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The Effect of Visual Obstructions on the Sexual Behavior of Guppies: The Importance of Privacy

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Summary

Sexual selection is the outcome of behavioral interactions within and between the sexes. Numerous studies show how individuals modify their behavior in response to ecological or social conditions, and such changes may therefore affect the evolutionary outcome of sexual selection. This study examined the effect of habitat structure on the sexual behavior of male and female guppies (*Poecilia reticulata*). I wished to determine whether the ability of males to observe other courting males would affect the rates of male courtship, courtship interference, and the sexual responsiveness of females. Specifically, I manipulated visibility using opaque barriers in laboratory aquaria and found that there was less male interference behavior in aquaria containing visual obstructions than in aquaria without barriers, regardless of whether the male was courting virgin ("responsive") or non-virgin ("unresponsive") females. In addition, the sexual responsiveness of virgin females to male displays was significantly increased, and the frequency of male displays when courting virgin females was significantly reduced in aquaria with barriers. The presence of visual barriers, however, did not appear to affect the rates of male courtship displays to non-virgins or the responsiveness of non-virgins.

Evidently, the barriers impede visibility enough that males are less likely to observe and interfere with the courtship activity of other males, and therefore, females are less likely to flee or lose interest in a courting male. Such habitat related changes in male-male competition and female responsiveness could potentially affect female choice and the evolutionary outcome of sexual selection.

Introduction

Sexual selection is an evolutionary process that was suggested by Charles Darwin to account for the evolution of secondary sexual traits. Under sexual selection, a category of natural selection, individuals with traits that improve their ability to obtain proportionately more mates than others will pass on genes to the next generation, leading to evolutionary elaboration of those heritable traits. The evolutionary outcome of sexual selection is determined by behavioral interactions within and between the sexes. Numerous studies have shown that individuals modify their behavior in response to ecological or social conditions, such as population density, sex ratio, predation regime, and the visual environment, and interactions between individuals may differ as well. Thus, any variation in these behaviors or interactions between individuals may affect the pattern of sexual selection and its evolutionary consequences. Therefore, in order to gain a better understanding of the evolution of specific, elaborate secondary sexual characteristics, it is important to study the ecological factors and behavioral mechanisms that may influence the outcome of sexual selection (Endler, 1993).

Gaining insight on how the sexual behavior of natural populations is influenced by alterations in their ecological and social environment is crucial if we as a human society are to understand and predict how our activities may affect the process of sexual selection and its evolutionary consequences for individual populations. Considering that the sexual behaviors and mating systems of natural populations interact in complex and flexible ways with the physical and biotic environment to produce the traits and behaviors we see in different species, we must be aware of the vulnerability of such interactions to change. If we plan on conserving the natural state of our world's populations, we need to expand our understanding of how our actions specifically affect the operation of natural and sexual selection processes in different populations and how we can coexist in an undisruptive manner.

Guppies (*Poecilia reticulata*) are small live-bearing fish well known for their conspicuous color patterns and for the incessant courtship of males and females. The nature of the guppy mating system makes guppies especially amenable to studies of sexual selection and mate choice, and previous work on guppy sexual behavior has already provided much needed information on how sexual selection may operate in natural populations (Houde, 1997). Guppies are native to northeastern South America, and are especially abundant and widely studied in Trinidad, West Indies. Males are brightly colored and vary greatly in their color patterns within and between populations, whereas females are not colored (Houde, 1997). Males in most populations devote much of their time and effort to pursuing females and performing courtship displays (Houde 1997).

Male guppies exhibit two alternative mating tactics. The courtship display or "sigmoid" display is a very obvious and showy behavior in which a male positions himself in front of a female and arches his body into an S-shape while extending his fins and quivering (Baerends et al., 1955; Liley, 1966). This is a courtship behavior directed toward females in which the male attempts to gain the female's attention and eventual copulation. Alternatively, a male may attempt to "sneak" copulate with the female using gonopodial thrusting without displaying or receiving a receptive response from her. Male guppies achieve internal fertilization using the gonopodium, a modified anal fin, to transfer sperm, and compete with one another for the opportunity to mate with females by interfering on the courtship of other males. Female guppies are responsive to courting males only when sexual maturity is first reached or following the birth of offspring.

Female choice, a mechanism of sexual selection, has been implicated in the evolution of conspicuous color patterns in the guppy (Anderson, 1994, as cited in Houde, 1997). Female guppies display preferences among males, choosing males based partly on color pattern. While females tend to prefer those males with a greater degree of orange coloration, variation from population to population in preference for orange coloration does exist, with some females preferring large amounts of orange coloration, and others showing preference for other colors, distastes for certain colors, or no preference at all (Houde, 1988a; Houde & Endler, 1990; Endler & Houde, 1995). In addition, males

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vary in the intensity and extent of orange coloration between populations, with a general trend for the degree of female preference for orange coloration in a population to match the degree of expression of orange coloration in males in that population (Houde & Endler, 1990).

Some studies of guppies suggest that mate choice by female guppies and its evolutionary outcome can vary depending on ecological or social factors that may differ between populations (Eakley & Houde, 2004; Godin & Briggs, 1996; Gong, 1997; Gong & Gibson, 1996; Houde & Hankes, 1997; Hughes et al., 1999; Magurran & Seghers, 1994; Rosenqvist & Houde, 1997). For example, when predators are present, female guppies reduce their level of choosiness relative to situations when predators are absent (Godin & Briggs, 1996; Gong, 1997; Gong & Gibson, 1996). Further, the color patterns of males previously experienced by females affect both the degree of choosiness (Rosenqvist & Houde, 1997) and the specific male phenotypes females prefer (Eakley & Houde, 2004; Hughes et al., 1999). Finally, an apparent mismatch between preferences demonstrated by females and average color pattern characteristics expressed by males in one Trinidad guppy population suggests that environmental conditions in this population may be preventing females from mating with males they prefer (Houde & Hankes, 1997).

Courtship disruption is important in mating systems of a number of species. Specifically, males interfere in the copulation attempts of other males by attempting to court a female already attended by a male. Such courtship interference has been shown to affect the interaction of males and females and the outcome of sexual selection in a number of species including icterid birds (Webster & Robinson, 1999), dung flies (Krausharr & Blackenhorn, 2002), and the red spotted newt (Gabor et al., 2000). In addition, as is suggested by a study involving sand gobies (Kangas & Lindstrom, 2001), this harassment by intruding males may affect the sexual behavior of females and could constrain females' ability to exercise mate choice. In guppy courtship, a courting sequence will not proceed beyond a male's initial displays unless the female shows a sexual response. A receptive female may make a gliding approach to a displaying male, referred to as a "sexual response" (Liley, 1966; Houde, 1997), which is a clear indication of the female's sexual interest in that male and has been used to assess female mating preferences (e.g. Houde, 1987; Reynolds & Gross, 1992). Thus, female responsiveness is a necessary step toward copulation and is a prerequisite to mate choice. Hence, any factor that affects female responsiveness may in turn affect female choice.

