charge or lunge at NFF
Dart away from female	Agonistic	Count	Quick swim (*i.e.*, dart) from NFF whether or not NFF had lunged at focal female
Bite female	Agonistic	Count	Bite NFF
Simultaneous circling	Agonistic	Duration (sec)	Focal and NFF circle one another head to tail (tight chase)
Movement	General activity	Count	Number of grids traversed

Note: NFF means non-focal female

Table 1.2 Linear mixed model investigating the effects of the predictors on time spent in Zone 1. Q is the Benjamini-Hochberg adjusted p-value.

Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size at Testing	2.317	9.058	0.065	0.798	0.864
Treatment	**-80.86**	**18.513**	**17.325**	**0.000**	**0.000**
Age at Maturation	6.753	9.496	0.503	0.478	0.764
Random Effects	*Variance*	*SE*			
Focal Fish	74.519	8.558			
Family	0.000	0.000			

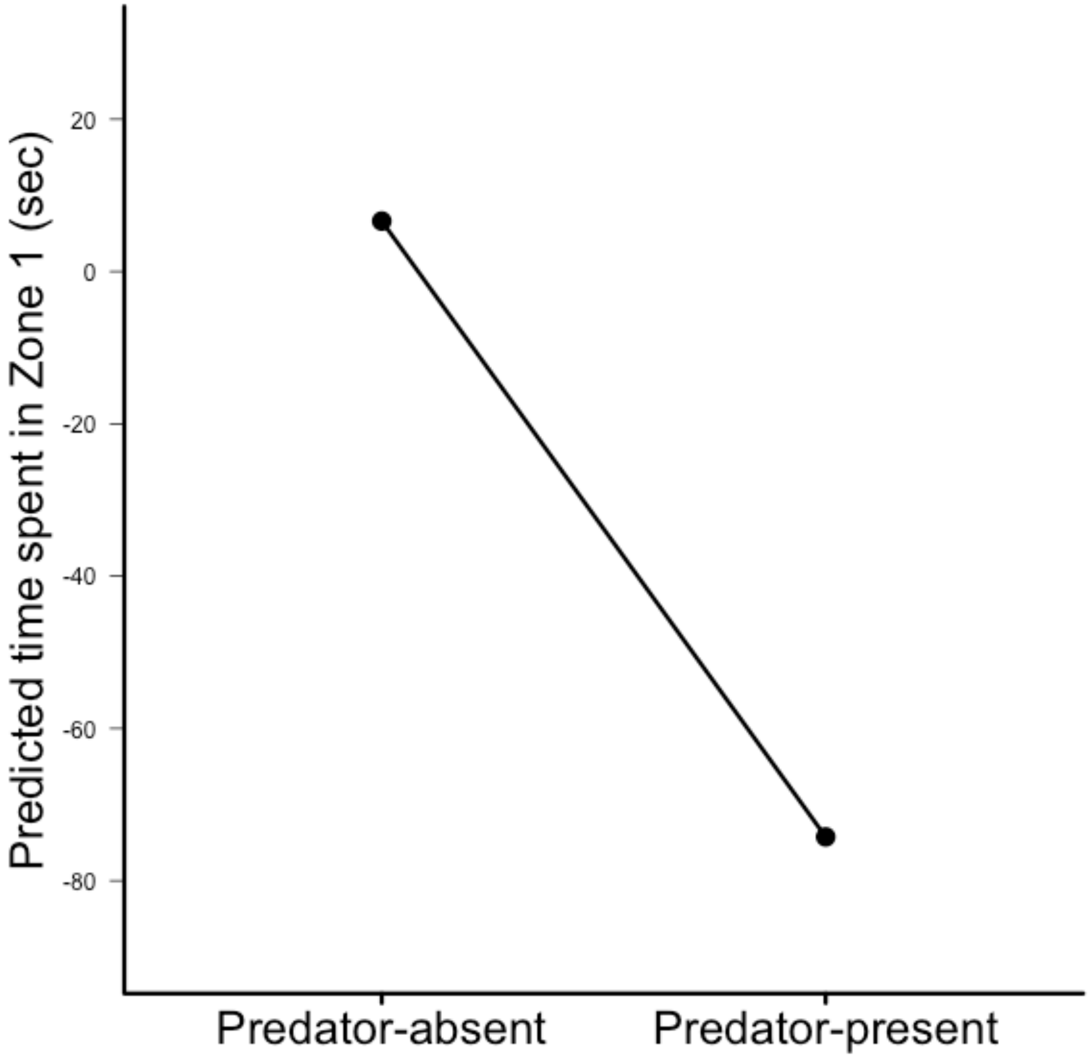

Figure 1.3 Behavioral reaction norm for the prediction of predator-related plasticity in the time spent in Zone 1 (third of the tank nearest to the monitor that displayed a stimulus) (n=20 small females, n=30 large females).

Table 1.3 Linear mixed model for investigating the effects of the predictors on number of darts from the stimulus. Q is the Benjamini-Hochberg adjusted p-value.

Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size at Testing	0.476	0.22	4.398	0.036	0.360
Treatment	0.324	0.422	0.585	0.444	0.764
Age at Maturation	**0.673**	**0.206**	**9.165**	**0.002**	**0.047**
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

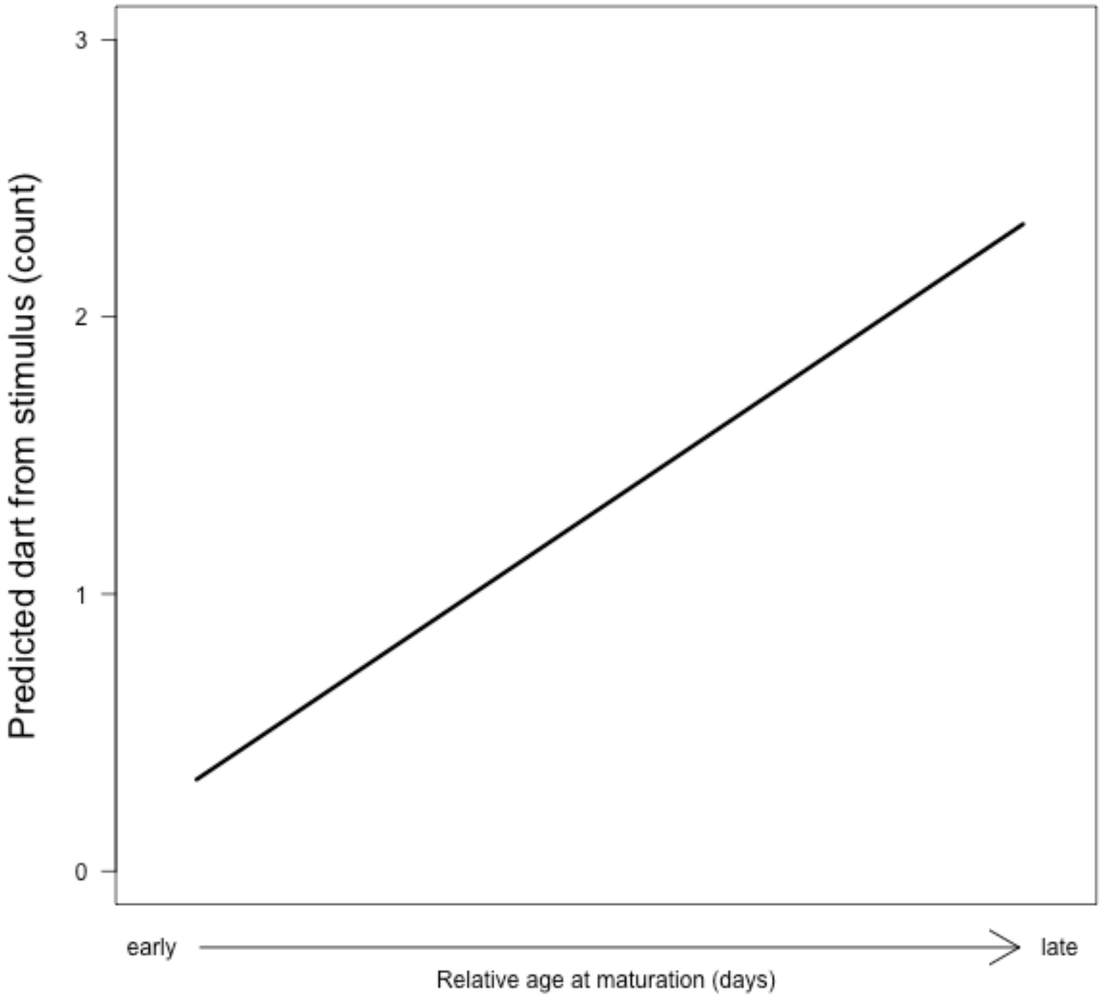

Figure 1.4 Predicted number of darts from stimulus as a function of age at maturation, regardless of treatment or body size at testing (n=13 small females, n=21 large females).

Table 1.4 Linear mixed model investigating the effects of the predictors on time spent transverse displaying toward non-focal females by focal females. Q is the Benjamini-Hochberg adjusted p-value.

Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size at Testing * Age at Maturation * Treatment	**-76.025**	**23.894**	**9.437**	**0.002**	**0.047**
Body Size at Testing * Treatment	33.54	22.61	2.166	0.141	0.487
Body Size at Testing * Age at Maturation	1.043	12.806	0.007	0.935	0.977
Treatment * Age at Maturation	38.242	21.17	3.187	0.074	0.398
Body Size at Testing	-3.245	11.651	0.078	0.781	0.864
Treatment	40.359	22.31	3.196	0.074	0.398
Age at Maturation	-19.982	10.909	3.275	0.070	0.398
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

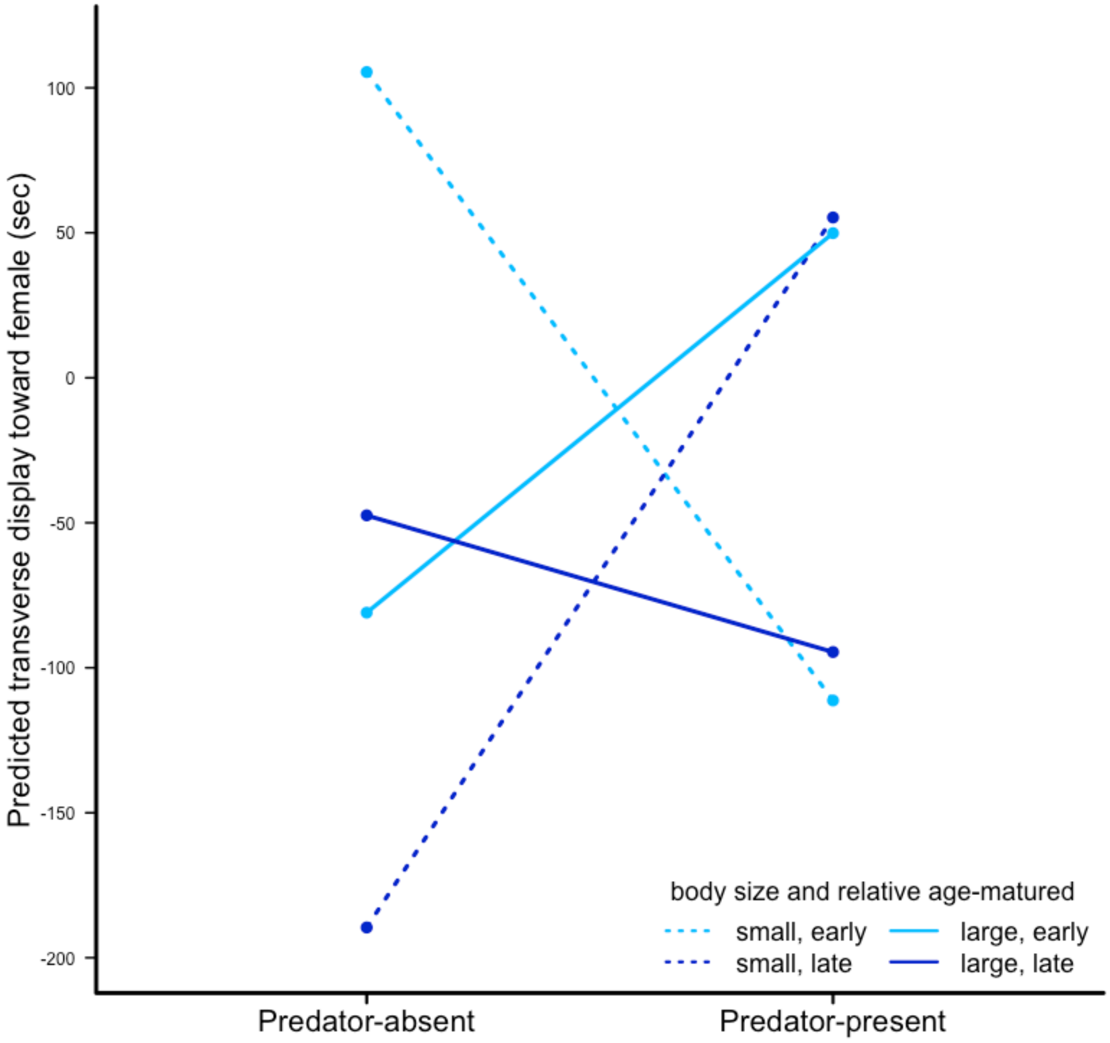

Figure 1.5 Behavioral reaction norm illustrating differences in the degree of predator-related plasticity in predicted time spent transverse displaying toward non-focal females by focal females of varying body sizes at testing and ages at maturation.

