2020

GEOGRAPHIC VARIATION IN AGONISTIC AND TERRITORIAL BEHAVIOR BY GENETICALLY DISTINCT GROUPS OF THE EASTERN RED-BACKED SALAMANDER (PLETHODON CINEREUS)

Jessica Ryan
John Carroll University, jryan21@jcu.edu

Recommended Citation

Ryan, Jessica, "GEOGRAPHIC VARIATION IN AGONISTIC AND TERRITORIAL BEHAVIOR BY GENETICALLY DISTINCT GROUPS OF THE EASTERN RED-BACKED SALAMANDER (PLETHODON CINEREUS)" (2020). *Masters Theses*. 45.
https://collected.jcu.edu/masterstheses/45

GEOGRAPHIC VARIATION IN AGONISTIC AND TERRITORIAL BEHAVIOR BY GENETICALLY DISTINCT GROUPS OF THE EASTERN RED-BACKED SALAMANDER (*PLETHODON CINEREUS*)

A Thesis Submitted to the
Office of Graduate Studies
College of Arts and Sciences of
John Carroll University
In Partial Fulfillment of the Requirements
for the Degree of
Master of Science

By
Jessica M. Ryan
2020

Table of Contents

Geographic variation in agonistic and territorial behavior by genetically distinct groups of the Eastern Red-backed Salamander (*Plethodon cinereus*)

Abstract

Geographic variation in agonistic and territorial behavior is largely unknown in terrestrial plethodontid salamanders, even though territoriality is widespread throughout this group. Using the color polymorphic Eastern Red-backed Salamander (*Plethodon cinereus*), I compared the aggressive and submissive behaviors of two genetically distinct groups in northern Ohio using male and female salamanders from six populations. As in other populations of *P. cinereus* studied, I expected to find agonistic and territorial behavior present in the populations in this study. Additionally, I predicted that the genetic group that is monomorphic for the striped morph would exhibit a higher degree of agonistic and territorial behavior compared to the polymorphic genetic group consisting of both striped and unstriped morphs as an adaptive consequence of the altered social dynamics in monomorphic populations. In laboratory trials, residents from the polymorphic group were significantly more aggressive than residents from the monomorphic group, in contrast to my hypothesis. This finding was corroborated by a strong residency effect in the polymorphic group, an effect that was not as apparent in the monomorphic group. In particular, female residents from the polymorphic group displayed a significantly greater degree of aggression compared to female residents from the monomorphic group and were much more aggressive and less submissive as residents than as intruders. My results imply that the individuals in the polymorphic group, particularly females, are more aggressive and territorial than the individuals in the monomorphic group, suggesting they are more likely to secure a territory and defend it against intruders. Studying geographic variation in behavior may be a

valuable avenue of continued research to determine if divergent selection is occurring among genetically distinct groups within this species.

Introduction

Population and quantitative geneticists have established that nonbehavioral traits, such as morphological, physiological, and molecular traits, vary geographically within a species, but behavioral traits have historically been recognized as unchanging (Foster 1999; Foster and Endler 1999). However, research across a wide variety of taxa has revealed that population differentiation exists for a multitude of behavioral traits including animal communication (Wilczynski and Ryan 1999), dietary preferences (Burghardt and Schwartz 1999), antipredator behavior (Brodie 1993; Coss 1999; Magurran 1999), and life history traits (reviewed in Morrison and Hero 2003). Moreover, divergence in phenotypes associated with behavior often has a genetic basis. For example, song repertoire size and recurrence interval of Marsh Wrens (*Cistothorus palustris*) differ in populations from San Francisco and New York when raised in controlled laboratory conditions and are therefore genetically based (Kroodsma and Canady 1985). Additional examples include mating behavior in *Drosophila paulistorum* (Koref-Santibanez 1972), feeding behavior in garter snakes (*Thamnophis elegans*; Arnold 1977), calling behavior in túngara frogs (*Physalaemus pustulosus*; Pröhl et al. 2006), rejection behavior of cuckoo eggs by Eurasian magpies (*Pica pica*; Soler et al. 1999), and agonistic behavior in coho salmon (*Oncorhynchus kisutch*; Rosenau and McPhail 1987). Genetically based geographic variation in behavior is therefore common and diverse and provides insight into adaptive behavioral divergence and the initial stages of speciation (Foster 1999).

