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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Experimental Evaluation of Territoriality and Associated Behaviors in the Spotted Dusky Salamander (*Desmognathus conanti)*

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Territoriality has been shown to meaningfully influence the distribution and population dynamics of several species of plethodontid salamander, but has never been formally evaluated in the spotted dusky salamander (*Desmognathus conanti)*. I assessed the territorial defense, tenacity, and advertisement of male and female adult *D. conanti* individuals in two laboratory behavioral experiments, using 82 wild-caught individuals from two shaded first-order streams in the oak-pine-hickory woodland of Lullwater Preserve in Atlanta, Georgia. The first experiment involved substrate choice trials to assess the role of chemical signaling and chemosensation in the advertisement and perception of individual home areas. The second experiment involved behavioral aggression trials featuring resident-intruder pairs to assess site tenacity, territorial defense, and the determinants of aggressive and submissive behavior. I considered the effects of subject sex, weight, snout-vent length, tail length, residency status, and origin subpopulation, both individually and in relation to an associated conspecific, on behavioral manifestations of territoriality. In accordance with the criteria for salamander territoriality posited by Gergits (1981), *D. conanti* qualifies as a territorial species, with both males and females exhibiting behavior indicative of site fidelity, defense, and territorial advertisement via the deposition and perception of chemical signals. Overall, sex, residency status, and origin subpopulation were the most meaningful characteristics in determining the context and occurrence of territorial behavior. Although further studies of *D. conanti* in a natural setting are needed to understand how

territorial interactions and behavior influence its wild distribution and populations, this study’s experimental evaluation of territoriality in an understudied semi-aquatic plethodontid species serves as a valuable foundation for conservation efforts and future investigations of stream salamander ecology.

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Introduction

Territoriality is defined in animals as the advertisement and aggressive defense of a fixed area with respect to intruders (Brown and Orians 1970). According to the concept of economic defendability (Brown 1964), territorial behavior evolves most often in situations when the benefits of maintaining a dominant status – generally in order to secure access to food, shelter, or mates – outweigh the costs of injury, energy expenditure, and increased vulnerability associated with confrontational interaction. The spatial distribution of many territorial species is principally shaped by their territoriality, with individuals spaced in accordance with the distribution of resources (Brown and Orians 1970). Because territoriality depends on a variety of biotic (inter-specific, intra-specific) and abiotic factors, which govern the desirability of available territories as well as the capacity for individuals to successfully defend them, determining the specific context in which a species may exhibit territorial behavior is essential to understanding its ecological role and the distribution of its wild populations (Maher and Lott 2000, Hinsch 2017).

The lack of knowledge concerning the territorial interactions and communication of many amphibian species is a pressing conservation concern in light of the unprecedented decline of amphibian populations worldwide that has accelerated over the past several decades (Blaustein and Wake 1996, Stuart et al. 2004, Beebee and Griffiths 2005). One third of the world's amphibian species have undergone severe population declines or extinctions, with over 5,700 species affected globally (IUCN Red List 2004). Although several factors, both biotic and abiotic, contribute to global amphibian decline, the destruction and alteration of habitat via anthropogenic activity is thought to be the primary threat to species today (Gardner et al. 2007). Given the increasing scarcity of viable amphibian habitat, especially for species that reside within and around urban environments, an increased understanding of amphibian territoriality is essential to target conservation efforts effectively (Van Buskirk 2005, Gardner et al. 2007).

While some plethodontid species are not territorial (e.g., Ovaska 1988, Ovaska and Davis 1992), terrestrial woodland salamanders of the family Plethodontidae have long been regarded as an ideal model for examining variation and determinants of agonistic territorial behavior (e.g., Jaeger 1984, Mathis 1990, Anthony and Wicknick 1993, Staub 1993, Gabor and Jaeger 1995, Anthony et al. 1997, Wise and Jaeger 1998, Marvin 1998, Camp 1999). Territoriality mediates both inter- and intraspecific competition in many salamander species (Hairston 1987, Hairston 1996, Jaeger and Forester 1993), thereby shaping their distributions in the wild and influencing the dynamics of their populations and communities. Several factors have been demonstrated to shape the nature of territoriality in plethodontids. Differences in body size often mediate territorial behavior (e.g., Fraser 1976, Keen 1982, Roudebush and Taylor 1987, Houck 1988, Mathis 1990, Townsend and Jaeger 1998), and individuals of similar body size are generally more likely to interact in a competitive context. Sex is also a meaningful determinant of behavior for several plethodontids (e.g., Jaeger et al. 1995, Lang and Jaeger 2000), with males of some species exhibiting behavioral aggression related to nest-guarding or competition over mates. The ability of salamanders to shed their tails provides an additional source of asymmetries that may meaningfully influence behavior (e.g., Gildemeister et al. 2017). Finally, geographic variation in territorial behavior has been demonstrated for plethodontid species, with populations evolving meaningfully different behavior in response to habitat-specific selective pressures (e.g., Maerz and Madison 2000, Wise and Jaeger 2016).

The Eastern red-backed salamander (*Plethodon cinereus*), in particular, has been the subject of numerous territoriality studies (e.g., Tornick 2010, Kohn et al. 2013, Wise and Jaeger

2016). One such study postulated specific criteria for territoriality in plethodontid salamanders, which are now widely accepted and referenced in studies of plethodontids: a species ought to be regarded as territorial if it exhibits site tenacity, a mechanism of territorial advertisement (pheromone signaling in salamanders), and site defense (a greater-than-random probability of expelling intruders and competitors) (Gergits 1981, Mathis et al. 1995). Therefore, a species may exhibit non-overlapping ranges and behavior associated with dominance without being considered as "truly" territorial (Brown and Orians 1970). Adult *P. cinereus* individuals of both sexes will establish and defend feeding territories beneath rocks and logs on the forest floor (Jaeger et al. 1983), either as male-female pairs (Jaeger et al. 1995) or singly with intersexually overlapping territories (Mathis 1990, 1991a, 1991b). *P. cinereus* will defend these feeding territories year-round, even throughout the non-courtship summer months (Jaeger et al. 1995), and advertise territorial ownership by means of complex chemical communication systems (Jaeger 1986, Mathis and Simons 1994, Kohn et al. 2013). It has been suggested that *P. cinereus* males will use their possession of high-quality feeding territories as leverage to attract mates (Walls et al. 1989, Mathis 1990, 1991b) in the autumn and spring, when courtship and insemination occurs (Sever 1997).

Although territorial behavior has been well-studied in *P. cinereus* and widely observed in other terrestrial plethodontids, our understanding of territoriality in semi-aquatic lungless species is limited. As territoriality depends on environmental context, the territorial behavior of terrestrial plethodontids cannot be generalized to species that occupy streams and began their lives in a fully aquatic larval state. Furthermore, semi-aquatic species are uniquely crucial members of their ecological communities. Because they occupy different habitats at different life stages, stream salamanders facilitate an important exchange of nutrients between aquatic and terrestrial communities (Burton and Likens 1975, Davic and Welsh 2004). Additionally, their vulnerability to a diversity of environmental stressors throughout their life cycles makes them an important bioindicator species, meaning that their presence and abundance can be used to assess the overall health of an ecosystem (Rocco and Brooks 2000, Pfleeger et al. 2016). Given the well-evidenced importance of territoriality for terrestrial plethodontids, investigations of territoriality in theinr semi-aquatic relatives are likely to uncover meaningful information regarding the wild distributions and population dynamics of this understudied clade.

In this study, I assessed the territoriality of the spotted dusky salamander (*Desmognathus conanti*), which has never before been formally evaluated (Lannoo 2005). *D. conanti* occurs in seepage areas, cold springs, and at the edges of small rocky streams in deeply shaded, heavily wooded ravines throughout several natural regions of the Southeastern United States (Duellman 1999, Green et al. 2014). Despite their presumably large geographic distribution, *D. conanti* is one of the most poorly understood amphibians in Eastern North America (Lannoo 2005). *D. conanti* is adversely affected by urbanization-related stream disturbance (Orser and Shure 1967), and siltation and sedimentation of their small stream habitats has extirpated or severely reduced several populations throughout their range (Green et al. 2014). The reproduction of *D. conanti* is semi-aquatic, and the development of their gilled larvae may take as long as 13 months in water, but may be greatly accelerated in drier habitats or streams with inconsistent hydroperiods (Mount 1975). The age and size at reproductive maturity is unstudied for *D. conanti*. No breeding migrations have been recorded for *D. conanti,* and females deposit eggs in secretive sites beneath rocks, matted leaves, moss, and in cavities of rotting logs near the streams and seeps within their habitat (Lannoo 2005). During daylight hours, *D. conanti* adults can be found beneath rocks, leaf litter, and in burrows or rotting logs (Mount 1975). With the exception of populations in the vicinity of Atlanta (Orser and Shure 1967), no detailed population surveys or studies of the ecology of *D. conanti* have been conducted. Because *D. conanti* does not migrate, adults would be expected to defend territory, or at least choice hiding spots and feeding sites.

Studies of territoriality in small, cryptic animals like salamanders are difficult to successfully conduct in the field; observing the behavior and interactions of wild-caught individuals in captivity is the most practical and productive approach for such investigations (Mathis et al. 1995, 1998). Because of its relative abundance and hardy nature (Kirchberg et al. 2016), *D. conanti* is a well-suited model for investigations of behavior in a laboratory setting. I evaluated the territoriality of *D. conanti* in accordance with the criteria that Gergits postulated in his 1981 study of *P. cinereus* through two experiments featuring three cohorts of wild-caught adult individuals. In the first experiment, I conducted choice trials to assess the function of chemical signaling in territorial advertisement. In the second experiment, I conducted behavioral trials with paired *D. conanti* individuals to assess site tenacity, site defense, and the determinants of specific territorial behaviors. I evaluated the results of these two experiments in relation to several hypotheses regarding the constituents of territoriality in plethodontids; specifically, I attempted to determine whether *D. conanti*'s territorial behaviors and interactions were associated with body size, sex, tail length, or subpopulation.

Methods

Field Collections

I collected *D. conanti* individuals from two subpopulations at two similar streams, within Lullwater Preserve (33°47’52.4” N, -84°18’55.5” W), a 50+ hectare oak-pine-hickory forest on Emory University’s campus in the metro Atlanta area and known habitat of several stream salamander species (Committee on the Environment 2002). These first-order streams reside at approximately the same elevation (~280m) and exhibit homogenous bank flora composition (predominately Chinese privet (*Ligustrum sinense)* and English ivy (*Hedera helix*), and hydroperiod. These two streams constitute spatially separated subpopulations of the *D. conanti* metapopulation within Lullwater.