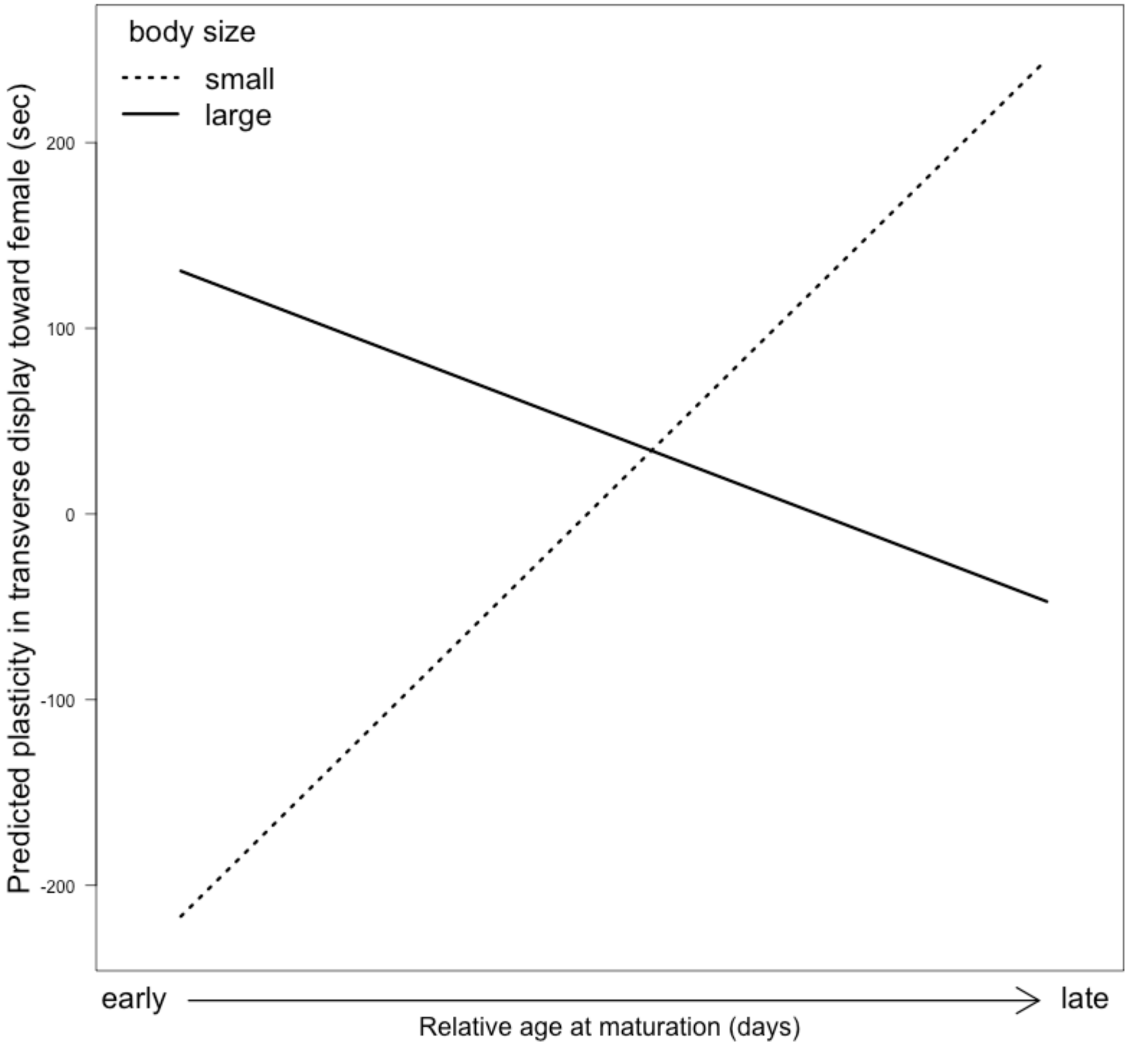

Figure 1.6 Predicted plasticity in time spent transverse displaying at non-focal females by focal females as a function of body size at testing and age at maturation (n=13 small females, n=21 large females).

CHAPTER 2. Lack of individual consistency in predator-related behavioral plasticity expressed by female green swordtails (*Xiphophorus hellerii*) across social contexts

ABSTRACT

Behavioral plasticity is a type of phenotypic plasticity in which one genotype expresses multiple behavioral phenotypes, under differing environmental conditions. The expression of behavioral plasticity can vary among individuals based on individual traits, such as body size or age at maturation. Few studies have investigated the extent of inter-individual variability in the expression of behavioral plasticity, and even fewer have considered whether inter-individual differences in the degree of behavioral plasticity are consistent across contexts. We used female green swordtails (*Xiphophorus hellerii*) to test how body size at testing and age at sexual maturation affect the degree of predator-related behavioral plasticity expressed. We also tested to what extent individual plasticity is consistent across two social contexts: (1) intrasexual (two females) and (2) intersexual (two females and a male). We found that all females, regardless of body size at testing or age at maturation, expressed predator-related plasticity in spatial positioning. For two agonistic behaviors, the interaction of the individual traits examined (body size at testing and age at maturation) affected the degree of predator-related plasticity expressed. Further, the effect of the interaction of the two individual traits on the expression of predator-related plasticity in these two behaviors was different across contexts. There was no evidence of individual consistency in behavioral plasticity across contexts. Individuals that were more plastic for a particular behavior in one context were not consistently more

plastic for that behavior in another context. Within a context, however, individuals that were more plastic for one behavior tended to be more plastic for other behaviors. This study shows that the effect of individual traits on the expression of behavioral plasticity by individuals is complex. Environmental change alone may not be a sufficient predictor of behavior, and individual traits should be considered when investigating individual variation in behavioral plasticity within a population.

INTRODUCTION

Phenotypic plasticity is the ability of one genotype to express different phenotypes across varying environments (West-Eberhard 1989; DeWitt *et al*. 1998). Behavior is among the most labile of all phenotypes that an organism expresses (Hazlett 1995). An organism that modulates or changes behavior in response to changes in environmental conditions is said to exhibit behavioral plasticity. Because environmental conditions can change rapidly, behavioral plasticity allows individuals to quickly adjust to changing environmental conditions (Komers 1997; Pigliucci 2001).

Behaviors and behavioral plasticity are typically measured at the level of the individual, but interpreted at the level of the population (Sih and Bell 2008). However, the behavioral response expressed as a population average does not capture inter-individual differences in behavior (Bolnick *et al*. 2003; Nussey *et al*. 2007). For example, female common gulls (*Larus canus*) express plasticity in egg-laying date in response to temperature, but the individual change in egg-laying date is highly variable among females within a population, with some individuals showing no plasticity (Brommer *et al*. 2008). As recent studies of behavioral plasticity have suggested, considering the extent to which individuals within a population differ in the degree of behavioral plasticity expressed is increasingly important (Dingemanse and Dochtermann 2013; Alonzo 2015). This line of investigation can provide greater insight into how phenotypic plasticity can evolve, and how different degrees of plasticity can be maintained within a population (Nussey *et al*. 2007; Dingemanse and Wolf 2013). This may be especially true if the optimal response to environmental perturbations differs among individuals that differ in phenotypes (Wolf *et al*. 2008), such as when variation in individual traits like sex and body size influence the fitness effects of plasticity.

Plasticity in the expression of behavior by individuals under the threat of predation, or *predator-related plasticity*, can be affected by individual traits such as body size (Peters 1986; Lima and Dill 1990). For example, after exposure to a predator, small brown rockfish (*Sebastes auriculatus*) forage sooner and more readily than large rockfish, suggesting that large rockfish are more plastic in response to predation risk (Lee and Bereijikian 2008). Other studies have also suggested that small individuals may be more likely to engage in risky behavior, and that this is potentially attributable to the greater metabolic needs of small compared to large individuals (Clark 1994; Brown and Braithwaite 2004). Another explanation for differences in behavioral plasticity based on size is that large individuals may incur a greater risk of predation by predators (Lima and Dill 1990; Rosenthal *et al*. 2001). For example, female guppies (*Poecilia reticulata*) are larger, but duller (thus less conspicuous) than male guppies, yet females are preferentially attacked by cichlid predators (Pocklington and Dill 1995). As a result, large individuals may be more cautious than small individuals.

In some environments, however, larger individuals may be less susceptible to predation than small individuals, and thus may express fewer risk-sensitive behaviors. For example, in some species, predator-presence leads to the evolution of larger body size. In an experimental study of southern platyfish (*Xiphophorus maculatus*), male and female size evolved over time in populations. Prey body size increased in populations when a piscivorous fish predator was present, and decreased in populations when a predator was not present. Furthermore, genotypes for later maturation increased in the predator–present populations, but decreased in predator-absent populations (Basolo 2008). Field studies of wild populations of the southern platyfish *X. maculatus* and the related green swordtail *X. hellerii* (also known as *Xiphophorus helleri;* Froese and Pauly 2016) have found that adult size is greater at sites with piscivorous fish compared to sites at which piscivorous fish were absent (Basolo and Wagner 2004; Basolo unpublished data). These inter-population body size differences are thought to be, in part, a result of gape-width limitations of predators, for whom large prey are too cumbersome to capture and consume (and therefore are less susceptible to predation than smaller prey) (Basolo and Wagner 2004; Basolo 2008). Thus, large individuals may behave differently than small individuals when they co-occur with piscivorous fishes. Body size therefore is an important phenotype to consider when investigating the effect of individual traits on behavioral plasticity. This is particularly true for studies of predator-related behavioral plasticity when individuals of different size have different susceptibilities to predation.

The expression of predator-related plasticity may also be affected by age. In a study of larval blue-tailed damselflies (*Ischnura elegans*), older, but not younger, larvae were plastic and reduced activity in response to predator presence (Mikolajewski *et al.* 2015). How age at maturation affects the expression of predator-related plasticity is less well studied compared to the effects of age. Age at maturation is a life history trait associated with a life history strategy that only some individuals will share (Flatt and Heyland 2011), whereas age is a set of life stages through which many individuals will progress. As a result, individual differences in age at maturation could profoundly affect the expression of predator-related behavioral plasticity, in a way that differs from the effect of age differences, but this warrants investigation.

Predator presence can also have pronounced effects on female behavior in mating contexts. It can be costly for females to associate with males that exhibit conspicuous traits that attract predators (Lima and Dill 1990; Dill *et al.* 1999; Martin and Wagner 2010). To mitigate such costs, females may express plasticity in social behaviors within a mating context. For example, female guppies reduce their preference for conspicuously colored males in the presence of a predator (Godin and Briggs 1996; Gong and Gibson 1996) because these males are more likely to attract the attention of predators (Pocklington and Dill 1995; Godin and McDonough 2003). This pattern is evident in populations with high predation risk (Godin and Briggs 1996) and populations with low predation risk (Godin and Briggs 1996; Gong and Gibson 1996).

A similar pattern has been shown in a fish species related to the guppy, the green swordtail (*Xiphophorus hellerii*). Male green swordtails express a set of conspicuously colored rays at the base of the caudal fin that extend beyond the lower caudal margin known as a ‘sword’ (Basolo 1996). For some populations, male sword length approaches the length of the male’s body. Female green swordtails have been shown to prefer males with longer swords (Basolo 1990a; Basolo 1990b) and larger body sizes (Basolo 1998; Rosenthal and Evans 1998). In a study investigating the effect of predation on the female sword response, females preferred a digital male with a long sword to a digital male with sword coloration, but lacking the extension component of a sword. Yet, after witnessing a piscivorous predator consuming a male with a long sword, females no longer expressed a preference for the digital male with a long sword (Johnson and Basolo 2003). In a subsequent study, female green swordtails switched their preference for a long-sworded male to a short-sworded male after viewing a piscivorous predator consuming a short-sworded male (Melie and Basolo *in revision*). In a similar study, female green swordtails also preferred a short-sworded male to a long-sworded after viewing a swimming piscivorous predator (Pilakouta and Alonzo 2013).

