Agonistic behavior in zebrafish (*Danio rerio*)

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Introduction

Ethology is the study of animal behavior, mainly concerned with behaviors which are instinctive. These instinctive behaviors may be attributed to the genetics of the organism, rather than to learned or programmed responses. Instinctive behaviors are advantageous due to the fact that they often aid in the survival of the species, but instinctive behaviors do not require much synaptic input. It is not necessary for an animal to store much information to engage in innate responses. Simple animals, therefore, take frequent advantage of these types of responses. However, instincts are, by definition, inflexible, and thusly, may result in a species' inability to adapt to environmental change. All species must rely on innate behaviors for such diverse activities as feeding, nesting, self-preservation and sexual displays. Yet another example of instinctive behavior is communication. Animals communicate using various methods, including mechanical, visual and chemical forms of communication.

Aggressive (agonistic) behavior in animals is necessary in that it allows them to establish a dominance hierarchy.[1] This type of innate behavior, however, may be influenced by the members of their community. In the presence of a predator, animals will exhibit a differing frequency of agonistic behaviors than in the presence of members of their own species.

The zebrafish (*Danio rerio)* has emerged as a model species for behavioral genetics studies, as well as for biomedical research. Due to the transparent nature of the egg, zebrafish embryology has been extensively studied and is well understood. In addition, zebrafish have a low maintenance cost, are hardy, and have a rapid life cycle, with large numbers of offspring. [3] The entire, relatively small, [4] zebrafish genome has been elucidated and hundreds of mutations have been discovered.[2] Many of these mutations have been described, including those that effect the shape of the embryo, those that interrupt the differentiation of the germ layers, those that effect organ systems, those that effect various cell types, those that disrupt the organization of the brain, those that interrupt the normal vascular development, and those that impede normal neuronal circuitry.[2] However, little is understood about zebrafish behavior, therefore it is a fertile ground for behavioral genetics research. The exciting elucidation of the molecular mechanisms of zebrafish behavior will be applicable in other vertebrates[3] in the normal as well as the diseased state.

We chose to investigate agonistic behavior in zebrafish in the presence of a predator and in the presence of a shoal. The fish in the lab are a TM1 lab strain, an SH lab strain, and a Nadia wild strain. The Nadia strain originates from ponds, flood plains and rice fields in an area east of Calcutta, India.[5] In an early demonstration, we were shown their differing reactions to taps on the aquarium. The lab strains immediately swam together to the top of the tank, while the wild type scattered and swam away. Upon observation, differences in aggression between the strains were quite apparent, with the TM1 displaying the most agonistic acts. We were assigned the F2 cross between the SH and the Nadia strains. We took some time narrowing down our questions to one: Are zebrafish of the F2 hybrid strain (SH/Nadia) more aggressive in the presence of a predator, or in the presence of a shoal of their own species? Following journal research, we formulated our hypothesis: zebrafish of the F2 strain will be more aggressive in the presence of a predator than in the presence of a shoal.

Experimental Conditions

The lab room temperature was approximately 30^{o}C, while the temperature of the tanks was 26^{o}C. The home tank had a 5 gal. capacity, with a population of 8-10 fish. The experimental tank was a 115L tank, measuring 91x31x43 cm, divided into three sections. The dimensions of the sections were as follows: the middle compartment was 55x31x43cm, while each of the two side sections were 18x31x43 cm. Black Plexiglas blinds could be lowered to hide the side compartments. A model predator was made of epoxy and painted with black and white stripes, representing a *Etroplus canarensis* and was suspended with fishing line in the right compartment of the triple tank. A shoal of 4 zebrafish was placed in the left compartment. The light/dark cycle was of 14:10h duration, mimicking the natural daylight hours during breeding season in the wild.[5] The 40 zebrafish that were used in this experiment were approximately 30mm in length and ranged in age from 7-8 ½ months The water filtration system included a biological system and a carbon filtration system. Tanks were aerated with bubblers. The fish were fed dry flake food once a day following behavioral observations.

Ethogram

We originally developed an ethogram consisting of four behavior; nip, bite, charge and chase. However, after discussion, narrowed our choices down to two. The reasoning behind this unification was that from the researchers' vantage point it would be nearly impossible to distinguish a bite from a nip or a charge from a chase.

BIT: bite or nip; moving toward another fish, mouth opens and closes, contact may or may not be made with the other fish.
CHS: chase or charge; moving toward a second fish, increasing acceleration, while the second fish avoids the first.

