Single gene mutations causing exaggerated fins also cause non-genetic changes in the display behavior of male zebrafish

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Summary
We show that the exaggerated changes in fin lengths of zebrafish (*Danio rerio*) caused by a single gene mutation also resulted in changes in the lateral display rate. However, the increase in this threat display rate only occurred when both fish had very long fins. Fish with shorter fins did not show such an increase when they met fish with longer, same size, or shorter fins. Cutting the long fins to resemble the shorter fin types reduced the display rate, demonstrating that genetic differences do not account for the differences in behavior. These results suggest that species differences in the performance of a display may reflect only the physical differences in the display structure.

Keywords: *Danio rerio*, threat display, lateral display, communication signals.

Introduction

Visual communication signals are typically composed of a display structure/morphology and a display behavior. This close relationship between the structure and the behavior gives the impression that both evolve together. Indeed, when considering the evolution of communication signals, early ethologists rarely separated the two, perhaps expecting a close genetic relationship (see reviews in: Brown, 1975; Hinde, 1966; Smith, 1977). For example, Hinde (1966, p. 432) suggests that the display behavior and the display

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structure evolve in 'parallel' resulting in a ". . . correlation between the evolutionary development of the structure and that of the movement". In a recent review, West-Eberhard (2003) observed that behavior and morphology often appear to evolve together, although there are indications that either may 'lead' the process. Examining the relationship between display behavior and display structure is difficult because of intra- and inter-individual variation in the performance of the signal. Such variation is typically attributed to the complex association between an individual's motivation and physical ability, and the intended recipient of the signal (see review by Bradbury & Vehrencamp, 1998). Thus, differences in display behavior may be caused by a host of factors unrelated to the display structure.

Here we examine the relationship between the display structure and the display behavior using the lateral display behavior of the zebrafish in relation to genetic and surgical alterations in the display structure (i.e., fin length). The zebrafish is one of the few vertebrates where the genome sequencing project is nearly complete (http://www.sanger.ac.uk/Projects/D_rerio/), where exaggerated structures occur (i.e., extremely long fins), and where the mutations causing exaggerated fins are well understood. Although Plaut (2000) illustrated that exaggerated fins do influence the general swimming behavior of zebrafish, there are no studies that consider how the fish might use such exaggerated fins under social conditions. Kitevski & Pyron (2003) suggest that the exaggerated fin signal in zebrafish is not more or less attractive to females suggesting that the fin mutation has no effect on this aspect of social behavior. This study did not indicate whether the fin mutation affects the male's display of the fin. To examine the relationship between an individual's display behavior and its display structure, we monitored the lateral display. This behavior is a threat display involving the spreading of all unpaired fins while swimming slowly, or stopping, broadside to another fish. Thus, the lateral display includes not only the use of the fins but also the positioning of the body. This display is found in many vertebrates (often called the 'broadside display' in terrestrial species) and has been interpreted as a threat posture in which the individual attempts to maximize its body size. We propose here that the lateral display behavior is independent of the display structure. Thus, changing the structure of the fins involved in the display should not change the display behavior. The alternative hypothesis is that the lateral display behavior is not independent of the structure. Thus, individuals with exaggerated structures will also have modified display patterns.

To test the above hypothesis (i.e., that the display structure is independent of the behavior display), we monitored the display behavior in the dominant *long fin* (*lof*) mutant, which carries a single gene mutation that causes the overgrowth of all fins (Figure 1). For comparison, we also examined the recessive *short fin* (*sof*) mutant, which carries a single mutation that causes the undergrowth of all fins (Figure 1). If the lateral display and attacks are genetically controlled and both independent of fin length, we expected that groups of male zebrafish with the *lof* mutation would behave no differently than either wild-type or those with *sof* mutations. Alternatively, if fin length does influence the lateral display, it is possible that the mutation affecting length also causes a pleiotropic effect on the behavior. If this were the case, only fish with the *lof* mutation would show the exaggerated display behavior.

Methods

Zebrafish were raised at 25°C with a 14 h light:10 h dark photoperiod. Wild-type animals are from the C32 strain background. The lof^{Dt2} mutant arose spontaneously in the pet trade (Tresnake, 1981). The sof^{b123} mutant arose spontaneously in the lab of Charlene Walker (Johnson & Bennett, 1999). Both mutants have been maintained in the C32 strain background (Iovine & Johnson, 2000). The mutations causing the *lof* and *sof* phenotypes have been mapped and assigned independent linkage groups (Iovine & Johnson, 2002; Iovine et al., 2005), revealing that the phenotypes are each the result of a single mutation and furthermore, that *lof* and *sof* phenotypes are not the result of opposing mutations to the same gene. Animals used in this study were limited to males 12-14 months of age.