Although individual consistency in personality across contexts has garnered more attention in recent years, investigations of consistency in behavioral plasticity across contexts remain comparatively scant (Dingemanse *et al*. 2010; Stamps 2015; but see Przybylo *et al*. 2000; Mathot *et al*. 2011; Han and Brooks 2013). Investigating potential correlations in the expression of plasticity across contexts will further expand our understanding of costs and benefits of expressing behavioral plasticity, Further, understanding the extent to which individual expression of plasticity within a population differs can better inform the evolutionary trajectory of plasticity within a population (Dingemanse and Wolf 2013; Stamps 2015).

We used female green swordtails (*X. hellerii)* to evaluate: (1) how body size at testing and age at maturation pertain to the expression of predator-related behavioral plasticity, and, (2) to test whether there is consistency in the degree of plasticity exhibited across social contexts. Members of the genus *Xiphophorus*, *i.e.,* swordtails and platyfish, comprise a monophyletic clade of Neotropical, freshwater fish in the family Poeciliidae (Heckel 1848; Rauchenberger *et al*. 1990; Kang *et al*. 2013). These sexually dimorphic livebearers are considered model organisms for studies concerning size and behavior, because previous research has revealed a genetic mechanism that influences body size and age at sexual maturity for a number of species within the genus (Kallman 1989). First identified in the southern platyfish (*X. maculatus*), and later established in swordtails, alleles associated with the sex-linked P-locus (pituitary locus) affect the production of the gonadotropin-releasing hormone (Kallman *et al*. 1973; Bao and Kallman 1982; Kallman 1983). Hormones induced by these ‘P-alleles’ in turn initiate the development of the gonads, thus determining size and age at maturity (Schreibman *et al*. 1973; Kallman and Borkoski 1978; Zimmerer and Kallman 1988; Kallman 1989). It has previously been shown that in some swordtails, P-alleles have a genetic link with alleles that affect plasticity in mating behavior. For example, in one swordtail species *Xiphophorus multilineatus* (formerly *X. nigrensis*; Rauchenberger *et al*. 1990), small individuals are plastic in mating strategy, but large individuals are not. In the presence of a large, courting male, small males exhibit a ‘sneaker’ mating strategy, in which they attempt to force copulations with females, but in the absence of a large, courting male, small males will court females. Large males are not plastic in mating behavior, and will court females in the presence and absence of small males (Zimmerer and Kallman 1989). The *Xiphophorus* P-allele system provides a unique opportunity to investigate the relationship that a genetic polymorphism for size and age at maturity has with behavior expression.

Female green swordtails (*Xiphophorus hellerii*) are model candidates for testing the possibility that alleles that determine size and age at maturation may have a genetically associated relationship with alleles that affect expression of behavior. It has been suggested that differences in body size and age at maturation in green swordtails are both heritable and correlated (Campton 1992). These differences may be attributable to variation in alleles present at the P-locus (Kallman 1989; Campton 1992; Basolo and Wagner 2004). The presence of P-alleles in this species, however, has yet to be empirically shown. Male green swordtails grow continuously until maturation, and then grow little after maturation (Campton 1992; Basolo and Wagner 2004; Basolo 2008). As a result, early-maturing males mature at a small size and remain small, while later-maturers mature at larger sizes and remain large. According to Kallman (1989), female green swordtails mature at a size and age similar to that of the small, early-maturing males. This would suggest that females are fixed for early maturation and small size. However, female green swordtails in the population with which we worked matured at a range of ages and sizes. This suggests that the P-like-alleles are not fixed for early maturation (and small size) in females. Moreover, our female green swordtails, unlike their male counterparts, have indeterminate growth after sexual maturity (Royle *et al.*

2006; Basolo 2008; Basolo and DiSciullo personal observation). This means that a small, relatively early-maturing female can, over time, grow to become similar in size to a larger, later-maturing female (see Figure 2.1 reproduced from Basolo 2008). By comparing the behavior of females similar in body size but dissimilar in age at maturation, we can parse out the possible effects of the P-like-alleles on behavior from the effect of body size by itself.

We tested the hypothesis that body size at testing and age at maturation affect the degree of predator-related behavioral plasticity expressed by female green swordtails (*X. hellerii*) in two different social contexts: (1) intrasexual (two females) and (2) intersexual (two females and a male). Because green swordtails tend to be larger in environments in which they co-occur with piscivorous fish (Basolo and Wagner 2004), we assumed that small individuals are more susceptible to predation than large individuals. Thus, we predicted that the small females would express a greater degree of predator-related plasticity than large females in both social contexts. Moreover, female green swordtails may be more susceptible to predation risk when in the presence of an elaborated male, as in other species with conspicuous male (Lima and Dill 1990). For example, in a species closely related to *X. hellerii*, *X. multilineatus*, piscivorous fish predators are more likely to detect and orient toward relatively larger, sworded males (Rosenthal *et al.* 2001). In green swordtails, sworded males are more likely to be attacked by cichlid predators, possibly because these males are more conspicuous (Hernandez-Jimenez and Rios-Cardenas 2012), or because males have a larger apparent size as a result of the sword (Rosenthal and Evans 1998). Predators, however, may be more capable of consuming females associated with males due to gape-width limitations (Basolo and Wagner 2004) and females may thus suffer predation more often in the presence of a male. Therefore, we predicted that in the intersexual context, relatively small females (likely the most susceptible phenotype based on size; Basolo and Wagner 2004) would express a higher degree of predator-related plasticity than expressed in the intrasexual context (by reducing social behaviors in the presence of a predator). Large females may be less vulnerable in general to gape-width limited predators, and thus should express a lesser degree of plasticity across contexts if predator presence is not perceived as a threat.

Within the intrasexual social context (in which a focal female was paired with a non-focal female), we found that there was an effect of the interaction between body size at testing and age at maturation on the degree of predator-related plasticity in focal female transverse displays (see Chapter 1). Thus, we considered that, as in the intrasexual social context, individual dissimilar in both body size at testing and age at maturation would express similar degrees of behavioral plasticity in the intersexual social context as in the intrasexual social context, for the transverse display and other social behaviors. Evaluating the effects of these individual traits (body size at testing and age at maturation) allowed us to test the possibility that the P-allele-like mechanism for size and age at maturity is genetically associated with alleles that affect the expression of behavioral plasticity, as found in other species of swordtails. We predicted that older, relatively early-maturing females that have grown in standard length (early/large) to become similar in size to younger, relatively late-matured females (late/large) would express a similar degree of behavioral plasticity as a result of a genetically-linked effect of the P-allele-like genes, rather than the size of the individual when tested.

We also tracked plasticity expressed by individual females to test the hypothesis that individual expression of predator-related behavioral plasticity is correlated across contexts. We predicted that individuals that show a high degree of plasticity in an intrasexual social context (two females) would show a high degree of plasticity in an intersexual social context (two females and a male). By incorporating the individual traits of body size at testing and age at maturation into the analyses, we also were able to test whether females of similar phenotypes were consistent in the degree of predator-related plasticity expressed across contexts.

METHODS

Source of Test Fish

Green swordtails, *Xiphophorus hellerii*, were collected from Little Barton Creek near Unitedville, Belize, at approximately mile marker 59 on the Western Highway (N 17° 12’ 28’’/W 88° 56’ 36”) in March 2013 and 2014. These Generation 0 (G0) male and female fish were transported to the University of Nebraska—Lincoln and held individually in 20.8 L glass tanks with a handful of the freshwater plant Java moss, an under-gravel filter, and a loose gravel bottom (depth of gravel=1.5 cm). G0 females that were brought back to the laboratory, but did not have offspring, were mated in the laboratory to G0 males collected from the same location. Twenty-five G0 females produced between one and five broods with 1 to 89 Generation 1 (G1) offspring per brood. When broods were born, up to 14 newborn G1 fish were collected within 10 hours of birth.

G1 offspring were held individually in a 6.6 L plastic “home” tank (29.9 x 19.7 x 20.3 cm) with a loose gravel bottom (depth of gravel=1 cm), 10-15 small snails, and a handful of Java moss to help maintain the quality of the environment in the home tanks. All fish were housed in a room in which available light (12L:12D) and temperature (26-28°C) were controlled. G1 offspring were physically, but not visually isolated from one another throughout rearing.

To standardize experience during rearing of G1 offspring, each individual’s home tank was rotated weekly within (same height) and across (different heights) shelving rows, and between shelving units, in order to standardize any effects due to variation in light, temperature, and disturbance levels across the room. To further standardize conditions, we fed all individuals at the same hour, four times daily with Tetra brand TetraMin® tropical fish flakes at 0900 h and 1400 h, and hatched Brine Shrimp Direct live brine shrimp nauplii at 1200 h and 1600 h.

Further, every four weeks, each G1 offspring was temporarily removed from its home tank for 2-4 min in order to clean the tank. This involved emptying the tank (including all snails and Java moss), rinsing the gravel, and refilling the tank with clean water. The snails, Java moss, and G1 fish were then returned to the home tank, and the tank was placed back on the shelving unit.

To track the stages of sexual maturation, G1 offspring were measured when they reached 77 +/- 7 days old, and measured every 17.5 +/- 5.5 days thereafter until they reached sexual maturity. Four months after the date G1 females were identified as sexually mature (*i.e.*, gravidity spot present indicative of the presence of yolked eggs per Basolo and Melie unpublished data; fully developed gonopodium for males per Grobstein 1940), they were considered ready to be used in test trials.

Individual Traits of Test Fish Relevant to Analyses

From the pool of mature G1 females, focal individuals were categorized as either small or large, and non-focal females were categorized as intermediate-sized. Size classes attributed to the test fish were relative to the mean body sizes of the pool of G1 females at testing (standard length ranged from 38.80 to 53.75 mm; body mass ranged from 1.16 to 3.86 g). Our measure of standard length was defined as the length of the body from the tip of the rostrum to the midway point of the caudal vein. In our laboratory pool of test fish, as found in field populations (Basolo and Wagner 2004), standard length (mm) and body mass (g) are highly correlated (Pearson's correlation coefficient $r= 0.984$, $n= 71$, $p< 0.001$). Because of this correlation, our proxy for size class is based on standard length alone. Size classes were created by comparing the mean and range of female standard lengths within the pool of test fish, and by then choosing representative focal females from both ends of the range.

Body sizes at testing for each size class were as follows: small focal females (standard length ranged from 38.80 to 45.25 mm; mass ranged from 1.16 to 2.47 g; n= 13), large focal females (standard length ranged from 49.18 to 60.14 mm; mass ranged from 2.84 to 5.68 g; n= 22), intermediate non-focal females (standard length ranged from 41.05 to 53.75 mm; mass ranged from 1.55 to 3.86 g; n= 68), and intermediate non-focal males (standard length ranged from 34.04 to 42.07 mm; mass ranged from 0.74 to 1.33 g n=37). Intermediate non-focal females were chosen based on their body size relative to the focal individual, and were representative of the middle of the range of standard lengths within the pool of test fish at testing. On average, non-focal females differed in standard length from the focal female by 7.67 mm (standard length range of difference was 2.52 to 17.92 mm; mass range of difference was 0.21 to 4.08 g). Intermediate non-focal females were used as non-focal individuals between 1-7 times, with a mode of 4 tests per non-focal female.

Test fish within both size classes represented a wide range of ages at sexual maturation (in days). Because individuals were grouped into size classes based on the standard length measured six days before the trials began, age and size at testing was not necessarily close to age and size at maturation. This means that some individuals in the population that had been relatively small, early-maturers were ultimately grouped within the large size class, as a result of continued post-maturation growth. Focal females of the small size class had matured between 112 and 229 days old, and focal females of the large size class had matured between 112 and 279 days old.

Some individuals tested were half- or full-siblings (sibs). A total of 21 families were represented in this study. Of the focal females tested, at least one small and one large individual were represented from 5 of the 21 families. Individuals within the small size class came from 10 different families, and individuals within the large size class came from 16 different families. Non-focal intermediate-sized females came from 23 different families. Of these 23 non-focal female families, 17 families were also represented by focal individuals, though neither half- nor full-sibs were tested with one another. Non-focal males came from 17 different families. Of these 17 non-focal male families, 12 families were also represented by focal individuals, though neither half- nor full-sibs were tested with one another.

