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**GEOGRAPHIC RANGE SIZE AS A PREDICTOR OF DISPERSAL-  
DEPENDENT BEHAVIORAL TRAITS IN TWO CLADES OF A  
TERRESTRIAL SALAMANDER**

Teah Evers

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GEOGRAPHIC RANGE SIZE AS A PREDICTOR OF DISPERSAL-DEPENDENT  
BEHAVIORAL TRAITS IN TWO CLADES OF A TERRESTRIAL SALAMANDER

A Thesis Submitted to the  
Graduate School of  
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for the Degree of  
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By  
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## ABSTRACT

Animal movement has the potential to affect diverse processes within ecology and evolution including range expansion, gene flow, adaptation, and speciation. Two aspects of animal personality that are germane to dispersal are exploratory and aggressive behavior. These behavioral categories may represent a trade-off such that energy invested in territorial defense leaves little energy for movement and dispersal. The Eastern Red-backed Salamander (*Plethodon cinereus*) is a wide ranging, dispersal limited, terrestrial salamander with well documented phylogeographic divisions. I examined dispersal-relevant behavioral traits within two clades of *P. cinereus* with disparate geographic ranges. The Northern Clade (NC) has a range extending from the mid-Atlantic coast upward into Canada, across the northern reaches of the Great Lakes and south into Indiana. The Ohio Clade (OC) is largely restricted to Ohio. Previous research suggests that differences in aggression and territorial behavior exists between the clades. The OC is known to be aggressive and territorial while some evidence suggests that the NC lacks territorial behavior. I therefore predicted that members of the NC would be more exploratory and less aggressive and that members of the OC would be less exploratory and more aggressive. Salamanders from each clade were tested in two separate laboratory experiments to assess 1) aggressive behavior and 2) exploratory behavior. In experiment 1, resident salamanders of both clades spent significantly more time in the behavior Look Toward compared to control trials when no intruder was present. This response suggests that salamanders recognized intruders as potential threats. However, residency status (resident or intruder) did not influence the aggressive or submissive behavior for either clade. Additionally, in contrast to my prediction, no differences in submissive or aggressive behaviors by residents were detected between clades when intruders were present. Intruders from each clade also behaved similarly when interacting with residents.

In experiment 2, clade members differed in their exploratory behavior, with members of the OC being more exploratory, counter to my predictions. Specifically, members of this clade were quicker to leave their territory, and they crossed over barriers more often. I compared aggressive and exploratory behavior from experiments 1 and 2 in a correlational analysis to investigate trade-offs between these behaviors. Only in the NC was aggressive behavior “All Trunk Raised” correlated with latency to the exploratory chamber’s final zone, suggesting that, in this clade, aggressive individuals were more cautious during exploration. My results suggest that there are clear exploratory differences between the NC and OC but that these differences are not easily explained by geographic range size and historical range expansions.

## INTRODUCTION

Ecologists have long been fascinated with territoriality and dispersal, as the study of these behaviors contributes to our understanding of key ecological processes (Brown 1969, Ostfeld 1990, Sih et al. 2012). For instance, territoriality has been closely associated with species distribution, abundance, density, and mating systems (Carpenter 1987, Hixon 1987, Stamps et al. 1987), and dispersal has been shown to affect population dynamics (Gill 1978, Peterman and Semlitsch 2013), genetic structure (Shields 1982, Peterman et al. 2015, Cameron et al. 2019), and life history strategies (Horn 1983). Behavioral ecology is complex, given that behaviors can interact and may do so across or within contexts and situations. Context is defined as a functional behavioral category such as mating, aggression, and feeding, whereas situation refers to a given set of conditions at one point in time (Sih et al. 2003, 2004). When behaviors are consistently correlated within or across contexts, the suite of traits is referred to as a behavioral syndrome (Sih et al. 2004). In particular, understanding within-situation correlations across different contexts can have important evolutionary implications due to time budget conflicts (Sih et al. 2003, 2004). An animal's energetic demands limit how much time is spent on various activities (behavioral categories) and results in associated traits that are negatively correlated (Sih et al. 2004). For example, there are trade-offs associated with territoriality and predator avoidance since the time that an animal spends avoiding a predator is time that is not being spent defending a territory (Diaz-Uriarte 2001). Both behaviors may independently increase fitness, but they cannot be maximized simultaneously (Sih et al. 2004). Behavioral trade-offs allow for variation within and among populations of a species such that some individuals may spend more time performing one behavior (or activity) while other individuals may spend more time performing a second behavior (or activity). Behavioral phenotypes may also arise within a situation in the

same context. For example, in the polymorphic Eastern Red-backed Salamander (*Plethodon cinereus*), the striped morph often utilizes a territorial tactic whereas the unstriped morph often utilizes a floater tactic (unable to hold territories, Mathis 1991, Reiter et al. 2014). In this example, aggression (context) varied within a given set of conditions at one point in time.

Territoriality is a behavior in which an animal actively defends an area (Noble 1939). There are several reasons why holding a territory is beneficial, as defended areas often hold high quality food, shelter, or mates (Gergits and Jaeger 1990). Brown and Orians (1970) described four requirements for an animal to be considered territorial: site tenacity, advertisement, aggression, and expulsion of intruders. (1) Site tenacity refers to remaining in or returning to the same area over several seasons. (2) Advertisement refers to communicating ownership of the territory in which the animal will exhibit body displays, vocalizations, pheromonal cues, or a combination of these, to nearby conspecifics (or congeners). (3) Aggression is defined as the active defense of an area against intruders with the use of threatening postures, physical attacks, or chasing, and is often the measure of territoriality in many studies. (4) Expulsion of intruders refers to successfully defending the area that results in expelling the intruder from the resident animal's territory. Early literature on territoriality addressed the behavior in many vertebrates (reptiles, Evans 1938, Stamps 1977; birds, Nice 1941; mammals, Burt 1943; fishes, Gerking 1953; amphibians, Martof 1953, Wiewandt 1969) and invertebrates (Baker 1983), but variation in territoriality within a species is not as well-studied.

In contrast to territoriality, which is associated with site fidelity, dispersal is a permanent movement away from the natal site that increases gene flow (Ronce 2007, Lowe and McPeck 2014) and has been associated with a suite of traits such as exploration, boldness, neophilia, and aggression in the context of behavioral syndromes (Duckworth and Kruuk 2009, Canestrelli et al.



2016, Gruber et al. 2017a). Competition, inbreeding avoidance, and habitat variability are a few reasons why an animal may be inclined to disperse from its natal site (Bowler and Benton 2005). The process consists of three stages: an animal will leave its current population (emigration), move across a matrix (migration), and settle into an established population or colonize a new population (immigration; Ims and Yoccoz 1997, Bowler and Benton 2005). Dispersal can be condition-dependent and vary within and among populations depending upon selective pressures. Dispersal has also been shown to be heritable and repeatable in some species (Dingemanse et al. 2002, Clobert et al. 2004, Van Dyck and Baguette 2005, Baguette and Van Dyck 2007, Doligez et al. 2009). For example, Gruber et al. (2017a) found significant differences in exploratory behavior (a trait of dispersal) between populations of the Cane Toad (*Rhinella marina*) in its non-native range. They found that in both field and laboratory conditions, individuals from range-edge populations were more exploratory than their range-core counterparts, suggesting that the populations experiencing different selective pressures have evolved divergent behavioral phenotypes.

Few studies have explored the potential trade-offs between behaviors associated with territoriality and dispersal, whereas those that have, have primarily identified a positive relationship. Individuals of wild Great Tits (*Parus major*) that were identified as fast explorers were also more aggressive in that they started and won more fights than slow explorers (Verbeek et al. 1996, Drent and Marchetti 1999). In the Western Blue Bird (*Sialia mexicana*), dispersing males in new habitat were significantly more aggressive than philopatric males in older habitat (Duckworth and Badyaev 2007, Duckworth 2008). In contrast, Stapley and Keogh (2004) found evidence of a negative correlation in the Yellow-bellied Water Skink (*Eulamprus heatwolei*) in the laboratory. During exploratory trials, nonterritorial individuals were less likely to spend time

under the retreat, had higher movement rates, and took longer to return to their basking site than territorial individuals. More research is needed to fully comprehend the intricacy of these behaviors such as how they interact and how variation in these two behaviors among populations influences our understanding of ecological processes and evolutionary relationships. For example, conflict can arise through trade-offs between territoriality and dispersal, which may influence population abundances and distributions. In addition, behavioral variation among individuals of a species has long-term benefits because it may allow the species to exploit a wider biogeographical range and reduce the chance of local extinction (Sih et al. 2012). Alternatively, populations that exhibit divergent behavioral phenotypes may be on separate evolutionary trajectories, potentially leading to speciation.

Many amphibians present ideal systems in which to study behavior, as they are exceptionally abundant (Burton and Likens 1975, Gibbons et al. 2006, Semlitsch et al. 2014), diverse (Pough et al. 2016), ecologically important (Wyman 1998, Walton 2005, 2013, Hickerson et al. 2017) and serve as indicators of environmental change (Welsh and Droege 2001). The Eastern Red-backed Salamander (*Plethodon cinereus*) is the most abundant amphibian in the Eastern United States (Burton and Likens 1975) and has been used as a model system in many disciplines of biology (Anthony and Pfungsten 2013). This direct-developing salamander is lungless and is therefore constrained to moist environments for cutaneous respiration (Spotila 1972, Feder 1983, Anthony and Pfungsten 2013). This species feeds on small, leaf litter-dwelling arthropods (Cochran 1911, Bondi et al. 2019) and competition over prey and mates has resulted in territorial behavior, during which natural cover objects are actively defended against conspecifics (Jaeger and Gergits 1979, Jaeger 1981, Jaeger et al. 1982, Gergits and Jaeger 1990, Mathis 1991). This species' current geographic range is the largest of all members of the genus *Plethodon* (Petranka 1998,

Radomski et al. 2020). Given that nearly three quarters of its current range was glaciated roughly 18,000 years ago (Radomski et al. 2020), this small-bodied species must have been capable of rapidly dispersing over considerable distances. One study estimated that *P. cinereus* would have had to travel 80 meters per year to re-populate glaciated regions of its range following retreat of the last Pleistocene cycle (Cabe et al. 2007). With the waxing and waning of these glaciers that resulted in modifications of landscapes, this species fragmented into divergent allopatric and parapatric groups while expanding its range (Kozak et al. 2006, Rundell and Price 2009, Kuchta et al. 2018, Radomski et al. 2020). Recent work has characterized these groups into six genetically distinct lineages based on mtDNA (Northern, Southern, Virginia, Pennsylvania, Ohio and North Carolina Clades, Radomski et al. 2020, Fig. 1). The Northern Clade (NC) originated in Virginia's Piedmont region and has since expanded down the Eastern Coast as far south as North Carolina, up the Eastern Coast into Canada and westward down into Michigan and Indiana (Radomski et al. 2020, Fig. 1). In contrast, the other five clades exhibit a much more limited range with considerably less dispersal from their inferred ancestral origin (Radomski et al. 2020). Ecologists are interested in the dispersal of the NC because such a range expansion may provide insight into evolutionary divergence among clades as well as movement in response to human-induced climate change.