It has become increasingly evident that in many species, including guppies, sexual selection is likely to be affected by factors in the ecological and social environment in which they live and mate. My study examined the influence of habitat structure on the sexual behavior of male and female guppies (*Poecilia reticulata*) in the laboratory. Specifically, I wanted to determine the effect of the presence of obstructions to visibility on the frequency of male courtship and interference behavior and female sexual responsiveness. I predicted that the ability of males to observe the courtship activities of other males might have an effect on the frequency of courtship interference behaviors by male guppies, and that this might in turn affect the sexual behavior of virgin females interacting with these males. I tested these predictions by manipulating the structure of guppy habitats in the laboratory using opaque barriers and observing the effect of such visual obstruction on male courtship and interference behavior, as well as on female sexual responsiveness to male displays in experimental groups of guppies. Males had direct and unobstructed visual contact with other courting males in aquaria without barriers, while visual contact was reduced in aquaria with barriers. My specific prediction was that an environment in which fewer visual obstructions are present would allow for more male-male competition and interference behavior to occur relative to an environment with more visual obstructions. I also predicted that such increased courtship interruption would lead to a decrease in the frequency of courtship sequences that reach the point where females show a positive response to male display. Ultimately, the structure of the habitat could potentially affect the ability of females to exercise choice and the mating success of preferred males, therefore influencing the outcome of sexual selection.

Literature Review: The Vulnerability of Sexual Selection to Environmental and Social Factors

Sexual selection is a special case of natural selection, in that certain characteristics of organisms are selected for which do not appear to have survival value. For example, secondary sexual characteristics, such as the elaborate colors and plumes of tropical birds, as well as the showy courtship displays of many species do not appear to enhance survival in any way and seem to be ornamental in nature. In fact, these characteristics most likely have physiological trade-offs, such as increased energy expenditure or reduced maneuverability, and may actually detract from survival perhaps by increasing the animal's risk of predation or reducing time available for foraging.

Darwin (1871) suggested that sexual selection arises due to an unequal distribution of mating success among individuals of the same sex. Hence, the elaborate secondary sexual characteristics exhibited by many species must have evolved as a result of their enhancement of the mating success of individuals. In this case, individuals with particular characteristics obtain more matings than individuals with different characteristics. In other words, phenotypic variation in a character in one sex (usually males) leads to differential mating success. If the variation in that character is heritable, then the differences in mating success of individuals will lead to evolutionary change of the character from generation to generation. Hence, this explanation defines sexual selection as the process by which differential mating success can lead to the evolution of elaborate secondary sexual characteristics.

The ability of certain individuals to obtain proportionately more matings than others could be due either to preferences expressed by the opposite sex, known as mate choice or intersexual selection, or occur as a result of competition within a sex, termed intrasexual selection (Houde, 1997). In intrasexual selection, members of one sex, usually males, compete among one another for matings with members of the opposite sex. In intersexual selection, members of one sex, usually females, exhibit preferences for certain traits among mates of the opposite sex and choose their mates based on those traits (Houde, 1997). Therefore, if a character improves an individual's chance of being chosen as a mate by the opposite sex, that character can evolve, becoming prevalent in the population.

Because mate choice and competition can be observed and quantified, we may be able to gain a better understanding of the evolution of elaborate secondary sexual characteristics and hence the evolutionary outcome of sexual selection by studying these behavioral mechanisms. Considering that in most species males are the competitors and females are the choosers (Kangas & Lindstrom, 2001), many studies have evaluated the contributions of female mate choice and male-male competition as mechanisms of sexual selection. In the broadest sense, female choice operates whenever a female influences what male will sire her offspring regardless of the means by which she brings this about. Positive female responses, such as moving toward the male or soliciting him, as well as negative responses, such as moving away from a suitor or rejecting him, are both indicators of female choice and may be instrumental in determining with whom a female will mate. Therefore, any factor that affects these response behaviors will in turn influence female choice. Male-male competition operates when a male interferes in the courtship of or attempts to solicit the attention of a female away from another male in an effort to win or maintain the female for himself.

The strength of mate choice and the intensity of sexual competition may be determined by factors that affect these behaviors. Female choosiness and male-male competition vary as a result of genetic and facultative behavioral responses to environmental conditions (Houde, 1997). The intensity of sexual selection, resulting from female mate choice and male-male competition, depends on the environmental conditions experienced by a population and the extent to which these conditions affect the sexual behavior of males and females in that population.

Many studies have been conducted in an attempt to identify ecological and social factors that may impact sexual selection. For example, costs associated with the often conspicuous activities of courtship and copulation, such as increased risk of predation, should affect the behavioral decisions of individuals. In fact, studies suggest that females respond adaptively to the risk of predation by altering their mating preferences. Godin and Briggs (1996) confirmed that following predator exposure, female guppies tend to reduce their overall level of sexual activity and switch their mating preferences in favor of duller (i.e. less conspicuous) males. More specifically, their findings suggest that females can adaptively adjust their degree of choosiness in response to a perceived increase in predation risk by reducing their association with the more brightly colored, actively courting males generally preferred by females (Houde, 1987) and instead choose to associate with duller males expected to draw less attention from predators. As a result, costs associated with mate choice are able to constrain evolution by leading females to alter patterns of mate choice thus changing the outcome of sexual selection.

Predators have also been shown to mediate changes in male courtship behavior. For example, male guppies exhibit responsiveness to risk and have been shown to switch from a mating strategy of cooperative courtships with females to a less conspicuous coercive mating strategy, known as gonopodial thrusting, under increased risk of predation (Magurran & Seghers, 1990). While this behavioral transition has been interpreted as a risk-sensitive response by males designed to make them less conspicuous to predators (Endler, 1987), Evans et al. (2002) demonstrated that the switch to coercive mating by male guppies in high risk situations is actually mediated by adjustments in female behavior rather than directly by the predator. In high-risk environments, females show more enhanced anti-predator responses, including predator inspections, increased schooling and less attentiveness to males, all of which may result in coercive mating attempts being more profitable (Houde, 1997). Therefore, in this case, the altered sexual behavior of males appears to be an attempt to exploit the females' preoccupation with observing and avoiding predators rather than a method of predation avoidance. In addition, considering that a successful gonopodial thrust completely circumvents female choice in the mating process, an increase in such uncooperative or forced matings in a population in effect contributes to the undermining of female choice. Therefore, while the earlier study demonstrated a reduction in female choosiness as a result of predation, predation in this case presents the potential to completely eliminate the contribution of female choice to sexual selection (i.e., to counteract the effects of sexual selection).