I collected by hand adult *D. conanti* individuals from the two study streams, searching primarily beneath rocks along the stream bank and in shallower areas. Only fully metamorphosed adult individuals were captured for this study, as lab setup and husbandry would be complicated by the inclusion of juveniles, which are gilled and fully aquatic. Furthermore, only individuals that appeared to be in good health (healthy apparent weight, lacking major injuries or obvious external signs of parasite infection) were collected. To avoid further stressing any individuals who had recently shed their tail, only individuals whose tail length measured at least 70% (approximated by eye) of their snout-vent length (SVL) were captured for the study (Wise and Jaeger 1998), as the earliest stages of tail regeneration are the most energy intensive (Jamison and Harris 1992, Maginnis 2006). Captured individuals were stored temporarily in moistened plastic deli cups and carried back to the lab on foot (an approximately 20-minute journey) immediately after the collection effort was concluded.

To account for seasonal variation in activity or behavior, three cohorts were collected in the months of November, December, and February (Table 1). Each of these collections took place over 2-3 separate nights between 19:00 and 23:00 (~4 hours per collection effort). Although friends and colleagues often accompanied me in my collection efforts and assisted with capturing individuals for pilot experimentation, I was the sole collector of all salamanders analyzed in the study proper. I collected no more than 25 individuals from each stream, collecting every other individual found to prevent oversampling the population.

Table 1. Record of salamanders captured from streams one and two during each collection, including overall totals for individuals captures and ultimate trial participants.

	Collection Date	Stream One	Stream Two	Total # Captured	Total # Tried
Cohort One	11/15/18	4	0	24	22
	11/16/18	7	3		
	11/17/18	0	10		
Cohort Two	11/30/18	0	18	41	38
	12/1/18	23	0		
Cohort Three	2/18/19	0	12	22	22
	2/22/19	10	0		
All Cohorts		44	43	**87**	**82**

Of the 24 individuals captured in the first sampling effort in mid-November (Table 1), 22 ultimately participated in trials and were released back into their streams of origin at the approximate location of capture. One individual died within the first week of captivity due to unknown causes, and one individual was released prematurely because it would not eat in captivity.

Of the 41 individuals captured in the second sampling effort in late-November/early-December (Table 1), 38 ultimately participated in trials and were released back into their streams of origin at the approximate location of capture. Two individuals died within the first week of captivity due to unknown causes, and one individual was released prematurely because it would not eat in captivity.

All of the 22 individuals captured in the third and final sampling effort in mid-February (Table 1) ultimately participated in trials and were released back into their streams of origin at the approximate location of capture.

After being captured and allowed to habituate in the lab for at least three nights, the sex, weight, and length measurements were recorded for every individual. The presence of any minor physical handicaps that may impact an individual's capacity for aggressive or defensive behavior, including ongoing limb, digit, and tail regeneration, were noted. The salamanders were sexed visually based on the presence of testis, ova, and relative snout length (pers. com. Wilson 2019). Weight was consistently measured one day after all individuals had been fed, using an analytical balance. Individuals were photographed in lidded petri dishes over ½ centimeter grid paper, and all length measurements (SVL, tail length, full length) were taken from these photos in the ImageJ software.

Husbandry

Captive salamanders were kept individually in 24.5-liter IRIS stackable storage bins made from BPA-free, acid-free, PVC-free, lignin-free plastic with drilled holes for ventilation, transparent sides, and an opaque lid. Enclosures were lined with one square of moistened dye-free paper towel, which was replaced with a clean sheet as needed (one to two times weekly). Each enclosure contained a hide constructed from an overturned plastic petri dish with a drilled entryway. The hides featured a removable opaque cover constructed from a slightly larger overturned plastic petri dish lined with blue painter's tape. These removable covers allowed the salamanders to be viewed with minimal disturbance. All stacked enclosures were covered by a beige 50% shade cloth at all times to simulate the low-light conditions of the salamanders' natural habitat. In addition, the laboratory wherein the enclosures were kept contained several large and unobstructed windows, subjecting the salamanders to a largely natural photoperiod. The lab room temperature and humidity was monitored continually using a HOBO device. Enclosures were misted with de-ionized water daily to maintain a relative humidity at or above 70%. All salamanders were habituated and monitored for two to three weeks before the commencement of trials to minimize the influence of capture stress on their behavior. All individuals were fed tropical house crickets (*Gryllodes sigillatus)* ad libitum, and any uneaten crickets were removed during daily routine spot cleaning. Enclosures were deep-cleaned between occupancy by different individuals using a 10% bleach solution.

This study was approved by the Emory University Institutional Animal Care and Use Committee (IACUC) (PROTO201800055) on 9/24/2018, and animals were monitored weekly by rounding veterinary staff from the Division of Animal Resources.

Experiment 1: Choice Trials

To evaluate the capacity for territorial advertisement of *D. conanti* and role of olfaction and pheromones in territorial advertisement and communication, I conducted choice tests to assess individuals' preference for their own chemical markings versus the markings of conspecifics. Trial enclosures were identical to "home" enclosures in make and dimensions. At the beginning of each trial, I placed an individual beneath a habituation cover in the center of the trial enclosure (Figure 1). The basal area of each long end of the trial enclosure was covered by a folded paper towel that extended approximately five centimeters out from the wall in length and spanned the entire width of the enclosure. The paper towel on one end of the enclosure was sourced from the trial subject's own "home" enclosure, and the paper towel on the opposing end was sourced from the enclosure of a random conspecific. Pairings were randomized in Excel. I randomly allocated these "marked" paper towels to either end of the enclosure for each trial. I did not replace the substrate in the enclosures of trial participants for four days preceding these trials in order to allow for the accumulation of relevant chemical markings and signatures. After habituating the trial subjects for three minutes, I removed the cover dishes and replaced the enclosure lids. I left the subjects in darkness for 20 minutes to explore the trial enclosure and eventually choose a substrate to hide beneath. At the end of each trial, I recorded whether the individual had ultimately chosen to conceal beneath the paper towel sourced from its own enclosure or that from the enclosure of the random conspecific.

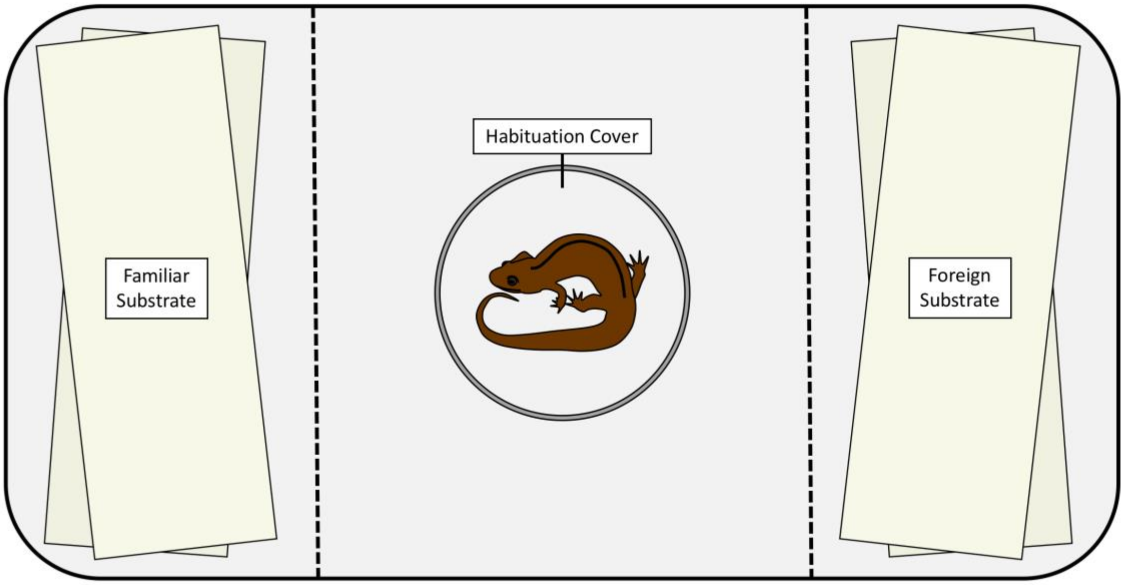

Figure 1. Schematic of enclosure layout for choice trials.

I used the statistical software R for all data analysis (R Core Team 2018). I used generalized linear models specifying binomial error distribution to relate the dependent variable of substrate choice (0 = choose foreign, 1 = choose familiar) to several independent variables. I constructed a "full" model encompassing all relevant independent variables, which included the weight difference of subject and foreign substrate owner, SVL difference of subject and foreign substrate owner, tail length difference of subject and foreign substrate owner, sex matchup of subject and foreign substrate owner, origin stream (subpopulation) difference of subject and foreign substrate owner, and cohort. I then ran this model through a backward stepwise selection using the 'step' function in base R to generate a "best" model, which ultimately accounted for the most variation using the fewest predictive variables and was regarded as the most accurate model for predicting whether a salamander would select to conceal beneath a familiar substrate sourced from its own enclosure versus a foreign substrate sourced from the enclosure of a random conspecific. I also constructed a “null” model including no independent variables. I specifically selected the predictor variables for inclusion in three additional candidate models based on my hypotheses regarding what might affect an individual’s choice of substrate; I created logistic regressions describing the impact of stream difference (implemented as subject stream * foreign substrate owner stream), subject body size (weight, SVL), and subject tail length on substrate choice. I generated a ranking of all six models according to their corrected (second-order) Akaike’s Information Criterion value (AICc) using the ‘aictab’ function in the *AICcmodavg* package (Mazerolle 2019). I reported the parameters, ΔAICc value, and Akaike’s weights (AICc and cumulative) for all models, and the coefficients, standard errors, Wald z-statistic, and associated p-values for each term in the “best” model. I also calculated the odds ratio (OR) by exponentiating the beta estimates for all terms included in the “best” model to quantify the strength of association with substrate choice for each. In addition, I conducted a chi-squared test to determine whether there was a significant difference in choice trial outcomes across the three collection cohorts using ‘the chisq.test’ function in base R. I created visualizations for the predictor variables in the “best” model using the *ggplot2* package (Wickham et al. 2018).