Pre-trial Acclimation of Test Fish

To acquaint test subjects to the environmental conditions experienced during testing, focal and non-focal fish experienced a series of pre-trial acclimation periods. Five days prior to the first test day, fish were individually introduced to a conspecific “socializer” female of a smaller size in a tank (60 x 30 x 30 cm) with a bottom white board partitioned into a 3 x 6 grid of equally-sized squares (9 x 9 cm), and covered with a thin layer of gravel (depth=3 mm) secured with silicon aquarium sealant (American Sealant, Inc.). The tank was partitioned into two sections of equal size (30 x 30 x 30 cm) by a clear, permeable plastic divider placed in the middle. Thus fish were physically, but not visually or chemically isolated from one another for 20 min.

On the fourth evening prior to when trials began, an empty foraging ring, identical to the foraging ring of the testing tank, was placed in their home tanks overnight. Finally, three days prior to trial commencement, focal and non-focal fish were individually placed in a tank (60 x 30 x 30 cm) with the 3 x 6 gridded gravel board bottom and two sides (one 60 x 30 cm and one 30 x 30 cm) covered with blue felt. On the other 30 x 30 cm side of the tank was a monitor (Truetech 2-in-1 17” LCD TV + DVD Model No. PVS21175S1) that played a 34-minute film sequence of a predation event by a large Jack Dempsey cichlid predator, *Rocio octofasciata* (known at various times by nine other species names; Froese and Pauly 2016), on a male conspecific (*X. hellerii*). Monitor side was randomly determined for each individual.

To standardize the relative length of the sword, non-focal males were modified six days prior to the start of the trial series. At this time, the sword was trimmed to a point to simulate the natural appearance of the sword, and the altered length was approximately 55% of the individual’s standard length. Female green swordtails have been shown to prefer males with longer swords (Basolo 1990a). Thus, standardizing male sword lengths relative to body size controls for potential differences in strength of preferences within and among females across trials. The cells of the trimmed caudal fin extension (*i.e.*, the sword), showed varied regeneration rates across males over the trial period. At the end of the testing period, some males had nearly completely regrown the originally trimmed sword length, while others had not (mean growth +/- SE: 4.60 mm +/- 0.41; n=35). Trimming of the sword does not appear to affect behavior or swimming ability (Basolo and Alcaraz 2003).

Because pregnant females may exhibit different behavior from virgin females, we reduced the likelihood of insemination of focal females by non-focal males by modifying the sexually developed anal fin (gonopodium) of every non-focal male. Specifically, we trimmed the hook and claw on the third and fifth ray, respectively, at the distal tip of the gonopodium (approximately 1-3 mm from the tip) (Figure 2.2). The hook and claw aid the male in securely placing the tip of his gonopodium at the female's genital opening (gonopore; Clark *et al*. 1954), which allows the sperm packet to be introduced into the female. It has previously been shown that the ray segments (including the hook and claw segments) of a mature gonopodium in another poeciliid, *Gambusia sp*., in addition to *X. hellerii,* do not regenerate readily after trimming (Turner 1941; Clark *et al*. 1954). Over the testing period, there was no evidence of segment or hook/claw re-growth in the modified test males (personal observation).

Experimental Design

A testing framework was developed in which each focal female was tested twice per week over a four-week period (with three to four days in between tests). Within each test week, a female was tested within one context in two different treatments. The treatments for this study were two predator environments: (1) predator-absent and (2) predator-present. Context order was randomized separately for each focal female prior to the start of a set of trials. Within each context, treatment order was also randomized.

For Chapter 1, females were tested in an intrasexual social context, in which a focal female was paired with an intermediate-sized non-focal (NF) female. For Chapter 2, females were tested in an intersexual social context, in which a focal female was paired with a NF female and a NF male. In this chapter, the results for the two social contexts are compared. Finally, for a separate study, the females were tested in two non-social contexts: general activity and foraging (Coit and Basolo *in prep*). Of the four contexts described above, both the intrasexual and intersexual social contexts are addressed in this chapter.

Each trial was 30-min in duration. Trials were divided into three 10-min periods: acclimation (P0), pre-exposure (P1), exposure (P2). For the predator-absent treatment, one of 13 film stimuli was randomly designated and displayed on a monitor at one end of the test tank during a trial. In the predator-absent treatment, P1 and P2 were identical to the acclimation (P0) period because in these periods, a film stimulus showed an environment devoid of predators for the entire 30 min. For the predator-present treatment, one of 12 film stimuli was randomly designated and displayed on a monitor at one end of the test tank during a trial. In the predator-present treatment, the third 10-min period (P2) differed from the predator-absent treatment P2 in that the film stimulus showed a large, field-collected fish, a Jack Dempsey cichlid (*Rocio octofasciata*), swimming across the monitor, in and out of view, in varying depths-of-field. Jack Dempsey cichlids are piscivorous predators that co-occur with green swordtails in the wild (Basolo and Wagner 2004). In the laboratory, *R. octofasciata* consume *X. hellerii* (DiSciullo personal observation). The film stimuli used in this study were created for previous experiments with green swordtails (Melie and Basolo *in revision*). Monitor side was randomly designated for each trial.

Testing Chamber Set-up

Trials were conducted in one of four designated testing chambers in the Basolo Laboratory. A testing chamber included a 60 x 30 x 30 cm test tank with a monitor

(DELL UltraSharp 2005FPW 51.05-cm Wide Aspect Flat Panel LCD Monitor) randomly placed at one of the two ends of the test tank (30 x 30 cm) to display a film stimulus. The glass at the other end of the tank (30 x 30 cm), opposite the monitor, was covered on the outer side with blue felt. One of the long sides of the tank (30 x 60 cm) was randomly designated the back of the tank, and, was covered (on the outer side) with blue felt. The other long side of the tank (30 x 60 cm) was designated the front.

We created a visually distinct grid 3 x 6 of 18, 9 x 9 cm squares on a white plastic panel by siliconing (American Sealants, Inc.) a thin layer of gravel across the panel, except at the borders of the 18 squares. The grid panel was situated on the bottom of the tank during experimental trials, allowing us to precisely track the position of test subjects throughout a trial. The six 9 x 9 cm squares closest to the monitor displaying a stimulus were designated Zone 1, the six 9 x 9 cm squares in the middle of the tank were designated Zone 2, and the six 9 x 9 cm squares farthest from the monitor displaying a stimulus were designated Zone 3 (Figure 2.3). Two plastic plant refuges (Imagine Gold Ambulia Green, 18 cm in length) were suspended in the middle of the tank, at the border of Zones 1 and 2, and, at the border of Zones 2 and 3. A clear plastic foraging ring was suspended across the tank from front to back, and equidistant to the ends of the tank.

Two cameras were positioned in the chamber: one 33.8 cm above the tank (top-facing; DCR-Sony SR47 Handycam or Sony DCR-SR68 Handycam; see Figure 2.3) and one in front of the tank, 130.8 cm away from the tank (front-facing; DCR-Sony SR47 Handycam, Sony DCR-SR68 Handycam or Panasonic 5100HS WV-PS03). Filmed sequences of trials from these cameras were recorded onto Fuji Pro VHS tapes or Sony

Memory Stick PRO Duo 4GB memory cards. In addition, trials were viewed remotely via two monitors located outside of the test chamber. A light fixture suspended above the tank was equipped with two 40 Vita light™ broad-spectrum lamps. To reduce glare on the surface of the tank water, the light fixture was fitted with a sheet of vellum.

Conducting and Scoring Trials

Fish were not fed for a two-hr period prior to testing. Prior to the start of a trial, the testing chamber was fully prepared, including starting and pausing the film stimulus, such that the monitor displayed a predator-absent environment (P0) when test subjects were introduced into a test tank. For each trial, the focal individual was placed in the tank, then the non-focal female (in both the intra- and intersexual contexts), the non-focal male (in the intersexual context), and finally a red TetraMin flake was placed on the surface of the water in the middle of the test tank, to ensure that females were not hungry. Then, the film stimulus was resumed and the cameras started recording the fish in the tank.

At the end of the 30-min test period, all test subjects were returned to their home tanks. If the trial series was not yet complete, the next trial was run three to four days after the previous trial. Once a focal fish had completed all trial types, she was removed from the study and monitored for five weeks to assess health and reproductive status (no females were impregnated during the trial series).

Following each trial, the tank was drained immediately and the tank and its components were rinsed with an 80% denatured ethyl alcohol solution to eliminate any trace of the previous occupants. Trials were conducted between 0900 and 1700 h.

Behaviors (including anti-predator and social agonistic behaviors) were scored from the filmed trials that were uploaded to a computer (see Table 1.1). Behaviors exhibited during the pre-exposure (P1) and exposure (P2) periods were scored and compared within and across treatments. The social behaviors that we scored had previously been described as expressed by members of the genus *Xiphophorus*. In these previous studies, behaviors were exhibited by males toward other males and females, and, by females toward males (Clark *et al*. 1954; Franck 1964; Beaugrand *et al*. 1984; Basolo 1995). In this study, we found that many of these same behaviors were exhibited by focal females toward non-focal females. A freeware program, Solomon Coder (version beta 16.06.26; Péter 2016) was used to track frequency (number of times a behavior was exhibited, *i.e.*, count) and duration (the time spent exhibiting the behavior from start to finish, sec) of the behaviors scored.

ANALYSES

Effect of Context, Treatment, and Individual Traits on Behavioral Plasticity

We used linear mixed models with a Gaussian distribution to assess the effects of predator treatment and the covariates on the response variables. Data were analyzed in R (version 3.2.2; R Core Team 2015). We used the *lmer* function for linear mixed modeling in package *lme4* (version 1.1-12; Bates *et al.* 2015).

Because we were interested in the possible effect of each predictor on a number of different behaviors, we used the same global model for each response variable. To compare behavioral responses across contexts, the global model included: the fixed effects of context (intrasexual social or intersexual social) and treatment (predator-absent or predator-present environment); focal individual body size at testing (standard length in mm) and age at maturation (number of days old when determined to be sexually mature) as covariates; and all possible two-, three-, and four-way interactions. Focal fish ID and family to which the fish belonged were included as random effects to account for the repeated measures design and the lack of independence of fish from the same family. Fish ID was nested within family ID. The response variable was defined as the difference in behavior expressed (*i.e.*, the number of times or length of time a behavior was exhibited) during the exposure period and the pre-exposure period. Our measure of plasticity was the change in that response from the predator-present treatment (P) to the predator-absent treatment (NP).

In this study, if there was a significant effect of treatment (*i.e.*, predator environment), or a significant effect of the interaction of treatment with body size at testing and/or age at maturation on the response variable, then predator-related plasticity was expressed.

To determine the effect of each predictor on the response variables, we used the *drop1* function to run chi-squared likelihood ratio tests and obtain p-values by comparing models with and without the predictors of interest. To validate our models, we checked all fixed/random effects and covariates for collinearity, and checked the residuals of all models for violations of assumptions of normality and homoscedasticity.

Consistency in Plasticity Across Contexts

To address the question of potential consistency in behavioral plasticity, we ran a Pearson's product-moment correlation in R using the function *cor.test.* This correlation compared the change in the behavioral response when the predator was present with the response when the predator was absent in the intrasexual social context (*i.e.*, plasticity: the change in response from P to NP), with that same measure of change in responses between treatments in the intersexual social context. Significant correlations indicate that for a given behavior, individual females are consistent in the degree of plasticity exhibited across the two contexts.

Alpha Correction for Multiple Tests

To account for multiple comparisons, we applied the Benjamini-Hochberg (B-H) procedure to control the false discovery rate across the multiple complex models applied to data from the same dataset (Benjamini and Hochberg 1995). We set the false discovery rate (FDR) at Q = 0.05 and adjusted critical p-values (P) to q-values (Q), by considering the effects of all predictors across the 10 models simultaneously. Each of the 150 significance values were then ranked and adjusted per the B-H procedure.

RESULTS

Effect of Predator on Plasticity in Anti-Predator Behaviors

There were no effects of the four- or any three- or two-way interactions on predator-related plasticity in anti-predator behaviors after controlling the false discovery rate (FDR). As a result, these interactions were dropped from the models for all anti-predator-related behavioral response variables (all $Q \geq 0.15$).

Time spent in Zone 1. There were no significant effects of context, body size at testing, or age at maturation on female time in Zone 1 (the third of the tank closest to the monitor displaying the stimulus) after controlling the FDR. Treatment significantly affected time spent in Zone 1 (Table 2.2); females spent less time in Zone 1 in the predator-present treatment compared with the predator-absent treatment (Figure 2.4). Females thus expressed predator-related plasticity in spatial positioning, but this was independent of context, body size at testing, and age at maturation.