In addition to guppies, male tungara frogs curtail conspicuous activities associated with reproduction when in the presence of predators by reducing the intensity of their mating calls in response to simulated attacks by model bats (Ryan, 1985, as cited in Evans et al., 2002). Similar examples of the predator-mediated changes in courtship behavior exhibited by male guppies have also been reported in insects, crustaceans, and a number of fish species (Sih, 1988; Koga et al., 1998; Forsgren & Magnhagen, 1993; Chivers et al., 1995; Candolin, 1997; as cited in Evans et al., 2002), and predation risk has been experimentally shown to directly influence preference and mate choice in the cricket (Hedrick & Dill, 1993; as cited in Evans et al., 2002) as well as fish species including gobiid fish, pipefishes, and sand gobies (Forsgren, 1992; Berglund, 1993; Forsgren & Magnhagen, 1993; as cited in Houde, 1997). It is evident that predation is one factor that has the ability to counteract the effects of sexual selection.

While ecological factors such as predation risk have the potential to significantly influence male and female sexual behavior and alter the outcome of sexual selection, variation in social environment may produce selection on morphological and behavioral traits as well (Hettyey & Pearman, 2003). It has been noted that although males defend females in all colonial blackbirds studied to date, the specific mating tactics employed by males differ across species (Webster & Robinson, 1999). In some species, males defend whole groups of females, whereas in others males defend single females. By studying the mating system of these colonial blackbirds, Webster and Robinson (1999) were able to shed light on the forces that lead to variation in individual mating strategies across and within species. Data collected in their comparison of two blackbird species, the Montezuma oropendola and yellow-rumped cacique, revealed that variation in male blackbird mating tactics occurs as a result of adaptive responses to increased colony size. They found that the frequency of disruptions by male blackbirds increases with greater colony size (the number of females nesting there), and that successful copulations are consequently less likely to occur at large colonies relative to small colonies. Therefore, as colony size increases males respond to their social environment by spending less time at the colony defending multiple females, and switch to consorting individual females away from the colony instead. This adoption by male blackbirds of strategies that reduce the likelihood of courtship disruption is a clear demonstration of behavioral plasticity in response to social environmental factors and an indication that factors affecting the social environment of a population, such as density, may affect mating success and the outcome of sexual selection.

Similarly, Hettyey and Pearman (2003) investigated the role of reproductive interference in generating variation in demography by examining the relationship among social environment, reproductive interference, and female reproductive success in an anuran amphibian. In a study of the agile frog *Rana latastei*, they demonstrate that an increased density of heterospecific males (*R. dalmatina*) relative to conspecific males (*R. latastei*) in a given population leads to a breakdown of sexual isolation and an increase in reproductive interference. Specifically, the presence of heterospecific sexual partners leads to a reduced frequency of conspecific amplexus (clasping of the female: a necessary stage of copulation), oviposition, and embryo viability of *R. latastei* females. Decreases in each of these phenomena constitute reproductive interference. Hence, the social environment in which a species lives and mates can have a predictable influence on courting interference and reproductive success.

The results of my study on courtship interference and female responsiveness in guppies lends a comparable conclusion concerning mating success in that an environment conducive to visual contact between individuals created more competitive interaction between courting males (altered social environment) and led to a reduced tendency of female guppies to respond to courting males. Although mating success was not directly measured (it was not feasible to obtain data on mating success, considering that molecular paternity analysis was beyond the scope of the project), it is reasonable to predict that this socially induced significant change in female sexual responsiveness could alter the pattern of male mating success.

The study of guppies has also shed light on the influence of social environment on sexual behavior in that sex ratios within guppy populations have been demonstrated to affect the mating strategies of male guppies and female mate choice. According to Rodd and Sokolowski (1995), male guppies from both high and low predation localities increase their rate of gonopodial thrusting as well as the frequencies of performing sigmoid displays as the density of mature males in their home tank increases. Thus, as the number of potential competitors for mates increases, males respond by increasing their sexual activity and utilizing a forced mating strategy known to undermine female choice. In addition, male guppies originating from the high predation locality decrease their rate of display to a given stimulus female as the density of mature females in their home tank is increased, and as the number of females per male is increased. The authors suggest that this decreased rate of male displays may reflect an increase in male choosiness given an increase in female density. The social environmental factor, density in this case, directly affects male courtship behavior and may indirectly affect female mate choice by preventing females from mating with their preferred males.

The physical habitat in which a species lives and mates also has the potential to affect their sexual behavior. For example, in guppy populations effective courting by a male requires visual contact with the female and male color pattern is important in mate choice. Therefore, fluctuations in lighting may disrupt visual communication and affect females' ability to see the finer pattern components which aid in pattern contrast and mate choice decisions. Considering that changes in the total intensity (irradiance) or spectral composition of light can change the visual scene, one would expect such changes to have a pronounced effect on behavior. Long and Rosenqvist (1998) demonstrated that male guppies do in fact exhibit behavioral plasticity in variable light environments by adjusting their courting distance to maximize their conspicuousness to females in any one light environment. Specifically, they found that male guppies court at exact and predictable distances from the female given a particular light level, both in field and in laboratory studies, with males decreasing initial courtship distance at lower light levels. This study suggests that variation in courtship behavior may be constrained and predicted by changes in environmental irradiance because of its effect on both the signaling and perceiving processes and the interplay between selective pressures on males and females (Long and Rosenqvist 1998).

In addition to the light intensity of a habitat, the structure of the habitat has been observed to affect visibility and hence mate encounter. The three-spined stickleback breeds in shallow coastal waters where males establish territories and build nests, preferring to nest in vegetated habitats concealed from predators and competing males (Candolin, 2003). However, lower visibility in dense algal growth may reduce the encounter rate of males and females at a nesting site and the opportunity for mate choice. Candolin (2003) noted that while density of territorial males was higher in habitats with a denser growth of filamentous algae, variation in the number of eggs among male nests significantly decreased. This more equal distribution of eggs in more densely vegetated habitats implies less variation in mating success among males in such habitats. Candolin's findings may indicate that changes in vegetation structure could bring with it changes in sexual selection through changes in the intensity or outcome of male-male competition or female mate choice. It has also been suggested that courtship intrusions, which influence female mate choice by allowing for comparison of competing males, are more frequent in open habitats (Sargent & Gebler, 1980; Sargent, 1982; as cited in Candolin, 2003). Thus, increased vegetation could permit less attractive or less competitive males to mate more often because of less distraction from other competing males during courtship, which would relax the strength of sexual selection (Candolin, 2003). Further, reduced female choice could also lead to reduced variation in male mating success if the ability of females to compare males, and therefore their choosiness, is reduced in more vegetated areas.