Experiment 2: Territorial Aggression

To evaluate and characterize territorial behavior in adult *D. conanti* individuals, I experimentally simulated the conditions under which territorial aggression has been previously observed for plethodontid taxa (Rollinson and Hackett 2015, Lynn 2018). This involved introducing a randomly assigned “intruder” individual into an enclosure occupied by a paired “resident” individual and observing their behavior. I conducted these trials after a minimum of three days following the choice trials and tried the same participants. Within each collection cohort, I tested conditionally randomized resident-intruder pairs of similar body weight both within and between subpopulations and sexes. Individuals were paired based on body weight so that the potential effects of variables other than size difference could be observed, as a substantial size difference may overwhelmingly determine whether individuals interact (Townsend and Jaeger 1998). I conducted these trials between 7:00pm and 11:00pm, as wild salamanders are most active in the evening, and tried each pair only once. Indirect illumination of the lab bench via a small desk lamp was dimmed such that the distinguishing markings of individuals were barely discernable. At the beginning of each trial, I removed the intruder from its enclosure and placed them beneath an overturned transparent petri dish within the enclosure of the paired resident (Figure 2). To prevent handling bias, I similarly removed the resident from their enclosure and also covered them beneath a dish. I left both individuals beneath the dishes for a five-minute habituation period. After removing the dish covers and replacing the enclosure lid, I observed and recorded all ensuing behavior through the enclosure’s transparent sides for 15 minutes. I terminated trials early if an individual was at risk of injury or if both the resident and intruder were concealed simultaneously and had remained still for at least one minute.

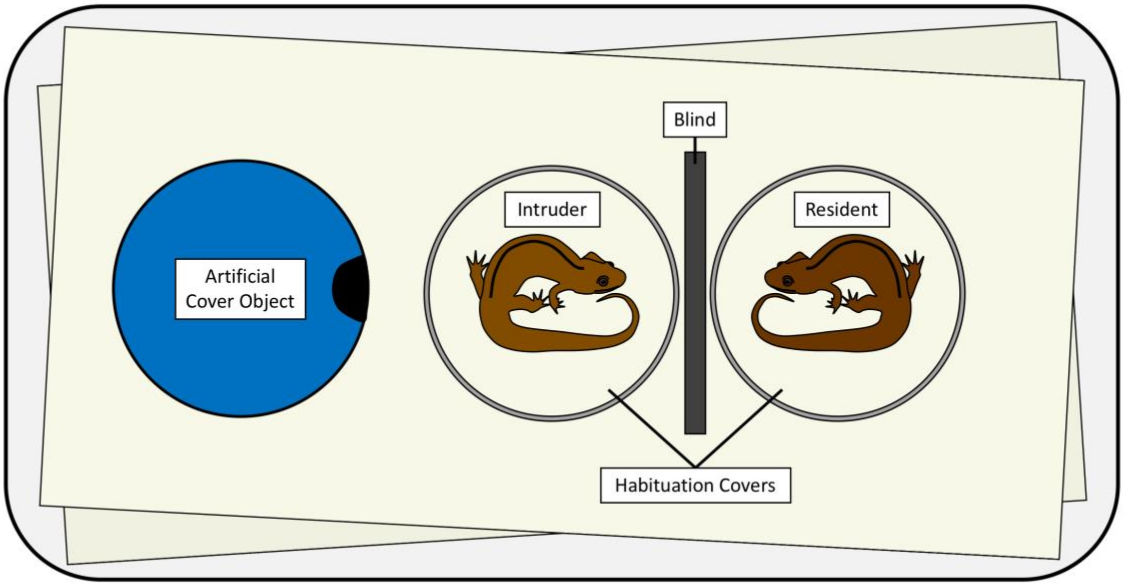

Figure 2. Schematic of enclosure layout for territorial aggression trials.

Behaviors recorded for this study were either selected from a thorough literature search (e.g., “Edge” behavior) or newly defined from repeated observation (Table 2). Interaction between individuals was defined as: all behavior involving physical contact, any action directed toward a conspecific, or any action in response to the behavior of a conspecific. I categorized any interaction between the two individuals as either “confrontational”, “casual”, or “courtship”. Courtship interactions featured behavior that could be misinterpreted as overt aggression (i.e. biting), although distinct (Verrell 1995, Arnold et al. 2017). Confrontational interactions were those which included aggressive behaviors (charging, biting, and expulsion from concealment) as well as submissive behaviors (flight, “Edge” behavior (Wise and Jaeger 1998)), with aggressive and submissive behaviors usually co-occurring in paired individuals. At the end of the trials, I returned all participants to their original enclosures and supplied them with fresh moistened substrate.

Table 2. Inventory of behaviors recorded during observation of territorial aggression trials.

Behavior		Description
Charge	Aggressive	Aggressor advances rapidly towards conspecific, often with body trunk fully or partially elevated.
Bite	Aggressive	1) Aggressor uses mouth to deliver one or several bites to any region of conspecific's body, OR 2) Defending individual delivers retaliatory bite to any region of conspecific's body *in response to* the action of an aggressor.
Expel	Aggressive	Aggressor physically intimidates conspecific in such a way that results in that individual vacating a cover object that it had previously occupied. Most often observed in conjunction with "Charge" and "Bite" behaviors.
Flight	Submissive	Defending individual moves rapidly away from Aggressor in an apparent attempt to avoid or escape *the individual*. Usually observed in response to any of the above aggressive behaviors.
"Edge"	Submissive	Previously defined by Wise and Jaeger (1998). Individual circumnavigates inner wall of enclosure, often scaling the wall itself, in an apparent attempt to avoid or escape *the substrate or enclosure*. Frequently observed without any interaction having occurred.

I used generalized linear models specifying binomial error distribution to relate the dependent variable of occurrence of confrontation (0 = no confrontation, 1 = yes confrontation) between paired individuals to several independent variables. I constructed a "full" model encompassing all relevant independent variables, which included sex matchup, weight difference, SVL difference, tail length difference, and origin stream (subpopulation) difference. I then conducted backwards stepwise selection on the "full" model to generate a "best" model for predicting whether a confrontation would occur between a resident-intruder pair. I also constructed a "null" model including no independent variables. I specifically selected the predictor variables for inclusion in four additional candidate models based on my hypotheses regarding what might affect the likelihood of a confrontational interaction between two individuals; I created logistic regressions describing the impact of resident-intruder sex matchup, stream difference, and body size difference, and tail length difference on confrontation. I generated a ranking of all seven models according to their corrected Akaike's Information Criterion value (AICc). I reported the parameters, ΔAICc value, and Akaike's weights (AICc and cumulative) for all models, and the coefficients, standard errors, Wald z-statistic, and associated p-values for each term in the "best" model. I also calculated the odds ratio (OR) for all terms included in the "best" model to quantify the strength of association with confrontation occurrence for each. In addition, I conducted a chi-squared test to determine whether there was a significant difference in confrontation across the three collection cohorts. I created visualizations for the predictor variables in the "best" model.

I used generalized linear models specifying binomial error distribution to relate two dependent variables – occurrence of individual aggressive behavior (charging, biting, expelling from concealment; 0 = no aggression, 1 = aggression) and individual submissive behavior (flight, "Edge" behavior; 0 = no submission, 1 = submission) – of all trial participants to several independent variables. For both aggression and submission, I also constructed a "full" model encompassing all relevant independent variables, which included the individual's randomly assigned role (resident or intruder), cohort, stream of origin, sex, weight, SVL, tail length, and whether or not the individual was handicapped. I then conducted backwards stepwise selection on both "full" models to generate "best" models for predicting the occurrence of aggressive and submissive behavior. I also constructed "null" models including no independent variables for each candidate model set. For both aggression and submission, I specifically selected the predictor variables for inclusion in four additional candidate models based on my hypotheses regarding what might affect an individual's behavior; I created logistic regressions describing the impact of sex, origin stream (subpopulation), body size (weight, SVL), and tail length on an individual's behavior. I generated a ranking of all seven models regarding aggression and submission according to their corrected Akaike's Information Criterion value (AICc). I reported the parameters, ΔAICc value, and Akaike's weights (AICc and cumulative) for all models, and the coefficients, standard errors, Wald z-statistic, and associated p-values for each term in the "best" model. I also calculated the odds ratio (OR) for all terms included in the "best" models to quantify the strength of association with aggression and submission. In addition, I conducted chi-squared tests to determine whether there was a significant difference in behavior across the three collection cohorts. I created visualizations for the predictor variables in each of the two "best" models.

Results

Eighty-seven adult *D. conanti* were collected from two streams across seven collection dates and three cohorts. Forty-three of the 82 trial participants were sexed as female, and 39 as male. The weight of trial participants ranged from 0.21 – 4.49 g, with a mean weight of 2.4 g. The SVL of trial participants ranged from 3.45 – 5.69 cm, with a mean SVL of 4.65 cm. The tail length of trial participants ranged from 4.16 – 7.37 cm, with a mean tail length of 5.73 cm.

Experiment 1: Substrate Choice

Of the 82 choice trial participants, ~67% (n = 55) chose to conceal beneath a familiar substrate that had been sourced from their own enclosure. The remaining individuals (n = 27) chose to conceal beneath a foreign substrate that had been sourced from the enclosure of a random conspecific from the same collection cohort. Substrate choice did not vary significantly across the three collection cohorts (χ^2 (2, N = 82) = 0.16, p = 0.923).

The best model for predicting substrate choice solely included sex matchup (s ex of subject + sex of foreign substrate owner) as the independent variable (AICc = 105.70); however, the predictive quality of this model was not meaningfully different (ΔAICc < 2) from the null model or the tail length model (Table 3).

Table 3. Ranking of several linear models describing substrate choice, according to AICc calculations. The best (most parsimonious) model for predicting whether an individual would choose to conceal beneath a familiar substrate (sourced from their own enclosure) versus a foreign substrate (sourced from the enclosure of a random conspecific) solely included the variable sex matchup (subject sex, conspecific sex, subject sex * conspecific sex). K is the total number of estimated parameters. ΔAICc is the difference in AICc between a given model and the "best" model. The Akaike weights (AICc weight and cumulative weight) indicate the level of support (weight of evidence) for a model being the most parsimonious among the candidate model set.

Model	**K**	**AICc**	**ΔAICc**	**AICcWt**	**CumWt**
Sex Matchup	4	105.70	0.00	0.37	0.37
Null	1	105.97	0.27	0.32	0.69
Tail Length	2	106.63	0.94	0.23	0.92
Body Size	3	110.15	4.45	0.04	0.96
Stream	4	110.51	4.81	0.03	1.00
Full	10	115.87	10.18	0.00	1.00

Table 4. Output of best (most parsimonious) model selected based on AICc, which solely included the variable sex matchup (subject sex, conspecific sex, subject sex * conspecifc sex)

term	**coef. est.**	**std. error**	**z-value**	**p-value**
subject sex (male)	-1.946	0.868	-2.242	0.025*
conspecific sex (male)	-0.965	0.895	-1.079	0.281
subject sex (male) x conspecific sex (male)	1.525	1.086	1.404	0.160

Female subjects selected the familiar substrate in a greater proportion of trials when the foreign substrate had been sourced from the enclosure of another female (87.5%) versus the enclosure a conspecific male (72.73%), and male subjects selected the familiar substrate in a greater proportion of trials when the foreign substrate had been sourced from the enclosure of another male (63.64%) versus the enclosure of a conspecific female (50%) (Figure 3). The odds of a female subject selecting the familiar substrate were seven times greater (OR = 7) than those for a male subject, regardless of the sex identity of the foreign substrate marker (p = 0.025).

