



Territoriality is some of the most complex behavior displayed by herpetofauna. Like many other aspects of animal behavior, territoriality is poorly understood due to the inherent difficulties of its study. Although the territorial behavior of the Strawberry Poison Dart Frog (*Oophaga pumilio*) has been studied extensively, much remains to be learned. In the following article, the author set out to better understand the factors at play in male territorial interactions within this species. Pictured here is a pair of adult *O. pumilio* in a tree cavity near a field station in the province of Bocas del Toro, in northwestern Panama.

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When it counts, size does matter: complex territoriality in *Oophaga pumilio* (Schmidt, 1857)

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ABSTRACT: Although territoriality is one of the most interesting and complex aspects of animal behavior, its nuances remain poorly understood. This lack of knowledge appears to have led to conflicting observations of the factors that influence the ability of an animal to control a territory. Two of the most common and important factors are individual size and defensive status. In this study, I investigated which of these factors is more important for success in territorial interactions. I created situations where two male *Oophaga pumilio* were induced to interact over a given territory on Isla Colón, Bocas del Toro Archipelago, Panama. I observed two types of territorial interactions: physical interactions (high-energy) and vocal interactions (low-energy). An analysis of my observations revealed that the defender was more likely to succeed when both interaction types were combined, as well as in low-energy interactions (67.3% and 70.6%, respectively). When examining the high-energy interactions, however, the larger male was successful more often (69.2% of the interactions). These results support the theory that the perceived asymmetry in success found in many taxa might result from different types of interactions, and not from increased resource holding potential in defending males.

Key Words: Anuran, behavior, defense, *Dendrobates pumilio*, probing interaction, territoriality

RESUMEN: Aunque la territorialidad es uno de los aspectos más interesantes y complejos de la conducta animal, sus matices siguen siendo poco conocidos. Esta falta de conocimiento parece haber llevado a observaciones contradictorias de los factores que influyen en la capacidad de un animal para controlar un territorio. Dos de los factores más comunes e importantes son el tamaño individual y el estado defensivo. En este estudio, yo investigué cuál de estos factores es más importante para el éxito en las interacciones territoriales y creé situaciones en las que dos machos de *Oophaga pumilio* fueron inducidos a interactuar en un territorio dado en Isla Colón, Archipiélago de Bocas del Toro, Panamá. Observé dos tipos de interacciones territoriales: interacciones físicas (alta energía) e interacciones vocales (baja energía). Un análisis de mis observaciones reveló que el defensor tenía más probabilidades de éxito cuando ambos tipos de interacción se combinaban, así como en las interacciones de baja energía (67,3% y 70,6%, respectivamente). Sin embargo, al examinar las interacciones de alta energía, el macho mayor tuvo éxito con más frecuencia (69,2% de las interacciones). Estos resultados apoyan la teoría de que la asimetría percibida en el éxito que se encuentra en muchos taxones podría resultar de diferentes tipos de interacciones, y no de un mayor potencial de retención de recursos en la defensa de los machos.

Palabras Claves: Anuro, comportamiento, defensa, *Dendrobates pumilio*, pruebas de interacción, territorialidad

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INTRODUCTION

Substantial evidence shows that individuals defending their territory are more successful than those attempting to gain control of it (Wells, 1978; Cooper, 1987; Greenberg, 1994; Crofoot et al., 2008). Nonetheless, superior physical attributes, usually quantified by body size, are the primary factors in determining success in agonistic interactions (Cuadrado, 2001; Tsuji, 2004; Kokko et al., 2006). A theory exists that accounts for these seemingly contradictory findings; defensive status may appear to bestow an increased resource holding potential (RHP) because the invader might not always be making an actual attempt to gain control of the territory, but might be probing to gain information (Grafen, 1987). This supposition might occur for two reasons: (1) the invader could be attempting to locate a vacant territory to occupy; or (2) the invader could be probing the occupied territories in the area to ascertain which is defended by the least fit male, thus making that territory the easiest to control (Grafen, 1987). In a probing interaction the invader likely would devote a small amount of energy, and thus easily could be driven out by the defender. If probing interactions are not correctly identified, studies may provide evidence leading to an incorrect understanding of the species' territorial interactions and the factors that drive them. I believe the simplest way to distinguish probing behavior from true territorial conflicts is a multi-stage interaction, consisting of an escalation. Thus, I chose to use a population of *Oophaga pumilio* to study this behavior, because I found that males of this species demonstrate escalating interactions.