Dart away from stimulus. There were no significant effects of context, treatment, body size at testing, or age at maturation on female darts from the stimulus after controlling the FDR (all $Q \geq 0.764$)

Remain motionless facing stimulus. There were no significant effects of context, treatment, body size at testing, or age at maturation on female time spent facing the monitor displaying the stimulus after controlling the FDR (all $Q \geq 0.638$).

Effect of Predator on Plasticity in Social Behaviors

Transverse display toward female. There was a significant effect of the four-way interaction between context, treatment, body size at testing, and age at maturation on female transverse displays after controlling the FDR (Table 2.3). Female body size at testing and age at maturation affected the expression of predator-related plasticity in transverse displays, and, the degree and direction of this plasticity differed across contexts (Figure 2.5). In the absence of a male, small females increased the time spent transverse displaying as age at maturation increased (Figure 2.6a). In the presence of a male, small females decreased the time spent transverse displaying as age at maturation increased (Figure 2.6b). Large females expressed the opposite trends of small females with respect to the relationship of body size at testing and age at maturation when transverse displaying in the absence (Figure 2.6a) and presence (Figure 2.6b) of a male.

Displace from female. There was a significant effect of the four-way interaction between context, treatment, body size at testing, and age at maturation on female displaces from non-focal female after controlling the FDR (Table 2.4). Female body size at testing and age at maturation affected the expression of predator-related plasticity in displacements from female, and, the degree and direction of this plasticity differed across contexts (Figure 2.7). In the absence of a male, small females increased the number of displacements as age at maturation increased (Figure 2.8a). In the presence of a male, small females decreased the number of displacements as age at maturation increased (Figure 2.8b). Large females expressed the opposite trends of small females with respect to the relationship of body size at testing with age at maturation when displacing from the non-focal female in the absence (Figure 2.8a) and presence (Figure 2.8b) of a male.

Other social behaviors. After controlling the FDR, there were no significant effects of any interactions, or of any individual fixed effects or covariates (all Q ≥ 0.171) on the other social behaviors tested (*i.e., charge at female, dart away from female, bite female, simultaneous circling*).

Effect of Predator on Plasticity in General Activity

Movement. After controlling the FDR, there were no significant effects of any interactions, or of any individual fixed effects or covariates (all Q ≥ 0.441) on movement throughout the trial.

Consistency in Individual Plasticity Across Contexts

Predator-related plasticity in the intrasexual context was not significantly correlated with predator-related plasticity in the intersexual context for any of the behaviors recorded in this study (Table 2.5).

DISCUSSION

Individual females expressed predator-related behavioral plasticity in three out of ten behaviors analyzed: time spent in Zone 1 (the third of the tank closest to the monitor playing the stimulus) (Table 2.2), transverse displaying toward the non-focal female (Table 2.3), and displacing away from the non-focal female (Table 2.4). The interaction between body size at testing and age at maturation affected the expression of predator-related plasticity in transverse displaying toward the non-focal female, and displacing from the non-focal female, but not time spent in Zone 1. Females did not express consistency in the degree of plasticity exhibited in a given behavior across contexts (Table 2.5). However, plasticity in some behaviors was positively correlated with other behaviors within contexts (Tables S1 and S2), such that individuals that expressed a high degree of predator-related plasticity in one behavior within one context also expressed a high degree of predator-related plasticity in another behavior in the same context. These results thus suggest that, within individuals, there is consistency in plasticity among some behaviors within social contexts, but that expression of behavioral plasticity is not consistent across social contexts.

Females expressed predator-related plasticity in the time spent in Zone 1 (Table 2.2, Figure 2.4). However, there were no effects of body size at testing or age at maturation on the expression of behavioral plasticity of time spent at Zone 1. On average, this plasticity was expressed to a similar degree and in the same direction in both the intrasexual social context and in the intersexual social contexts. Simply put, individuals moved away from the area with the predator when it was present, regardless of social context or individual traits. The effect of predator-presence on habitat-use behavior, such as spatial positioning, has been well documented in a variety of taxa (reviewed in Lima and Dill 1990), including males of this study species in the same laboratory setting

(Melie and Basolo *in revision*). Thus, our finding confirms that females perceived the presence of the predator on the video stimulus, and adjusted their behavior in order to reduce the perceived risk of predation.

There was an effect of the four-way interaction effect between social context, treatment, body size at testing, and age at maturation, on two agonistic behaviors: transverse display and displace from female (Tables 2.3 and 2.4). This means that female predator-related plasticity in these two agonistic behaviors was affected by the interaction between body size at testing and age at maturation, and this effect differed across social contexts. The first behavior, transverse display toward the non-focal female, is considered to be a threatening behavior (Beaugrand *et al*. 1984). The second behavior, displace from the non-focal female in response to an attack or lunge, is considered a defensive behavior (Beaugrand *et al*. 1984). Expression of the displace behavior followed very similar patterns to expression of the transverse display behavior, with respect to each of the four predictors of interest. This is because there was a strong correlation between expressions of plasticity for the two behaviors within each context; females that exhibited a high degree of plasticity in transverse displays toward non-focal females also exhibited a high degree of plasticity in displacements from non-focal females (Tables S1 and S2).

Overall, small females expressed a greater degree of plasticity in transverse displays and displaces from female in response to the predator than did large females, although not in a consistent manner across social contexts (Figures 2.5 and 2.7). In the intrasexual context (two females), both the small, early-maturing females and the small, late-maturing females expressed a greater degree of plasticity in transverse displays

(Figure 2.5a) and displacements (Figure 2.7a) than did large females (regardless of large female age at maturation). In the intersexual context (two females and a male), the degree of plasticity expressed in both transverse displays (Figure 2.5b) and displacements (Figure 2.7b) by small and large females (regardless of age at maturation) was similar in magnitude (*i.e.*, slope), but not in direction or elevation. Thus, it appears that age at maturation is strongly affecting small female plasticity in the intrasexual context, but is doing so to a lesser degree in the intersexual context, and, age at maturation is having less an effect on large female plasticity.

In the absence of a male (*i.e.*, in the intrasexual social context), small, early-maturing females reduced the expression of transverse displays and displacements in the presence of a predator, while small, late-maturing females' expression increased. This difference in response may be explained by varying motivations of these small individuals. Young individuals that have yet to reproduce should take fewer risks in order to best ensure survival in order to produce offspring (Bell 1980). The small, early-maturing (*i.e.*, relatively younger) females are among the smallest of the early-maturing genotype. If small individuals are at a greater risk of predation, we would expect small focal females to reduce time spent engaging with the relatively larger non-focal female, in order to reduce the risk of predation and ensure reproductive success. Small, late-maturing females (*i.e.*, relatively older) are among the smallest of the late-maturing genotype. Yet, these small females increased time spent interacting with non-focal females in the presence of a predator, despite their presumed susceptibility to predation as small individuals (Basolo and Wagner 2004). This difference in response between small, early-maturing females and small, late-maturing females may thus be attributable to the P-like-alleles that are determining age and size at sexual maturity. Females that are of the late-maturing phenotype, regardless of current body size, may be behaving as we would have expected large individuals to behave (*i.e.*, individuals that are invulnerable to predation are less risk-sensitive). This unexpected and potentially mismatched response could therefore be due to genetic association of the P-like-alleles with behavioral expression, rather than of current female body size.

In the presence of a male (*i.e.*, in the intersexual social context), the degree of plasticity expressed by small females was more similar to that of large females regardless of age at maturation, than in the absence of a male (intrasexual social context). The degree of plasticity expressed overall was reduced, as evidenced by the reduction of the slopes of the reaction norms (Figures 2.5 and 2.7). Male presence may make an environment more risky for females (Lima and Dill 1990; Martin and Wagner 2010), particularly in species like the green swordtails in which the male is highly elaborated. Because of this, we had predicted that all females would reduce behavioral expression when in the presence of both a predator and a male, but small females would do so to a greater degree. Instead, we found that predator-related plasticity in female-female social behaviors exhibited by all focal females was expressed to a lesser degree. These findings suggest that male presence may dampen female response to other females, as well as female response to a predator regardless of female size (and therefore regardless of female vulnerability to predation).

While we found it surprising that predator presence did not overall reduce behavioral expression, it is clear that expression of two agonistic behaviors, transverse display and displace, changed in the presence of a predator. As predicted, small females expressed a greater degree of predator-related plasticity than large females, though not always by reducing behavior in the presence of a predator. Because females that were similar in body size at testing but dissimilar in age at maturation expressed different degrees and directions of plasticity, we posit that there may be associated genetic effects of alleles that determine age and size at maturity on behavior expression for individuals of the early-maturing genotype. To our knowledge, this is the first evidence of such a possible genetic link of P-like-alleles with alleles that affect the expression of behavioral plasticity in this species.

Although the results suggest that females exhibit within-individual consistency in plasticity across behaviors within contexts (Tables S1 and S2), we found no evidence of within-individual consistency in plasticity within behaviors across contexts (Table 2.5). In fact, for seven of the ten behaviors that we tested, females, on average, did not express any notable degree of predator-related plasticity. However, when we consider the individual expression of behavioral plasticity for these same behaviors, we appear to see abundant inter-individual variation in both the direction and degree to which plasticity is expressed (Figures S1-S3). If significantly different, this apparent underlying variation in plastic responses among individuals within the same population is important to measure and evaluate. Individual variation in the expression of traits, such as plasticity in behavior, may affect the direction of evolution within a population (Nussey *et al*. 2007;

Dingemanse *et al*. 2010). An understanding of these individual differences in plasticity expression may better inform the ecological and evolutionary consequences of plasticity (Dingemanse and Wolf 2013).

The lack of intra-individual consistency in behavioral plasticity expressed across contexts may be explained by several reasons. First, focal females may differ in how highly they value the non-focal male in the intersexual social context. If a female is highly interested in mating with a male, her predation-risk sensitivity may be reduced in the male's presence, and increased in his absence. If a female is uninterested in a male, her risk sensitivity should be consistently high across contexts because we might expect more of her attention would be focused on assessing the environment rather than assessing a potential mate. Second, female-female competitive interactions in the presence of a male may also be informed by female interest in the male, male interest in either female, or both. Further, if some pairs of females are closer in body size to one another than other pairs, female interest in intrasexual competition may be higher in the presence of a male than in the absence, if closeness in body size increases the amount of intrasexual competition between the females. Thus, risk sensitivity in the presence of a male may be lower than in the absence. Third, male interest in either female may also inform female risk sensitivity, depending on a female's degree of reciprocal interest. Finally, there are a number of other individual traits that may affect the expression of behavioral plasticity across social contexts that we did not analyze, such as age at testing. Future analyses could address the impact of such traits.

Continuing to elucidate the extent to which there are intra- and inter-individual differences in behavioral plasticity within a population will allow researchers to gain a more thorough understanding of the evolution and maintenance of behavioral plasticity (Dingemanse and Dochtermann 2013). Future research regarding the amount of intra- and inter-individual consistency in behavioral plasticity that is expressed within a population should address how complex interactions of individual traits underlie the expression of such plasticity, in order to shed light on the answers to these questions.

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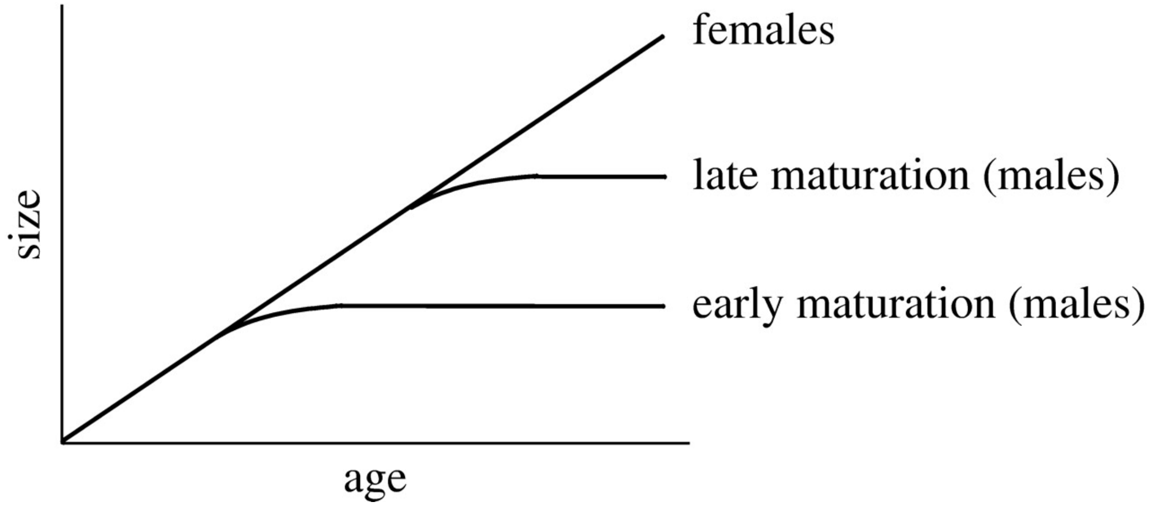


Figure 2.1 Antagonistic pleiotropy results in trade-offs between age and size at sexual maturation in platyfish, with alternate P-alleles affecting life histories differently (Reproduced from Basolo 2008).

