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Fin-mutant Female Zebrafish (Danio rerio) Exhibit Differences in Association Preferences for Male Fin Length

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Fin-mutant female zebrafish (*Danio rerio*) exhibit differences in association preferences for male fin length

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Abstract

Females often choose to associate with males that have exaggerated traits. In fishes, this may reflect an overall preference for larger size in a potential mate. Female zebrafish (*Danio rerio*) prefer males with larger bodies but not longer fins. The availability of mutant and transgenic strains of zebrafish make this a unique model system in which to study the role of phenotypic variation in social and sexual behavior. We used mutant strains of zebrafish with truncated (*short fin*) and exaggerated (*long fin*) fins to further examine female preferences for fin length in dichotomous association tests. Wild type females showed no preferences between wild type males and *short fin* mutant males or between wild type males and *long fin* mutant males. *short fin* females also showed no preference for *short fin* males or wild type males while *long fin* females preferred to associate with *long fin* males over wild type males. These results suggest that the single gene *long fin* mutation that results in altered fin morphological may also be involved in a related female association preference.

Keywords

association preference; *Danio rerio*; *long fin*; *short fin*; zebrafish

1. Introduction

Females often prefer to associate and mate with males that are larger in body size or have exaggerated traits (reviewed in Andersson, 1994). In fishes, exaggerated fins may allow males to appear larger while avoiding the high cost of increasing the total body size. These exaggerated fins may be used in aggressive interactions between males (e.g. *Betta splendens*: Simpson, 1968; *Cyathopharynx furcifer*: Karino, 1997; *Eviota prasina*: Sekiya and Karino, 2004; *Xiphophorus helleri*: Benson and Basolo, 2006; *Danio rerio*: Itzkowitz and Iovine, 2007) or may be the target of female choice (*Poecilia reticulata*: Bischoff et al., 1985; Karino and Kobayashi, 2005; *X. helleri*: Basolo, 1990a; *P. mexicana*: Jordan et al., 2006; but see *X. birchmanni*: Fisher and Rosenthal, 2007; *X. helleri*: MacLaren and Daniska, 2008). In some species, exaggerated fins may exploit existing female preferences for larger size (*X. helleri*: Basolo, 1990b; Rosenthal and Evans, 1998; *P. latipinna*: MacLaren et al., 2004; *P. mexicana*: MacLaren and Rowland, 2006). Thus, it is not the total body size that females prefer, but the largest overall apparent size (Ryan and Keedy-Hector, 1992), a hypothesis that may be particularly relevant in species that exhibit exaggerated fins.

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The use of mutant and transgenic strains of zebrafish (*Danio rerio*) has shed light on the role of phenotypic variation in social behavior (Engeszer et al., 2004; Snekser et al., 2006; Itzkowitz and Iovine, 2007) and this species could provide further insight to the function of exaggerated traits. Visual cues are important to zebrafish shoaling decisions (McCann et al., 1971; Turnell et al., 2003; Engeszer et al., 2004; Rosenthal and Ryan, 2005; but see Snekser et al., 2006) and shoaling may be advantageous in a mate-choice context due to the increased opportunity for mating and the probability of choosing a high quality, conspecific mate (Pitcher and Parish, 1993; reviewed in Krause and Ruxton, 2002). Despite this, there have been relatively few studies of mate association preferences of female zebrafish.

Wild type female zebrafish prefer to associate with larger males (Pyron, 2003, however, wild type females do not prefer to associate with males with exaggerated fins over wild type males (Kitevski and Pyron, 2003). Our objective was to further examine association preferences of female zebrafish by utilizing mutant strains of zebrafish with truncated and exaggerated fins. Using choice tests between wild type and *long fin* males, we tested the hypothesis that females prefer males with longer fins. Wild type females were not predicted to prefer *long fin* males over wild type males, as shown previously (Kitevski and Pyron, 2003). To determine if fin length plays any role in association preferences, wild type females were also given a choice to associate with wild type or *short fin* males. The *short fin* phenotype has truncated fins which reduce overall size, thus we predict that wild type females would associate with wild type males over *short fin* males.

