Animal Behavior Final Project: Male Dominance between Alternate Reproductive Strategies in *Xiphophorus nigrensis*

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Abstract

Xiphophorus nigrensis males have three distinct phenotypes, which dictate the reproductive strategy employed to gain copulations. Previous studies have indicated that large, courting males have greater reproductive success than small males that mate coercively. We suggest that this pattern will lead to a greater amount of aggressive dominance interactions between males of the same strategy, as there is a greater amount of direct competition for access to females between these males than between males of different reproductive strategies. Our hypothesis was not supported by experimental results; however, we found that of the dominance interactions between males of different strategies, a significantly higher proportion were initiated by large males. This finding aligns with previous studies and indicates that males are competing with all reproductive strategies for access to females. Further investigation is needed to determine potential differences in the manner of aggressive interactions between different male phenotypes and further elucidate the role of dominance in sexual selection.

Introduction

To live means to compete. From microorganisms to megafauna, everything on earth seeks resources equally sought by others, including food, shelter, centrality in a herd, or access to mates.[1] The result of competition is often resolved via aggressive interactions, which, when repeated over time, develop into dominance structures within a population.[2] Dominance structures are ubiquitous across the animal kingdom and can have important implications for reproductive success and health.[3]

This study examines dominance relationships in a species of Poeciliid fish, the Panuco swordtail (*Xiphophorus nigrensis)*.[4] In this species, female swordtails prefer large males, which enjoy greater reproductive success.[5] Natural selection favors the evolution of alternative mating strategies under these conditions.[6] Within this species, there are three male strategies, delineated by distinct male phenotypes controlled by differential expression of traits on the Y chromosome.[6] Large males (L), that possess a sword like extension of their tail, court females, and small males (S) that do not have a sword mate coercively, while intermediate males (INT), that have a reduced sword, both court and coerce.[4]

In this study, we examine dominance interactions in swordtails by investigating aggressive interactions to determine whether males exhibit differing levels of dominance interactions based on differences in reproductive strategy. Alternative reproductive strategies in this species indicate that males are potentially directly competing for access to females more with males of the same reproductive strategies than with males of different reproductive strategies. We hypothesize that there are statistically more dominance interactions between males of the same strategy (L and L, S and S, INT and INT), and males of different strategies that are not directly competing (L and S) will exhibit less dominance interactions. From this hypothesis, we propose two predictions.

<u>Prediction #1:</u> There is a significantly higher average number of dominance interactions per tank in tanks containing only males of the same reproductive strategy than tanks with a mixture of large and small males.

Prediction #2: Within the tanks containing both large and small males (LS treatment group), there is a significantly higher number of dominance interactions between males of the same reproductive strategy than males of different reproductive strategies.

Our null hypothesis is that there is no difference in the amount of interactions between males of the same reproductive strategy and males of different reproductive strategies. Alternatively, if a significant relationship is observed, this could be the result not of competition over direct access to reproductive females, but rather of the defense of a resource or territory. In gobies, dominance is virtually absent from interactions between males, which have large, ornamented tails to attract females.[7] Female choice therefore has the biggest effect on reproductive success, and dominance is not observed within males.[7] For male swordtails that court females, female choice also affects reproductive success, which would predict an absence of dominance interactions between large males if the males are competing only for direct access to females. Therefore, data indicating differential dominance interactions between the reproductive strategies may imply that males are defending territories or resources instead of direct access to females.

Sexual selection is one of the most influential theories in modern biology. This theory drives the evolution of alternative mating strategies, but we know relatively little about how the mechanisms of sexual selection interact with natural selection in group dynamics of social animals.[6] In particular, the relationship between alternative mating strategies and behavioral dominance remains largely unexplored, and this research has the potential to expand upon existing knowledge of the well-studied mating system of *Xiphophorus nigrensis* to alleviate this gap in knowledge.[8]

Methods

Female *Xiphophorus nigrensis* were raised in a laboratory under uniform conditions. Within each tank, there were four females and four males. The treatment group of each tank dictated the phenotype of the four males: all large males (LL), all intermediate males (INT), all small males (SS), or half large and half small males (LS). Lab assistants recorded videos of the tanks during the day for a range of fifteen to twenty minutes. We analyzed 15-minute segments from a total of 51 videos, 13 from each treatment group, and observers were randomly assigned videos to reduce bias. Observers recorded dominance interactions, defined by aggressive behavior, such as biting or approaching, between two males (ethogram based on Damore *et al* 2019 and Sopinka *et al* 2010). [9,10] The size of the aggressor male, type of interaction (approach or bite), size of receiver male, and behavior of the recipient male (stay or displacement) was recorded for each interaction. There were no tanks where intermediate males were combined with large or small males, so none of these interactions were able to be evaluated.