There is a wide array of literature on *P. cinereus* demonstrating the four aspects of territoriality described earlier (reviewed in Jaeger et al. 2016). Following displacement studies, *P. cinereus* has been shown to exhibit homing behavior (Kleeberger and Werner 1982, Ousterhout and Liebgold 2010, Martin and Hantak 2011) and has been found in the same area (sometimes under the same cover object) over several seasons, indicating site tenacity (Kleeberger and Werner 1982, Mathis 1991, Sutherland et al. 2016). With the use of chemical

communication and visual displays, this species will advertise its presence and mark its territory with pheromones (Jaeger and Gergits 1979, Simons et al. 1994). In addition to aggressive displays, *P. cinereus* may also use biting, which has been shown to lead to the expulsion of conspecific intruders (Jaeger 1981, 1984, Jaeger et al. 1982). In contrast, dispersal in *P. cinereus* is poorly understood. Some ecologists consider them poor dispersers due to limited observed gene flow (Cabe et al. 2007) and physiological constraints (Spotila 1972, Feder 1983, Anthony and Pflingsten 2013), despite their substantial range. Mark-recapture studies support this claim by showing that *P. cinereus* will remain within the same area over several seasons (Kleeberger and Werner 1982, Mathis 1991, Sutherland et al. 2016) and have used single cover objects for up to 3.5 years (Reiter et al. 2014). Additionally, they have small home ranges and low gene flow (Cabe et al. 2007), all suggestive of low dispersal capability. Conversely, researchers who have displaced territorial residents have found that they can return home from considerable distances. One displacement study found a 25% return rate when salamanders were displaced 90 meters away (Kleeberger and Werner 1982), and another study found *P. cinereus* movement up to 143 m (Sterrett et al. 2015). These are substantial distances for a salamander with a home range of typically only a few square meters (Mathis 1991, Liebgold and Jaeger 2007). In addition, Marsh et al. (2004) found evidence of colonization when artificial patches of forest habitat were placed into a field, indicating that *P. cinereus* had left its current population to traverse across a seemingly inhospitable matrix. They also found that there was no difference in return rates when individuals were displaced into the forest (control) and field, indicating that moisture constraints may not restrict dispersal as previously thought. These studies, along with the extensive dispersal and range of the NC (Radomski et al. 2020, Fig. 1), suggest that *P. cinereus* may have better dispersal capabilities than are currently understood.

Territorial behavior in *P. cinereus* has been shown to diverge geographically (Wise and Jaeger 2016). A study conducted in Michigan (most likely the NC), found that salamanders tended to aggregate in their space use in the field compared to populations in Virginia which displayed uniform distribution (likely the Southern Clade, Quinn and Graves 1999). This could be a result of differences in habitat distribution (Brown and Orians 1970), although, salamanders also aggregated in laboratory enclosures (Quinn and Graves 1999), which suggests differences in social or territorial behavior. Mark-recapture studies have found differences in home ranges at different localities. In Michigan, female and male minimum polygon home ranges are 123-fold and 66-fold higher than those found in Virginia, respectively (Kleeberger and Werner 1982, Mathis 1991). This difference in home range size could suggest that populations from Michigan are not as territorial, although Brown and Orians (1970) state that territory and home range are not necessarily synonymous. It may also suggest that Michigan populations consist of floaters rather than residents with fixed territories. In comparison, there are small differences in aggressive behavior across Ohio (most likely the Ohio Clade). Aggressive values in the central region of the state are only 1-2-fold higher than the northeastern region of the state (Hickerson et al. 2004, Deitloff et al. 2009, Ryan et al. unpublished). In addition, Hickerson et al. (2004) found that 97 % of OC salamanders occurred alone under cover objects which suggests a non-aggregated distribution.

In this study, I compared aggression (a trait of territoriality) and exploration (a trait of dispersal) in two genetically distinct clades of *P. cinereus* to test the hypothesis that the Northern and Ohio Clades differ in these behaviors. I compared the NC, which has been shown to have a larger geographic range (Radomski et al. 2020, Fig. 1), larger home ranges (Kleeberger and Werner 1982), and co-occurrence of individuals under cover (Quinn and Graves 1999), to the

Ohio Clade (OC), which is largely confined to the state of Ohio (Radomski et al. 2020, Fig. 1) and is known to be territorial (Hickerson et al. 2004, Deitloff et al. 2009, Ryan et al. unpublished). I predicted that members of the OC would be more aggressive than members of the NC, while NC members would be more exploratory than members of the OC. Additionally, behaviors associated with territoriality and dispersal should be negatively correlated within each clade as a consequence of time-budget conflicts (within situation across contexts) wherein salamanders that invest time in maintaining territories would invest less time in exploratory activities.

## **METHODS**

### *Study Sites and Salamander Collection*

Salamanders were collected in April 2021 from four localities in Indiana, representative of the NC, and four localities in Ohio, representative of the OC (consistent with Radomski et al. 2020 map, Fig. 1). Indiana collection sites were located in Yellowwood State Forest (YW, 39.209572, -86.345260), Brown County State Park (BC, 39.172388, -86.260434), Morgan-Monroe State Forest (MM, 39.3219463, -86.4251579), and Griffy Lake Nature Preserve (GL, 39.2002871, -86.5088572). Ohio collection sites were located in Strouds Run State Park (SR, 39.3391292, -82.0638438), Baker's Preserve (BP, 39.3319067, -81.9907056), Hocking Hills State Park (HH, 39.4174571, -82.5270965) and Airplane Rock (AR, 39.465562, -82.552710). Maximum interpopulation distance within Indiana was 36.2 km and 70.5 km within Ohio. In addition to clade membership, localities in each state were selected with similar latitudes to reduce temporal bias in habitat variability (Fig. 1). A total of 111 individuals (Indiana = 52; Ohio = 59) were collected for the purposes of these experiments. Most studies examining territoriality

have excluded females to prevent mistaking territorial behavior with mating, although both sexes are territorial (Jaeger et al. 1982, Horne 1988, Mathis 1991, Ryan et al. unpublished). Juveniles are often considered nonterritorial floaters (Mathis 1990, Anthony and Pfingsten 2013), and the striped morph is more territorial than the unstriped morph at polymorphic sites (Anthony et al. 2008, Reiter et al. 2014, Stuczka et al. 2016), therefore, only adult striped males (snout-vent-length > 32 mm, Sayler 1966) were collected. Males were characterized following the Anthony et al. (2008) method of sex identification.

### *Salamander Housekeeping and Maintenance*

Salamanders were transported individually in 50 mL polypropylene centrifuge tubes to John Carroll University, University Heights, OH, USA, where they were individually kept in 470 mL (11.5 x 5 cm) glass Pyrex containers. Animals were fed approximately 25 *Drosophila melanogaster* once per week until the start of experimentation, after which they were fed 15 flies every 2-6 days (mode = 5) in between trials. However, individuals were always fed 5-6 days before their scheduled trials (at the time of their territory set up). Any uneaten flies were removed before experimentation. Animals were maintained at approximately 16°C under a natural photoperiod. Containers were visually separated in the laboratory by opaque barriers to avoid interactions and were spatially rearranged occasionally during each experiment to avoid spatial bias in laboratory conditions. All experimental procedures were approved by John Carroll University IACUC (Protocol No. 2103).

### *Experiment 1: Aggression*

Individuals were transferred from their Pyrex containers to 15 x 1.5 cm polystyrene petri dishes lined with damp filter paper. I used aggression as a proxy for territoriality and recorded a number of individual behaviors as components of aggression for both resident and intruding

salamanders. Each of 46 individuals from each clade was used three times: once as a resident (R), once as an intruder (I), and once as a resident with a control (RC, moistened paper towel rolled and shaped into a salamander, Jaeger et al. 2016) for a total of 92 trials per clade. The order of status (R, I, RC) was randomized throughout the experiment. Individuals were randomly paired with a conspecific from their own clade within 2 mm of SVL difference to account for size asymmetries that may influence the outcome of contests (Mathis et al. 1998, Mathis and Britzke 1999). Intruders were paired with smaller residents, when possible, as residents already had the advantage of an established territory (Wise and Jaeger 2016). Animals collected in close proximity to each other were not paired together, and no salamanders were paired together more than once to decrease the likelihood of familiarity which may reduce aggression in contests (Jaeger 1981). Residents, including those in the control treatment, were allowed to establish a territory (deposition of fecal pellets and pheromones onto new filter paper) for five to six days prior to testing, as research has shown this is an adequate amount of time for territory ownership (Nunes and Jaeger 1989). During trials, both resident and intruder (whether another salamander or control paper towel) were marked dorsally, posterior to pectoral girdle with Day-Glo™ fluorescence for individual identification (Lynn et al. 2019). Blaze orange and signal green were used, and colors were alternated between residents and intruders each trial night to avoid bias, as studies have demonstrated that some Urodeles can discriminate between certain colors (Przyrembel et al. 1995). All trials were conducted blind with respect to clade membership of opponents. Intruder salamanders were transferred into the territory of a resident and restricted for a five-minute acclimation period using an opaque acclimation cover (6 x 1.5 cm polystyrene petri dish) before behaviors were recorded. Resident salamanders were also lifted and put back into their territory to control for handling. A number of individual behaviors of both resident and



intruder salamanders were recorded for 900 s after removal of the acclimation cover. Aggressive and submissive behaviors described by Jaeger (1984) were recorded in real time using TrueBasic Event-PC 3.0 data collection software. Aggressive behaviors included “All Trunk Raised” (ATR), in which a salamander extended its legs to lift its body off the substrate; “Look Toward” (LT), defined as turning its head in the direction of the other; “Move Toward” (MT), in which a salamander decreased the distance between itself and the other individual, which would result in eventual contact if movement continues; and lastly, BITE, in which a salamander successfully grasped or attempted to grasp another with its open mouth. The three submissive behaviors included: EDGE, defined as roaming the periphery and pressing its snout and limbs against walls or in crevices (this can also be interpreted as escape behavior, Horne 1988, Wise and Jaeger 1998); “Look Away” (LA), in which a salamander turned its head away from the other; and lastly, “Move Away” (MA), in which a salamander moved as to increase the distance between itself and the other individual. In addition to submissive and aggressive behaviors, the number of Nose Taps (NT, chemosensory behavior), defined as lowering the head so that the nasolabial cirri come into contact with the substrate, were recorded. A few specific behaviors could be recorded simultaneously (example: ATR and MT). However, other behaviors could not be recorded simultaneously, and the preceding behavior was terminated following a subsequent behavior (example: MT following LT). All continuous variables were terminated after twenty seconds of inactivity except for ATR, since this behavior is metabolically costly (Bortosky and Mathis 2016) and requires energy even when stationary. Additionally, I calculated an aggression index (AI) for each individual (Mathis et al. 2000, Reiter et al. 2014):  $[MT+LT]-[MA+LA]$ . All aggression trials were conducted at night between the hours of 1900 and 0400 with minimal light between May 10 and June 10, 2021. Three trials were rerun: two due to software failure and one

due to accidental partial tail loss during acclimation (9.32 mm, given 10 days to recover from potential stress). Equipment was cleaned with 70 percent ethyl alcohol in between trials to reduce disease transmission and the introduction of conspecific scent. Between aggression and exploration experiments, all animals were used in a separate study conducted from June 19 to August 2, 2021 (Garner et al. unpublished). Four salamanders (1 NC; 3 OC) died in between the two experiments. These individuals were included in aggression analyses but not exploration or correlation analyses. Extra salamanders (3 NC; 5 OC) were used for exploratory trials to redeem sample size.