We observed homogeneous groups of 3 male fish that were either all wild type, *lof*, or *sof* mutants. Differences among these groups would provide support for the hypothesis that the display behavior was dependent on genotype but continues to allow for the possibility that the phenotype is responsible. To overcome this ambiguity, we added a fourth group consisting of *lof* mutants in which all of their fins were shortened to the approximate size of the *sof* mutants (i.e., 'cut *lof*'). For all groups, fish were anesthetized, a random sampling of individuals was measured using calipers, and in the case of cut *lof*, also had their fins *shortened* using scissors. These fish were placed in separate aquarium for 24 h before testing. Finally, using the same methodology as above, we used 'mixed groups' consisting 1 *lof* and 2 wild-types,

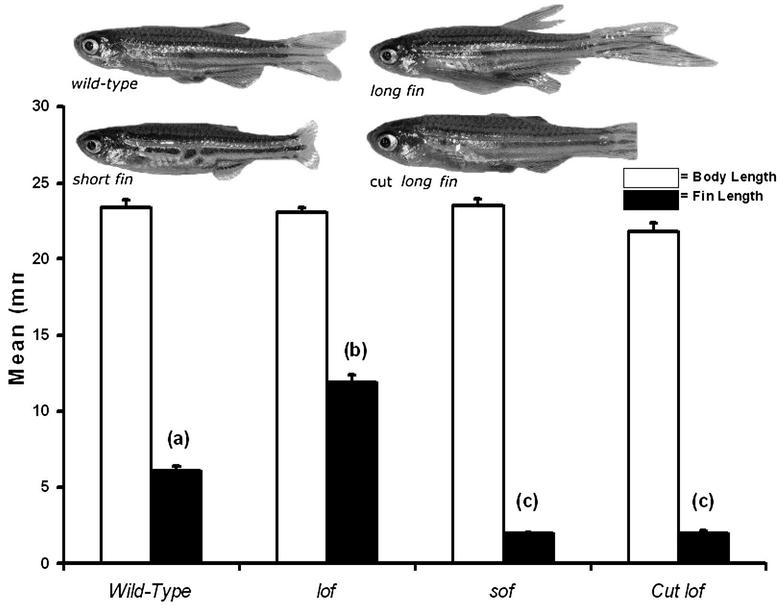

Figure 1. Body and fin lengths. Means (+ SE) body length (white bars) and caudal fin length (black bar) for wild-types ($N = 17$), long fin mutants (*lof*) ($N = 28$), short fin mutants (*sof*) ($N = 18$) and cut *lof* ($N = 8$). Because we selected for fish with similar standard lengths, it was not surprising that there was no significant difference among the four groups (ANOVA, df = 3, 67; $F = 1.9$; $p =$ N.S.). Fin lengths were significantly different (ANOVA, df = 3, 67; $F = 138.0$, $p < 0.001$). The Student Newman-Keuls Test revealed that *Lof* has caudal fins significantly longer than the other 3 groups) (SNK; $p < 0.001$) and wild-types have caudal fins longer than *sof* and cut *lof* (SNK; $p < 0.001$). Sof and cut *lof* are not significantly different in caudal fin length (SNK; $p > 0.05$). Letters above bars indicate significant differences.

2 *lo*f and 1 wild-type, 1 *lof* and 2 *sof*, and 2 *lof* and 1 *sof*. At the end of the experiments, all fish were returned to stock tanks.

Our intent was for all fish to have similar body lengths while differing in caudal fin lengths; Figure 1 illustrates that the *lof* mutants have caudal fins about twice the length of wild-types and about 6 times the length as *sof* mutants. To prevent inadvertently changing the signal patterns, we did not mark individual fish. All groups consisted of 3 male fish that that were placed in a 76 l tank and 3 min behavioral observations occurred 30 min, 4 h and 24 h after placing the fish in the tank. The average number of lateral displays was calculated for each replicate.

We also recorded the number of bites. A bite occurs when one fish swims rapidly toward another fish and often makes contact with its mouth open. Typically the other fish quickly moves away. Bites are commonly observed in male zebrafish groups (pers. obs.) and we recorded the number of bites to judge if the surgery affected other aspects of aggressive behavior. Thus, for example, if cut *lof* exhibited levels of biting lower than intact *lof*, any reduction in lateral displays may have been the result of surgical trauma.