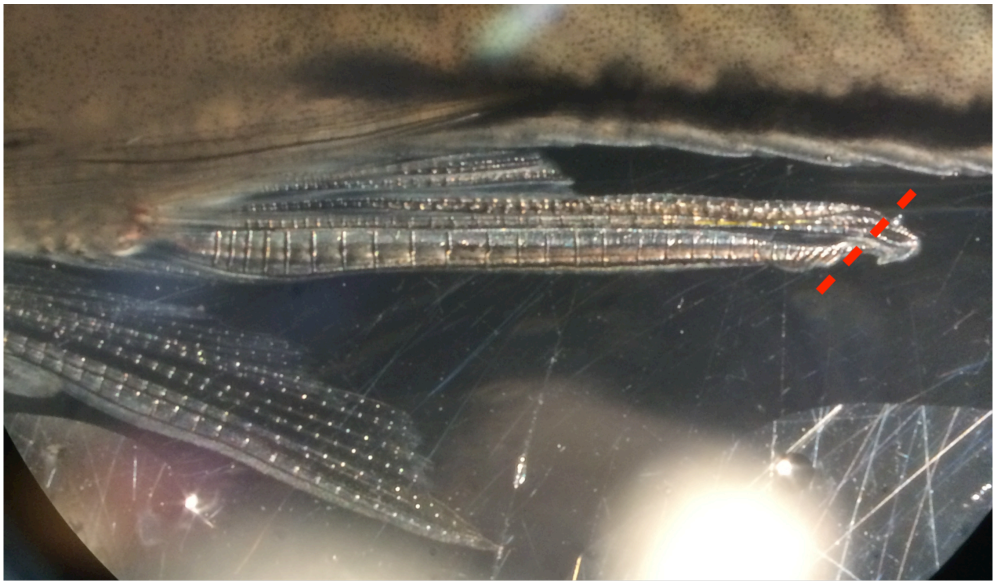


Figure 2.2 Fully developed gonopodium of a sexually mature green swordtail male. Dashed red line indicates location at which the distal hooks and claws were trimmed.

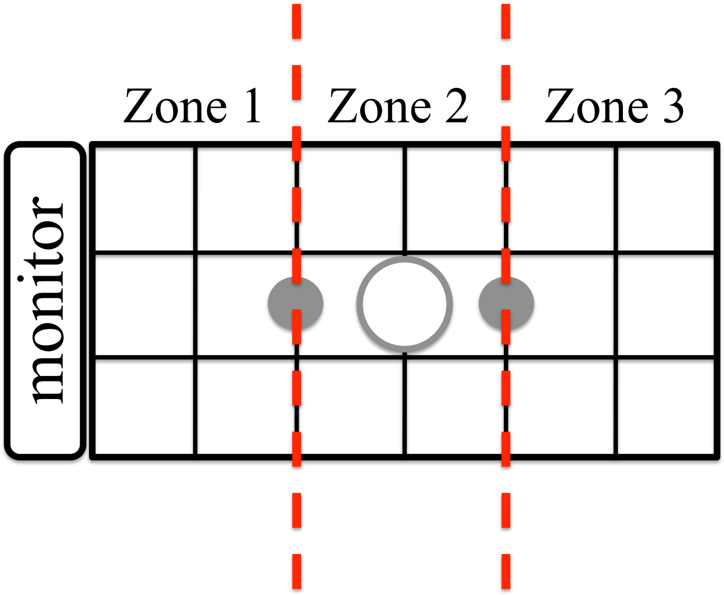


Figure 2.3 Top view of test tank with Zones demarcated.

Table 2.1 Behavioral responses measured in both contexts.

Behavior name	Type of behavior	Measurement method	Description of behavior
Time spent in Zone 1	Anti-predator	Duration (sec)	Amount of time spent in close proximity to the monitor, regardless of predator environment
Dart away from stimulus	Anti-predator	Count	Quick swim (*i.e.*, dart) from stimulus (*i.e.*, monitor displaying the stimulus) whether or not predator was on screen
Remain motionless facing stimulus	Anti-predator	Duration (sec)	Fish is unmoving at the bottom, top, or middle of the water column, body is oriented toward the monitor displaying the stimulus, regardless of predator environment
Transverse display toward female	Agonistic	Duration (sec)	Body shaped in an "S" or "C" curve, or, held straight perpendicularly to NFF
Displace from female	Agonistic	Count	Move or displace body/part of body in response to lunge or bite from NFF
Charge at female	Agonistic	Count	Charge or lunge at NFF
Dart away from female	Agonistic	Count	Quick swim (*i.e.*, dart) from NFF whether or not NFF had lunged at focal female
Bite female	Agonistic	Count	Bite NFF
Simultaneous circling	Agonistic	Duration (sec)	Focal and NFF circle one another head to tail (tight chase)
Movement	General activity	Count	Number of grids traversed

Note: NFF means non-focal female

Table 2.2 Linear mixed model investigating the effects of the predictors on time spent in Zone 1. Q is the Benjamini-Hochberg adjusted p-value.

Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size at Testing	3.811	8.330	0.209	0.647	0.932
Treatment	**-78.559**	**15.951**	**22.321**	**0.000**	**0.000**
Age at Maturation	5.333	7.799	0.467	0.494	0.911
Context	-9.294	15.951	0.339	0.560	0.932
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

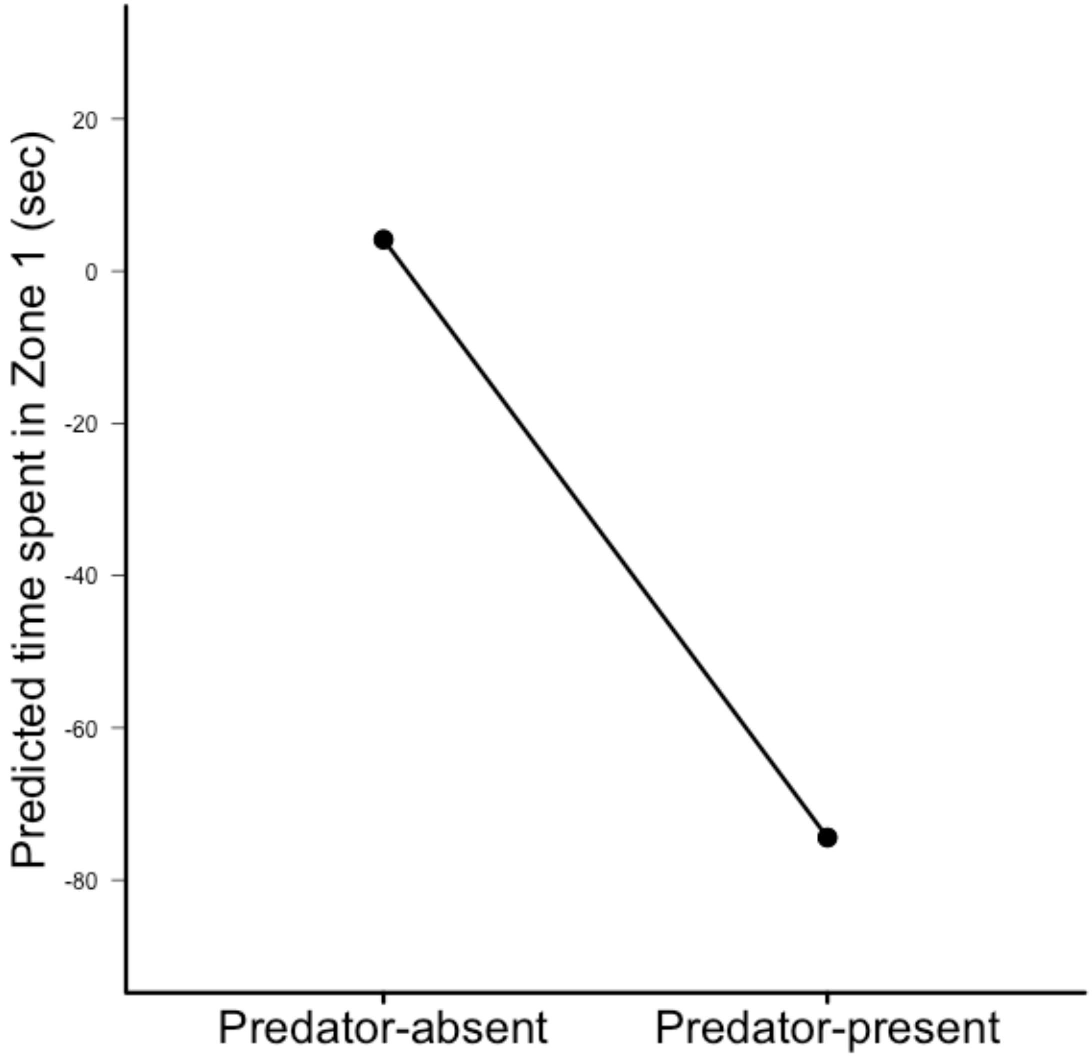


Figure 2.4 Behavioral reaction norm for the prediction of predator-related plasticity in time spent in Zone 1 (third of the tank nearest to the monitor that displayed a stimulus) (n=13 small females, n=21 large females).

Table 2.3 Linear mixed model investigating the effects of the predictors on time spent transverse displaying toward non-focal females by focal females. Q is the Benjamini-Hochberg adjusted p-value.

Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size at Testing * Treatment * Age at Maturation * Context	**111.774**	**28.571**	**14.260**	**0.000**	**0.000**
Body Size at Testing * Treatment * Age at Maturation	-20.138	15.320	1.713	0.191	0.702
Body Size at Testing * Treatment * Context	-52.884	27.049	3.752	0.053	0.442
Body Size at Testing * Age at Maturation * Context	-8.115	15.320	0.280	0.597	0.932
Treatment * Age at Maturation * Context	-49.582	25.327	3.762	0.052	0.442
Body Size at Testing * Treatment	7.098	14.036	0.255	0.613	0.932
Body Size at Testing * Age at Maturation	-3.015	8.722	0.119	0.730	0.932
Body Size at Testing * Context	14.383	14.036	1.045	0.307	0.818
Treatment * Age at Maturation	13.451	13.142	1.042	0.307	0.818
Treatment * Context	-46.836	26.879	2.992	0.084	0.525
Age at Maturation * Context	40.371	13.142	9.025	0.003	0.113
Body Size at Testing	3.947	7.714	0.261	0.610	0.932
Treatment	16.941	14.305	1.393	0.238	0.760
Age at Maturation	0.203	7.222	0.001	0.978	0.985
Context	10.553	14.305	0.543	0.461	0.910
Random Effects	*Variance*	*SE*			
Focal Fish	608.650	20.204			
Family	0.000	0.000			

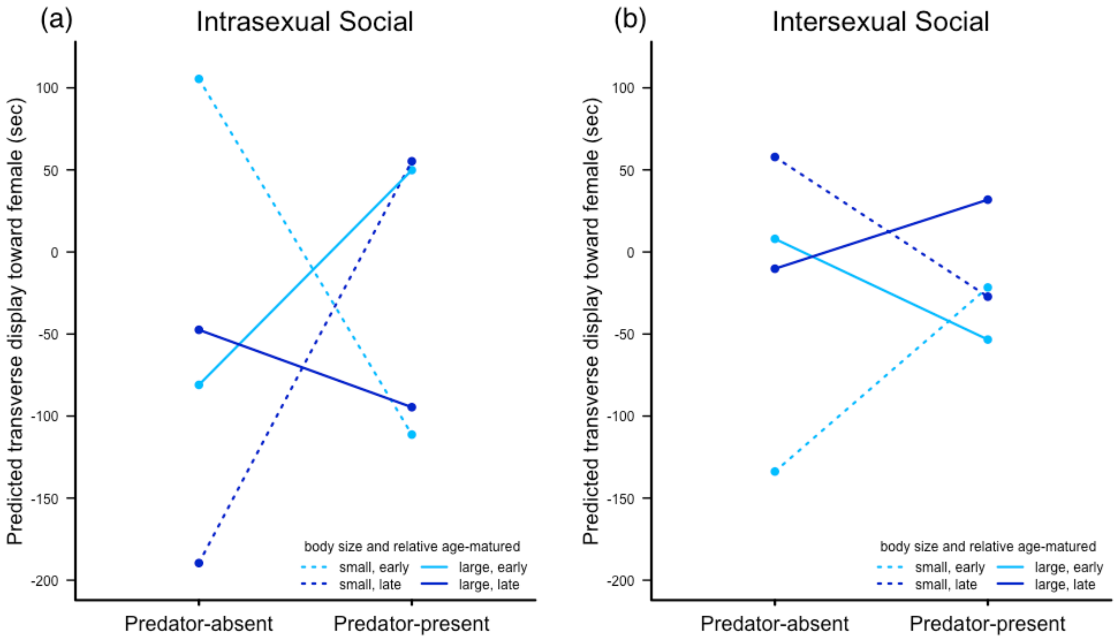


Figure 2.5 Behavioral reaction norms illustrating differences in the degree of predator-related plasticity in time spent transverse displaying toward non-focal females by focal females of varying body sizes at testing and ages at maturation in two contexts: (a) intrasexual social and (b) intersexual social.