### *Experiment 2: Exploration*

Feeding, housing and salamander identification for the second experiment was as described for the aggression experiment apart from the size of the petri dish (10 x 1.5 cm). I used exploratory behavior as a proxy for dispersal wherein each individual ( $n = 48$ ) was used once in an exploratory-barrier test modified from Lynn et al. (2019) and Reeder (2013). An arena containing rings acted as surmountable barriers to exploration (Fig. 2). The order of trials for each individual was randomized throughout the experiment. Salamanders, along with their marked filter paper and petri dish were then transferred to the center area of the arena (hereafter zone one or territory, Fig. 2). The wall of the petri dish served as the first surmountable barrier. Salamanders were restricted for a 5-minute acclimation period with an opaque 10 cm acclimation cover (the petri dish lid). Trials were started immediately following removal of the acclimation cover and movement behavior was recorded using an HD Infrared Camcorder for 1800 s while salamanders were allowed to move outside of zone one (their territory) into unmarked areas (zones 2-6, Fig. 2) lined with damp paper towels. During video analysis, latency to move (LTM, the time it took an individual to take its first step, not head movement); latency to cross out of

their territory (LTC, zone 1 into zone 2, the point at which the pelvis crossed); number of crosses (NX, from one zone into another, the point at which the pelvis crossed); number of reversals (NR, head and front limbs positioned above a ring but pelvis did not cross and individual moved back into the preceding zone) were recorded. I only included situations in which the individual had not already been in that zone. In addition, farthest zone reached (FZR, zones 1-6) and latency to the sixth and final zone (LT6, the time it took an individual to reach zone six, if achieved) were recorded. An individual with a short LTM and LTC, large NX, small NR and one that achieves zone six in a short amount of time would indicate an exceedingly exploratory individual. All exploratory trials were conducted at night between the hours 1900 and 0400 with minimal light between August 9 and October 1, 2021. Although two individuals escaped during trials, I did not rerun these salamanders because familiarity with the arena would likely influence their exploratory behavior. Equipment was cleaned with 70 percent ethyl alcohol in between trials to reduce disease transmission and the introduction of conspecific scent.

#### *Correlation of Experiments 1 and 2*

I examined relationships between behaviors measured in the aggression study and behaviors exhibited in the exploration study using correlation analysis between individual behaviors. Specifically, relationships between MT/LTC, LT/LTM, ATR/LT6, and AI/NX were analyzed. MT/LTC were analyzed together as they both involve movement and LT/LTM were paired as they both represent preliminary behaviors that are often followed by subsequent behaviors. In addition, ATR/LT6 as well as AI/NX were paired as ATR and AI are two commonly used measures of aggression in plethodontids for territorial studies and LT6 and NX signify an animal willing to explore areas outside of its territory.

### *Statistical Analyses*

I analyzed, separately, the following using R Studio (RStudio Team 2021): aggression between clades, aggression within clades (residency effect), exploration between clades, and how aggression and exploration are correlated in each of the clades. There were no significant interpopulation differences within clades for either the aggression (Table 1) nor exploration (Table 2) experiments, therefore, localities were pooled for all analyses. For comparisons of aggression between clades, data were first converted into Euclidean Dissimilarity Matrices using the `vegdist` function to account for non-independency concerns between dependent variables. Data did not meet assumptions of parametric tests, therefore, PERMANOVAs using `adonis2` were utilized to separately compare differences in ATR, MT, LT, EDGE, MA, LA, and NT between clades. AI was removed from analyses due to collinearity of dependent variables. BITE was removed from analyses due to infrequent occurrences. The Bonferroni-Holm correction method was used for chemosensory, aggressive, and submissive behaviors (alpha was reduced collectively according to these categories). For comparisons of aggression within clades (residency effect), statistical analyses were as stated above for comparisons of individual behavior between clades with the addition of the `strata` function incorporated into the model, as each individual was used three times. For comparisons of exploration between clades, statistical analyses were as stated above for aggression between clades where NX and LTC were separately analyzed. LTM and LT6 were removed from exploratory analyses due to collinearity of dependent variables. Alpha was reduced for all variables as part of the exploration category. In addition, for individuals that did not move or cross out of zone one, a Pearson's Chi-Square with Yate's Correction for Continuity was used with the `chisq.test` function. NR could not be analyzed due to infrequent occurrences, therefore, a Chi-Square as stated above was used to determine

differences in whether reversal behavior was exhibited or not between the clades. Lastly, for correlation of the individual aggressive and exploratory behaviors in each salamander in their prospective clade, a Spearman's Rank Correlation was used on non-matrix data using the `cor.test` function in R.

## RESULTS

### AGGRESSIVE BEHAVIOR

#### *Resident to Resident with Control Comparisons in each Clade*

When paired with conspecifics, resident salamanders of each clade spent significantly more time in LT behavior compared to controls (NC,  $F_{1,91} = 0.14$ ,  $P_{\text{adj}} = 0.003$ , Table 3; OC,  $F_{1,91} = 9.31$ ,  $P_{\text{adj}} = 0.005$ , Table 3). However, residents did not spend more time in other typical aggressive behaviors when paired with conspecifics (NC, Table 3; OC, Table 4). Instead, residents tended to behave more aggressively via individual behaviors MT and ATR and also displayed more chemosensory behavior in control trials (Northern Clade, MT,  $F_{1,91} = 2.51$ ,  $P_{\text{adj}} = 0.11$ ; ATR,  $F_{1,91} = 2.95$ ,  $P_{\text{adj}} = 0.11$ ; NT,  $F_{1,91} = 2.98$ ,  $P_{\text{adj}} = 0.08$ ; Ohio Clade, MT,  $F_{1,91} = 26.64$ ,  $P_{\text{adj}} = 0.002$ ; ATR,  $F_{1,91} = 15.98$ ,  $P_{\text{adj}} = 0.002$ ; NT,  $F_{1,91} = 7.1$ ,  $P_{\text{adj}} = 0.011$ ). All submissive behaviors (EDGE, MA, LA) did not differ among residents from either clade (NC, Table 3; OC, Table 4).

#### *Resident Comparisons between Clades*

I detected no differences in any aggressive (ATR, MT, LT) or submissive (EDGE, MA, LA) behaviors when comparing resident behaviors between clades (Table 5, Fig. 3). Number of NT tended to be higher in the NC individuals ( $F_{1,91} = 2.32$ ,  $P_{\text{adj}} = 0.13$ , Table 5). BITE was removed from analyses due to infrequent occurrences, though, one resident from the NC bit a conspecific

eight times while maximum bites in the OC was one.

#### *Intruder Comparisons between Clades*

No significant differences were detected in chemosensory (NT), aggressive (ATR, MT, LT) or submissive (EDGE, MA, LA) behaviors when comparing intruder behavior between clades.

#### *Resident with Control Comparisons between Clades*

Members of the OC spent significantly more time in MT, LT and MA (MT,  $F_{1,91} = 12.16$ ,  $P_{adj} = 0.008$ ; LT,  $F_{1,91} = 6.49$ ,  $P_{adj} = 0.02$ ; MA,  $F_{1,91} = 7.60$ ,  $P_{adj} = 0.02$ ) when paired with a control than did members of the NC. Increased time spent in ATR approached significance for the OC as well ( $F_{1,91} = 3.60$ ,  $P_{adj} = 0.06$ ). NT, EDGE and LA were not significantly different between clades ( $P_{adj} > 0.05$ ).

#### *Resident to Intruder Comparisons in each Clade (Residency Status)*

I found no evidence of a residency effect in the Northern (Table 6, Fig. 3) or Ohio Clades (Table 7, Fig. 3). Salamanders were not more aggressive (ATR, MT, LT) or less submissive (EDGE, MA, LA) as residents compared to when they were tested as intruders. The number of NT also did not differ by residency status, despite intruders being placed on an unfamiliar substrate. BITE was removed from analyses due to infrequent occurrences, although there were generally more bites from residents than from intruders in both clades.

### EXPLORATORY BEHAVIOR BETWEEN CLADES

I found differences in exploratory behavior between the clades. Notably, members of the OC had significantly more NX than members of the NC ( $F_{1,95} = 8.43$ ,  $P_{adj} = 0.004$ , Fig. 4, Table 8). When individuals that did not leave their territory were excluded, OC members still had significantly more NX than NC members ( $F_{1,95} = 8.43$ ,  $P_{adj} = 0.04$ ). Similarly, the OC individuals had a shorter latency to cross out of zone 1 (their territory) than the NC individuals ( $F_{1,95} =$

10.05,  $P_{\text{adj}} = 0.004$ , Fig. 5, Table. 8). Twelve animals did not move at all (NC = 10; OC = 2) and 26 individuals did not cross out of zone 1 (NC = 20; OC = 6) during the experiment. The latency to move and the latency to cross out of zone one was recorded as 1800 s for these 38 individuals. In addition, I found that there were significant differences for both movement ( $X^2_1 = 4.70$ ,  $P_{\text{adj}} = 0.03$ , Fig. 6) and crossing ( $X^2_1 = 9.06$ ,  $P_{\text{adj}} = 0.003$ , Fig 7) activity in which members of the OC were significantly more likely to move and cross compared to members of the NC. In addition, the OC individuals were also significantly more likely to exhibit reversal behavior ( $X^2_1 = 10.67$ ,  $P_{\text{adj}} = 0.001$ , Fig. 8). FZR was removed from analyses as all but one individual from the OC who left zone one achieved zone six.

#### CORRELATION OF AGGRESSION AND EXPLORATION WITHIN EACH CLADE

I correlated individual aggressive and exploratory behaviors MT/LTC, LT/LTM, ATR/LT6, and AI/NX. As anticipated, ATR and LT6 were significantly moderately correlated ( $R^2 = 0.3$ ,  $P = 0.047$ , Fig. 9, Table 9) for the NC members. Aggressive individuals of this clade took longer to reach the farthest zone in the exploratory arena. However, these variables in members of the OC were not correlated ( $R^2 = -0.03$ ,  $P = 0.84$ , Fig. 9, Table 10). All other behavioral pairs were not significantly correlated in either clade (NC, Table 9; OC, Table 10).