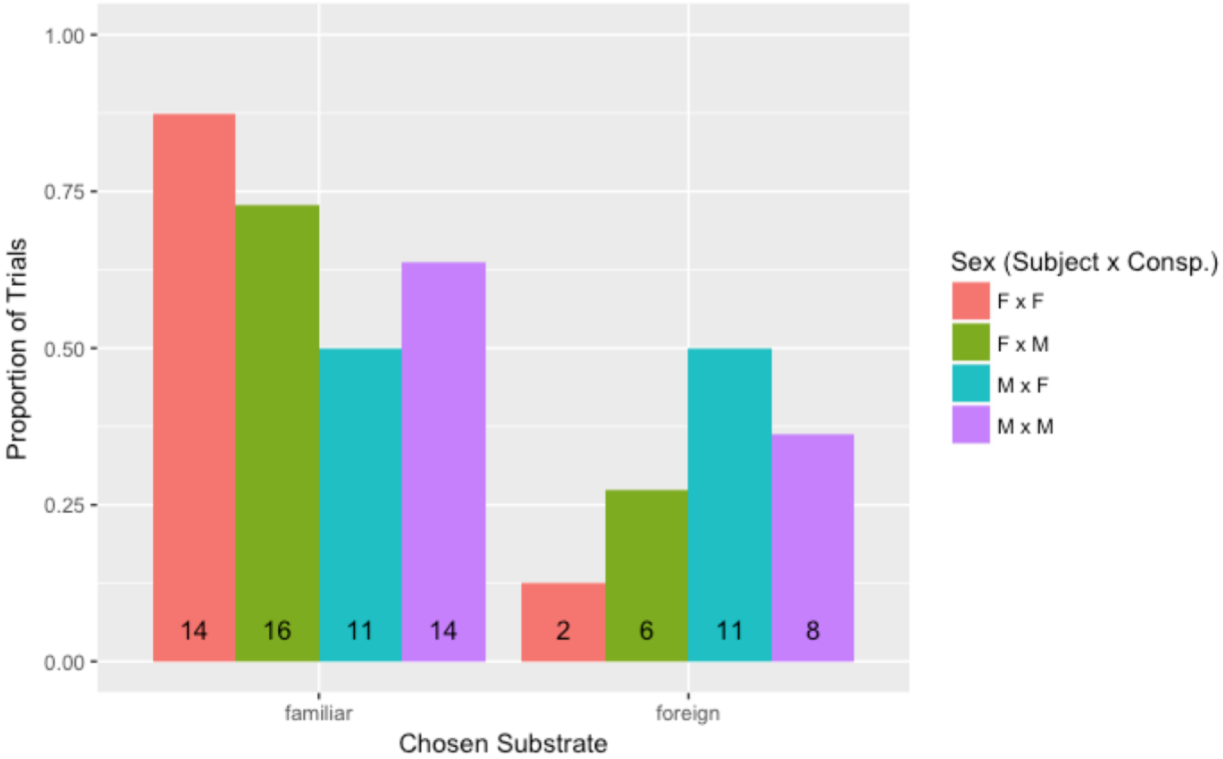

Figure 3. Substrate choice of adult *D. conanti* individuals in choice trials based on sex matchup of subject and paired foreign substrate owner. X-axis represents substrate choice (familiar or foreign). Y-axis represents the proportion of trials featuring each sex matchup (subject sex + foreign substrate owner sex) for each recorded substrate choice. The four possible sex matchups of the subject and foreign substrate owner (conspecific) were Female x Female, Female x Male, Male x Female, and Male x Male, with the subject's sex ordered first. Number labels on bars indicate the total number of trials in which each result was recorded.

The tail length of trial subjects that selected the foreign substrate (5.58 ± 0.97 cm) was shorter on average compared to the tail length of trial subjects who selected the familiar substrate (5.80 ± 0.67 cm) (Figure 4).

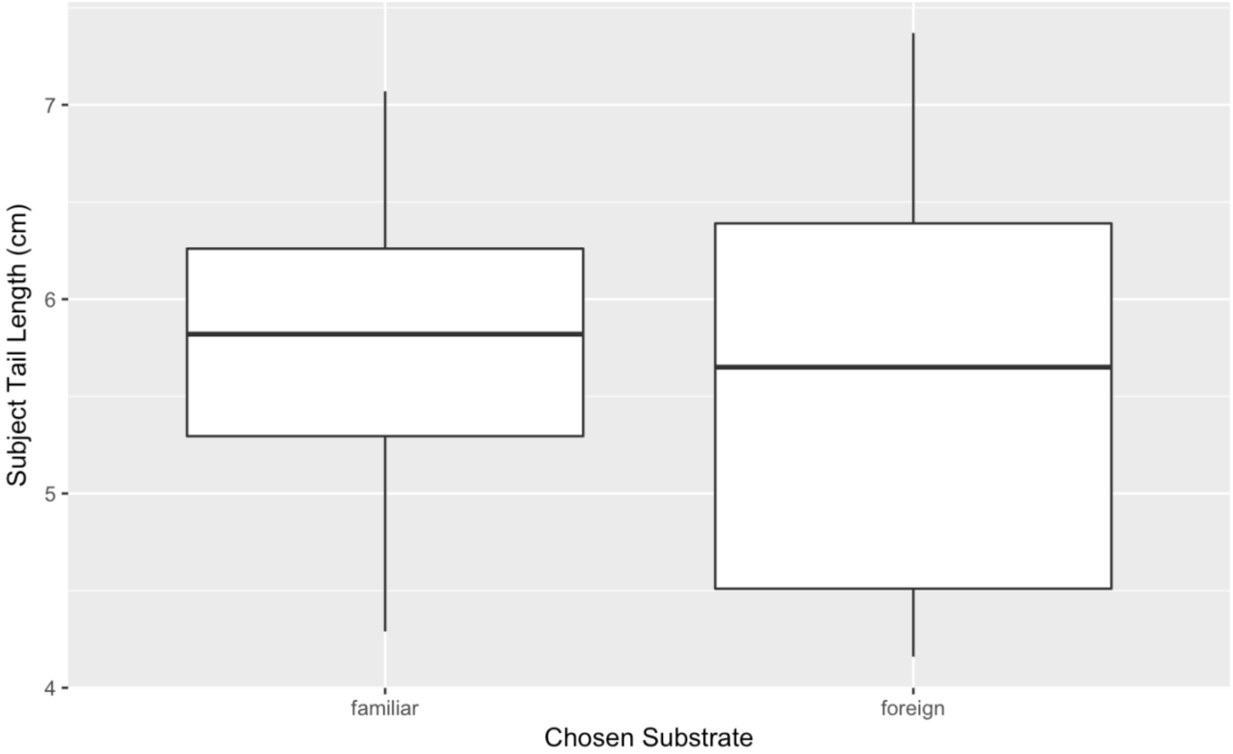

Figure 4. Relationship between subject tail length and sex. X-axis represents the substrate choice of *D. conanti* trial subjects. Y-axis represents the subject's tail length in centimeters. Boxes represent the bulk of conceal time values for each substrate choice. Bold horizontal lines represent median SVL for each choice.

Experiment 2: Confrontation

Forty-one resident-intruder pairs participated in territorial aggression trials, wherein I observed the nature of their interaction and noted the behavior of each individual participant. Of these 41 trials, ~29% (n = 12) resulted in a confrontational interaction between participants. The remaining trials either resulted in a casual interaction (n = 9), courtship interaction (n = 2), or no interaction (n = 18). The occurrence of confrontation did not vary significantly across the three collection cohorts (χ^2 (2, N = 41) = 0.09, p = 0.955).

The best model for predicting confrontation solely included the variable sex matchup (sex of resident * sex of intruder) (AICc = 50.63); however, the predictive quality of this model was not meaningfully different (ΔAICc < 2) from the null model (Table 5).

Table 5. Ranking of several linear models describing confrontation, according to AICc calculations. The best (most parsimonious) model for predicting whether a confrontational interaction would occur between paired individuals solely included the variable sex matchup (resident sex * intruder sex). K is the total number of estimated parameters. ΔAICc is the difference in AICc between a given model and the "best" model. The Akaike weights (AICc weight and cumulative weight) indicate the level of support (weight of evidence) for a model being the most parsimonious among the candidate model set.

Model	**K**	**AICc**	**ΔAICc**	**AICcWt**	**CumWt**
Sex Matchup	4	50.63	0.00	0.53	0.53
Null	1	51.67	1.05	0.31	0.84
Sex Matchup + SVL dif.	7	54.17	3.54	0.09	0.93
Stream dif.	4	55.22	4.60	0.05	0.98
Tail Length dif.	4	57.46	6.83	0.02	1.00
Body Size dif. (Weight + SVL)	7	60.66	10.03	0.00	1.00
Full	16	84.46	33.83	0.00	1.00

Table 6. Output of best (most parsimonious) model selected based on AICc, which solely included the variable sex matchup (resident sex * intruder sex).

term	**coef. est.**	**std. error**	**z-value**	**p-value**
resident sex (male)	-1.386	1.225	-1.132	0.258
intruder sex (male)	-1.504	1.219	-1.234	0.217
resident sex (male) x intruder sex (male)	3.989	1.740	2.293	0.022*

Of the 12 trials that resulted in a confrontation, half featured two male participants (N = 6), and one-third featured two female participants (N = 4), with the remaining trials featuring participants of differing sex (N = 2). The proportion of trials that resulted in confrontation was greatest for male-male pairings (60%), followed by female-female pairings (33.33%), male(resident)-female(intruder) pairings (11.11%), and female(resident)-male(intruder) pairings (10%) (Figure 5). The odds of confrontation occurring were highest between paired males and paired females (OR = 54). The odds of confrontation occurring between mixed-sex pairings were comparatively much lower (p = 0.022).