Oophaga pumilio (Schmidt, 1857), commonly referred to as the Strawberry Poison Dart Frog, is a small dendrobatid [19–24 mm snout–vent length (SVL)] that engages in reproductive activity year round in areas without a pronounced dry season (Leenders, 2001). The frogs maintain their territory by engaging with encroaching same-sex conspecifics. Males interact with conspecific males that physically enter their territory, or whose call is perceived by the defending male (Bee, 2003). *Oophaga pumilio* has been observed to participate in heightened territory maintenance from 0700 to 1700 h (Haase and Pröhl, 2002; Pröhl, 2002; Bee, 2003), and two distinct territorial interactions have been documented: vocal interactions between two males, and physical interactions, which are exhibited by both sexes (Haase and Pröhl, 2002; Meuche et al., 2012). My observations of this species suggest that male physical interactions actually are escalations of vocal interactions. This finding was extremely important, because it allowed me to categorize the vocal interactions as low-energy interactions (LEI), and the physical interactions as high-energy interactions (HEI). I believe that the majority of the probing interactions were conducted as LEI, and the remaining HEI mostly consisted of the actual territorial interactions, which facilitated my examination of the factors that influenced both types of interactions.

The territorial behavior of *O. pumilio* has been studied extensively (McVey et al., 1981; Haase and Pröhl, 2002; Pröhl, 2002; Bee, 2003; Meuche et al., 2012). Previous research, however, has relied upon audio recordings of male frogs. While this experimental method is reasonable for testing vocal interactions, I argue that it contains four important drawbacks:

- (1) Physical interactions cannot be tested.
- (2) This method cannot determine whether size or defensive status is more important in determining the RHP.
- (3) The resident males were marked by toe clipping, which can negatively affect the individual (Pröhl, 2002; Narayan et al., 2011).
- (4) Distortions or other alterations of the vocalization might occur during the recording and playback.

In an attempt to avoid these concerns, I developed an alternative testing method. I set out to determine whether the larger male or the defending male was successful in a disproportionate number of interactions. By analyzing the results from the HEI and LEI separately, I tried to differentiate probing from the actual territorial interactions. Additionally, I examined whether the degree of size difference in the frogs influenced the latency or duration of the interactions.

MATERIALS AND METHODS

I conducted my experiment in lowland, closed canopy, broadleaf, rainforest in the vicinity of the Institute of Tropical Ecology and Conservation's (ITEC) field station in Bocas del Toro, Provincia de Bocas del Toro, Panama (9.4066°N , 82.3134°W ; datum WGS 84; elev. ca. 0–25 m; Fig. 1). I collected male frogs, and performed my experimental observations in an area that measured roughly $3,000\text{ m}^2$. To induce an interaction, I placed two male *Oophaga pumilio* inside a glass aquarium measuring $50\text{L} \times 25\text{W} \times 30\text{H}$ cm, with the open side placed downward on the forest floor (Fig. 2). I chose to place the frogs directly on the forest floor in order to increase the chance that the defender would recognize it remained within its territory, and the invader would recognize that it was not. While currently we are unaware exactly how individuals of *O. pumilio* recognize their territory, some salamanders rely on chemical marking to recognize their territories (Gautier and Miaud, 2003). Some anurans also are capable of detecting and acting on chemical cues left by conspecifics (Schulte and Rössler, 2003; Pizzato et al., 2016). To prevent the frogs from escaping, I lined the perimeter of the open side of the aquarium with about 15 cm of mosquito netting (Fig. 2). The territory of a male *O. pumilio* has been documented to encompass an area of 17 to 39 m^2 (Haase and Pröhl, 2002). Accordingly, I collected the invader from a minimum distance of 10 m from the location where the “defender” was found, to ensure that the “invader” was removed from its territory (based on the methods of Savage, 2002; Bee, 2003). I also placed the enclosure within 30 cm of the location where the defender initially was found, to ensure that the defender remained within its territory.