#### DISCUSSION

Contrary to predictions, evidence of aggressive differences was not observed between the Northern (NC) and Ohio (OC) Clades. Both clades exhibited a similar degree of aggression and submission in individual behaviors ATR, MT, LT, EDGE, MA, and LA when residents were allowed to mark their territory and defend it against a conspecific intruder. Additionally, a residency effect was not observed for either clade. When salamanders were allowed to

investigate the barrier arena, members of the OC showed greater exploratory tendencies than members of the NC in behaviors NX and LTC. This result was contrary to my predictions based on geographic range differences between the two clades. Members of the OC had both greater number of crosses over barriers and shorter latencies to cross out of their territory. Finally, as anticipated, there was evidence indicating a negative relationship among individual behaviors associated with territoriality and dispersal, although this evidence was limited and only exhibited in the NC individuals. All Trunk Raised was correlated with Latency to Zone Six, suggesting a trade-off between aggression and exploration.

Many studies have investigated agonistic and territorial behavior in *Plethodon cinereus* throughout its extensive geographic range (in Ohio, Gall et al. 2003, Hickerson et al. 2004, Deitloff et al. 2008, Reiter et al. 2014; in Michigan, Kleeberger and Werner 1982, Quinn and Graves 1999; in Virginia, Jaeger 1981, Jaeger et al. 1982, 1995, Horne 1988; in New York, Jaeger 1981, Jaeger 1984; in Canada, Rollinson and Hackett 2015). In most cases, salamanders from the above studies can be assigned to the six genetically distinct mitochondrial clades recognized by Radomski et al. (2020). However, only two studies have intentionally incorporated comparisons of agonistic behavior among genetically distinct groups of *P. cinereus* (Wise and Jaeger 2016, Ryan et al. unpublished). Ryan et al. (unpublished) compared individual behaviors of aggression, similar to my study, between the OC and the Pennsylvania Clade (PC). They reported that resident males from the OC tended to have higher AI values (more aggressive) than males from the PC. Additionally, they found a significant clade effect in resident females in which members of the OC had higher AI, ATR, MT, and LT values. Ryan et al. (unpublished) also detected a clear residency effect, wherein residents were more aggressive than intruders, but only in the OC. They suspected these differences were due to genetic



dissimilarity, morph composition, and biological factors in local environments (Ryan et al. unpublished). Other studies comparing resident and intruder behavior have also found a residency effect (Hickerson et al. 2004, Reiter et al. 2014). Territorial residents have more to lose after investing time and energy into their territory and therefore should be more aggressive compared to intruders (Smith and Parker 1976). The above three studies, conducted on northern Ohio populations, and my study, conducted on southern Ohio and Indiana populations, used similar methodology, and so it is unclear why my populations did not exhibit this residency effect. The second study to intentionally compare agonistic behavior in genetically distinct groups of *P. cinereus* was Wise and Jaeger (2016). They compared genetic groups II and III described by Hass (1985), which likely contain more than one clade (sensu Radomski et al. 2020). They found that group II males were more submissive (increased EDGE behavior) than group III males, but they did not differ in the aggressive behavior ATR.

Despite a general failure to detect strong behavioral differences among genetic groups in my study, geographic differences are known across this species range. Territorial behavior evolves when defensible patches can be monopolized (Brown and Orians 1970, Mathis 1990), therefore, geographic differences in this behavior may arise via adaptation to local environmental pressures. Previous studies in *P. cinereus* have revealed how environmental factors can influence the degree of territoriality in populations. For example, northern populations in Upper Peninsula Michigan and North Bay, Ontario, may not be as territorial or aggressive as southern populations due to their non-uniform distribution (Quinn and Graves 1999, Rollinson and Hackett 2015) and exceptionally low ATR values (Rollinson and Hackett 2015). Northern populations may not benefit from territoriality as evapotranspiration rates are lower and rainfall is higher, producing more favorable conditions for foraging activity (Jaeger 1980, Rollinson and Hackett 2015). In

contrast, southern populations of this species range are more restricted in foraging which may explain their higher levels of territorial behavior (Jaeger 1979, 1980, Jaeger et al. 1995, Quinn and Graves 1999, Rollinson and Hackett 2015). I found no differences in any of the individual behaviors for the aggressive nor submissive categories between the Northern and Ohio Clades, and this may result from environmental similarities of temperate forest habitats at similar latitudes in Ohio and Indiana.

In short, geographic differences in behavior are likely a result of local adaptation to biotic and abiotic conditions rather than genetic differences among groups in this species. This is a reasonable assumption as time spent in ATR as residents varies greatly within and among mitochondrial clades. For the OC, average ATR (s) in the southeastern region is 152 (this study) compared to 437 in the central region of the state (Deitloff et al. 2009). Even across counties within the same region (northeastern Ohio), average ATR differs (344 in Cuyahoga Co., Hickerson et al. 2004; 171 in Lorain and Cuyahoga Co., Ryan et al. unpublished). For the NC, average ATR (s) in southcentral Indiana is 160 (this study) compared to 272 in southeastern New York (Jaeger 1984) and 34 in North Bay, Ontario (Rollinson and Hackett 2015). However, these differences may be a result of observer bias in how ATR was quantified during trials (see gradation of behavior, Jaeger and Schwarz 1991).

Despite plethodontid salamanders serving as a model system in conservation biology and evolutionary ecology (Anthony and Pfingsten 2013), the specific behaviors relevant to dispersal across landscapes have been understudied. Instead, previous research has used gene flow as an indirect measure of dispersal among populations of this species. Wilk et al. (2020) found significant genetic differentiation among populations of the Ohio Clade near Columbus, Ohio, suggesting low dispersal among populations ( $F_{st}$  range 0.029 - 0.247). Alternatively, Wuerthner

et al. (2021) did not find significant genetic differentiation among populations of the Northern Clade in southcentral Indiana, suggesting high dispersal among populations ( $F_{st}$  range 0.0002 - 0.003). Yet, my behavioral findings suggest members of the OC are generally more exploratory in NX and LTC than NC individuals. However, this interpretation is subject to two caveats. First, context and spatial scale are important to consider. Dispersal is the permanent movement away from the natal site to a new place (Howard 1960, Clobert et al. 2012) and occurs over short to long distances (leptokurtosis). Alternatively, exploration is considered a response to an unfamiliar area (Reale et al. 2007) and is typically used in the laboratory on a very small scale. Thus, exploration used as a proxy for dispersal in the laboratory may not be able to fully capture the dynamics of this process to the extent that it occurs in nature. Few studies consider the speed at which exploration occurs and how this relates to dispersal distance (Dingemanse et al. 2003). Exploration speed (fast and superficial versus slow and thorough) may be important to consider in the context of dispersal (Dingemanse et al. 2003, Reale et al. 2010), especially in animals that are at high risk of desiccation and predation, such as terrestrial salamanders (Spotila 1972, Bonte et al. 2012). Although Dingemanse et al. (2003) demonstrated that faster birds dispersed over longer distances, avians may not experience the above risks to the same extent as salamanders. In my study, members of the NC were slower to explore the novel arena. A slower, more thorough exploratory strategy might allow individuals to locate suitable microhabitat along dispersal routes, thus decreasing predation and desiccation risk. Connecting exploration speed to distances traveled by *P. cinereus* on a large-scale, in their natural environment, would be a valuable contribution to future researchers using exploration as a measure of dispersal. For example, comparing homing behavior between the Northern and Ohio Clades would be an effective approach in upcoming studies. Second, the relationship between dispersal and age class is

probably important to consider. Although juveniles are theorized to be the dispersing life history stage in amphibians (Smith and Green 2006), only adult salamanders were used in my study. Evers et al. (unpublished) found differences in limb length among the Northern, Southern and Virginia Clades in which NC juveniles had significantly longer limbs. Longer limbs in some amphibians enhance dispersal ability (Phillips et al. 2006, Lowe and McPeck 2012) but it is not currently known if this is the case in juvenile *P. cinereus*. If dispersal primarily occurs in *P. cinereus* juveniles, longer limbs in NC juveniles may have assisted with their range expansion as glaciers retreated. Future research may benefit from comparing adult and juvenile exploratory behavior in this species in the context of dispersal capability (morphology) and propensity (behavior).

The range expansion of the Northern Clade may have been a condition-dependent response of the waning of the last glacial maximum (Clobert et al. 2009, 2012) in which enhanced dispersal traits were lost after expansion ceased. This could help explain why NC members were not highly exploratory as predicted. After glacial retreat, dispersing animals would have pursued more favorable circumstances, such as the lack of competition in post-glacial habitat (Travis and Dytham 2002, Clobert et al. 2012). However, travelling 80 m per year to recolonize this land probably exceeds conventional dispersal rates for this species (Cabe et al. 2007). Termed Reid's paradox, expansion rates can exceed normal dispersal rates during colonization events (Clark et al. 1998, Radomski et al. 2020). A possible explanation for this paradox is spatial sorting (also termed spatial selection) at the range-edge whereby exceedingly exploratory individuals mate with one another and pass these traits on to their offspring (Travis and Dytham 2002, Phillips et al. 2008, Shine et al. 2011, Chuang and Peterson 2016, Gruber et al. 2017a, 2017b). This concept has notably been recognized in invasive Cane Toad populations (Phillips et al. 2008, Gruber et

al. 2017a, 2017b). Phillips et al. (2008) used a common garden experiment to demonstrate the likely genetic basis behind spatial sorting. Toads at the front of the expansion dispersed farther, moved more often, and followed straighter paths than toads from center populations. However, to determine what happens to dispersal traits after colonization, Gruber et al. (2017b) compared Cane Toad populations that differed in time since post-colonization. Not surprisingly, there were significant differences in exploratory behavior between the oldest (76 yr) and newest populations (<5 yr). Interestingly though, intermediate populations (11 yr) tended to be less exploratory than newest populations and significantly more exploratory than oldest populations, suggesting a gradual decrease in exploratory behavior over time. In addition, Simmons and Thomas (2004) observed a rapid reduction in morphological dispersal traits in wing-dimorphic bush crickets only 5-10 years after colonization. Colonized range-edge populations that were previously highly dispersive (high dispersal frequencies, long wings) later displayed similar traits to old range-core populations (low dispersal frequencies, short wings). Dispersal traits are costly to maintain (Roff 1984, Duckworth 2008, Cosentino and Droney 2016, reviewed in Bonte et al. 2012) and there is a consequence of lower reproduction in dispersing individuals (Bonte et al. 2012), therefore, post-colonization favors philopatric individuals with high reproductive rates (Hanski 1999, Olivieri et al. 1995, Travis and Dytham 2002, Duckworth 2008). After colonization, it is possible that the dispersing enhancing traits in the ancestors of the NC were reduced over generations due to dispersal costs or were a plastic condition-dependent response to the newly available habitat.