Statistical analyses were performed using the program *Statistica* (Release 7, StatSoft, Tulsa, OK, USA). Data were analyzed using parametric statistics after we determined that they were not significantly different from a normal distribution (Kolmogorov-Smirnov Test, $p > 0.20$).

Results

Comparing among the homogeneous groups (Figure 2) revealed a significant difference in the number of lateral displays (ANOVA; $F = 5.25$, df $= 3$; $p < 0.01$) with *lof* producing more lateral displays than *sof* (Student-Newman-Keuls test, $p < 0.01$) and cut *lof* (SNK, $p < 0.05$) and no group was significantly different from wild-type (SNK, $p > 0.05$). In spite of a large increase in the number of bites generated by cut *lof* compared to intact *lof*, there were no significant differences in the number of bites among the

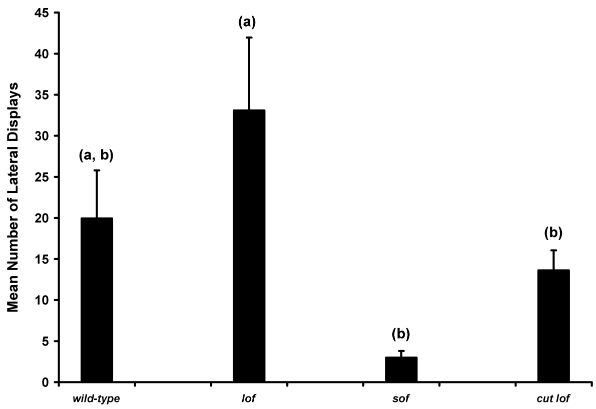

Figure 2. Mean (+ SE) number of lateral displays for the homogenous groups. $N = 7$ for each group. *lof* = long fin mutant, *sof* = short fin mutant, cut *lof* = long fins with all fins cut to resemble *sof*. *Lof* and *sof* are significantly different but they are not significantly different from the other groups.

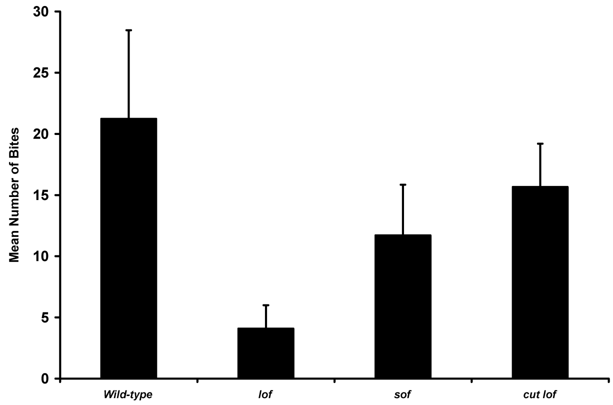

Figure 3. Mean (+ SE) number of bites for the homogenous groups. $N = 7$ for each group. *lof* = long fin mutant, *sof* = short fin mutant, cut *lof* = long fins with all fins cut to resemble *sof*. There are no significant differences among the four groups.

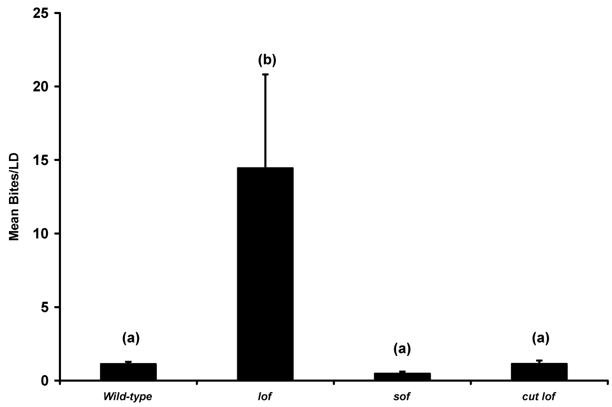

Figure 4. Mean (+ SE) ratio of bites/lateral displays for the homogenous groups. $N = 7$ for each group. *lof* = long fin mutant, *sof* = short fin mutant, cut *lof* = long fins with all fins cut to resemble *sof*. *lof* is significantly different from the other three groups.

four groups (Figure 3; ANOVA; $F = 2.42$; df = 3; $p > 0.05$). However, the bites/lateral displays ratios were significantly different (Figure 4; ANOVA; $F = 4.48$, df = 3; $p < 0.02$) with *lof* groups exhibiting significantly more lateral displays per bites than the other three homogeneous groups (Student-Newman-Keuls test, $p < 0.05$). The reduced bites/lateral display ratios for the cut *lof* indicate a close similarity to the shorter-finned types (Student-Newman-Keuls test, $p > 0.05$).