Fig. 1. Location of the Institute for Tropical Ecology and Conservation in Bocas del Toro, Provincia de Bocas del Toro, Panama. All data collection was conducted in lowland rainforest in the vicinity of the station.

I conducted daily observations from 0900 to 1100 h and from 1300 to 1600 h, from 29 July to 8 August 2013, and from 1 July to 12 August 2014, for a total of 42 days of sampling. I chose the above-mentioned observation times to ensure the sampling occurred when the frogs were most active in their territories (Haase and Pröhl, 2002; Pröhl, 2002; Bee, 2003).

Both the “defending” and “invading” males were held in separate one-gallon plastic Ziploc bags until placed in the aquarium. The two males were placed in the aquarium within 30 sec of one another, with the defender introduced first and the invader last. Individuals of *O. pumilio* display unique dorsal spot patterns, which I photographed and visually compared to estimate the number of frogs that inadvertently were reused in the experiment (Leenders, 2001).



Fig. 2. The sampling aquarium I used to contain the frogs and help induce agonistic interactions. The green mesh along the bottom edge prevented the frogs from escaping.  © Justin K. Clause

In previous research, Meuche et al. (2012) indicated a latency of up to 3 min for the observed interactions to take place, and thus I allowed a total observation time of at least 10 min in my experiment. If I did not observe agonistic behavior from either individual after 10 min, I concluded there was no interaction and released both frogs.

I collected the following data during each encounter:

1. Snout–vent length (SVL).
2. Latency: how long it took for at least one of the frogs to interact agonistically, either vocally or physically, after both were placed in the enclosure.
3. Duration: length of the agonistic interaction.
4. Type of interaction: vocal contests (low-energy interactions: LEI) or physical contests (high-energy interactions: HEI). A vocal contest consisted of the frogs facing each other and calling repeatedly; a physical contest involved the two frogs calling repeatedly and physically wrestling.
5. Confrontation winner: after the frogs engaged one another, either vocally or physically, I deemed the individual that fled first as unsuccessful.

I created and analyzed two regressions, comparing the duration in relation to the size difference, and the latency in relation to size difference, using GraphPad Software, QuickCalcs®. This analysis allowed me to test for differences in the time it took for an interaction to begin, or how long the interaction lasted, if the size of the frogs was drastically different.

RESULTS

I observed a total of 139 separate interactions, of which 26 consisted of neither frog engaging in an interaction and thus the individuals were removed from the analysis. In the remaining 113 interactions, at least one frog visibly interacted. After inspecting the spot patterns of the first 78 frogs, I positively identified only one frog as a recapture. Assuming that the rate of recapture remained constant, approximately 4 frogs were recaptured ($0.01282 \text{ recapture rate} \times 278 \text{ captures} = 3.56 \text{ predicted recaptures}$). The size of the defender was larger in 55 interactions (49%), and that of the invader was larger in 53 interactions (47%), and the frogs were similar in size in 5 interactions (4%). I performed chi-squared tests using two-tailed P -values to analyze the interaction results (Table 1). Many of the interactions involved one frog interacting while the other did not exhibit a detectable response. Out of 113 total interactions, the defender was not detected engaging in 23 interactions, and the invader was not detected engaging in 47. I removed these interactions and analyzed the remaining 43 interactions separately, as above, and recorded the results in Table 1 under “Both Frogs Detected Interacting.” I conducted this test to determine if the higher number of invaders that were not detected interacting was the sole driver of my observed asymmetry in success rates. Four results were statistically significant. The defending male was successful more often when considering all of the interactions together (in interactions where one or both frogs were detected interacting). The defending male also was successful more often in low-energy interactions with at least one frog detected interacting. The larger male, however, was successful more often in the high-energy interactions, with at least one frog being detected interacting.