Correlated behaviors can have important evolutionary and ecological implications. As correlated behaviors often result in trade-offs, maladaptive or suboptimal behavior can emerge which ultimately influences patterns in population and community ecology (Sih et al. 2004). Additionally, correlated behaviors that confer a fitness advantage can maintain individual

variation in populations (Sih et al. 2004). Individual behaviors associated with territoriality and dispersal were correlated in members of the NC. Salamanders that spent more time in ATR (more aggressive) concurrently had longer latencies to reach the farthest zone in the arena (less exploratory), suggesting a trade-off between the two behavioral categories. Similarly, NC individuals that spent less time in ATR were concurrently more exploratory with shorter latencies to zone six. Correlations between behaviors associated with territoriality and dispersal may arise through time budget conflicts, which regulates the time that is spent on certain activities. Salamanders investing time and energy in territorial defense are predicted to spend less time exploring or dispersing from their territory. This is reasonable, as by definition, territoriality requires site fidelity (Brown and Orians 1970) and dispersal requires site departure (Ims and Yoccoz 1997, Bowler and Benton 2005). However, it is unclear why ATR and LT6 was not also correlated in members of the OC. Therefore, more research is needed to fully understand how individuals of *P. cinereus* employs aggressive and exploratory behavior in a functional manner and how this may relate to genetic lineage.

Ecological processes, such as geographic distribution, population density and abundance, individual fitness, and genetic structure, can be mediated by behavioral variation and behavioral correlations (Marvin 1998, Sih et al. 2003, 2012, van Oers et al. 2004, Biro and Stamps 2008, Smith and Blumstein 2008, Dochtermann et al. 2015, Gruber et al. 2017a, 2017b). For example, studying variation in dispersal and territorial phenotypes can facilitate ecologists' understanding of how genetic divergence occurs within a species. Additionally, anthropogenic effects, such as global climate change and forest fragmentation, influence species abundance, richness, and diversity (Franklin and Forman 1987, Gustafson et al. 2001, Marsh et al. 2008, Watling and Donnelly 2006, 2008) and understanding territoriality and dispersal can provide insight into how

species may respond to habitat changes. For instance, habitat alterations may affect certain lineages of *P. cinereus* more-so than others depending on their dispersal tendencies and their degree of territorial site attachment. Amphibians are at high-risk of human-related activities and are largely declining (Wyman 1990, Blaustein et al. 1994, Duellman and Trueb 1994, Gardner 2001, Hels and Buchwald 2001, Marsh et al. 2005). They are an essential component of ecosystems and declines have both ecological and human-related implications (Gardner 2001). *Plethodon cinereus*, specifically, is an important bio-indicator of environmental change that regulates top-down effects on trophic hierarchies (Welsh and Droege 2001, Hickerson et al. 2017).

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Table 1. Interpopulation differences in the Northern (NC) and Ohio (OC) Clades in the aggression experiment. A matrix of all continuous response variables for both aggressive (AI, LT, MT, ATR) and submissive (LA, MA, EDGE) behaviors was used for each status (as a resident, as an intruder, and as a resident with a control). NC localities include Brown County State Park (BC), Griffy Lake Nature Preserve (GL), Morgan-Monroe State Forest (MM), and Yellowwood State Forest (YW). OC localities include Airplane Rock (AR), Baker’s Preserve (BP), Hocking Hills State Park (HH), and Strouds Run State Park (SR). Sample sizes are included for each locality. There were no significant interpopulation differences, therefore, data were pooled for all aggression analyses.

Clade	Locality	n	F, P		
			Resident	Intruder	Control
NC	BC	7	1.60, 0.15	1.92, 0.1	1.23, 0.31
	GL	10			
	MM	12			
	YW	17			
OC	AR	15	0.58, 0.73	1.23, 0.32	1.24, 0.30
	BP	3			
	HH	14			
	SR	14			

Table 2. Interpopulation differences in the Northern (NC) and Ohio (OC) Clades in the exploration experiment. A matrix of all continuous response variables for exploratory behavior (LTM, LTC, LT6, NX) was used. NC localities include Brown County State Park (BC), Griffy Lake Nature Preserve (GL), Morgan-Monroe State Forest (MM), and Yellowwood State Forest (YW). OC localities include Airplane Rock (AR), Baker’s Preserve (BP), Hocking Hills State Park (HH), and Strouds Run State Park (SR). Sample sizes are included for each locality. There were no significant interpopulation differences, therefore, data were pooled for all exploratory analyses.

Clade	Locality	n	F, P
NC	BC	7	1.26, 0.29
	GL	12	
	MM	11	
	YW	18	
OC	AR	15	1.14, 0.35
	BP	2	
	HH	17	
	SR	14	

Table 3. Behavior of salamanders from the Northern Clade as residents compared to their behavior in the control. Mean  $\pm$  SE of chemosensory, aggressive, and submissive behaviors are included. Chemosensory behavior includes number of Nose Taps (NT). Aggressive behaviors include Look Toward (LT), Move Toward (MT) and All Trunk Raised (ATR). Submissive behaviors include Look Away (LA), Move Away (MA) and Edge (EDGE). Asterisks indicate significant differences using the Bonferroni-Holm correction method. n = 46 per clade.

Behavior	Resident	Control	F	P <sub>adj</sub>
LT	66.83 $\pm$ 7.08	35.35 $\pm$ 4.05	14.89	0.003**
MT	15.31 $\pm$ 2.34	20.60 $\pm$ 2.39	2.51	0.11
ATR	159.51 $\pm$ 23.86	221.58 $\pm$ 27.12	2.95	0.11
LA	18.52 $\pm$ 2.56	14.73 $\pm$ 2.75	1.02	0.34
MA	10.76 $\pm$ 1.77	7.96 $\pm$ 1.03	1.87	0.34
EDGE	371.15 $\pm$ 33.25	428.55 $\pm$ 38.26	0.78	0.34
NT	7.89 $\pm$ 1.19	11.28 $\pm$ 1.56	2.98	0.08

Table 4. Behavior of salamanders from the Ohio Clade as residents compared to their behavior in the control. Mean  $\pm$  SE of chemosensory, aggressive, and submissive behaviors are included. Chemosensory behavior includes number of Nose Taps (NT). Aggressive behaviors include Look Toward (LT), Move Toward (MT) and All Trunk Raised (ATR). Submissive behaviors include Look Away (LA), Move Away (MA) and Edge (EDGE). Asterisks indicate significant differences using the Bonferroni-Holm correction method. n = 46 per clade.

Behavior	Resident	Control	F	P <sub>adj</sub>
LT	73.58 $\pm$ 6.41	50.21 $\pm$ 4.20	9.31	0.005**
MT	16.15 $\pm$ 2.02	32.37 $\pm$ 2.41	26.64	0.002**
ATR	151.84 $\pm$ 21.69	296.99 $\pm$ 29.12	15.98	0.002**
LA	19.62 $\pm$ 3.32	19.17 $\pm$ 3.52	0.01	0.94
MA	10.30 $\pm$ 1.43	12.81 $\pm$ 1.43	1.54	0.57
EDGE	387.16 $\pm$ 35.51	429.09 $\pm$ 31.42	0.78	0.57
NT	5.65 $\pm$ 0.86	10.26 $\pm$ 1.50	7.09	0.011*

Table 5. Comparison of Northern and Ohio Clade residents in mean  $\pm$  SE chemosensory, aggressive, and submissive behaviors. Chemosensory behavior includes number of Nose Taps (NT). Aggressive behaviors include Look Toward (LT), Move Toward (MT) and All Trunk Raised (ATR). Submissive behaviors include Look Away (LA), Move Away (MA) and Edge (EDGE). Asterisks indicate significant differences using the Bonferroni-Holm correction method. n = 46 per clade.

Behavior	Northern Clade	Ohio Clade	F	P <sub>adj</sub>
	Resident	Resident		
LT	66.83 $\pm$ 7.08	73.58 $\pm$ 6.41	0.50	0.83
MT	15.31 $\pm$ 2.34	16.15 $\pm$ 2.02	0.07	0.83
ATR	159.51 $\pm$ 23.86	151.84 $\pm$ 21.69	0.06	0.83
LA	18.52 $\pm$ 2.56	19.62 $\pm$ 3.32	0.07	0.84
MA	10.76 $\pm$ 1.77	10.30 $\pm$ 1.43	0.04	0.84
EDGE	371.15 $\pm$ 33.25	387.16 $\pm$ 35.51	0.11	0.84
NT	7.89 $\pm$ 1.19	5.65 $\pm$ 0.86	2.32	0.13

Table 6. Residency effect in the Northern Clade including mean  $\pm$  SE chemosensory, aggressive, and submissive behaviors. Chemosensory behavior includes number of Nose Taps (NT). Aggressive behaviors include Look Toward (LT), Move Toward (MT) and All Trunk Raised (ATR). Submissive behaviors include Look Away (LA), Move Away (MA) and Edge (EDGE). Asterisks indicate significant differences using the Bonferroni-Holm correction method. n = 46 per clade.

Behavior	Resident	Intruder	F	P <sub>adj</sub>
LT	66.83 $\pm$ 7.08	57.31 $\pm$ 6.40	1.00	0.45
MT	15.31 $\pm$ 2.34	13.74 $\pm$ 1.76	0.29	0.60
ATR	159.51 $\pm$ 23.86	199.93 $\pm$ 26.99	1.26	0.45
LA	18.52 $\pm$ 2.56	15.94 $\pm$ 2.86	0.45	0.75
MA	10.76 $\pm$ 1.77	11.07 $\pm$ 1.75	0.02	0.88
EDGE	371.15 $\pm$ 33.25	460.17 $\pm$ 35.66	3.33	0.23
NT	7.89 $\pm$ 1.19	7.43 $\pm$ 0.87	0.10	0.78

Table 7. Residency effect in the Ohio Clade including mean  $\pm$  SE chemosensory, aggressive, and submissive behaviors. Chemosensory behavior includes number of Nose Taps (NT). Aggressive behaviors include Look Toward (LT), Move Toward (MT) and All Trunk Raised (ATR). Submissive behaviors include Look Away (LA), Move Away (MA) and Edge (EDGE). Asterisks indicate significant differences using the Bonferroni-Holm correction method. n = 46 per clade.

Behavior	Resident	Intruder	F	P <sub>adj</sub>
LT	73.58 $\pm$ 6.41	57.37 $\pm$ 5.80	3.52	0.19
MT	16.15 $\pm$ 2.02	16.00 $\pm$ 2.76	0.002	0.97
ATR	151.84 $\pm$ 21.69	208.99 $\pm$ 30.34	2.35	0.19
LA	19.62 $\pm$ 3.32	20.39 $\pm$ 4.04	0.02	0.88
MA	10.30 $\pm$ 1.43	11.36 $\pm$ 1.57	0.25	0.88
EDGE	387.16 $\pm$ 35.51	461.29 $\pm$ 34.68	2.23	0.47
NT	5.65 $\pm$ 0.86	5.80 $\pm$ 0.84	0.02	0.92

Table 8. Comparison of exploration between the Northern and Ohio Clades in mean  $\pm$  SE Number of Crosses (NX) and Latency to Cross (LTC) behaviors. Asterisks indicate significant differences using the Bonferroni-Holm correction method. n = 48 per clade.