It has become increasingly evident through the studies described above that the two selection components (male-male competition and female choice) often interact in complex ways that can complicate the interpretation of factors influencing sexual selection in a population. Therefore, behaviorally plastic changes in male and female sexual behavior in response to ecological or social environmental factors may overlap, making it difficult to determine whether directional changes in sexual selection are the result of male-male competition, female choice, or a combination of the two. For instance, as Evans et al. (2002) pointed out, changes in the mating strategy of male guppies under predation risk may actually be mediated by changes in female behavior in response to the presence of predators. On the other hand, male behavior may mediate female behavior, as in the case of reduced female responsiveness in light of increased male interference behavior in the guppy (my study). Considering that any change in male sexual behavior may affect female sexual behavior and vice versa, the possibility for complex interactions between the two components of sexual selection is great. In some cases, experiments can be designed to separate the mutual interactions between males and females.

For example, male competition has been shown to hamper female choice in a number of species where females do not prefer dominant males (Wong, 2004). In the fish *Pseudomugil signifer*, commonly referred to as Pacific blue-eyes, courtship, not competitive success, appears to be important in signaling fitness benefits to females in the form of higher egg hatching success, with females preferring males that spend a greater proportion of time in courtship (Wong, 2004). In fact, earlier in 2004, Wong showed that competitive success has no bearing on paternal abilities because dominant males make only average fathers. However, Wong (2004) also demonstrated that aggressive interactions between competing males are common when males are able to interact and that such competition is disruptive to courtship. Specifically, courtship bouts were shown to be longest when males were not in physical or visual contact with other males, intermediate when males were in visual contact with other males, and shortest when males were in physical contact with other males. The alteration in courtship bout length illustrates that frequent interruption by competing males prevents courting males from accurately signaling their quality to females, which in turn prevents females from accurately assessing the quality of that male. This possibility that competition may hamper female choice is also consistent with results reported for sand gobies and tiger salamanders.

Kangas and Lindstrom (2001), in a study of a small marine fish known as sand gobies, also examined the ability of male-male interactions to constrain female choice by comparing the expression of female preferences under three conditions that varied the degree of male-male interaction with matings that resulted from free interactions. After establishing which male out of two potential mates was preferred by a female, female choice was assessed in the three conditions. They found that females spawned with their preferred male when there was no initial contact between males. However, when visual contact and full interaction between males was allowed, females were inconsistent in their spawning choices, suggesting that male-male interaction may affect the ability of females to evaluate males. Thus, it seems that male-male interaction and competition impede female choice and may consequently alter the outcome of sexual selection.

In addition, male competition and female choice may interact in complex ways. Studies of newts (Gabor et al., 2000) and tiger salamanders (Howard et al., 1997) clearly indicate the importance of attempting to separate these two components of sexual selection. In the red-spotted newt, bigger males with deep tails are significantly more successful at mating with females than are smaller males with shallow tails (Gabor et al., 2000). However, Gabor et al. (2000) found no evidence of female choice, and in fact, when males were not competing to mate with females, tail height and body size did not influence male mating success. Instead, the mating success of deep tailed males appears to be a result of competition between males for access to reproductive females, considering that deep tailed males may be more likely to follow and capture these females (Able, 1999, as cited in Gabor et al., 2000). Thus, male-male competition not only dictates with which males females mate, but also appears to produce a “false mate selection” for particular traits (Gabor et al., 2000). The ability of larger, deep tailed males to monopolize females when competition is occurring may provide strong selective pressure in the evolution of male behavior and demonstrates that male-male competition is a major factor in the mating system of red-spotted newts.

The ability of male-male competition to alter selective pressures is also demonstrated in a study of the eastern tiger salamander. Howard et al. (1997) demonstrated that the two components of sexual selection, female choice and male-male competition may favor different traits in the same species and hence have differing effects on the direction of sexual selection. Specifically, they found that while male competition in the eastern tiger salamander appeared to favor increased male body length, mate choice seemed to select for greater male tail length. This dual selection of traits is due to the fact that larger male salamanders display advantages in almost every aspect of mate competition, usually gaining sole access to the female in the event of an interruption, yet males with greater than average tail length perform significantly more successful or attempted sperm transfers than the average male encountered. Although females do not prefer the larger males, the dominance of such males when competition arises may prevent “more attractive,” longer tailed males from displaying. Therefore, the absence or presence of male-male competition in a population at a given time will affect the degree to which female choice contributes to the selection of male secondary traits.

Previous research has shown that environmental and social conditions affect sexual behavior and the sexual selection components of competition and mate choice. However, it is important to consider that these effects could be the result of adaptive plasticity in behavior or could arise simply out of necessity or circumstance. Adaptive plasticity refers to the adaptive adjustment of behavior by individual organisms in response to environmental conditions (Houde, 1997). Although various environmental and social conditions may lead to the same evolutionary outcome, the actual mechanism that produces that outcome may not be the same. For instance, guppies and other species demonstrate behaviorally plastic responses to environmental factors, such as predation, sex ratio, and density, and are thus actively adjusting their behavior to their perceived environmental condition or social situation. On the other hand, in the case of my study on the effects of visual obstruction on the sexual behavior of male and female guppies, the altered environment appears to cause an inevitable change in male sexual behavior through a change in group interaction rather than a plastic response to the variation on the male’s part. In other words, males are not directly adjusting their propensity to interfere with other males they see courting females. Instead, the presence or absence of visual barriers affects the frequency with which males can observe other courting males, and this circumstance affects the rate of interference between males. Therefore, both the environmental and social circumstances of the individual as well as its active behavioral decisions may ultimately affect sexual selection.

Results

All experimental groups showed plenty of courtship behavior, with the exception of one of the 20 groups tested with non-virgin females in which males did not interact, interfere, or court, suggesting that these fish may have been sick. Therefore, only 19 groups were included in the statistical analysis for non-virgin comparisons. Our prediction that interference behavior would be reduced when visual obstructions were present was supported by the results from both virgin and non-virgin trials. The frequency of chases and fend-offs differed markedly between the two treatments, with significantly more chases and fend-offs occurring in blank tanks than in barrier tanks (for chases, Fig 2, non-virgin trials: t=5.73, df=18, P<0.001; virgin trials: t=2.49, df=19, P=0.022; for fend-offs, Fig 3, non-virgin trials: t=4.58, df=18, P<0.001; virgin trials: t=3.69, df=19, P=0.0016).