I show the regression comparing the magnitude of size difference and the duration of the interaction in Fig 3A, and the regression comparing the magnitude of size difference and latency of the interaction in Fig. 3B. For these regressions, I used only the interactions where both frogs visibly interacted ($n = 43$). Neither test showed statistical significance.

Table 1. Results of the chi-squared tests investigating the proportion of interactions where the defender or larger frog was successful. Bold numbers indicate the statistically significant results ($P < 0.05$). *The number of interactions is different between “Defending Male Successful” and “Larger Male Successful” calculations due to three pairs of frogs being the same size.

At Least One Frog Detected Interacting	All Interactions	Low-energy Interactions	High-energy Interactions
Number of Interactions	113, 108*	85, 82*	28, 26*
Defending Male Successful	67.3%	70.6%	57.1%
Chi-Square value, <i>df</i>	13.460, 1	14.412, 1	0.571, 1
<i>P</i> -value	0.0002	0.0001	0.450
Larger Male Successful*	56.5%	52.4%	69.2%
Chi-Square value, <i>df</i>	1.815, 1	0.195, 1	3.486, 1
<i>P</i> -value	0.178	0.659	0.0499
Both Frogs Detected Interacting	All Interactions	Low-energy Interactions	High-energy Interactions
Number of Interactions	43, 40*	18, 17*	25, 23*
Defending Male Successful	67.4%	72.2%	64.0%
Chi-Square value, <i>df</i>	5.233, 1	3.556, 1	1.960, 1
<i>P</i> -value	0.0222	0.0593	0.162
Larger Male Successful*	62.5%	58.8%	65.2%
Chi-Square value, <i>df</i>	2.500, 1	0.529, 1	2.130, 1
<i>P</i> -value	0.114	0.467	0.144

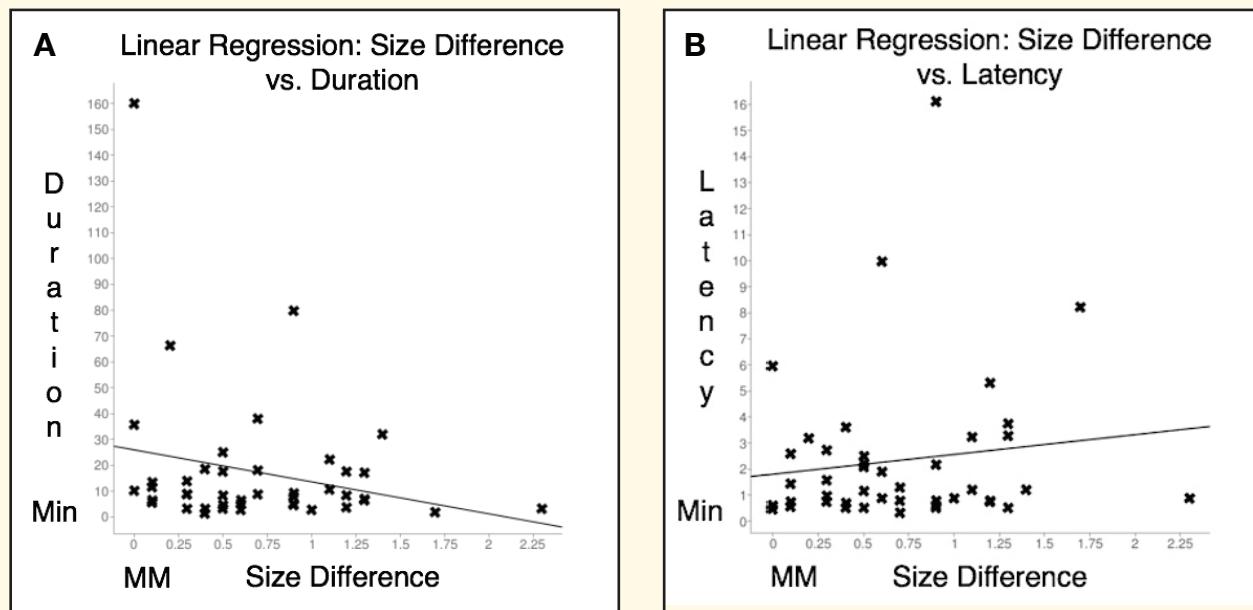


Fig. 3. Linear Regression. (A). Size difference vs. the duration of the interaction. $F(1,41) = 2.27$, $P = 0.140$, $R^2 = 0.0524$; and (B). Size difference vs. latency of the agonistic interaction. $F(1,41) = 0.692$, $P = 0.410$, $R^2 = 0.0166$.