Relatively little attention has been given to the study of geographic variation in agonistic and territorial behavior, despite this being a potential tool for determining how different communities are structured and how local ecological factors might shape behavior (Jaeger and Forester 1993). For example, *Ensatina eschscholtzii*, a salamander belonging to the family of lungless salamanders, Plethodontidae, is distributed as a ring species on the west coast of the United States. Wiltenmuth and Nishikawa (1998) found that agonistic and sensory behavior varies geographically among genetically distinct coastal and inland *Ensatina* populations, with a convergent reduction in aggression among inland populations attributed to either reduced territoriality and competition or similar environmental conditions encountered by the inland populations (Wiltenmuth and Nishikawa 1998). Plethodontid salamanders can serve as model organisms with which to examine this type of geographic variation, as they are an ecologically diverse group in which territoriality is common and have been a focus of study for several decades (reviewed in Mathis et al. 1995). In particular, the Eastern Red-backed Salamander, *Plethodon cinereus*, represents an ideal model with which to test hypotheses regarding geographic variation in agonistic and territorial behavior. This species is geographically widespread and experiences a variety of environmental conditions, is known to have limited dispersal and gene flow (Cabe et al. 2007) and exhibits a complexity of behavioral patterns that have been extensively studied for the past five decades (reviewed in Jaeger et al. 2016).

Plethodon cinereus is a small, completely terrestrial, direct-developing plethodontid. Because activity is typically constrained to moist environments (Feder and Londos 1984), individuals are often found under cover objects (e.g., rocks, logs) on the forest floor, as these provide preferable environmental conditions and greater access to prey, courtship areas, and nesting sites (Gergits and Jaeger 1990). It is the most abundant salamander species throughout many parts of its range (Anthony and Pfingsten 2013), which extends across northeastern North America southward to western and northeastern North Carolina, northwestward to western Minnesota, and as far north as parts of southern Canada (Petranka 1998). There are eight known color morphs, with the two most commonly occurring morphs being the red-backed, or striped morph, and the lead-backed, or unstriped morph (Moore and Ouellet 2014). The striped morph is characterized by a red dorsal stripe running from the head to the base of the tail and the unstriped morph, in comparison, appears uniformly dark (Anthony and Pfingsten 2013). There is a genetic basis for the observed phenotypic variation of the striped and unstriped morphs, and it is likely that multiple gene pairs control color expression (Highton 1975). Relative proportions of striped and unstriped individuals in a given population can vary greatly, to the extent that monomorphic striped populations appear to be continuous throughout the range of *P. cinereus*, whereas populations with only unstriped morphs are uncommon (Pfingsten and Walker 1978; Petranka 1998). In northern Ohio, populations are typically dimorphic, but sometimes only one morph might be present (Pfingsten and Walker 1978). Additionally, some populations exhibit clinal variation in morph frequency including localities in northern Ohio (Pfingsten and Walker 1978; Hantak et al. 2019), Long Island (Fisher-Reid et al. 2013, Fisher-Reid and Wiens 2015), and the Delmarva Peninsula (Highton 1977). According to McLean and Stuart-Fox (2014), if gene flow is low or absent, populations that differ in morph composition or along a morph-frequency cline might be subject to speciation with natural selection favoring a particular morph at either end of the selective cline. In northern Ohio, three genetically distinct groups have been identified (Hantak et al. 2019) that correlate with changes in morph frequency. From west to east: the Western Cluster consists of two sampled localities and is made up of mostly unstriped individuals (≥ 90% unstriped); the Central Cluster is made up of mostly dimorphic populations, with a noticeable transition from mostly unstriped morphs including one completely unstriped population, to populations that contain a high proportion of striped morphs including one completely striped population; and the Eastern Cluster, which is separated from the Central Cluster by the Cuyahoga River, is fixed for the striped morph (Hantak et al. 2019). The steep genetic differentiation found in populations of *P. cinereus* in northern Ohio can potentially lead to pronounced behavioral differences related to agonistic and territorial behavior that allow us to examine hypotheses regarding geographic variation in behavior with a genetic component. Numerous studies have been carried out that provide evidence for territoriality in *P. cinereus* in various portions of its range, although most have only looked at individuals from one local population (reviewed in Jaeger et al. 2016), making it difficult to extrapolate the results across the species' range (Selby et al. 1996).