Behavior	Northern Clade	Ohio Clade	F	P <sub>adj</sub>
NX	6.31 $\pm$ 0.97	10.85 $\pm$ 1.23	8.43	0.004**
LTC	888.75 $\pm$ 115.03	442.92 $\pm$ 80.93	10.05	0.004**



Table 9. Correlation analysis of behaviors for the Northern Clade from the aggression and exploration experiments. Aggressive behaviors include Aggression Index (AI), Move Toward (MT), All Trunk Raised (ATR), and Look Toward (LT) in mean seconds  $\pm$  SE. Exploratory behaviors include Number of Crosses (NX), Latency to Cross (LTC; s), Latency to Six (LT6; s) and Latency to Move (LTM, s). Asterisks indicate significant correlations. n = 45 per clade.

Aggressive Behavior	Exploratory Behavior	R <sup>2</sup>	P
AI	NX	-0.08	0.59
MT	LTC	0.01	0.97
ATR	LT6	0.30	0.047*
LT	LTM	-0.18	0.25

Table 10. Correlation analysis of behaviors for the Ohio Clade from the aggression and exploration experiments. Aggressive behaviors include Aggression Index (AI), Move Toward (MT), All Trunk Raised (ATR), and Look Toward (LT) in mean seconds  $\pm$  SE. Exploratory behaviors include Number of Crosses (NX), Latency to Cross (LTC; s), Latency to Six (LT6; s) and Latency to Move (LTM, s). Asterisks indicate significant correlations. n = 42 per clade.

Aggressive Behavior	Exploratory Behavior	R <sup>2</sup>	P
AI	NX	0.001	0.99
MT	LTC	0.02	0.92
ATR	LT6	-0.03	0.84
LT	LTM	-0.07	0.68

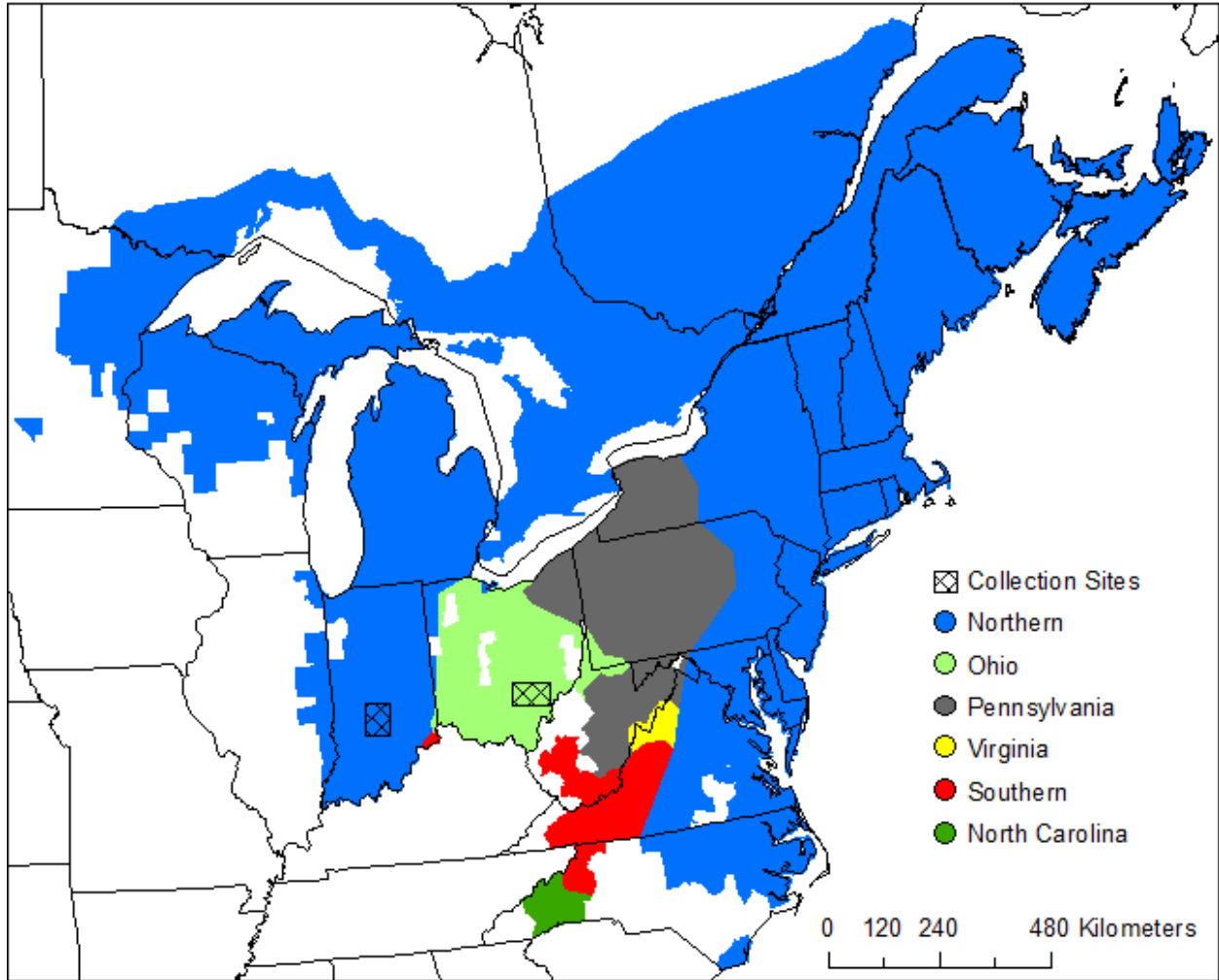


Figure 1. Map modified from Radomski et al. (2020) showing my collection sites and the distribution of the six mitochondrial clades in *P. cinereus*. Colors indicate different clades. The Northern Clade (blue) was collected from four localities within the black box in Indiana (left). The Ohio Clade (light green) was collected from four localities within the black box in Ohio (right).

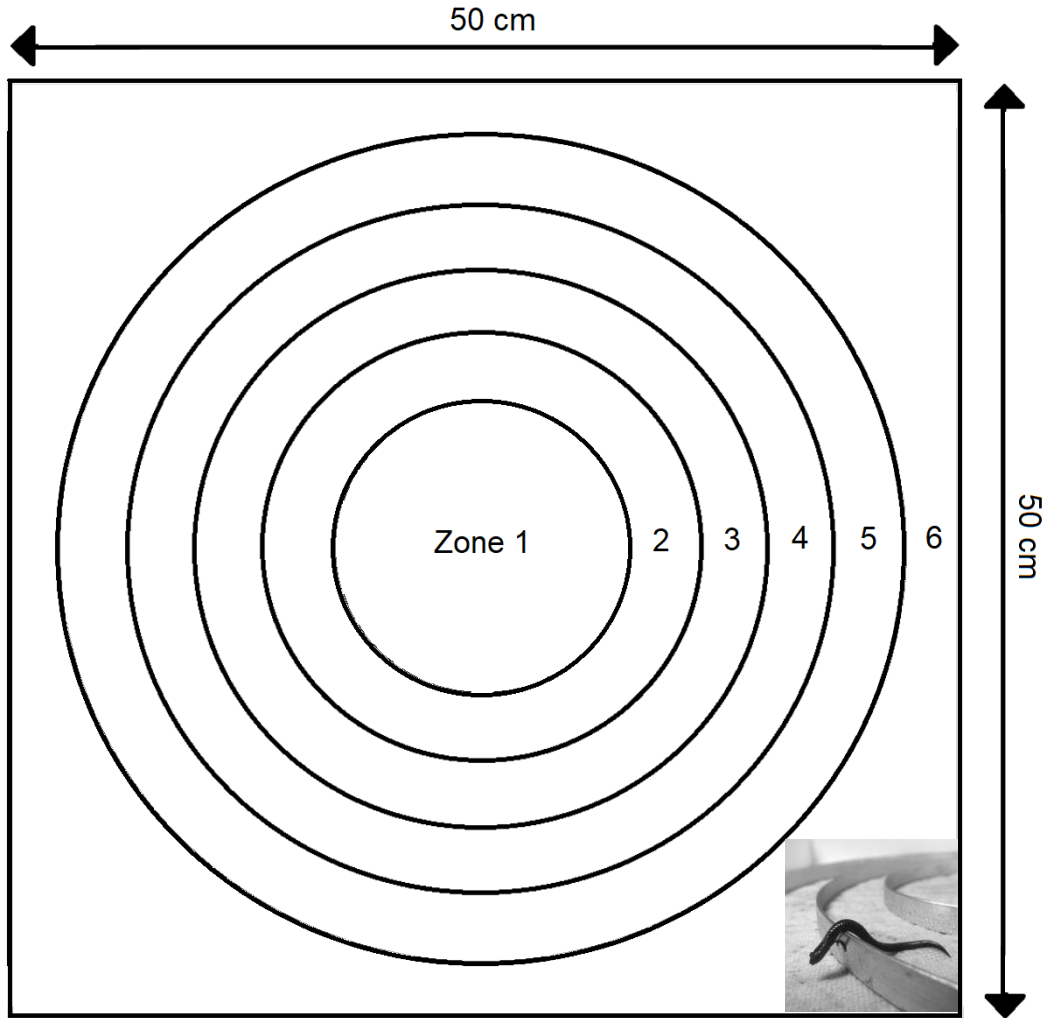


Figure 2. Exploratory barrier arena modified from Lynn et al. (2019) and Reeder (2013). Zone 1 is the salamanders marked territory comprising a petri dish (1.5 cm tall x 10 cm in diameter) which was transferred to the arena at the start of the trial. Zones 2-6 are unmarked areas lined with moist paper towels in which the salamander was allowed to explore after the acclimation period. Zone 6 is maximum. Rings were constructed from aluminum (1.5 cm tall x 1 mm thick x 18.5, 28.5, 38.5, 48.5 cm in diameter). Borders of the arena (5 cm tall x 1 cm thick x 50 cm length) were constructed from Lexan plastic. Glass (1 cm thick x 50 cm length) was placed on top of the arena walls at the start of the trial to prevent escape.