Protocol

We were interested in watching the aggressive behavior of zebrafish in the presence of a predator and in the presence of a shoal of their own species. We began by placing the black Plexiglas blinds between the center compartment and each of the side compartments. We then netted 4 zebrafish into the middle of the experimental tank. We then allowed an acclimation period of 10 minutes. Two of the experimenters stood approximately 1.83 M from the tank, hidden so as not to influence the behavior of the fish. By use of the scan method the two experimenters scored the number of bites and chases as described in our ethogram for a period five minutes. The experimenters held a counter in each hand keeping track of bites on one counter and chases on the other. We then recorded our data. The blind between the first compartment and the experimental shoal was raised, followed by a 1 minute waiting period. This was done in order to allow the fish to calm down from the blind raising. The above procedure was repeated with the blind raised between the first compartment and the shoal, followed by a two minute rest period and then repeated again when the blind was raised between the predator and the shoal. We repeated the procedure, randomly alternating blinds for a total of two trials, then returned the fish to their home tank. This was repeated for a total of 22 trials.

Results

The control (with both blinds down) resulted in a mean of 10.4 +/- SD 16.8, with a standard error of 2.4 and a 95% confidence interval ranging from 5.8 to 15.1. For the trials where the shoal of the same species was exposed, the mean was 5.5 +/-SD 6.2, with a standard error of 1.3 and a 95% confidence interval ranging from 2.9 to 8.1. For the trails where the predator was exposed, there was a mean of 11.5 +/-SD 19.7, with a standard error of 4.2 and a 95% confidence interval ranging from 3.3 to 19.7. A t-test was run to test for significant differences in the control, shoal and predator trials and it was found that $P = 0.85$ between the control and the predator, $P = 0.21$ between the control and the shoal, and $P = 0.185$ between the shoal and the predator. While the difference between the shoal and the predator does not represent a statistically significant difference, this researcher concludes that a larger sampling is called for and would likely result in a finding of a significant difference.

Observations and Discussion

After analyzing the graph, it became immediately apparent to this researcher that it was not the presence of the predator that increased aggression, as we had originally hypothesized, rather it was the presence of the other shoal that *decreased* aggression. This may have been due to the fact that the experimental shoal was investing so much energy and attention on getting to this other shoal, that they virtually ignored each other. The question remains, “Why were they more focused on the other shoal than on their own shoal?”

Some interesting observations are noteworthy. We noticed that under almost all circumstances, the experimental shoal tended to remain on the left side of the tank, nearest the shoal of their own species. This remained consistent whether both blinds were down, or either of the blinds was raised. During most of the trials, the shoal remained together, although at times, they paired off on opposite sides of the tank, and occasionally, one would venture off on its own. Every time that the other shoal was exposed, the experimental group became much more active. They faced the Plexiglas partition and swam much more vigorously, even biting at the Plexiglas at times.

Another interesting observation, which showed up in the data, was the highly increased agonistic behavior from the fish in tank #68. While there was no discernable difference in this tank, we did notice that one of the fish was missing its operculum and discussed the possibility of the injury resulting in the aggression.

We also discussed the feasibility of using the model as a predator. We were not convinced, by observing their behavior, that the zebrafish were at all intimidated by a motionless model. It would be interesting to run the trials again using a real, live, swimming *E. canarensis,* or if that is not feasible, to cause realistic movement in the model.

Each of these may be an area for future research. Interestingly, I come away with more questions than ever. And that is the nature of science.

Reflections

Dr. Martins lab is focused on behavioral genetics of animals. Which behaviors are learned? Which ones are instinctual and therefore genetically driven? What are the molecular mechanisms involved in guiding behaviors? Where are the genes that drive these mechanisms? Perhaps more importantly, how did these gene mutations evolve into the present behavior?

There appears to be a personality type that is required for researchers: tenacity, persistence, commitment, lack of concern with traditional time restraints, deep-seated curiosity, and patience. A vast majority of a researcher's time is used to review the published literature, refine the question to be investigated, formulate the hypothesis, design the experiment and analyze and draw conclusions from the data. The smallest amount of time is spent on the experiment itself. This is counter to what is the accepted perception of the layperson regarding scientists.

Peer review is a very important aspect of research. Issues are illuminated by peers that would not have come to light by a lone researcher. As it is unlikely that any one researcher will see their work come to fruition, even following a lifetime of investigation, the researcher must be satisfied with being a cog in the mechanism, while at the same time realizing that the mechanism would not function without that cog.

Unexpected interruptions and delays are a part of the job. When an undergrad changed the water, using tap instead of filtered, Jason lost a dozen of his fish and we lost three of our F2. While we were disappointed, I know that Jason, his post doc reputation on the line, must have been devastated. It is hard to say how much this will set him back. Of special consequence was the unequal distribution of males to females. This was an issue even before the undergrad mistake, but became even more critical afterward.

References

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