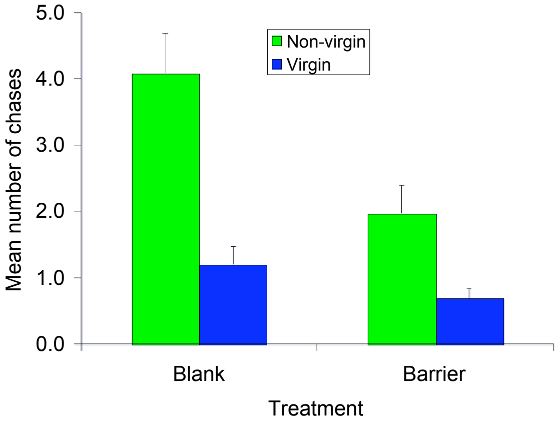

Figure 2. Mean “chases”
Number of “chase” interactions between males per 10 minutes in blank and barrier tanks. Data for virgin and non-virgin females were analyzed separately. Error bars represent standard errors.

Out of the 19 groups of males observed with non-virgin females, 18 showed an increase in the frequency of

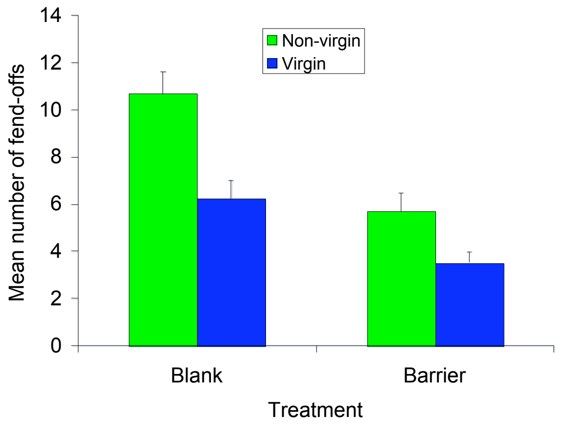

Figure 3. Mean "fend-offs"
Number of "fend-off" interactions between males per 10 minutes in blank and barrier tanks. Data for virgin and non-virgin females were analyzed separately. Error bars represent standard errors.

interference behavior (total number of chases and fend offs combined) in the blank treatment relative to the barrier treatment (binomial test, P<0.001). Only one group did not follow this trend, showing no difference in interference behavior between treatments. Eighteen out of the 20 groups of males observed with virgin females showed more interference behavior in the blank treatment relative to the barrier treatment (binomial test, P<0.001). In this case, two groups did not follow the trend, showing more interference behavior in the barrier treatment than in the blank treatment.

The total frequency of displays was significantly less in virgin trials compared to non-virgin trials in both the blank and barrier treatments (blank, Fig 4a: t=7.07, df=7, P<0.001; barrier, Fig 4b: t=6.00, df=7, P<0.001). In order to make a valid comparison of males courting virgin versus non-virgin females, data for interspersed trials only were used in this comparison. Further, data for all trials analyzed separately for virgin and non-virgin females showed that males courting virgin females reduced their rate of displays in barrier relative to blank treatments, with significantly fewer displays being performed by males courting virgin females in the barrier treatment than in the blank treatment (Fig 5, t=3.10, df=19, P=0.0059). The presence of barriers however did not appear to affect rates of courtship displays for trials in which males were observed with non-virgin females. Display rates were uniformly high and did not differ significantly between treatments (Fig 5, t=0.096, df=19, P=0.923).

In the trials in which less interference behavior occurred in the barrier tank, the fish seemed to be well dispersed throughout the tank and thus somewhat visually isolated from other males as predicted. However, quantitative data on the dispersion of fish were not recorded. Cases in which individual males showed more interference in the aquarium with barriers or no difference between treatments (3 out of the 39 groups of males included in the results) might be attributable to the occasional grouping of many fish in one area of the tank divided by barriers. In such cases, fish were indeed grouped with a male-biased sex ratio in one corner or visually separate area of the aquarium for the majority of the 10-minute trial which seemed to cause an overall increase in male interference behavior. In other cases, a high density of guppies persisted in one area of the aquarium and seemed to crowd the fish, causing them to get in each other's way during courtship and consequently instigating male-male competition and interference behavior.

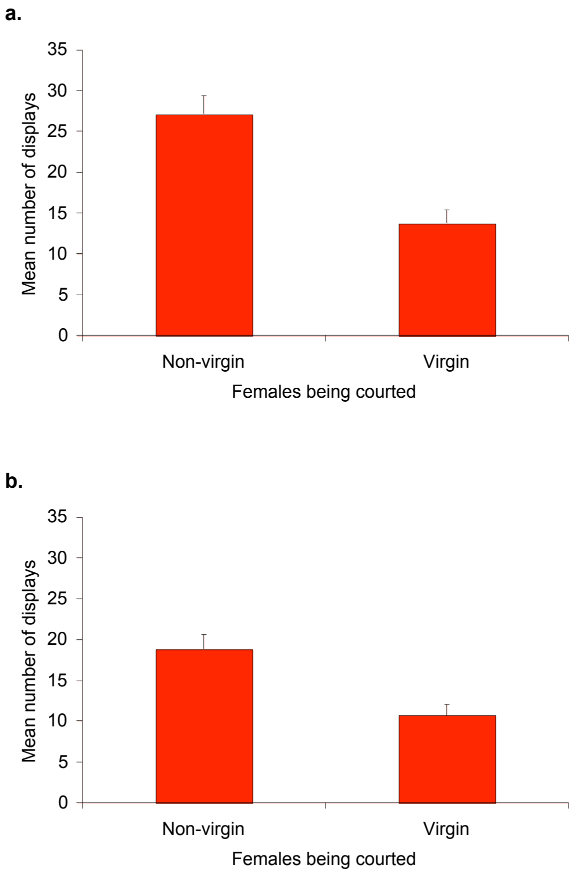

Figure 4. Male displays in barrier versus blank treatment
Number of male displays per 10 minutes to virgin and non-virgin females in blank (a) and barrier (b) treatments for "interspersed" trials in which virgin and non-virgin females were tested with the same males. Error bars represent standard errors.