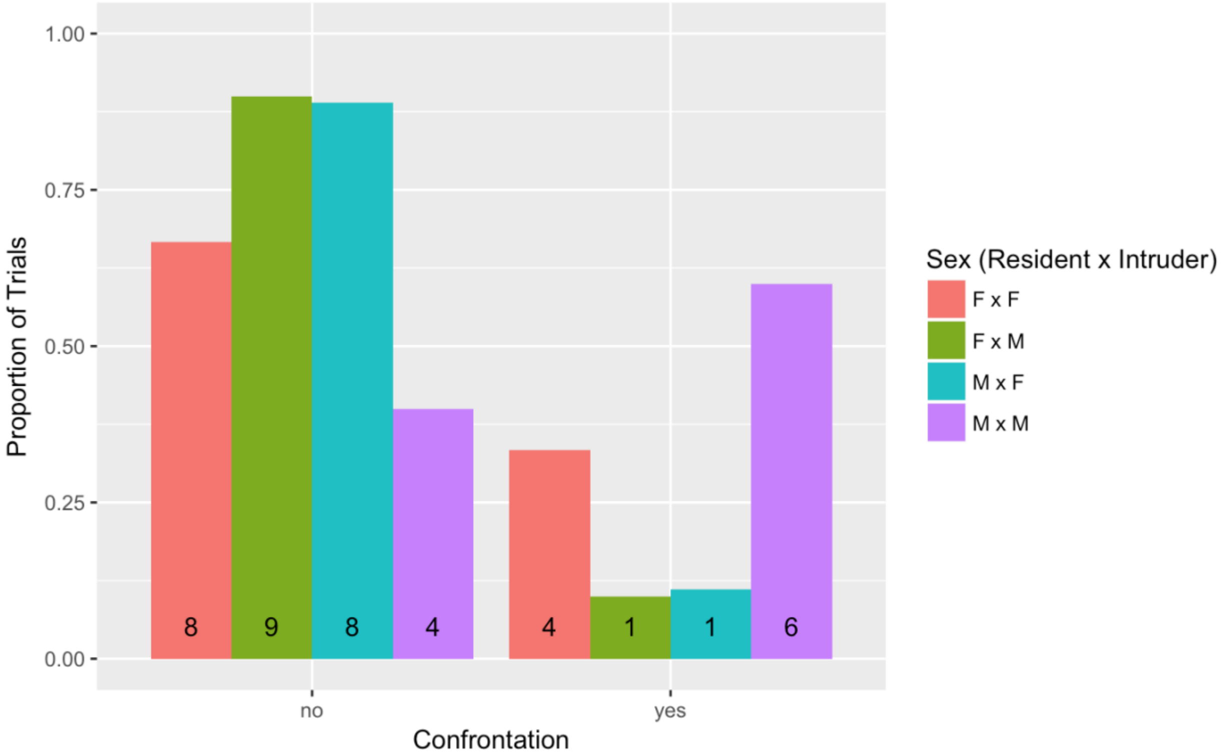

Figure 5. Occurrence of confrontation between paired *D. conanti* individuals in territorial aggression trials based on sex matchup of participants. X-axis represents occurrence of confrontation. Y-axis represents the proportion of trials featuring each sex matchup (resident sex + intruder sex) for either outcome. The four possible sex matchups of the resident and intruder were Female x Female, Female x Male, Male x Female, and Male x Male, with the resident's sex ordered first. Number labels on bars indicate the total number of trials in which each result was recorded.

Experiment 2: Aggressive Behavior

Of the 82 individuals who participated in the territorial aggression trials, ~16% (n = 13) displayed aggressive behavior, meaning that they either bit their paired conspecific, chased their paired conspecific, or expelled their paired conspecific from concealment. The occurrence of aggressive behavior did not vary significantly across the three collection cohorts (χ^2 (2, N = 82) = 0.171, p = 0.918).

The best model for predicting an individual's exhibition of aggressive behavior during a territorial aggression trial included the variables role (resident or intruder) and sex (AICc = 70.00); however, the predictive quality of this model was not meaningfully different (ΔAICc < 2) from the model which solely included role (Table 7).

Table 7. Ranking of several linear models describing *D. conanti*'s exhibition of aggressive behavior (biting, chasing, expelling from concealment) during territorial aggression trials, according to AICc calculations. The best (most parsimonious) model for predicting whether an individual would behave aggressively towards a conspecific included the variables role (resident or intruder) and sex. K is the total number of estimated parameters. ΔAICc is the difference in AICc between a given model and the "best" model. The Akaike weights (AICc weight and cumulative weight) indicate the level of support (weight of evidence) for a model being the most parsimonious among the candidate model set.

Model	**K**	**AICc**	**ΔAICc**	**AICcWt**	**CumWt**
Role + Sex	3	70.00	0.00	0.47	0.47
Role	2	71.17	1.17	0.26	0.73
Sex	2	72.90	2.90	0.11	0.84
Null	1	73.76	3.76	0.07	0.91
Tail Length	2	74.82	4.82	0.04	0.95
Stream	2	75.77	5.77	0.03	0.98
Body Size (Weight + SVL)	3	77.16	7.16	0.01	0.99
Full	8	78.62	8.62	0.01	1.00

Table 8. Output of best (most parsimonious) model selected based on AICc, which included the variables role (resident or intruder) and sex.

term	**coef. est.**	**std. error**	**z-value**	**p-value**
role (resident)	1.492	0.716	2.082	0.037*
sex (male)	1.174	0.670	1.754	0.080

Individuals who occupied the resident role behaved aggressively in a greater proportion of territorial aggression trials (24.4%) compared to intruders (7.32%) (Figure 6). The odds of a resident exhibiting aggressive behavior (OR = 4.444) were comparatively greater than those for an intruder (p = 0.037).

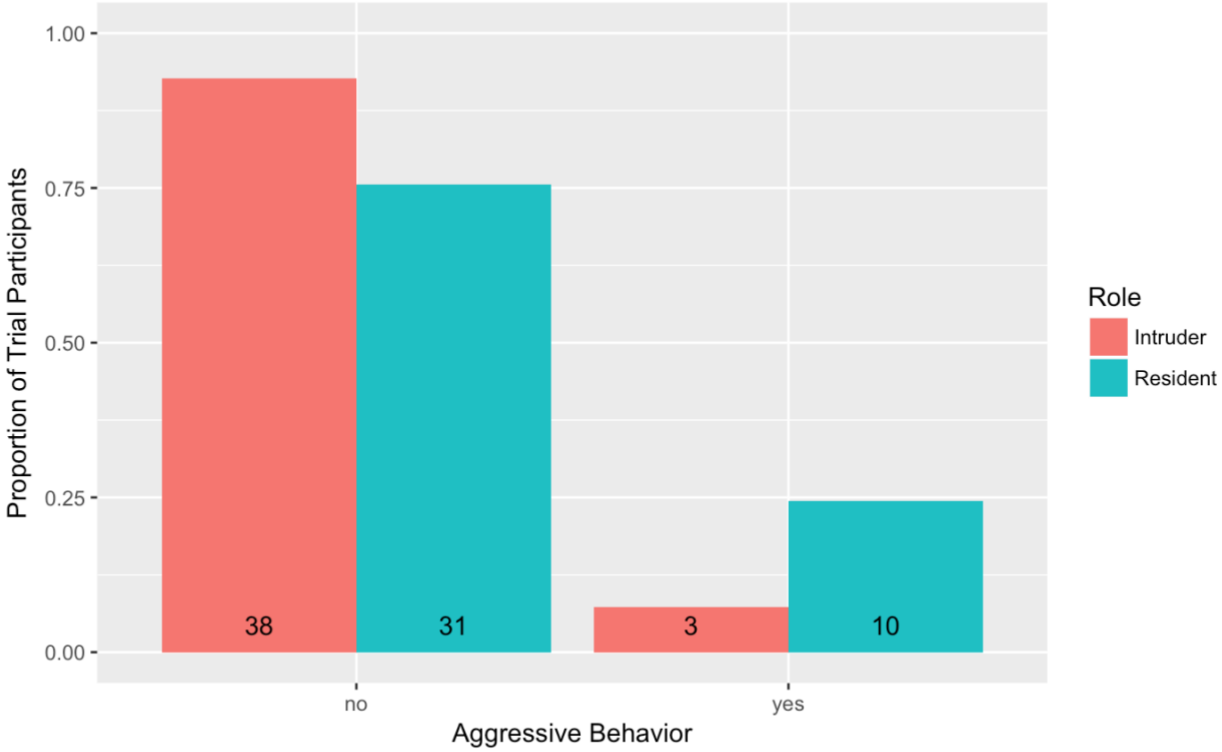

Figure 6. Exhibition of aggressive behavior (charging, biting, expelling) by adult *D. conanti* individuals in territorial aggression trials based on assigned role (resident or intruder). X-axis represents occurrence of aggressive behavior. Y-axis represents the proportion of individuals in each role for either outcome. Individuals occupied the role of resident or intruder. Number labels on bars indicate the total number of trials for each result.

Male individuals behaved aggressively in a greater proportion of trials (23.08%) compared to females (9.3%) (Figure 7). The odds of a male exhibiting aggressive behavior (OR = 3.236) were comparatively greater than those for a female, although the association of sex with aggression was not statistically significant (p = 0.080).

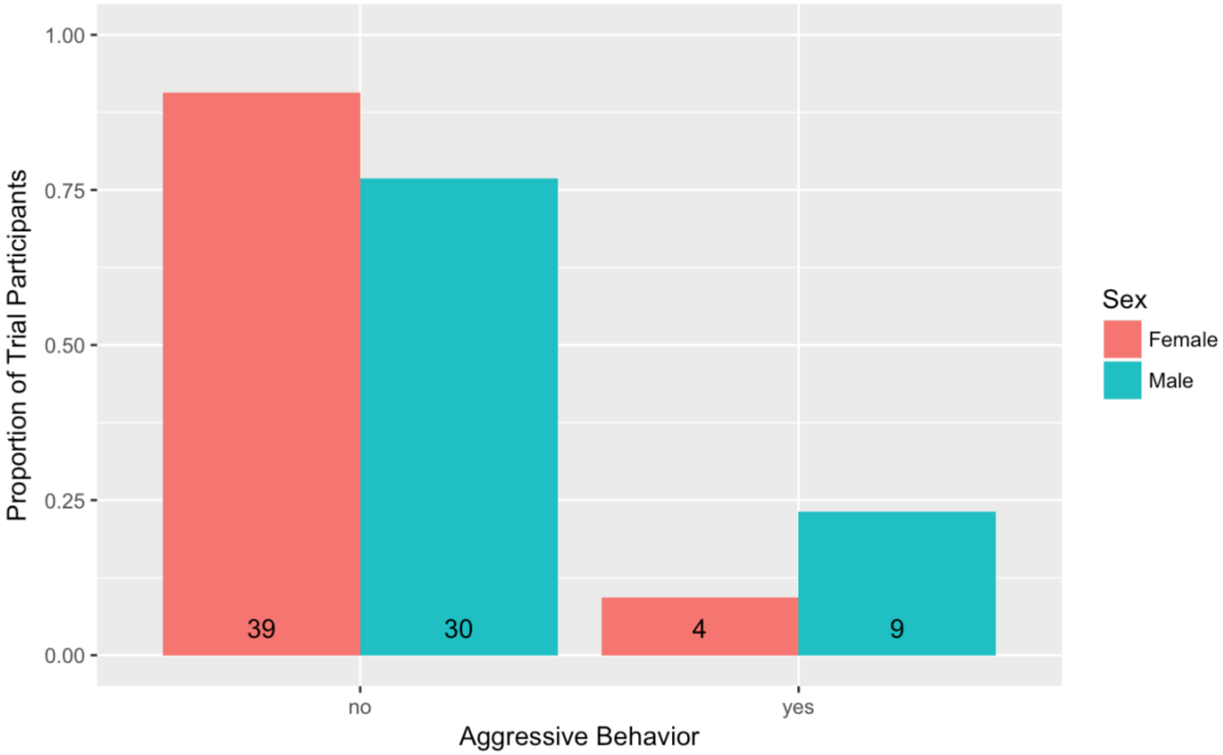

Figure 7. Exhibition of aggressive behavior (charging, biting, expelling) by adult *D. conanti* individuals in territorial aggression trials based on sex. X-axis represents occurrence of aggressive behavior. Y-axis represents the proportion of individuals of each sex for either outcome. Legend depicts whether individuals were male or female. Number labels on bars indicate the total number of trials for each result.