All statistical tests were conducted in R version 3.5.3..[11] To determine the variance in dominance interactions between treatment groups, we calculated the mean number of interactions per tank (in a fifteen-minute period), and conducted an ANOVA analysis of variance to determine significance. To investigate the variance in the number of dominance interactions between males of the same and males of different reproductive strategies, we analyzed only the LS treatment group, as this is the only group that contains males of different reproductive strategies. Within each tank, we calculated the average number of dominance interactions between males of the same reproductive strategy and different reproductive strategies. A two sample, independent t test was conducted to determine significance. To determine if dominance interactions were initiated by large or by small males more frequently, we calculated the mean number of dominance interactions initiated by each size of male within the LS treatment group. A two sample, independent t test was conducted to determine significance.

Results

The ANOVA analysis of variance in mean number of dominance interactions per tank between treatment groups resulted in a P value of 0.1384 ($P >> 0.05$ df = 3, N = 51) (**Figure 1**).

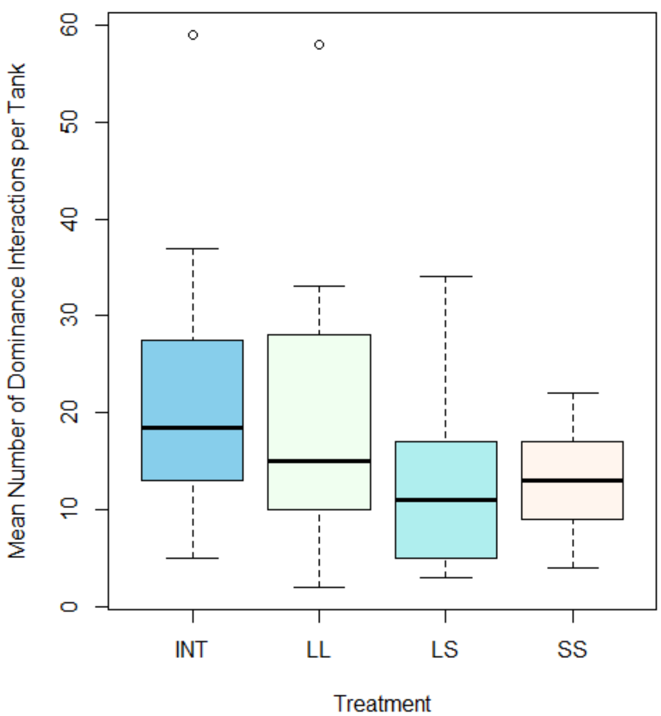

Figure 1*: Mean number of dominance interactions per tank by treatment group.*

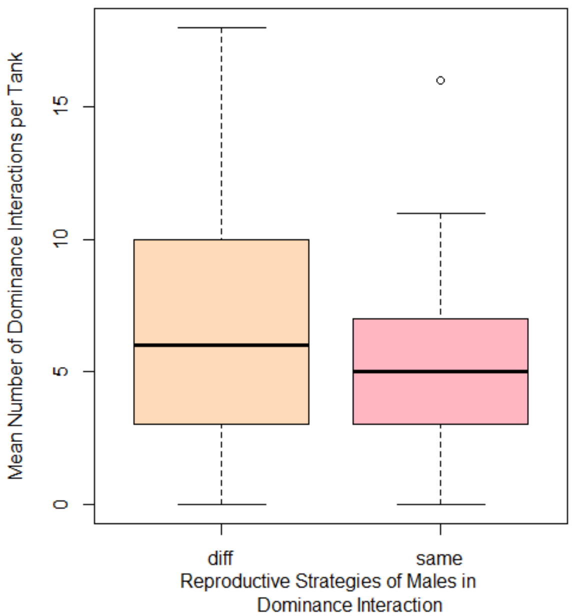

Figure 2*: Mean number of dominance interactions per tank within the LS treatment group by reproductive strategy*

A two sample, independent t test of the mean number of dominance interactions within LS tanks between males of the same reproductive strategy vs males of different reproductive strategy resulted in a P value of 0.8455 ($P >> 0.05$, df = 24, N = 13) (**Figure 2**).

A two sample, independent t test of the mean number of dominance interactions within LS tanks that were initiated by large or small males resulted in a P value of 0.0087 ($P << 0.05$, df = 20, N = 13) (**Figure 3**).