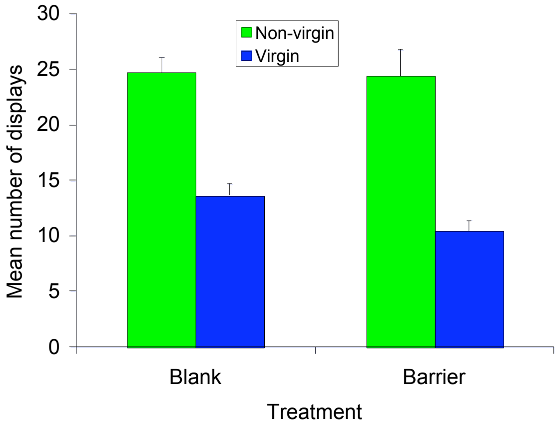

Figure 5. Male displays to virgin versus non-virgin females
Number of male displays per 10 minutes in blank and barrier tanks. Data for virgin and non-virgin females were analyzed separately. Error bars represent standard errors.

Our prediction that female responsiveness to male courtship displays (recorded for virgin trials only) would increase when visual obstructions were present was also supported by the results. Females responded more frequently to male sigmoid displays performed in the barrier treatment compared with the blank treatment. Specifically, females being courted by males in the barrier treatment responded to a significantly greater fraction of displays than in the blank treatment (t=-5.19, df=19, P=0.000052, Fig 6). In trials with non-virgins no sexual responses were noted in any trials.

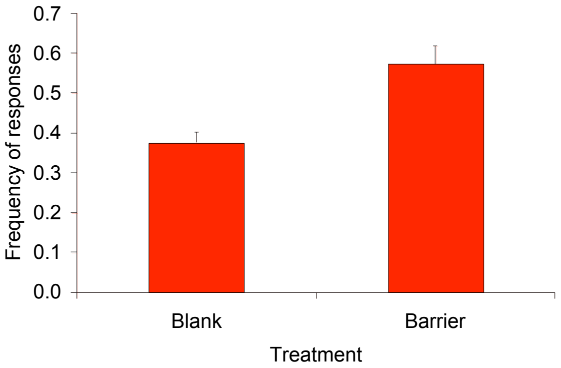

Figure 6. Female Responsiveness
Frequency of responses by virgin females to male displays in blank and barrier treatments. Error bars represent standard errors.

Discussion

The results support my predictions in that there was less male interference behavior and increased female responsiveness occurring in tanks with visual obstructions than in tanks with unobstructed visibility. On average, males engaged in fewer "chase" and "fend off" interactions when the barriers were present than in tanks lacking barriers. The likely explanation for this change in behavior and the basis for my initial prediction is that males intrude on the courtship activity of other males when they see a courting pair. Evidently, the barriers impede visibility enough that privacy is permitted and males are less likely to see and respond to the courtship activity of other males in this way.

Females were more responsive to courting males in the treatment containing visual barriers. In the absence of frequent interruptions by intruding males, females may actually have more of a chance to respond. In guppy courtship, the first few displays by the male may not receive a response from the female and appear to be performed to gain her attention. It follows that if intruding males are constantly breaking up a courting pair, the male may not get the chance to conduct a thorough display, and the female may not get to the point of responding to these displays. Any factor that decreases the tendency or ability of females to respond to male courting attempts may have significant implications for the mating success of "chosen males." In other words, such disruptions may undermine female choice and hence influence the direction of sexual selection.

A comparison of results from virgin and non-virgin trials for male display rates revealed that males in the presence of virgin females displayed significantly less than males courting non-virgin females. Houde (1988b) found a negative correlation between frequency of courtship displays and frequency of female responses and suggested that once a female responds to a male's display, the male follows that female more closely and becomes more particular about when he displays to her, waiting until no other fish are in the vicinity and the female is in a position to view the display. This has the effect of reducing the number of male displays performed for receptive females. Therefore, while males generally appear to pursue receptive females more persistently (Houde, 1997), they do so with a lower total rate of display than they do unresponsive females.

This slowed display rate in the presence of receptive females in the virgin study was exaggerated even more when barriers were present and less male interference occurred. As previously mentioned, the initial purpose of a display is to draw the attention of the female. Further more calculated and careful displays are meant to lure her in. It makes sense that males in a more private, uninterrupted setting would be better able to gain the full attention of the female and would not need to constantly display to her to regain her attention, especially after interruptions. Hence, the presence of visual barriers leads to a further reduced frequency of displays by males courting virgin females probably because more of these displays result in a response by females. In non-virgin trials, barriers had no effect on the frequency of displays. This is likely due to the fact that non-virgin females are not responsive to the courtship attempts of males.

Although the barriers I used were artificial and the fish were observed in laboratory aquaria, the structure of Trinidad streams containing guppies varies from location to location so that visibility varies in a way comparable to that seen in my experiment (Hibler TL, Houde AE, personal observations). In some locations, wide, deep pools have relatively long unobstructed views, while in others, shallower water is broken into many small pools by rocks and other obstructions that limit visibility. This leads me to suggest that the structure of the habitat in which guppy populations live and mate may have an effect on their sexual behavior, especially male-male competition, and this may in turn affect female choice and the evolutionary outcome of sexual selection.

Kangas and Lindstrom (2001) conducted a study similar to mine in which they manipulated visual contact between male sand gobies in the presence of a single female. The tank was divided into three sections: the two males placed in the rear compartments were either freely interacting, separated by a transparent barrier, or separated by an opaque barrier while the female in the third compartment had continual visual contact with the two males through a transparent divider. It was determined, as in my study, that frequency of male aggression increased when male sand gobies were in visual contact with one another. Additional results indicating a decrease in courtship duration of females by males as well as in the mate choice consistency of females were in line with my suggestion that male-male competitive interaction may affect the ability of females to evaluate males and possibly impede female choice.

Investigations of visual contact and interaction performed by Long and Rosenqvist (1998) as well as Wong (2004) also indicated the importance of visual cues between individuals in a population. Long and Rosenqvist demonstrated that changes in the visual scene, as a result of fluctuations in lighting, led male guppies to adjust the distance from which they courted females. In his study of the Pacific blue-eye, Wong (2004) demonstrated that aggressive interactions between males, occurring when males were able to interact visually or physically, caused courtship bouts to be shorter in duration than when male interaction and competition was absent. Thus, male courtship behavior may be significantly altered by changes in the visual environment. Considering that visual cues play an important role in female choice in guppies and that the quality and length of a courtship bout is important in signaling fitness benefits to female Pacific blue-eyes, the observed changes in male courtship behavior in these populations may affect female choice. Similarly, it may be reasonable to suggest that the changes in male courtship behavior observed in my study as a result of reduced visual interaction between individuals could affect the quality and success of male courtship attempts as well as females' ability to properly assess the attractiveness of courting males.