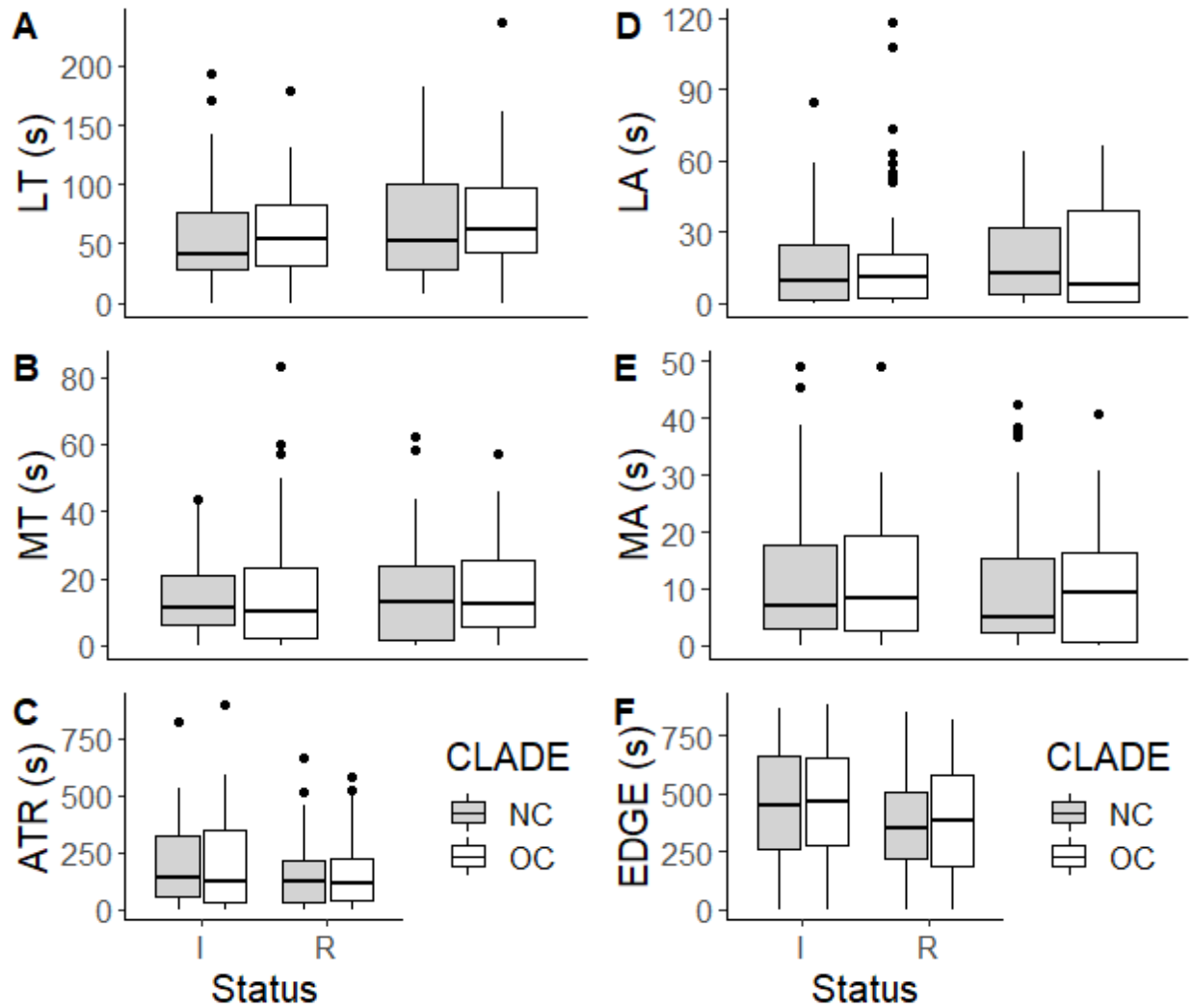


Figure 3. Differences in aggressive and submissive behaviors by status (Resident = R, Intruder = I) and clade (Northern = NC, grey boxes, Ohio = OC, white boxes). (A-C) Time spent (s) in aggressive behaviors: Look Toward, Move Toward, and All Trunk Raised. (D-F) Time spent (s) in submissive behaviors: Look Away, Move Away, and Edge. All comparisons (R between clades; I between clades, R versus I within clade, i.e. residency effect) were not significant using the Bonferroni-Holm correction method. Data are presented as nested boxplots with horizontal lines indicating medians, the boxes indicating interquartile range, and the solid circles signifying outliers. n = 46 per clade.

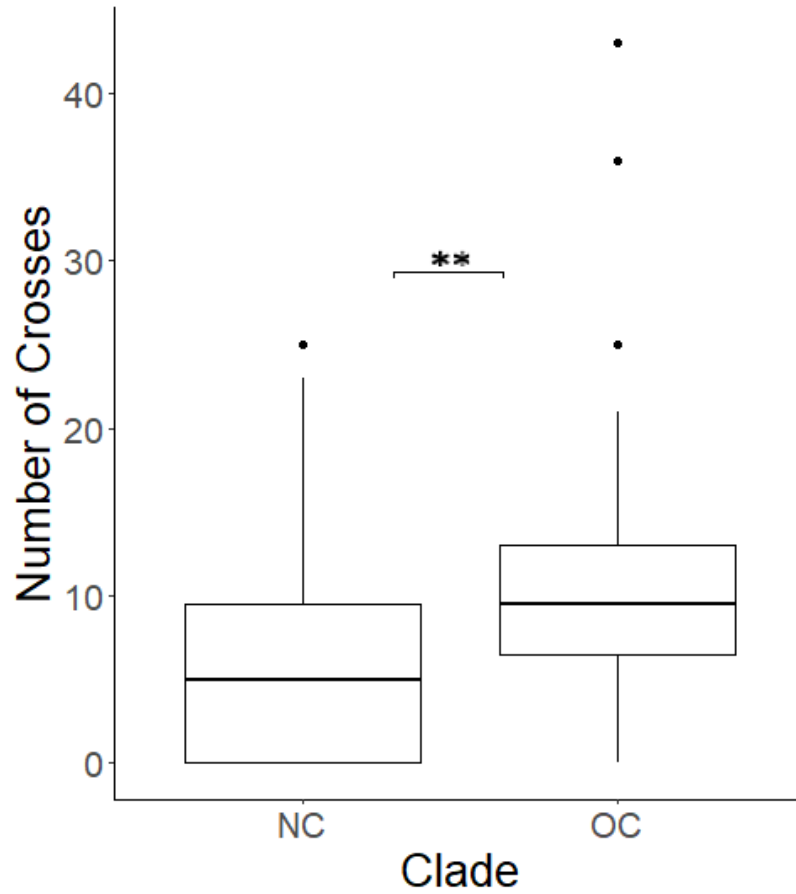


Figure 4. Differences in exploratory behavior Number of Crosses in the Northern (NC) and Ohio (OC) Clades. Asterisks denote significant differences using the Bonferroni-Holm correction method. Data are presented as boxplots with the horizontal lines in the boxes indicating medians, the boxes indicating interquartile range, and the solid circles signifying outliers. n = 48 per clade.

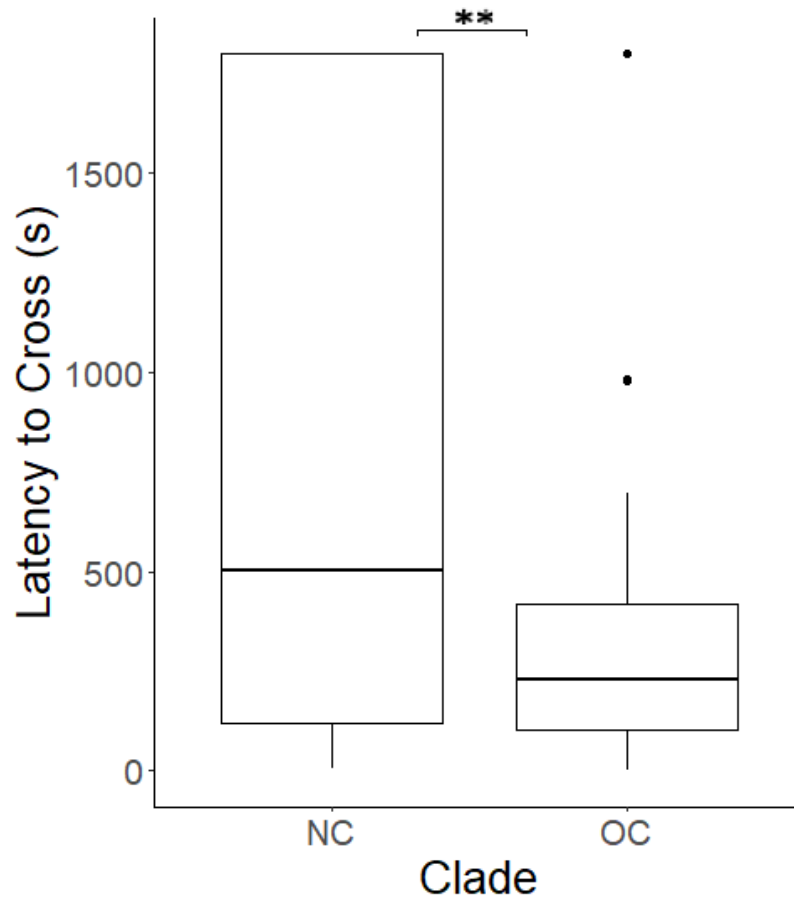


Figure 5. Differences in exploratory behavior Latency to Cross in the Northern (NC) and Ohio (OC) Clades. Asterisks denote significant differences using the Bonferroni-Holm correction method. Data are presented as boxplots with the horizontal lines in the boxes indicating medians, the boxes indicating interquartile range, and the solid circles signifying outliers.  $n = 48$  per clade.

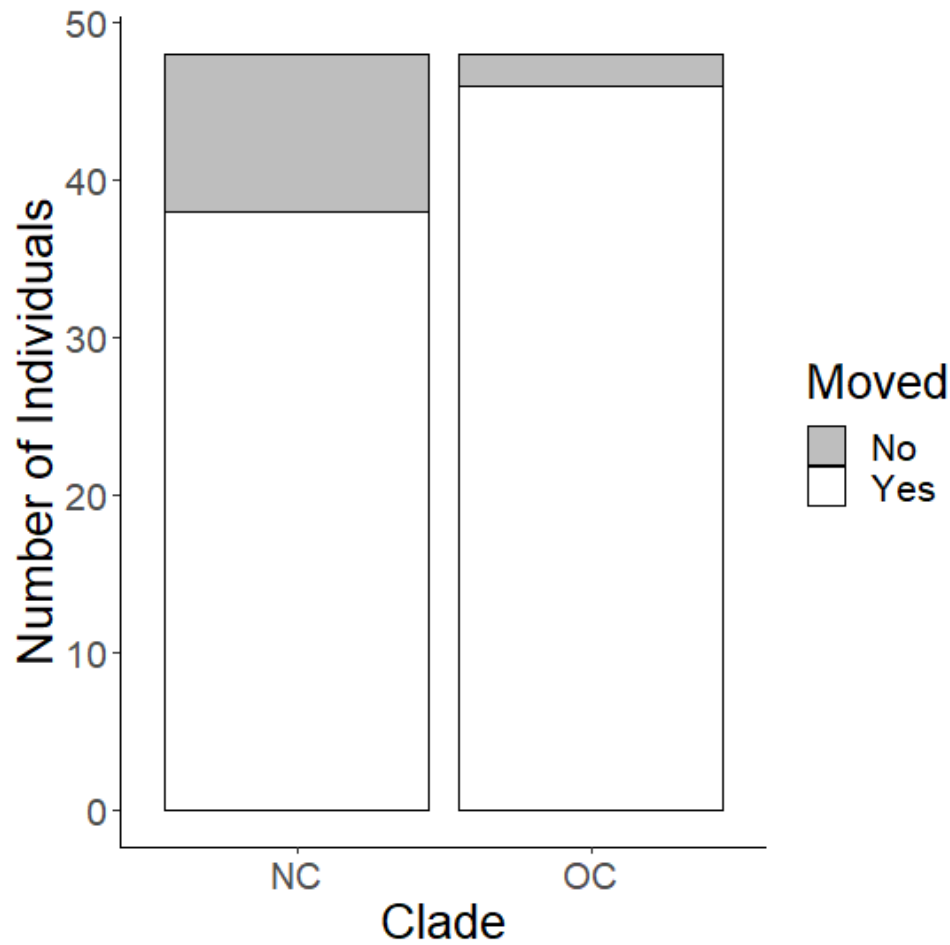


Figure 6. Number of individuals that moved (white) or did not move (grey) during the exploration experiment in the Northern (NC) and Ohio (OC) Clades. Observed data were significantly different than expected data using the Yate's correction method ( $P_{adj} = 0.03$ ). Members of the Northern Clade were significantly less likely to move during the experiment.  $n = 48$  per clade.