Figure 2.6 Predicted plasticity in time spent transverse displaying toward non-focal females by focal females as a function of focal female body size at testing and age at maturation, in two contexts: (a) intrasexual social and (b) intersexual social (n=13 small females, n=21 large females).

Table 2.4 Linear mixed model investigating the effects of the predictors on number of focal displaces from non-focal females. Q is the Benjamini-Hochberg adjusted p-value.

Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size at Testing * Treatment * Age at Maturation * Context	**27.807**	**7.091**	**14.324**	**0.000**	**0.000**
Body Size at Testing * Treatment * Age at Maturation	-2.361	3.803	0.385	0.535	0.922
Body Size at Testing * Treatment * Context	-12.310	6.715	3.306	0.069	0.477
Body Size at Testing * Age at Maturation * Context	-1.295	3.803	0.116	0.733	0.932
Treatment * Age at Maturation * Context	-10.788	6.288	2.902	0.088	0.528
Body Size at Testing * Treatment	2.473	3.444	0.514	0.473	0.910
Body Size at Testing * Age at Maturation	0.854	2.381	0.129	0.720	0.932
Body Size at Testing * Context	2.536	3.444	0.541	0.462	0.910
Treatment * Age at Maturation	5.018	3.225	2.393	0.122	0.634
Treatment * Context	-3.147	6.595	0.227	0.633	0.932
Age at Maturation * Context	7.907	3.225	5.842	0.016	0.245
Body Size at Testing	0.217	2.106	0.011	0.918	0.976
Treatment	5.015	3.436	2.108	0.147	0.638
Age at Maturation	0.272	1.972	0.019	0.890	0.976
Context	2.338	3.436	0.462	0.497	0.911

Random Effects	*Variance*	*SE*
Focal Fish	61.340	5.832
Family	0.000	0.000

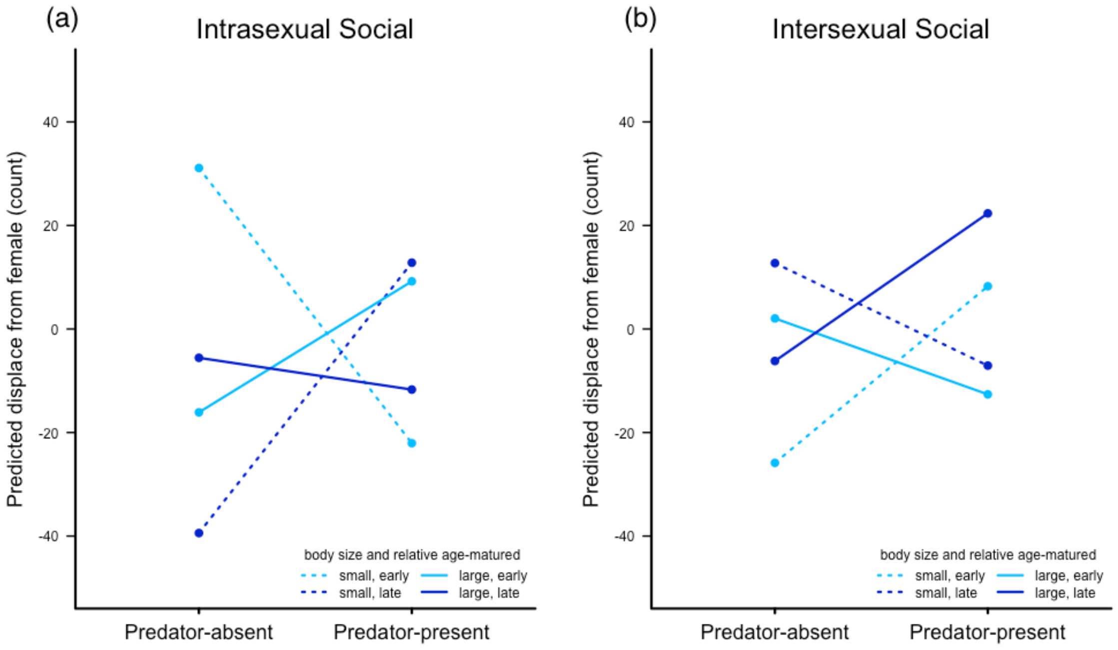


Figure 2.7 Behavioral reaction norms illustrating differences in the degree of predator-related plasticity in number of focal displaces from non-focal females of varying body sizes at testing and ages at maturation in two contexts: (a) intrasexual social and (b) intersexual social.

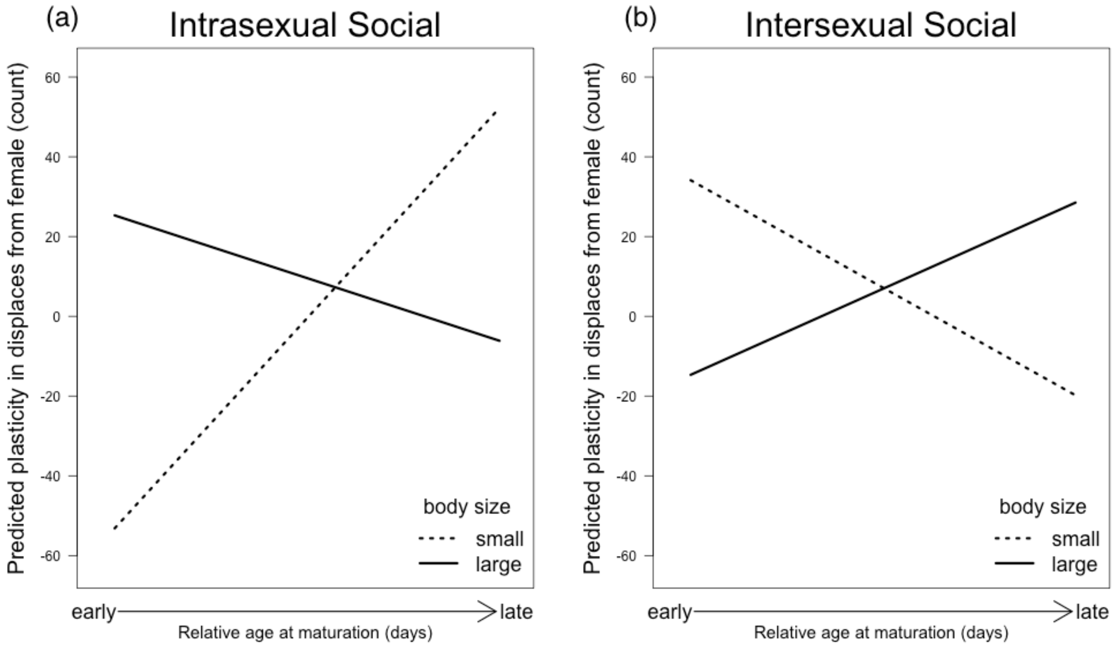


Figure 2.8 Predicted plasticity in number of focal displaces from non-focal females as a function of body size at testing and age at maturation, in two contexts: (a) intrasexual social and (b) intersexual social (n=13 small females, n=21 large females).

Table 2.5 Pearson's product-moment correlation between plasticity measures of the same behavior in two social contexts. Q is the Benjamini-Hochberg adjusted p-value.

Plasticity measures correlated	*df*	*P*	*Q*	*Pearson's correlation coefficient*
Time spent in Zone 1	32	0.353	0.832	-0.164
Dart away from stimulus	32	0.315	0.832	0.177
Remain motionless facing stimulus	32	0.745	0.832	0.058
Transverse display toward female	32	0.624	0.832	-0.087
Displace from female	32	0.614	0.832	-0.090
Charge at female	32	0.749	0.832	0.057
Dart away from female	32	0.989	0.989	0.002
Bite female	32	0.314	0.832	0.178
Simultaneous circling	32	0.107	0.832	0.281
Movement	32	0.533	0.832	-0.111

APPENDIX

Table S1 Pearson's product-moment correlation between plasticity measures of different behaviors within the intrasexual social context. Q is the Benjamini-Hochberg adjusted p-value.

Plasticity measures correlated		*df*	*P*	*Q*	*Pearson's correlation coefficient*
Dart away from stimulus	Displace from female	34	0.51	0.84	0.12
Dart away from stimulus	Simultaneous circling	34	0.32	0.79	0.17
Dart away from stimulus	**Remain motionless facing stimulus**	**34**	**0.00**	**0.00**	**0.56**
Dart away from stimulus	Transverse display toward female	34	0.63	0.89	-0.09
Dart away from stimulus	Movement	34	0.58	0.84	0.10
Dart away from stimulus	Time spent in Zone 1	34	0.65	0.89	0.08
Remain motionless facing stimulus	Transverse display toward female	34	0.01	0.06	-0.43
Remain motionless facing stimulus	Movement	34	0.96	0.98	0.01
Remain motionless facing stimulus	Time spent in Zone 1	34	0.82	0.94	-0.04
Transverse display toward female	Movement	34	0.99	0.99	0.00
Transverse display toward female	Time spent in Zone 1	34	0.48	0.84	-0.12
Displace from female	Simultaneous circling	34	0.05	0.23	0.35
Displace from female	Remain motionless facing stimulus	34	0.27	0.71	-0.19

Displace from female	**Transverse display toward female**	**34**	**0.00**	**0.00**	**0.84**
Displace from female	Movement	34	0.55	0.84	0.11
Displace from female	Time spent in Zone 1	34	0.44	0.84	-0.14
Charge at female	Dart away from female	34	0.75	0.89	0.06
Charge at female	Dart away from stimulus	34	0.24	0.71	-0.21
Charge at female	**Displace from female**	**34**	**0.00**	**0.00**	**0.58**
Charge at female	**Simultaneous circling**	**34**	**0.00**	**0.00**	**0.47**
Charge at female	Remain motionless facing stimulus	34	0.19	0.64	-0.23
Charge at female	**Transverse display toward female**	**34**	**0.00**	**0.00**	**0.66**
Charge at female	Movement	34	0.51	0.84	-0.12
Charge at female	Time spent in Zone 1	34	0.01	0.06	-0.46
Dart away from female	Dart away from stimulus	34	0.16	0.60	-0.24
Dart away from female	Displace from female	34	0.88	0.94	0.03
Dart away from female	Simultaneous circling	34	0.95	0.98	0.01
Dart away from female	Remain motionless facing stimulus	34	0.03	0.15	-0.38
Dart away from female	Transverse display toward female	34	0.87	0.94	0.03
Dart away from female	Movement	34	0.34	0.79	-0.17

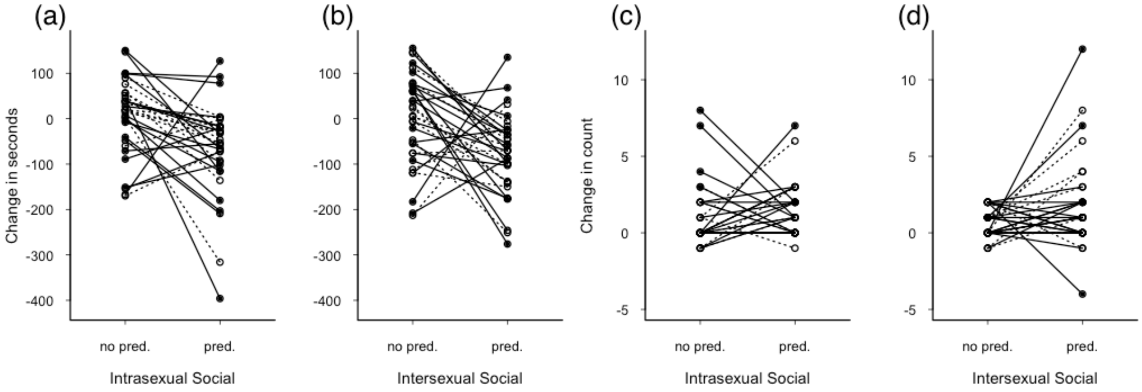
Dart away from female	Time spent in Zone 1	34	0.71	0.89	0.07
Bite female	Charge at female	34	0.27	0.71	0.19
Bite female	Dart away from female	34	0.69	0.89	-0.07
Bite female	Dart away from stimulus	34	0.13	0.53	0.26
Bite female	Displace from female	34	0.20	0.64	0.23
Bite female	Simultaneous circling	34	0.86	0.94	-0.03
Bite female	Remain motionless facing stimulus	34	0.71	0.89	0.07
Bite female	Transverse display toward female	34	0.54	0.84	0.11
Bite female	Movement	34	0.37	0.79	-0.16
Bite female	Time spent in Zone 1	34	0.75	0.89	0.06
Simultaneous circling	Remain motionless facing stimulus	34	0.58	0.84	-0.10
Simultaneous circling	**Transverse display toward female**	**34**	**0.00**	**0.00**	**0.54**
Simultaneous circling	Movement	34	0.54	0.84	-0.11
Simultaneous circling	Time spent in Zone 1	34	0.57	0.84	-0.10
Movement	Time spent in Zone 1	34	0.35	0.79	0.16

Table S2 Pearson's product-moment correlation between plasticity measures of different behaviors within the intersexual social context. Q is the Benjamini-Hochberg adjusted p-value.