Direct-developing, terrestrial salamanders like *P. cinereus* are constrained by temporal fluctuations in surface moisture, thereby dictating foraging activity and reproductive tactics (Jaeger 1981a). As the leaf litter dries, prey availability decreases, and competition for food becomes more intense (Jaeger 1972). Females typically produce smaller clutch sizes of larger eggs with more investment per offspring (Mathis et al. 1995). Furthermore, competition for suitably moist substrates (beneath cover objects) is high because they are considered a valuable and defendable resource for *P. cinereus* (Mathis 1990a). A territory can be defined as an exclusive area that is defended against intruders (Noble 1939; Tinbergen 1957; Brown and Orians 1970). To defend and maintain a territory, particularly one in an environment with limited resources (i.e., food and high-quality cover objects), an individual must experience a net fitness benefit. Consequently, a balance must be achieved between the benefits of mate and food acquisition, protection from predators, etc., and the costs of time lost and missed opportunities, energy expenditure, and risk of injury, known as economic defendability (Brown 1964). Jaeger and Gergits (1979) established four essential characteristics to describe a territorial species: site fidelity, advertisement, aggressive defense, and expulsion of intruders. Adults of *P. cinereus* exhibit each of these characteristics. They exhibit site attachment across seasons (Gergits and Jaeger 1990) and both males and females chemically advertise their presence with pheromones deposited on substrates in or on fecal pellets (Jaeger et al. 1986; Horne and Jaeger 1988; Mathis 1990b). Both actively defend sites during the courting and non-courting seasons through agonistic behavior, which involves threat postures, as well as aggressive and submissive displays, and may occasionally lead to biting (Jaeger 1984; Horne 1988; Mathis 1989, 1990a). Lastly, territorial residents have been shown to expel intruders from defended areas beneath cover, the fourth and final requirement of territoriality (Jaeger et al. 1982; Mathis 1990a). Conspecifics that are defeated and not able to secure and defend a territory may adopt a nonterritorial floater tactic (Brown 1969; Mathis 1991) that may result in lower fitness due to an inability to acquire mates, resources, or both (Anthony et al. 1997). At one locality in northern Ohio, it appears that striped morphs are more capable of territory ownership, as Reiter et al. (2014) found that striped males were significantly more aggressive and occupied more cover objects for longer time periods as territorial residents compared to unstriped males.

As outlined above, territorial behavior in *P. cinereus* is well-established and has been examined in many parts of its geographic range, particularly in the southern extent of its range in Virginia (reviewed in Jaeger et al. 2016). In the north, some studies have suggested that *P. cinereus* may not be as territorial. Quinn and Graves (1999) found that a population in northern Michigan displayed an aggregated spatial distribution rather than a uniform one. Uniform distributions have been observed in Virginia (Jaeger 1979; Mathis 1989, 1991) and are indicative of a territorial spatial distribution. Additionally, in southern Ontario, Canada individuals exhibit a random spatial distribution and low levels of aggression (Rollinson and Hackett 2015). Reduced aggression and territorial behavior in these areas can potentially be attributed to resource distribution and availability, as well as a continually wetter habitat. In contrast, populations in Ohio appear to exhibit levels of aggression comparable with those in Virginia and New York (Anthony and Pfingsten 2013). Clearly, there is variation in agonistic and territorial behavior across the range of *P. cinereus* and this phenotypic variation quite likely reflects genetic variation due to local adaptation. However, only one study to date has investigated genetically based geographic variation in territorial behavior (Wise and Jaeger 2016). In the current study, I experimentally compared the agonistic and territorial behavior of striped individuals from two genetic clusters in northern Ohio to examine geographic variation of behavior in genetically distinct groups.