Experiment 2: Submissive Behavior

Of the 82 individuals who participated in the territorial aggression trials, ~33% (n = 27) displayed submissive behavior, meaning that they either fled from their paired conspecific or scaled the wall of the trial enclosure ("Edge" behavior) in an apparent attempt at evasion or escape. The occurrence of submissive behavior did not vary significantly across the three collection cohorts (χ^2 (2, N = 82) = 0.464, p = 0.793).

The best model for predicting an individual's exhibition of submissive behavior during a territorial aggression trial included the variables role (resident or intruder), stream of origin, sex, and handicap (whether or not the individual possessed any physical handicap) (AICc = 96.75) (Table 9).

Table 9. Ranking of several linear models describing *D. conanti*'s exhibition of submissive behavior (fleeing, "Edge" behavior) during territorial aggression trials, according to AICc calculations. The best (most parsimonious) model for predicting whether an individual would behave submissively included the variables role (resident or intruder), stream of origin, sex, and handicap (whether or not an individual possessed a physical handicap). K is the total number of estimated parameters. ΔAICc is the difference in AICc between a given model and the "best" model. The Akaike weights (AICc weight and cumulative weight) indicate the level of support (weight of evidence) for a model being the most parsimonious among the candidate model set.

Model	**K**	**AICc**	**ΔAICc**	**AICcWt**	**CumWt**
Role + Stream + Sex + Handicap	5	96.75	0.00	0.84	0.84
Role	2	101.24	4.49	0.09	0.92
Stream	2	103.53	6.79	0.03	0.95
Full	8	103.60	6.85	0.03	0.98
Null	1	105.97	9.22	0.01	0.99
Sex	2	106.27	9.52	0.01	1.00
Tail Length	2	107.85	11.10	0.00	1.00
Body Size	3	109.16	12.41	0.00	1.00

Table 10. Output of best (most parsimonious) model selected based on AICc, which included the variables role (resident or intruder), stream of origin, sex, and handicap.

term	**coef. est.**	**std. error**	**z-value**	**p-value**
role (resident)	-1.527	0.558	-2.735	0.006**
sex (male)	-0.782	0.545	-1.436	0.151

stream (two)	-1.165	0.550	-2.118	0.034*
handicap (yes)	16.675	1583.657	0.011	0.992

Individuals who occupied the resident role behaved submissively in a lesser proportion of territorial aggression trials (19.51%) compared to intruders (46.34%) (Figure 8). The odds of an intruder exhibiting submissive behavior (OR = 4.602) were comparatively greater than those for a resident (p = 0.006).

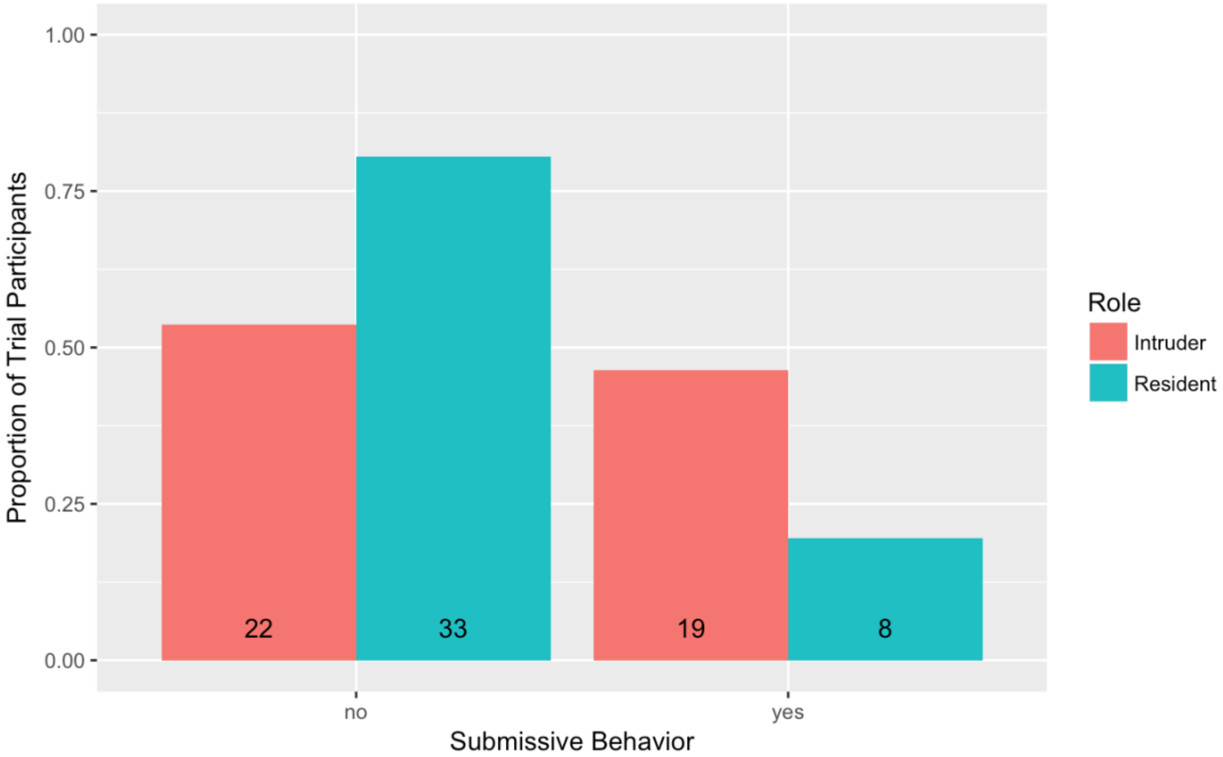

Figure 8. Exhibition of submissive behavior (flight, “Edge” behavior) by adult *D. conanti* individuals in territorial aggression trials based on assigned role (resident or intruder). X-axis represents occurrence of submissive behavior. Y-axis represents the proportion of individuals in each role for either outcome. Individuals occupied the role of resident or intruder. Number labels on bars indicate the total number of trials for each result.

Female individuals behaved submissively in a greater proportion of trials (39.53%) compared to males (25.64%) (Figure 9). The odds of a female exhibiting submissive behavior (OR = 2.186) were comparatively greater than those for a male, although the association of sex with submission was not statistically significant (p = 0.151).

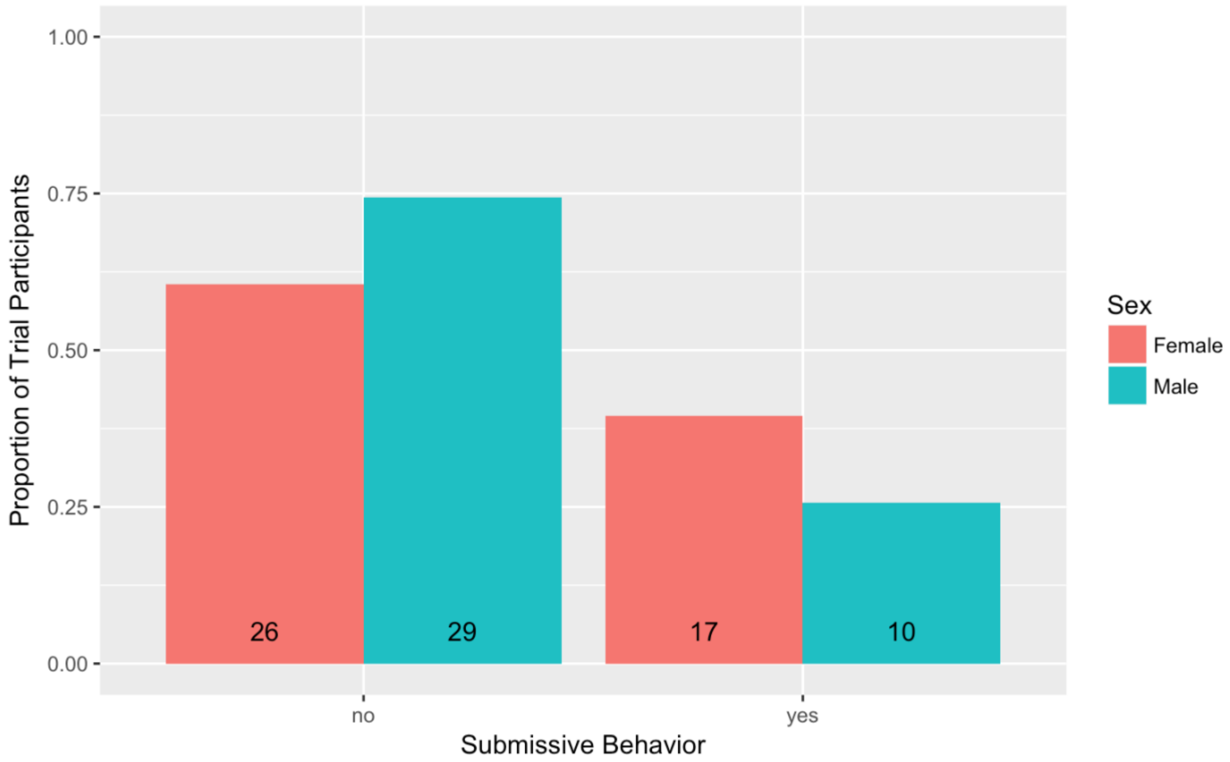

Figure 9. Exhibition of submissive behavior (flight, “Edge” behavior) by adult *D. conanti* individuals in territorial aggression trials based on sex. X-axis represents occurrence of submissive behavior. Y-axis represents the proportion of individuals of each sex for either outcome. Number labels on bars indicate the total number of trials for each result.