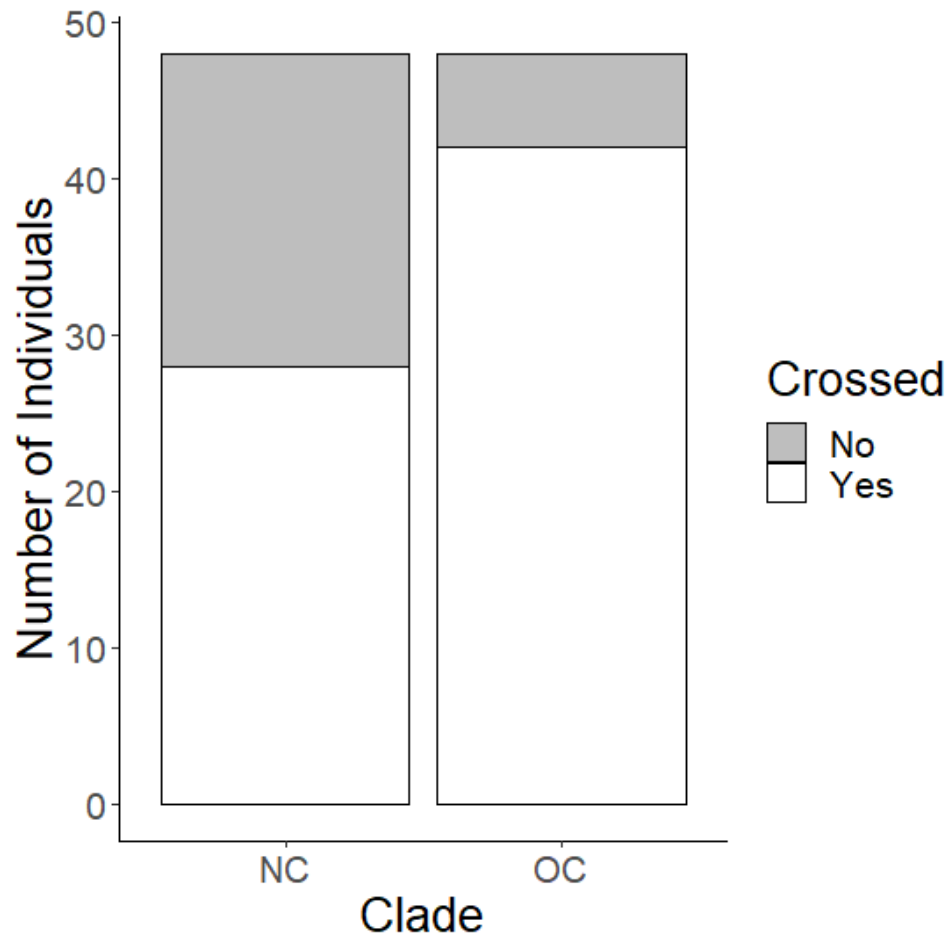


Figure 7. Number of individuals that crossed (white) or did not cross (grey) out of zone one (their territory) during the exploration experiment in the Northern (NC) and Ohio (OC) Clades. Observed data were significantly different than expected data using the Yate’s correction method ( $P_{adj} = 0.003$ ). Members of the Northern Clade were significantly less likely to cross out of their territory during the experiment.  $n = 48$  per clade.

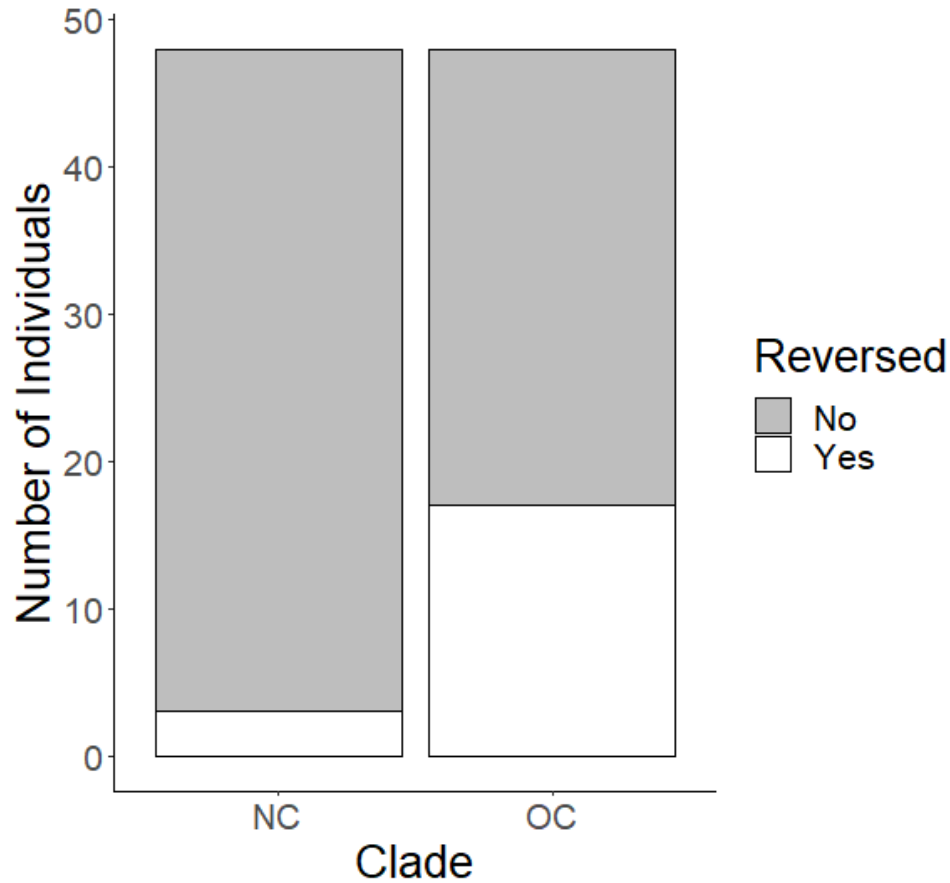


Figure 8. Number of individuals that did (white) or did not (grey) exhibit reversal behavior during the exploration experiment in the Northern (NC) and Ohio (OC) Clades. Observed data were significantly different than expected data using the Yate’s correction method ( $P_{adj} = 0.001$ ). Members of the Ohio Clade were significantly more likely to exhibit reversal behavior when allowed to explore the arena containing surmountable barriers.  $n = 48$  per clade.

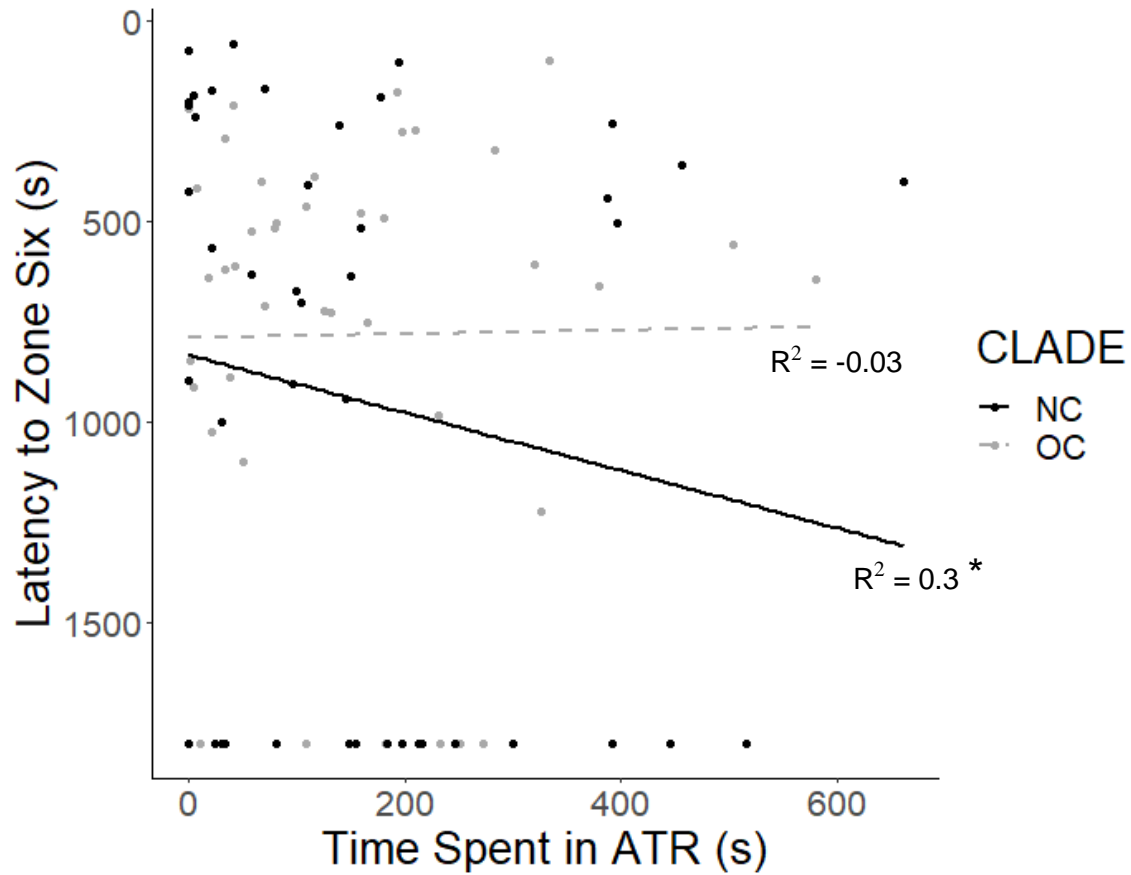


Figure 9. Correlation of aggressive behavior All Trunk Raised (s) and the inverse of exploratory behavior Latency to Zone Six (s) in the Northern (NC, solid black line) and Ohio (OC, dashed grey line) Clades.  $R^2$  value and degree of significance are displayed. Individuals that spent more time in ATR were less likely to quickly reach zone 6.  $n_{NC} = 45$ .  $n_{OC} = 42$ .