DISCUSSION

My study provides evidence that the factors at play in the territorial interactions of *Oophaga pumilio* are more complex than previously reported. The territorial interactions of male *O. pumilio* consist of two stages: a vocal interaction that can escalate into a physical interaction. The two-stage, escalating, interaction being displayed allowed me to test the relative importance of size and defensive status in the territorial interaction.

The defender was successful more often when examining all of the interactions together, and when only considering low-energy, vocal interactions (LEI). In high-energy, physical interactions (HEI), however, the larger male was successful more often. I found these results statistically significant ($P < 0.05$). If the defensive status provided an advantage in agonistic interactions, then the defenders should have been successful more often in the HEI and LEI. This, however, was not the case. These results suggest that some invaders might be probing a defender's territory, possibly to gain information, and that they are not always making an attempt to gain control of the territory. These interactions inflate the "win percentage" of defenders, thus perpetuating misleading evidence for the defensive status leading to improved fighting ability or RHP. I think that the HEI are much more likely to be true interactions over territory, and found that, in these interactions, body size is more important than defensive status.

I did not detect 70 frogs (25.1%) interacting in the complete interactions. The invader was more than twice as likely not to be detected interacting than the defender (47 invaders vs. 23 defenders), and I identified two possible explanations for this result: (1) the frogs were too stressed to interact; and (2) the frogs only interacted for a short time. Stress alone is not likely to be the main explanation for the frogs' lack of interaction, as I observed many exploring their surroundings and eating while in the enclosure. The more likely explanation is that the duration of the interaction was short and went unobserved. I found that many of the interactions, where both frogs interacted vocally, involved the unsuccessful frog only calling once or twice. This behavior could go undetected in the enclosure for three reasons: (1) the glass of the aquarium fogged up, thereby reducing visibility; (2) males often hid under leaves or other debris while in the aquarium, remaining out of sight for up to 10 min; and (3) the ambient noise in the forest could obscure one or two isolated calls. When I removed these interactions from the analysis, the results were not greatly altered; all three of the statistically significant success rates discussed above were within 4 percentage points of their previous value. This finding suggests that the disproportionate number of invaders that

were not detected interacting was not driving the results. A possible explanation for this asymmetry could be that the invaders more likely called once or twice in the probing interactions, so as to gain information about the defender while using minimal energy input.

The results of the two regressions, comparing latency and the duration of the interactions with size difference, were both inconclusive. The small sample size ($n = 43$) might have been a factor. Additionally, the sampling design also might have biased my results in the analysis of size difference vs. latency. I only observed pairs for 10–15 min without an interaction before removing the frogs; interactions that would have begun after that time could not be documented, thus biasing the interactions that occurred with a shorter latency.

CONCLUSIONS

This study supports the probing theory first proposed by Grafen (1987). My results suggest that the reason males defending their territories in LEI appear to have an increased RHP, is that the invading male does not always make an actual attempt to gain control of the territory. Perhaps, in many of the LEI, the invading male could be attempting gain knowledge about the defender of the territory. Once an interaction has escalated to a HEI, the invading male likely is attempting to gain control of the territory, and the larger frog usually is successful. When studying species where probing interactions cannot easily be differentiated from true territorial interactions, care must be taken. My findings suggest that many observations of the defensive status leading to higher RHP could be misleading. The perceived asymmetries in RHP could be caused by unknown nuances within the interactions, such as probing behavior. I recommend additional research with other species, as well as with *O. pumilio*, to better elucidate the factors involved in determining RHP.

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