Individuals collected from stream one behaved submissively in a greater proportion of trials (43.9%) compared to individuals from stream two (21.95%) (Figure 10). The odds of an individual from stream one exhibiting submissive behavior (OR = 3.206) were comparatively greater than those for an individual from stream two (p = 0.034).

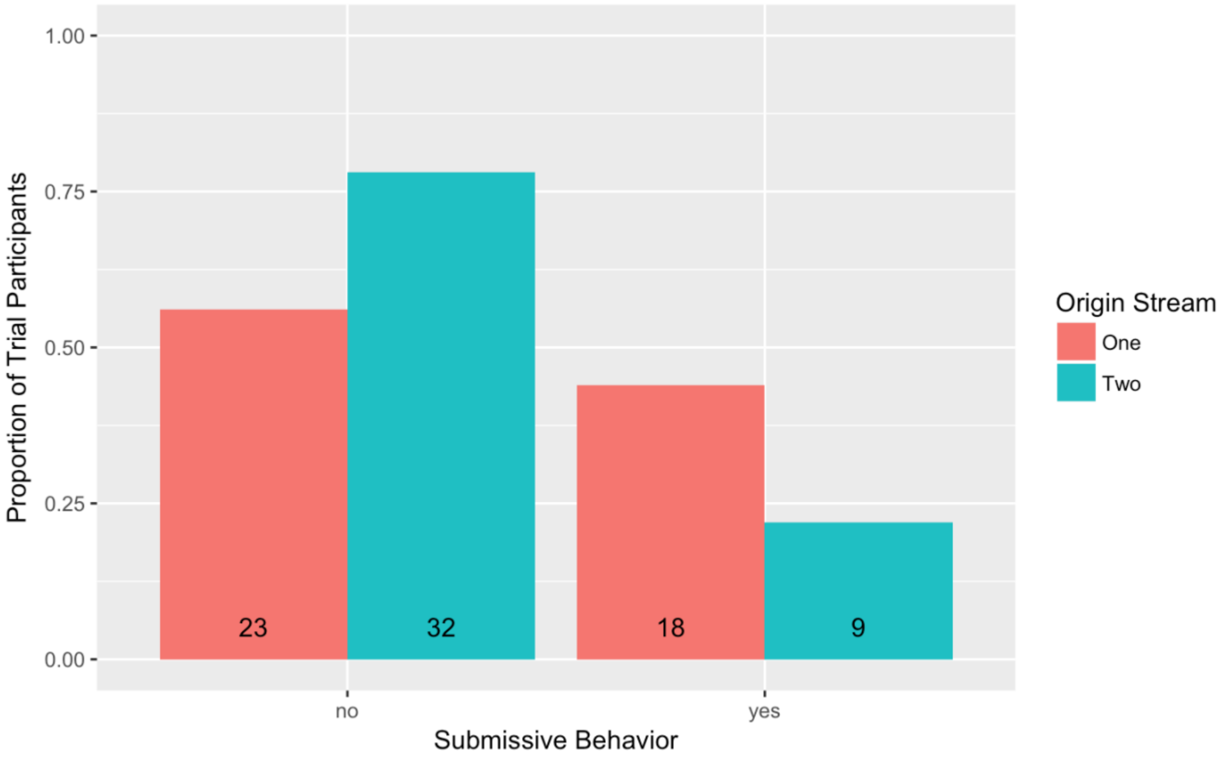

Figure 10. Exhibition of submissive behavior (flight, “Edge” behavior) by adult *D. conanti* individuals in territorial aggression trials based on stream of origin. X-axis represents occurrence of submissive behavior. Y-axis represents the proportion of individuals collected from each stream for either outcome. Paired individuals were either collected from different streams or the same stream. Number labels on bars indicate the total number of trials for each result.

Discussion

To evaluate the territoriality of *Desmognathus conanti*, I conducted two experiments featuring 82 wild-caught adults collected from two streams in Lullwater Preserve. The first experiment evaluated *D. conanti*'s use of chemosensation in advertising and interpreting territorial ownership by presenting trial subjects with a choice between familiar- and foreign-marked substrates. The second experiment evaluated the context and nature of *D. conanti*'s territorial behavior by simulating the encroachment of a conspecific intruder into the established home area of a paired resident. The results of these two experiments indicate that *D. conanti* adults from the population sampled in this study are indeed territorial, as their behavior accorded with the taxa-specific territoriality criteria of site tenacity, advertisement, and defense (Gergits 1981). For *D. conanti*, territoriality appears to be associated with cover objects rather than spatial area, and primarily influenced by asymmetries in sex, residency status, and subpopulation. To my knowledge, this is the first time that territorial behavior has been formally recorded for *D. conanti.*

Experiment One

All plethodontid species, including *D. conanti*, possess specialized naso-labial grooves and cirri for the purpose of detecting the pheromones of conspecific and congeneric individuals, as well as other chemical cues (Dawley and Bass 1989). These salamanders perceive chemical signals by pressing their naso-labial cirri onto marked surfaces (often fecal pellets), which then transport the chemical information to their vomeronasal organs. The chemosensory cues left by plethodontid salamanders are known to convey information about an individual's species, sex, and individuality (Dawley 1984, Gillette et al. 2000, Jaeger and Gergits 1979, Mathis 1990, Palmer

2004). In experiment one's choice trials, the best model for predicting whether an individual would choose to conceal beneath a familiar substrate (sourced from their own enclosure and marked by their own chemical signature) or beneath a foreign substrate (sourced from the enclosure of a random conspecific and marked by its chemical signature) solely included the variable sex (individual sexes of subject and foreign substrate owner, as well as sex matchup). However, this model was not significantly different from the null model in its predictive quality, likely due to the high variability and low sample size of my choice trial data. Furthermore, this model barely differed in its predictive quality from the model which included the variable of stream (subpopulation) difference. Despite the results of this experiment being inconclusive regarding *D. conanti* perception and interpretation of chemical signals, I will discuss here my theories concerning the potential effects of both sex and subpopulation on substrate choice.

Assuming that the sex of individuals is relevant in describing the observed trend in substrate choice from experiment one, both male and female *D. conanti* individuals are more likely to choose the familiar substrate if the foreign substrate had been marked by a conspecific male, although the association of sex matchup (specifically) with substrate choice was not statistically significant. This trend may indicate that individuals of either sex are less willing to trespass in territories with chemical indicators of ownership by a resident male. Furthermore, regardless of the foreign substrate owner's sex, females were seven times as likely to select the familiar substrate compared to males. This apparent avoidance of foreign-owned territories by *D. conanti* females is consistent with the observation from experiment two that females are generally more submissive in the context of territorial disputes, and with findings from studies of *P. cinereus* and other plethodontid species, which indicate that females will usually avoid or submit to residents in confrontations over territory (Horne 1988, Jaeger and Wise 1991, Wiltenmuth 1996). The apparent avoidance of male-owned territories by *D. conanti* males may indicate that individuals are aware of their ownership status and will avoid trespassing in the territory of a conspecific male unless necessary (e.g., seeking higher quality prey access or shelter).

Assuming that tail length is relevant in describing the observed trend in substrate choice from experiment one, individuals with shorter tails were more likely on average to conceal beneath the foreign substrate compared to individuals with longer tails. Like most species of salamander, *D. conanti* individuals engage in caudal autotomy, or the loss of their tail at a pre-formed breakage plane in order to distract or avoid predators (Stebbins and Cohen 1995, Downes and Shine 2001, Bateman and Fleming 2009, Marvin 2010). While this strategy is effective in reducing mortality associated with predation (Ducey and Brodie 1983, Maginnis 2006), tail loss is costly for individual fitness, as the tail is an important asset for locomotion, balance, and energy storage (Jamison and Harris 1992, Maginnis 2006). However, this cost is temporary, as salamanders will grow a new tail over time. In a study of Allegheny Mountain dusky salamanders (*Desmognathus ochrophaeus*), individuals that had recently undergone caudal autotomy were more motivated to forage despite elevated levels of threat indicated by predator kairomones and more efficient in doing so (Gildemeister et al. 2017). Assuming that individuals with shorter tails had autotomized more recently, this trend in substrate choice may indicate that *D. conanti* individuals investing energy in tail re-growth may be more willing to risk trespassing into territory marked by a conspecific because it is relatively more imperative that they secure access to refuge from predation and a steady source of prey.

Despite my inability to determine with any certainty the role of chemical signaling in *D. conanti*’s advertisement and interpretation of territorial ownership based on the results of experiment one, observations from the present study strongly indicate *D. conanti*'s ability to recognize and respond to the chemical signatures of conspecifics. The primary evidence of the importance of chemical signaling in communicating territorial ownership for *D. conanti* is the consistent observation of the submissive "Edge" behavior in experiment two's territorial aggression trials. This behavior, first described in a study of *P. cinereus* as an escape or avoidance behavior (Wise and Jaeger 1998), was consistently exhibited by intruders in experiment two and rarely by residents. Significantly, intruders often exhibited "Edge" behavior without having interacted with the resident individual at all, and in some cases even after the resident individual had concealed beneath the paper towel substrate or within the hide. This phenomenon has been observed in previous studies of plethodontid territoriality as well (e.g., Jaeger et al. 1982, Mathis et al. 1998). I interpret this observation as a strong indication of intruders' awareness of their role as interlopers in a conspecific's territory based on perception of a foreign chemical signature, and therefore, as evidence for *D. conanti*'s use of chemical signaling in territorial advertisement.

Experiment Two

All confrontational interactions observed in the territorial aggression trials of experiment two were associated with the defense or pursuit of a specific cover object within the enclosure. These interactions can be divided into two general contexts; confrontational interactions were either initiated by a concealed individual in response to the intrusion of a conspecific into its vicinity, or by an active individual as an apparent attempt to expel a conspecific from a site of concealment. The objects defended in these interactions were either the overturned petri dish hides positioned in each enclosure or sections of the paper towel substrate that had folded to create a nook wherein an individual could hide. The association of territorial behavior with specific objects rather than spatial area is consistent with observations of the importance of above-ground cover objects in defining high-quality habitat for plethodontid species (Keen 1984, Mathis 1990, Caruso 2016). Without lungs or gills, these salamanders must respire by means of cutaneous gas exchange and require cool, moist conditions to do so without desiccating (Spight 1967, Spotila 1972, Petranka 1998). In a natural setting, the microhabitats beneath rocks and logs serve as a concentrated source of invertebrate prey for adult salamanders (Fraser 1976, Jaeger 1980, Jaeger et al. 1981, Feder and Landos 1984) and offer protection from predation (Krzysik 1979). While I observed aggressive defense of cover objects by individuals of both sexes and from both streams, more work is needed to understand whether *D. conanti* individuals of differing size, sex, and subpopulation classes are utilizing these object territories for distinctive habitat needs.