To further test if fin length is important to zebrafish females in presenting an overall larger appearance, we additionally examined the association preferences of fin mutant females. If female preferences are consistent across phenotypic mutants, then we predict that *short fin* females will prefer wild type males that have significantly longer fins when given a choice between wild type males and *short fin* males. In the only study of fin-mutant zebrafish behavior, Itzkowitz and Iovine (2007) found that *long fin* males differ in social behavior from wild type zebrafish. Examination of aggressive interactions among *long fin* males revealed a complex relationship between the display signal and display behavior. *long fin* males perform more aggressive displays than wild type or *short fin* males but only when with other *long fin* males. Given the behavioral differences of male *long fin* mutants, we predicted that females with the *long fin* phenotype would differ from wild type zebrafish and exhibit a preference for *long fin* males.

2. Materials and methods

2.1 Maintenance of fish

Three phenotypes were used in these experiments, *long fin* mutants, *short fin* mutants and wild type zebrafish. All phenotype variants were of the C32 strain background (Iovine and Johnson, 2000). The fin mutations are independent of each other and all fins are affected by theses mutations: the *long fin*^{dt7} mutation causes changes in the number of bone segments (Iovine and Johnson, 2002), whereas the *short fin*^{b123} mutation causes changes in the length of bone segments (Iovine and Johnson, 2000; Iovine et al., 2005). When matched for body size, *long fin* fish have significantly longer fins than wild type while *short fin* fish have significantly shorter fins than wild type (Itzkowitz and Iovine 2007).

All fish were artificially spawned and raised to maturity in 10 l tanks on a recirculating system (Aquatic Habitats, Inc.) at 24–26° C. All fish were raised with others of their own phenotype so all are assumed to have had similar previous experience (McCann and Carlson, 1982; Engeszer et al., 2004; Spence and Smith, 2007). Fish were fed flake food (O.S.I.), supplemented with live brine shrimp daily, and kept on a 14:10 h light:dark cycle. After reaching maturity, fish were separated by phenotype and sex and kept in aerated, 38 l

aquaria ($54 \times 29 \times 33$ cm) containing gravel substrate for at least 7 days, kept at a constant temperature (24–26° C) on a 14:10 h light:dark cycle and fed commercial flake food daily, supplemented with frozen blood worms.

2.2 Preference trials

Trials were conducted in 76 l aquaria ($76 \times 32 \times 32$ cm) containing a gravel substrate and 20 cm of water. The sides and back of the aquaria were lined to visually isolate each aquarium and to minimize disturbance. All trials were conducted between 9:00 and 4:00 h. While spawning and courtship typically begin at sunrise (Westerfield, 2000), all trials were conducted after this time to reduce effects that intense male courtship behavior might have on overall female association preferences and therefore better understand the influence of the fin structures.

The test aquaria were divided into three compartments. Thin, clear plastic dividers were placed 9 cm from each end of the aquaria. Stimulus males were placed into these end compartments. The dividers allowed for visual and chemical communication, as chemical cues may be important in zebrafish social behavior (reviewed by Whitlock, 2006). Markings on the front of the aquaria further divided the middle compartment into three sections. The area closest to each end compartment (9 cm wide) was considered the choice section, while the middle section (40 cm wide) was considered a no choice area. The stimulus fish were randomly selected from stock tanks. Focal fish were used only once and stimulus fish were used only once per treatment.

Each focal fish was placed in the center compartment and allowed to acclimate for at least 10 min. Acclimation periods of various lengths are commonly used in zebrafish preference tests (Kitevski and Pyron, 2003; Engeszer et al., 2004; Ruhl and McRobert, 2005; Snekser et al., 2006). During this time, all focal individuals visited both choice sections. The time spent by the focal fish in each of the choice sections was recorded for 10 min. The positions (left/right) of the stimulus fish were reversed and the trial was conducted again after allowing the focal fish to re-acclimate for 10 min. This controlled for any potential side bias of the focal fish. Focal fish that spent more than 80% of the total time on one side of the aquarium were considered to have a side bias and these data were excluded from analysis.