The finding by Rodd and Sokolowski (1995), that male guppies increase their rate of gonopodial thrusting as the density of mature males and hence perceived competition for mates in their home tank increases, suggests investigation into the extent to which males alter their mating strategy in the visually manipulated habitat of my study. If the increase in male interference behavior observed in the absence of visual barriers translates to an increase in perceived competition for mates, such changes in the social environment may lead to changes in male mating strategy similar to those observed by Rodd and Sokolowski, with males possibly increasing their frequency of gonopodial thrusting in the visually unobstructed, more interruptive environment. Considering that gonopodial thrusting is a non-cooperative mating strategy known to undermine female choice, gaining information on this behavior in my experimental treatments would allow me to determine with greater conviction whether the observed increase in male interference behavior in visually unobstructed treatments is disruptive to female choice. However, such an investigation would require an alternate experimental guppy population considering that rates of gonopodial thrusting in the Paria River population I studied were too low for adequate analysis.

The additional implications drawn by researchers in the above studies about the affects of visually induced changes in male-male interaction and competition on female sexual behavior and mating cues are in line with my findings concerning female responsiveness and provide additional support for the suggestion that male-male interactions affect female choice. However, while the alterations in male-male competitive interaction and behavior are suggested to impede female choice in the cases of the guppy, sand goby, and pacific blue-eye, this effect should not be generalized for all species.

In the three-spined stickleback, interactions between males actually appear to be necessary to prevent less attractive or less competitive males from mating with females that would ordinarily not choose such males if they were distracted by other competing males during courtship (Candolin, 2003). As previously discussed, Candolin (2003) suggested that habitats with increased vegetation and reduced visual contact between individuals reduced the encounter rate of males and females at a nesting site, the ability of females to compare males, and the opportunity for mate choice. Therefore, although obstructions to visibility in the experimental habitats of Kangas and Lindstrom's study of the sand goby (2001), my study of the guppy, and Candolin's study of the three-spined stickleback (2003) all led to a decrease in male-male competitive interaction and interference, this environmentally induced change in male behavior is suggested to have opposing effects on female choice in these populations. Researchers must be aware of the specific selective mechanisms of the species in question when making conclusions about the effects of environmental factors on sexual selection.

Considering that female responsiveness to male courting is a necessary step toward copulation in the guppy, the documented change in female responsiveness in my study when male interference behavior is increased could affect the ability of females to mate with their preferred males. In extreme cases of male-male competition, such as water striders, the cost imposed on females by male-male interference results in a failure of females to avoid unwanted copulations by harassing males, thus undermining mate choice by females (Watson & Arnqvist, 1998). However, even if male harassment does not result in unwanted copulations, any increase in levels of harassment by males may lead females to reduce their responsiveness and even actively avoid males, as seen in my study. While such avoidance behavior may reduce some of the costs involved in mate choice, it may also constrain the ability of females to exercise their mating preferences, and alter the pattern of mating success in the population (Houde, 1997). In addition, Wong (2004) pointed out that female Pacific blue-eyes may be unable to fully assess the fitness of a courting male when courtship bouts are cut short as a result of male competitive interference. Candolin (2003) also suggested that the impeded ability of female three-spined sticklebacks to evaluate and respond to males of their choice in less interactive habitats led to a decrease in the variation of male mating success in more densely vegetated habitats.

Changes in the pattern of male mating success as a result of disruptions in female choice could alter the effect of sexual selection on secondary sexual characteristics. The observations by Gabor et al. (2000) that increased competition between male red-spotted newts appears to produce a "false mate selection" for particular traits expressed by competitively superior males in the population and by Howard et al. (1997) that male competition in eastern tiger salamanders appears to favor different male traits than those selected for through female mate choice, suggest that changes in the intensity of male competitive behavior are likely to alter male phenotypes in the population. It would therefore be interesting to expand my study to include paternity analyses and data interpretation of male offspring characteristics to determine whether the expression of those traits generally preferred by Paria River female guppies is affected by the observed changes in courtship interference behavior induced by the manipulated habitat structure.

In fact, the mechanism proposed by my study may provide a possible explanation for the previously documented mismatch between preferences demonstrated by females and average color pattern characteristics expressed by males in a guppy population from the Yarra River of Trinidad (Houde & Hankes, 1997). While females in this population show a strong preference for orange coloration in the laboratory, males show a reduced expression of orange (Houde & Hankes, 1997), which is surprising considering that in guppy populations expression of orange coloration in males tends to match the expression of female preferences for orange coloration (Houde & Endler, 1990; Endler & Houde, 1995). Although I am not ruling out the equally likely possibility that the presence of predators is contributing to the mismatch through a number of possible mechanisms suggested by Godin and Briggs (1996), Magurran & Nowak (1991), and Magurran & Seghers (1994), this population's visual and social environmental also may be preventing females from mating with preferred males. Houde and Hankes (1997) characterized the Yarra River collection site as being wide with few obstructions and noted highly cohesive schooling behavior. Hence, individuals may typically be in visual contact with many others (Houde AE, personal observation; Magurran et al., 1995), and as was seen in the present study and that of Kangas & Lindstrom (2001), this lack of visual obstruction could potentially limit Yarra females' ability to mate with preferred males if it leads to greater courtship interference.

The previously presented studies have provided additional information on the functioning of sexual selection in natural populations and defended the argument that factors in the environment in which individual species live and mate have the ability to alter the sexual behavior of males and females in a population as well as the specific selective mechanisms operating in different species. My study contributes to this knowledge base by indicating that environmental conditions can have a predictable effect on guppy sexual behavior and suggests that seemingly subtle differences in a guppy habitat might affect the mechanism of sexual selection. Nevertheless, further studies are needed to assess the full effect of male-male competition and interference behavior on female choice and, ultimately, on male mating success in the guppy.

Future studies need to examine patterns of actual mating success of males in order to determine if visual barriers affect the relative success of "preferred" males. I would predict that the relative mating success of such males would increase when visual obstructions are present because, in the absence of frequent interruptions by intruding males, females would be more likely to respond to and remain with preferred males long enough to successfully copulate. Generally, if a male is to be successful in mating, females must respond to his displays and cooperate in copulation (Houde, 1997). Finally, it would also be useful to document the frequency of gonopodial thrusts and sneak copulation attempts, perhaps in a population in which these are more frequent, in order to determine whether habitat differences cause any variation in these male behaviors known to undermine female choice.