Correlation of plasticities within the intersexual social context		*df*	*P*	*Q*	*Pearson's correlation coefficient*
Dart away from stimulus	Displace from female	34	0.72	0.93	-0.06
Dart away from stimulus	Simultaneous circling	34	0.09	0.34	-0.30
Dart away from stimulus	Remain motionless facing stimulus	34	0.05	0.25	0.33
Dart away from stimulus	Transverse display toward female	34	0.29	0.73	-0.19
Dart away from stimulus	Movement	34	0.81	0.93	0.04
Dart away from stimulus	Time spent in Zone 1	34	0.89	0.93	0.02
Remain motionless facing stimulus	Transverse display toward female	34	0.03	0.19	-0.36
Remain motionless facing stimulus	Movement	34	0.47	0.93	0.13
Remain motionless facing stimulus	Time spent in Zone 1	34	0.65	0.93	-0.08
Transverse display toward female	Movement	34	0.75	0.93	0.06
Transverse display toward female	Time spent in Zone 1	34	0.81	0.93	-0.04
Displace from female	Simultaneous circling	34	0.72	0.93	0.06
Displace from female	Remain motionless facing stimulus	34	0.26	0.69	-0.20
Displace from female	**Transverse display toward female**	**34**	**0.00**	**0.00**	**0.79**

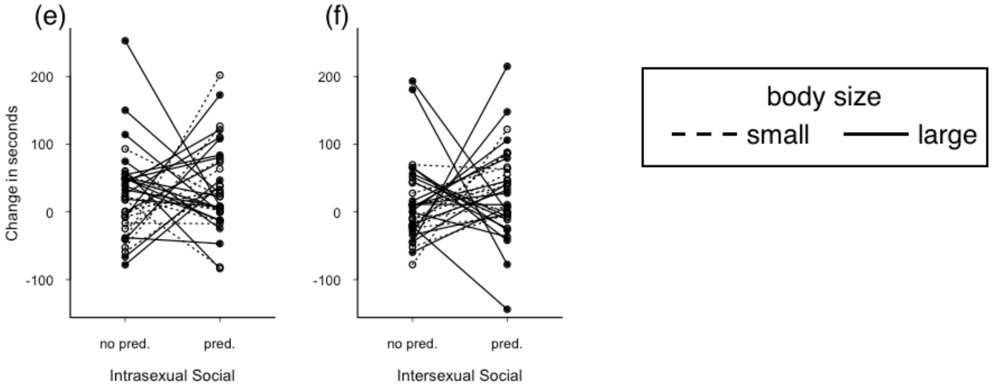
Displace from female	Movement	34	0.11	0.35	0.28
Displace from female	Time spent in Zone 1	34	0.83	0.93	-0.04
Charge at female	Dart away from female	34	0.55	0.93	-0.11
Charge at female	Dart away from stimulus	34	0.06	0.25	-0.33
Charge at female	**Displace from female**	**34**	**0.00**	**0.00**	**0.55**
Charge at female	Simultaneous circling	34	0.37	0.79	0.16
Charge at female	Remain motionless facing stimulus	34	0.04	0.23	-0.36
Charge at female	**Transverse display toward female**	**34**	**0.00**	**0.00**	**0.49**
Charge at female	Movement	34	0.74	0.93	-0.06
Charge at female	Time spent in Zone 1	34	0.90	0.93	0.02
Dart away from female	Dart away from stimulus	34	0.65	0.93	0.08
Dart away from female	Displace from female	34	0.23	0.65	-0.21
Dart away from female	Simultaneous circling	34	0.11	0.35	-0.28
Dart away from female	Remain motionless facing stimulus	34	0.88	0.93	-0.03
Dart away from female	Transverse display toward female	34	0.16	0.48	-0.25
Dart away from female	Movement	34	0.91	0.93	0.02
Dart away from female	Time spent in Zone 1	34	0.70	0.93	-0.07

Bite female	Charge at female	34	0.83	0.93	0.04
Bite female	Dart away from female	34	0.32	0.76	-0.18
Bite female	Dart away from stimulus	34	0.84	0.93	0.03
Bite female	**Displace from female**	**34**	**0.00**	**0.00**	**0.49**
Bite female	Simultaneous circling	34	0.60	0.93	-0.09
Bite female	Remain motionless facing stimulus	34	0.79	0.93	-0.05
Bite female	**Transverse display toward female**	**34**	**0.00**	**0.00**	**0.56**
Bite female	Movement	34	0.98	0.98	0.01
Bite female	Time spent in Zone 1	34	0.62	0.93	-0.09
Simultaneous circling	Remain motionless facing stimulus	34	0.55	0.93	-0.11
Simultaneous circling	Transverse display toward female	34	0.03	0.19	0.36
Simultaneous circling	Movement	34	0.81	0.93	-0.04
Simultaneous circling	Time spent in Zone 1	34	0.06	0.25	0.33
Movement	Time spent in Zone 1	34	0.36	0.79	-0.16



Time spent in Zone 1

Dart away from stimulus



Remain motionless facing toward the stimulus

Figure S1 Behavioral reaction norms illustrating inter-individual variation in predator-related plasticity in anti-predator behaviors across social contexts (n=13 small females, n=21 large females).

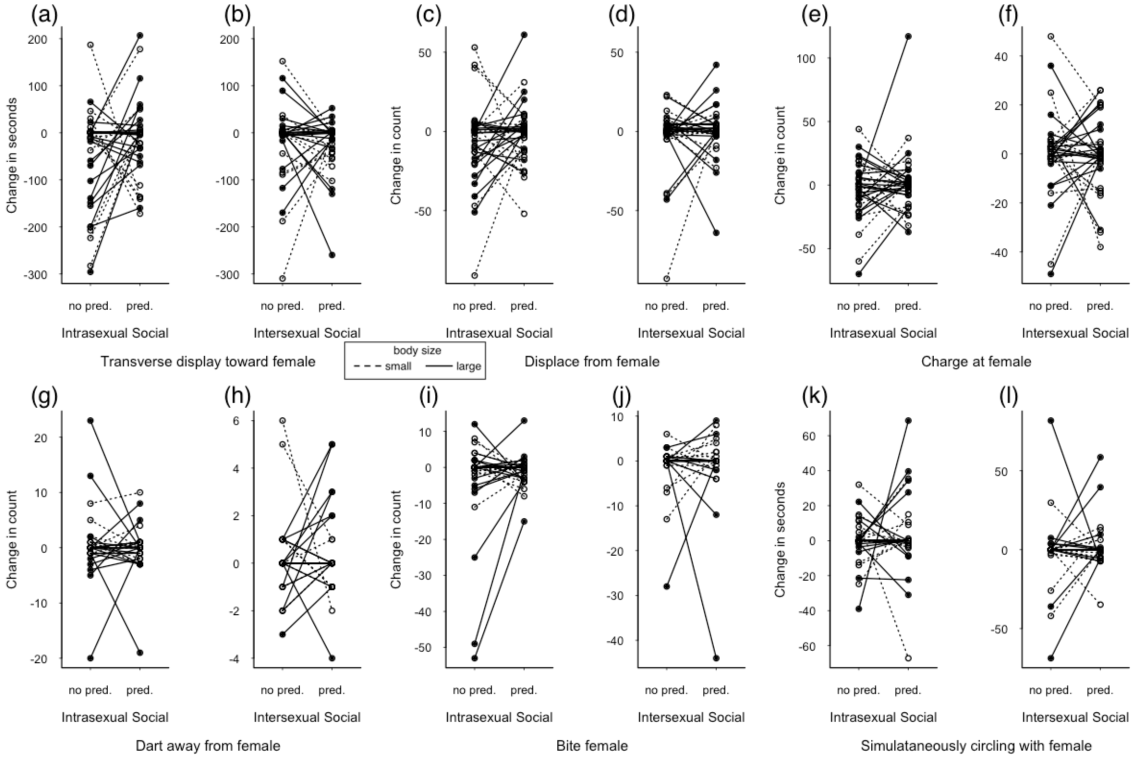


Figure S2 Behavioral reaction norms illustrating inter-individual variation in predator-related plasticity in social behaviors across social contexts (n=13 small females, n=21 large females).

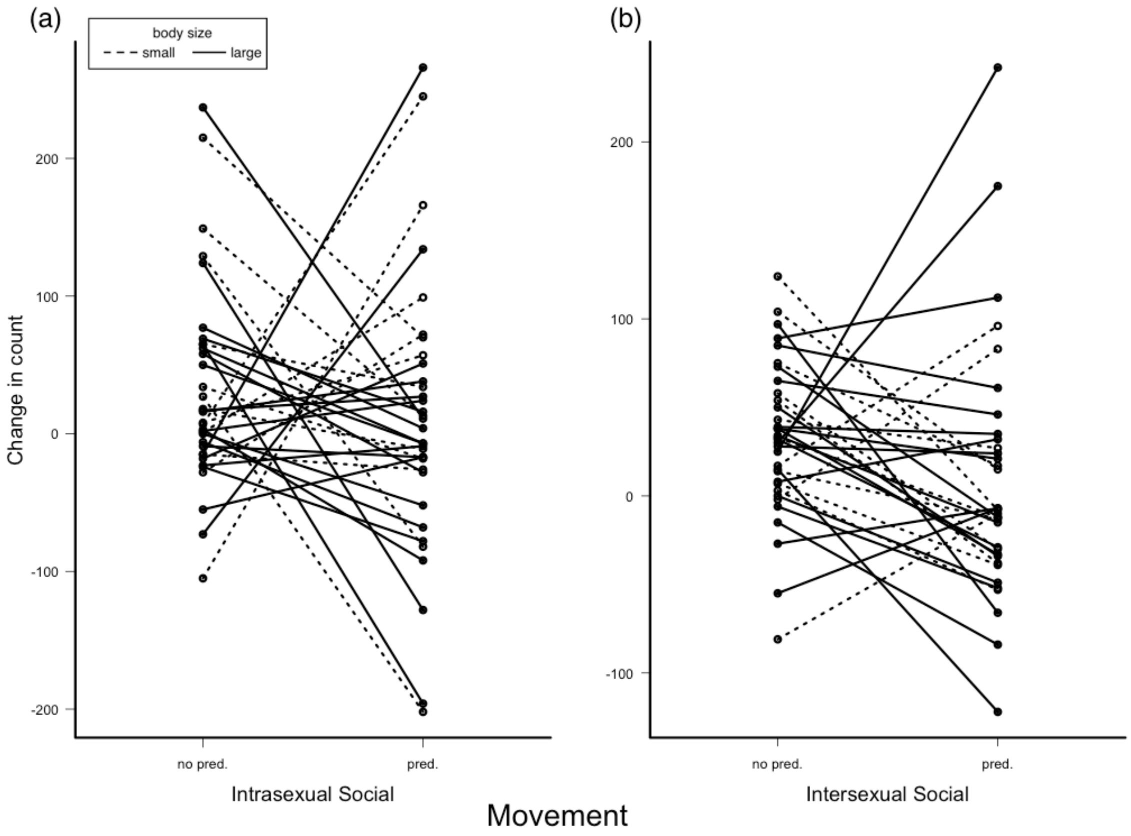


Figure S3 Behavioral reaction norms illustrating inter-individual variation in predator-related plasticity in movement across social contexts (n=13 small females, n=21 large females).