2.3 Experimental design

Females of three phenotypes (wild type, *long fin,* and *short fin*) were tested for preference to associate with males in 4 treatments: (1) wild type female (n = 19) preference of *short fin* males vs. wild type males; (2) wild type female (n = 20) preference of wild type males vs. *long fin* males; (3) *long fin* female (n = 20) preference of wild type males vs. *long fin* males; and (4) *short fin* female (n = 19) preference of *short fin* males vs. wild type males. Within each trial, males were matched for body size (difference in body size, mean ± S. E.: 6.7% ± 0.6%) based on standard length (rostrum to caudal peduncle; mean ± S. E.: wild type 23.56 mm ± 0.296; *long fin* 22.60 mm ± 0.243; *short fin* 23.58 mm ± 0.34) to avoid confounding effects of differences in fin lengths.

2.4 Statistical Analyses

The amount of time a focal individual spent in each choice section near a stimulus fish was summed across both observations in each trial. The data were not normally distributed after all attempts at transformation and thus, non-parametric statistics were used for within treatment analyses. Total time spent with each stimulus was compared within treatments with a Wilcoxon-Signed Rank test.

A responsiveness score was calculated as the total time spent in a 'choice area' per 1200 s (Gabor and Page, 2003). Responsiveness scores near 0 indicate that the focal fish spent little time associating with either stimulus while scores near 1 indicate either a strong preference for one stimuli or a high degree of movement between the stimuli with no preference for either. Responsiveness scores for all trials met the assumptions for parametric statistics and a one-way ANOVA was used to test for differences in female responsiveness.

3. Results

Wild type females had no significant association preferences for wild type or *long fin* males (Wilcoxon-Signed Rank test: n = 19, z = −0.181, p = 0.86). Wild type females also showed no significant preferences for *short fin* or wild type males (Wilcoxon-Signed Rank test: n = 20, z = −0.131, p = 0.90). *long fin* females spent significantly more time with *long fin* males over wild type males (Wilcoxon-Signed Rank test: n = 20, z = −2.427, p = 0.02) while *short fin* female zebrafish had no significant preferences for *short fin* or wild type males (Wilcoxon-Signed rank test: n = 19, z = −0.0765, p = 0.45, Fig. 1).

Additionally, females of all phenotypes spent more time associating with male zebrafish than in the no choice area of the aquaria. There were no differences in responsiveness across treatments (one-way ANOVA: $F_{(3,74)} = 0.658$, p = 0.5807). Wild type females expressed high responsiveness scores in both trials (wildtype vs. *long fin* males = 0.617 ± 0.035; wild type vs. *short fin* males = 0.624 ± 0.031) but no significant association preference. *short fin* females also expressed high responsiveness scores (0.616 ± 0.038), but no significant preferences. This indicates that these females were frequently sampling both stimuli. In contrast, *long fin* females had high responsiveness (0.605 ± 0.036) as well as a significant preference for *long fin* males. The combination of preference and high responsiveness indicates that *long fin* females were strongly associating with *long fin* males.

4. Discussion

The preference for males with longer fins was observed only in females that also have long fins. This unique preference for longer fins by *long fin* females may suggest that the mutation controlling the expression of the *long fin* trait is also playing a role in controlling female association preferences. As the *long fin* mutation is caused by a single point mutation (Iovine and Johnson, 2002), it is possible that the fin morphology and the preference are pleiotropic effects of this single gene. There is, however, generally little support for the hypothesis that trait and preference could be controlled by the same gene due to the differential pathways related to the phenotypes (trait = physical attribute; preference = neural processing) (Boake, 1991) and empirical evidence for such effects is sparse (*Drosophila melanogaster*: Marcillac et al., 2005; *Heliconius* butterflies: Kronforst et al., 2006). Further, the lateral display used in aggressive encounters between male zebrafish, has been shown to be genetically decoupled from the *long fin* phenotype. Surgically shortening the caudal fin of *long fin* males decreases the frequency of lateral displays to levels shown by *short fin* males, indicating that the frequency of this specific behavior was not linked to the genetics of *long fin*, but dependent on the possession of the long fin structure itself (Itzkowitz and Iovine, 2007). While our results suggest that the preference of *long fin* females for *long fin* males might be genetically controlled, the results from this previous study on *long fin* behavior indicate that expression of the longer fin alone might lead to differences in behavior.