Experimental Procedures

Experimental Fish

All experimental trials were conducted with descendents of wild guppies collected from the Paria River population in Trinidad and maintained under standard laboratory conditions. Virgin females were reared in same sex 40 liter aquaria divided into four sections containing six females each, and were used in experiments after they reached sexual maturity at about 12 weeks of age. Males and non-virgin females were raised in mixed sex, undivided aquaria, and were used in experiments between four and eight months of age. All experimental groups were composed of arbitrarily chosen males and females. All fish were fed twice a day with Tetramin flake food in the morning and freshly hatched brine shrimp nauplii in the afternoon and were kept on a 12:12 hour light-dark cycle. Aquarium temperature was maintained between 22 and 27° C using automatic aquarium heaters. All behavioral observations were conducted within two or three hours after the aquarium lights came on in the lab each morning.

We observed the behavior of male guppies in groups with both virgin females, which are sexually responsive to male displays, and with non-virgin females, which are unresponsive. In all trials, virgin females were introduced to the experimental group the afternoon prior to observing them and are likely to have mated prior to the observations. We refer to these females as "virgins" for convenience because they continue to be sexually responsive at the time of observations. A total of 128 males were arbitrarily separated into 32 groups of four males each. In an initial phase of the study, 12 groups of males were observed with non-virgin females. Subsequently, another 12 groups were observed with virgin females. Finally, to allow valid comparison of male behavior with virgin versus non-virgin females, eight groups of males underwent trials with both virgin and non-virgin females, which we refer to as the "interspersed" trials. Thus, the sample size for the comparison of aquaria with and without barriers was 20 male groups observed with non-virgins and 20 observed with virgins.

Male Interference Behavior and Female Responsiveness

Each group of four males in the initial phase of the study underwent two 40-minute observation sessions (10-minutes per male). One session was conducted in a 38-liter aquarium including only bottom gravel ("blank" treatment) in which all fish were continually visible to one another, whereas the other was conducted in a 38-liter aquarium containing bottom gravel and four opaque plastic sheets placed vertically throughout the tank and extending partially across the aquarium ("barrier" treatment) (Fig 1). Male groups in the "interspersed" virgin and non-virgin trials underwent four 40-min observation sessions (10-min per male per session). Two of these sessions were conducted with non-virgin females in each of the two treatments, whereas the other two were conducted with virgins in both treatments. The barriers created visual obstructions in the tank, in effect dividing it into partially separated "rooms." In an aquarium containing barriers, a fish in a given location could see other fish only in its immediate vicinity but could not see fish in other parts of the tank. In order to ensure that any unperceived difference between experimental tanks would not bias the results, barriers were switched between the tanks for each group of four males.

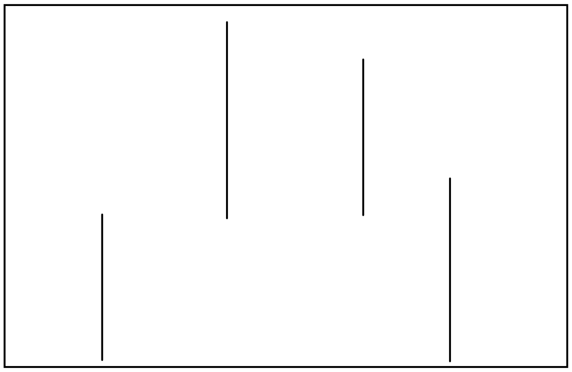

Figure 1. Tank design
In the barrier treatment visibility was manipulated by placing four opaque plastic sheets vertically throughout the tank and extending partially across the aquarium. In the blank treatment, all dividers were removed and all eight fish had free access to all parts of the tank.

For trials involving non-virgins, males and females were introduced to treatment aquaria the afternoon before an observation session. The following morning fish were observed in the initial treatment and were then immediately moved into the second treatment where they were given ten minutes to adjust to the new surroundings before the second set of observations began. The initial treatment, either blank or barrier, was alternated between experimental groups to ensure that time of observation and order of treatments encountered for each group would not bias the results. Observation sessions in the two treatments for a given group of four males were conducted with the same group of four non-virgin females.

For observations with virgin females, each observation session was conducted with a new group of four virgin females to ensure similar levels of receptivity. In this case, males and females were introduced to the treatment aquaria 24 hours prior to trials in order to allow time for adjustment to the surroundings and initial mating of the new females. Immediately following the trial, males were removed from the initial treatment, and placed in the second treatment with a new group of four virgin females, to be tested 24 hours after introduction to the new treatment and females. Therefore, only one trial was performed each day for male groups being observed with virgins. As was done for non-virgin trials, the order of initial treatment, either blank or barrier, was alternated for each experimental group of four males paired with virgin females. Small amounts of flake food were given to all experimental groups prior to timed observations in order to ensure maximum sexual behavior.

Males were observed for ten minutes each during a given observation session, during which occurrences of sigmoid displays and male interference behavior were recorded for each male. Female responsiveness to each male's courtship displays was recorded for virgin trials only. Because non-virgins showed sexual responses only very rarely, female responsiveness to male courtship displays was not recorded for non-virgin trials.

Following Houde (1988b, 1997), we scored interference behaviors as either "fend-offs" or "chases." A "fend-off" occurs when an intruding male attempts to court a female that is already attended by a male and thus elicits a defensive response from the original male. The original male then moves between the female and the other male and attempts to "fend off" the intruding male by whipping his tail at the other male. Original males remain with the female in the majority of "fend-offs." In the instance of a "chase," a second male intrudes on the courtship of another male, but instead of being fended off, both males pursue the female for several seconds, each attempting to remain with the female and to exclude the other male as she darts away and swims evasively. Under the experimental conditions of this study, too few sneak copulation attempts or gonopodial thrusts were observed for statistical analysis.

Female responsiveness to male courtship was measured using guidelines first described by Liley (1966) and by Houde (1997). Males perform courtship displays in an attempt to gain the attention and eventual copulation of a pursued female. However, in order for a male's initial display to proceed to courtship and copulation, the female must respond to the display. We recorded that a female was unresponsive to a male's display if the female appeared to simply ignore the male's display. A response was recorded if the female oriented toward the male and glided smoothly toward the male as he displayed. In the instance of a successful courtship sequence ending in copulation, the glide is followed by the male and female circling around one another, and the male thrusting his gonopodium. However, considering that females do not normally approach males in other contexts and that the gliding response is the first and necessary step toward copulation, the gliding response alone is a clear indication of the female's sexual interest in the male and may been used to assess female mating preferences (Houde, 1987; Reynolds and Gross, 1992; as cited in Houde, 1997).

Rates of interference behaviors and sigmoid displays for each male, as well as responsiveness to sigmoid displays for virgin trials only were compared between the control versus barrier tank treatments using paired t-tests (Microsoft Excel Software). Mean rates of behavior per trial (rather than per male) were used as the unit of analysis to avoid pseudoreplication. Rates of male courtship displays were also compared between virgin versus non-virgin trials.

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Disclaimer

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