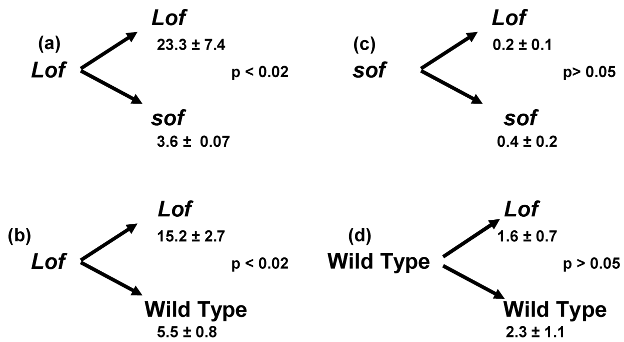

Figure 5. Lateral displays directed at same fin type or other type within a group of three fish. Means (± SE) are given below tail type. Because we were unable to tell individual fish apart, we presumed that each fish had an equal chance of laterally displaying to each of the other two fish. For example, in group (a), the three fish group consists of two *lof* and one *sof*. Together the two *lof* mutants produce a mean of 23.3 lateral displays per 3 min period while the two *lof* mutants combined to produce a mean of 3.6 against the *sof* mutant. $N = 7$ for groups (a) and (b) and $N = 6$ for groups (c) and (d). p values generated by paired t-test. See text for details.

The difference in behavior among the *lof* and cut *lof* is suggestive of an assessment system based on either having or seeing a *lof* mutant individual. As a test, we used mixed groups consisting of three fish composed of: 1 *lof* and 2 wild-types, 2 *lo*f and 1 wild-type, 1 *lof* and 2 *sof*, and 2 *lof* and 1 *sof*. The intent was to determine how *lof* mutants responded when housed with another *lof* mutant or with individuals with shorter fins. Figure 3 summarizes the lateral displays in the mixed groups. *Lof* displayed significantly more often to *lof* than to *sof* (Paired t-test: $t_6 = 2.81$; $p = 0.03$; Figure 3a) or to wild-type (Paired t-test: $t_5 = 3.8$, $p = 0.01$; Figure 3b) while *sof* did not increase their displays to *sof* compared to *lof* (Paired t-test: $t_6 = 1.98$, $p = 0.09$; Figure 3c) nor did wild-type increase their displays to other wild-types compared to *lof* (Paired t-test: $t_5 = 0.78$, $p = 0.47$; Figure 3d). This illustrates that *lof* mutants selectively increased their lateral displays to other *lof* mutants while neither the wild-types nor the *sof* mutants increased their lateral displays to their own types or to *lof* mutants.

Discussion

Because males with the *lof* mutation behaved differently, we reject the hypothesis that fin length has no influence on the lateral display. Surgically shortening the fins of the *lof* mutants caused them to display more similarly to *sof* than to *lof*, illustrating that the higher display rates of *lof* was not genetically controlled but dependent on the possession of the long fin. We reject the possibility that this reduction of lateral displays was caused by surgical trauma because biting behavior of cut *lof* showed a nonsignificant increase in frequency, more closely resembling *sof* than *lof*. The ratio of bites to lateral displays in cut *lof* also more closely resembled the shorter-finned non-surgical groups (*sof* and wild-types). Therefore, we also reject the pleiotropic hypothesis because *lof* mutation was not the only contributing factor to display rate.

When also considering the results of the mixed groups, we conclude that the increased use of the lateral display required two conditions: First, the individual must have long fins and, second, it must be competing with a similar phenotype. These results indicate that zebrafish assess not only the fin length of its opponent but also of itself. Furthermore, the homogeneous groups illustrate that the frequency of the lateral display is not dependent on individuals confronting those with similar fin sizes but is relates to having long fins and being opposed by individuals with long fins.

In conclusion, we demonstrate that the close association between an exaggerated structure and its prominent display may not be genetically coupled and that the exaggeration of the behavior may evolve independently of the structure's exaggeration. It is difficult to determine whether these results are widespread because so few species have had their genome sequenced and of these few, none seem to have phenotypically variable display structures. Thus it remains to be determined whether the exaggerated behavior used to display exaggerated structures in other species are genetically coupled with the display structure or based on an individual's assessment process.

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