Alternatively, the observed association preferences may be due to shoaling decisions and not female mate choice. Shoaling provides individuals with multiple benefits (Pitcher, 1983) including protection from predators (reviewed in Godin, 1986, Krause and Ruxton, 2002).

When a shoal is comprised of many phenotypically similar fish, predators may be unable to identify and attack any one individual due to the confusion and oddity effects (Milinski, 1979; Ohguchi, 1981; Landeau and Terborgh, 1986; Pitcher, 1986), thus shoaling fish tend to associate with similar fish. Zebrafish shoal based on a variety of visual cues (stripe pattern: McCann et al., 1971; Turnell et al., 2003; Engeszer et al., 2004, Rosenthal and Ryan, 2005; body size: Pyron, 2003; sex and shoal size: Ruhl and McRobert, 2005; but not body color: Snekser et al., 2006). *long fin* fish have swimming deficiencies due to the exaggerated fins (Plaut, 2000), thus may benefit by associating with one another whereas associating with stronger swimming fish (wild type or *short fin*) might negate the antipredatory benefits of shoaling. Wild type and *short fin* fish would not incur negative effects by associating with slower-swimming *long fin* fish and therefore lack specific association preferences.

In summary, our results provide additional evidence that the preference of wild type female zebrafish for larger males (Pyron, 2003) is not expressed when size is influenced by fin length (Kitevski and Pyron, 2003). *short fin* mutants also showed no preference for individuals with larger fins while *long fin* females preferred males with longer fins. The association preference of and for zebrafish expressing the *long fin* mutant phenotype and the differences in preference by the various strains further indicate the significance of utilizing phenotypic mutants to better understand complex behavioral systems.

Acknowledgments

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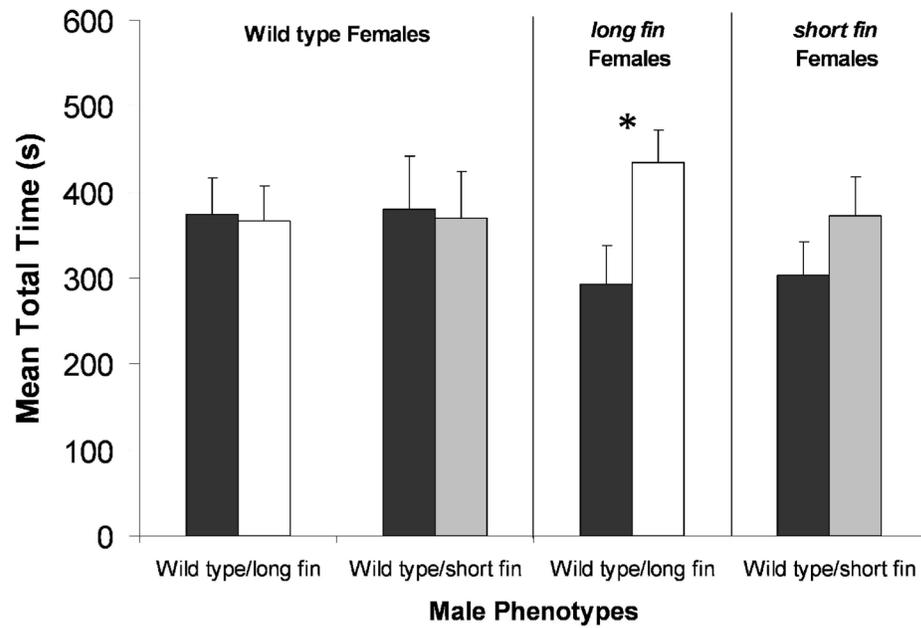
Fig. 1.
Mean association time (+ SE) of wild type, *long fin* mutant and *short fin* mutant female zebrafish when given a choice between two males with different length fins: wild type males (black) with *long fin* males (white) or wild type males (black) with *short fin* males (gray). *p < 0.05.