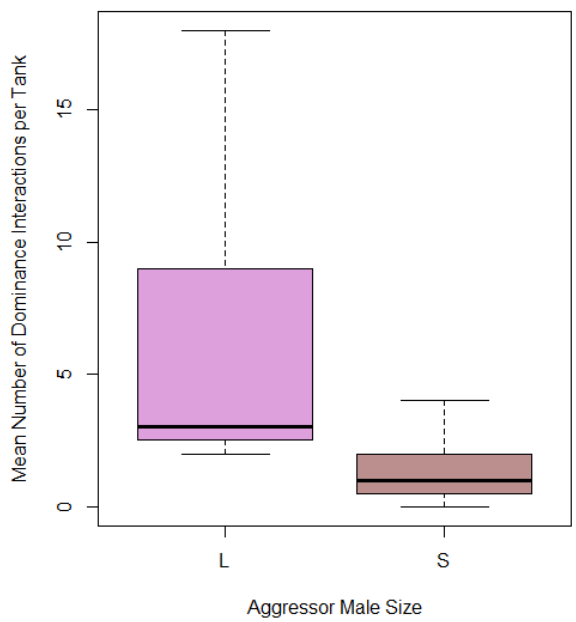

Figure 3: *Mean dominance interactions between males of different reproductive strategies in LS treatment by size of the aggressor male.*

Discussion

Prediction one stated that we expected to see significantly higher average numbers of dominance interactions per tank in a fifteen-minute period in the LL, SS, and INT treatment groups compared to the LS treatment group. The mean number of dominance interactions in the LS treatment group appears to be the lowest, but an ANOVA test revealed that this difference is not statistically significant ($P = 0.1384$) (**Figure 1**). Therefore, we conclude that no treatment groups significantly differed from one another in mean number of dominance interactions per tank. However, among the four males in the LS tanks, two are large and two are small, so dominance interactions solely between males of the same reproductive strategy in this treatment group may have confounded the results. To control for this, we looked at dominance interactions only within the LS treatment groups to test prediction two, which stated that we expected to see a significantly higher number of dominance interactions between males of the same reproductive strategy than between males of different strategies. A two-sample independent t test revealed that the difference in mean number of interactions per tank for each group is not significant ($P = 0.8455$) (**Figure 2**). Dominance interactions are occurring, but there is no effect of reproductive strategy on the frequency of occurrences. This, combined with our previous ANOVA test, tells us that neither males of the same reproductive strategy or males of different reproductive strategies exhibit notably higher levels of dominance interactions. From these results, we deduce two possible explanations: that males compete equally with all other males for access to females, or males are competing over a resource which is unaffected by reproductive strategy.

To further elucidate possible explanations, we examined the significance of the proportion of dominance interactions that were initiated by large versus by small males. Based on the first two tests that we conducted, we expect to see the same amount of dominance interactions initiated by large and by small males. We conducted a t test was to see if, within the LS treatment group, the mean number of dominance interactions initiated per tank by large males was different than the mean number of dominance interactions initiated by small males. The results of this test showed that there is a significantly higher number of dominance interactions initiated by large males ($P = 0.0087$) (**Figure 3**).

Previous studies support this finding: Morris *et al* 1992 found that in *Xiphophorus nigrensis*, males attempt to exclude one another from access to females, and large males are much more likely to block smaller males while smaller males were much more likely to be blocked.[5] Ryan 1988 found that large *X. nigrensis* males court females while patrolling an area where females are feeding on mossy rocks.[12] Our data is consistent with these results, and this pattern is found in other taxa as well. In *Zorotypus* insects, dominant males always win fights, and small males are more likely to avoid dominance interactions.[13] Additionally, in the goby *Neogobius melanostomus*, size is related to dominance rank, and large males are always ranked higher than smaller males.[1]

Morris *et al* 1992 also found that the dominance of larger males was not confined to interactions between males of different reproductive strategies but was also present within a reproductive strategy.[5] Even when both males interacting were L or INT, the male that was slightly larger was much more likely to successfully exclude the other male from access to females. As the size of males in our data was grouped into three discrete, rather than many continuous levels, we are not able to analyze this relationship. However, future studies replicating these results would provide insight on if this pattern is present across swordtail populations. Future directions may also include attempting to further tease out the difference in the type of dominance interaction between males. In a cursory look at other aspects of the data collected, we see that bites made up a smaller proportion of the behavior that initiated a dominance interaction, while direct approaches were much more common. 2.4% of the interactions between fish of the same reproductive strategy consisted of bites, while only 1.2% of the interactions between fish of different reproductive strategies were bites. Future studies investigating the significance of this relationship may provide insight into if there is a difference in type, rather than of scale, of the interactions between males of different reproductive strategies.

Our hypothesis that there are more dominance interactions between males of the same strategy is unsupported. We conclude that our data supports other studies' findings that males are competing with individuals of all reproductive strategies for access to females, and that some competitive interactions are unequal with large males more commonly initiating aggression against small males rather than vice versa. We can improve the confidence of future studies by modifying the methodology to include a greater number of videos from the LS treatment group. Thirteen videos from each treatment were scored, so analysis of only the LS group had low statistical power that can easily be increased by scoring a greater number of videos.

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