Overall, residency status (role) appears to be the most consistent and meaningful determinant of territorial behavior for *D. conanti*, appearing in the highest quality models for predicting both aggressive and submissive behavioral displays, while the sex matchup of paired individuals appears most meaningful in determining the interaction outcome. Subpopulation (stream of origin) also seemed to meaningfully affect territorial behavior, and was included in the best model for predicting submissive behavior. Specifics of the interactive effects of sex, subpopulation, and asymmetries in residency on territorial behavior in *D. conanti* require further study, particularly in a natural setting and in relation to courtship and breeding events, but the results of the present study suggest that territorial behavior in *D. conanti* is principally shaped by some combination of these three factors. Because the experimental design of the present study controlled for the effects of substantial body size difference, these results are not a complete exemplification of the territoriality of *D. conanti*. However, small body size differences (< 5%) between interacting salamanders have been demonstrated by previous assessments of plethodontid territoriality to have negligible effects on behavior relative to sex and residency status (Houck 1988, Wiltenmuth 1996). The observed trends in territorial behavior and interactions according to sex, residency status, and subpopulation provide a valuable foundation for more comprehensive investigation in future studies.

The territorial behaviors exhibited by *D. conanti* vary according to an individual's sex, and the outcome of territorial interactions is principally dictated by the sex matchup of interacting individuals. While both males and females of *D. conanti* exhibited territorial aggression, aggressive behavior was more common for males, and submissive behavior was more common for females, although neither trend was statistically significant. This asymmetry between the sexes has previously been observed both in studies of *E. eschscholtzii* and *P. cinereus*, which have found males to exhibit overt aggression more often than females (Jaeger and Wise 1991, Wiltenmuth 1996). Differences in territorial behavior between males and females likely reflect a discrepancy in selective pressures between the sexes, and in the costs and benefits of territory ownership (e.g., Lynn 2018). Relatively greater aggression may have evolved in *D. conanti* males as a result of intra-sexual competition over mates, or over possession of high-quality territory for the purpose of attracting mates, as seen in *P. cinereus* (Walls et al. 1989, Mathis 1991b). Because adult female plethodontids require several seasons to accumulate sufficient yolk for summer egg deposition, the operational sex ratio (Emlen and Oring 1977) of individuals engaged in territorial activity at any time is expected to be skewed towards males (eg. Thomas et al. 1989, Sayler 1966), which may facilitate increased competition and the evolution of more aggressive behavior. Further studies of behavioral asymmetries between the sexes of *D. conanti* in a natural setting are needed in order to elucidate differences in the evaluation and utilization of resources by males versus females.

Intra-sexual aggression has been commonly observed in plethodontid salamanders (reviewed in Mathis et al. 1995), and the results of the present study suggest that this pattern holds true for *D. conanti* as well. The outcomes of experiment two's territorial aggression trials were chiefly determined by the sex matchup of paired trial participants. Although the sex matchup ("best") model for predicting confrontation was not significantly different in quality from the null model, likely due to the high variability and low sample size of my choice trial data, studies of other plethodontid species have demonstrated that the sex matchup of two individuals is highly relevant to the nature of their interaction. Confrontation occurred between same-sex pairs substantially more often than mixed-sex pairs, and confrontation resulted most often from interactions between two males. The infrequency of agonistic interactions between mixed-sex pairings observed in the present study may indicate that *D. conanti* individuals will avoid confrontation with potential mates. This evolutionarily-stable territoriality strategy has been modeled for *P. cinereus* (Hom et al. 1997), and suggests that males compete with other males over territories that are most conducive to female fitness, such as abundant prey, while females selectively associate and breed with the males that possess these high-quality feeding territories; therefore, males who tolerate female intruders ultimately have higher fitness compared to males who expel intruders indiscriminately. Indeed, laboratory studies of *P. cinereus* have shown that territorial male residents will tolerate both gravid (courting) and non-gravid adult female intruders (Thomas et al. 1989), and non-gravid residents will often avoid and submit to male intruders (Horne 1988). Evidence for intra-sexual antagonism in plethodontid salamanders has also been recorded in a natural setting; in surveys of a wild population of *P.*

cinereus at the Mountain Lake Biological Station in Virginia, male-female pairs were found to cohabit cover objects substantially more often than male-male pairs (perhaps to secure future mating opportunities, as suggested in Jaeger et al. 1995), and no female-female pairs were recorded (Lang and Jaeger 2000). The results of the present study suggest a similar pattern of territorial interactions for *D. conanti*; however, further examination of courtship and cover object cohabitation in wild populations is necessary to determine the role of sex relations in shaping *D. conanti*'s territoriality and population distributions.

Residency status also appears to significantly influence the territorial behavior and interactions of *D. conanti*. In experiment two's behavioral trials, residents exhibited aggressive behavior more often than intruders, and intruders behaved submissively more often. This pattern of behavior is a strong indication of site tenacity in *D. conanti* and is consistent with previous findings regarding the role of residency status in territorial salamanders. In a study of territoriality in Southern red-backed salamanders (*Plethodon serratus*), which involved comparable behavioral trials with paired individuals of asymmetric residency status, only residents exhibited aggressive behavior, while intruders consistently avoided confrontation (Mathis et al. 1998). In a similar study of the common ensatina salamander (*Ensatina eschscholtzii*), residents also behaved aggressively more often than intruders, and asymmetries in residency status had comparably greater effect on aggression than body size (Wiltenmuth 1996). The results of the present study and previous studies of terrestrial plethodontids not only demonstrate that residents are capable of recognizing and defending a territory, but also that intruders are likely aware of their role as interlopers in other salamanders' territories.

The consistently heightened aggression of *D. conanti* residents relative to intruders indicates that residency may provide individuals an inherent advantage in territorial competitions that is independent of other factors. The persistence of this trend across different species of plethodontid salamander can be explained by the phenomenon of “resident advantage” (Maynard Smith and Parker 1976), which has been observed in other amphibians (e.g., Given 1988) and territorial animals (e.g., Davies 1978, Krebs 1982). Several factors may contribute to an inherent advantage for residents in territorial competitions (Parker and Rubenstein 1981); residents are likely to have a greater familiarity with the disputed territory and, depending on the length of their occupancy, have had more consistent access to food and shelter (Nunes and Jaeger 1989). Furthermore, because residents have likely taken the opportunity to advertise their ownership of the area via pheromonal markers, that territory may be of greater value to them relative to non-occupants (Maynard Smith and Parker 1976, Nunes and Jaeger 1989).

Submissive behavior appeared to vary between individuals that had been collected from different streams, which suggests that the subpopulations of *D. conanti* in Lullwater may have evolved different territorial behavior in response to disparate environmental or social pressures between stream habitats. While I did not measure the resource availability, population density, community richness, or any other potentially meaningful distinctions between the two stream habitats, these factors may drive differences in competitive pressure and consequently, the behavior of *D. conanti*. For species that occupy a sizeable geographic range with diverse habitats, success is determined largely by the ability of individuals to respond to environmental variation (Agrawal 2001). This certainly applies to territorial behavior, as territoriality is known to affect individual fitness and depends on social and environmental factors that may vary between habitats (Brown and Orians 1970, Courtene-Jones and Briffa 2014). Indeed, geographic variation in these factors has been shown to facilitate variation in agonistic behavior within and among populations (Wiltenmuth and Nishikawa 1998, Grether et al. 2009, 2013, Laiolo 2012, Gonçalves-de-Freitas et al. 2014). Geographic variation in territorial behavior has been demonstrated in *P. cinereus*, which found significant differences in submissive behavior between populations across an elevation gradient (Wise and Jaeger 2016) and between populations at sites 30km apart (Maerz and Madison 2000). That this pattern of variability in territorial behavior exists for *P. cinereus* suggests that such variation is possible for other plethodontid species, including *D. conanti*. Further field and laboratory studies are necessary to determine whether the behavioral differences observed between subpopulations in the present study are the result of social or environmental differences between habitats rather than random variation in the behavior of individuals.

Study Limitations

One limitation of these data is the implicit bias of my sampling efforts. Only individuals that were active above ground (generally beneath surface cover objects) were collected, which may have been the boldest or most competitively successful members of the population. Furthermore, each stream was only sampled on a handful of occasions, resulting in a small number of collected salamanders relative to longer- or larger-scale plethodontid studies. Both of the streams sampled in this study were subject to many years of frequent disturbance from recreational herpetofauna searches. Consequently, the behavior exhibited by individuals from these subpopulations could be meaningfully different from that of *D. conanti* individuals from elsewhere. The isolation of the Lullwater Preserve community within the urban environment of metro Atlanta limits the ability of *D. conanti* and other species to migrate, which may lead to pronounced differences in territorial behavior (e.g., Riechert and Maynard Smith 1989).

The results of my experiments are likely also biased by the various unavoidable differences of the laboratory setting from *D. conanti*'s wild habitat. The unnatural photoperiod, relative lack of temperature variability, enclosure setup, and generally disturbing sound and action of the laboratory setting may very well have affected the activity and behavior exhibited by the salamanders. In addition, the behavior of individuals in trials may have been influenced by the stress of physical handling as well as my mere presence as an observer. Finally, because salamanders of similar body size were paired for the territorial aggression trials, the observed frequency of confrontational interactions is likely to be much greater than that which would be observed in a wild setting.

Conclusion

Although the behavior of animals in captivity does not always accurately reflect their behavior in a natural setting, it provides a valuable point of reference for observations of wild patterns and activity. Therefore, the present study's evaluation of *D. conanti* behavior and territoriality in a laboratory setting serves as an essential foundation for subsequent investigations of the ecology and distribution of their wild populations. Furthermore, that *D. conanti* meets all criteria for plethodontid territoriality suggests that other semi-aquatic salamander species, especially congeners, may behave territorially under similar conditions. In light of the unprecedented decline of amphibian species facing our world today, our lack of knowledge regarding the forces that shape the distributions and population dynamics of plethodontid salamanders and other threatened amphibious taxa is a pressing conservation concern. Given the vulnerability of *D. conanti* to anthropogenic disturbances, the evidence that this study offers in support of their territoriality is highly valuable in informing efforts for the preservation of their habitat and wild populations. Conservation on behalf of *D. conanti* and stream habitat restoration initiatives within their geographic range ought to prioritize maintaining an abundance of the rocks and logs that adult individuals nest, forage, and reside beneath, as their active defense of comparable above-ground cover objects in experimental trials is a clear indication of their importance in constituting high-quality habitat. Hopefully, further investigations of the territorial behavior of spotted dusky salamanders under natural conditions will be undertaken to elucidate how the territoriality of *D. conanti* and other desmognathine species drives patterns in their wild distribution and shapes their populations and ecological communities.

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