I performed resident-intruder tests in the laboratory on equal numbers of male and female *P. cinereus* within a single genetic cluster to compare aggressive and submissive behaviors in intrasexual contests between the two clusters. My null hypothesis was that the clusters would display a similar degree of agonistic and territorial behavior, as has been reported in Jordan's Salamander (*Plethodon jordani*; Selby et al. 1996). Alternatively, I predicted that individuals in the monomorphic cluster would show a higher degree of agonistic behavior compared to individuals in the polymorphic cluster. Alleles that increase the fitness of striped morphs in polymorphic populations may be free to spread in monomorphic populations due to the absence of the unstriped morph, a process known as genomic character release (West-Eberhard 1986). This process may result in the evolution of increased agonistic behavior in the polymorphic cluster, following release from the evolutionary constraints of having to accommodate multiple phenotypes in polymorphic populations (West-Eberhard 1986). Furthermore, rapid phenotypic evolution associated with morph loss can lead to morphic speciation, such as in side-blotched lizards (*Uta stansburiana*; Corl et al. 2010). Lastly, I predicted that residents of both clusters should exhibit more aggressive and fewer submissive behaviors compared to intruders (i.e., residency effect) due to prior resident advantage, as well as an asymmetry in payoff for the resident (Maynard Smith and Parker 1976; Krebs 1982; Figler and Einhorn 1983). Residents should have more to gain by winning a contest because they have invested more time and energy into holding the territory; therefore, they can afford to suffer a greater fitness loss in a fight than an intruder (Parker 1974; Maynard Smith and Parker 1976). The difference in resident and intruder behavior may be more pronounced in the Eastern Cluster if this group exhibits a more aggressive phenotype.

Methods

Salamander collection

Salamanders were collected in the spring of 2019 from three populations in the Central Cluster and three in the Eastern Cluster. Central Cluster localities included Black River (41.41602, -82.10184), Rocky River (41.42073, -81.8592), and West Creek (41.39006, -81.6912) reservations. Eastern Cluster localities consisted of Tinkers Creek (41.37557, -81.5736), Doan Brook (41.49361, -81.593533), and South Chagrin (41.42324, -81.4207) reservation. Among populations in northern Ohio, F_{st} values derived from microsatellite markers range from 0.007 in continuous forest to 0.149 in urban fragmented habitat (Cameron et al. 2019), as well as a maximum F_{st} of 0.17 across a 20 km distance (Waldron et al. 2019). Therefore, sites were selected because each had a considerably high F_{st} (0.29–0.41) between it and a population in the adjacent cluster, thereby allowing us to maximize genetic differentiation among sampled localities. Additionally, populations in the Central Cluster were selected only if the proportion of striped morphs was no greater than 0.8 in order to minimize differences in color morph frequency among polymorphic populations. The proportion of striped morphs in Central Cluster populations ranged from 0.65–0.8.

Ten adults of each sex were collected at each of the three sampling localities within the two genetic clusters for a combined total of 120 individuals. The minimum size for sexually mature adult male *P. cinereus* in Ohio is approximately 32 mm SVL and for females 34 mm SVL (Anthony and Pfingsten 2013); as such, only males greater than 32 mm and females greater than 34 mm were collected. To determine the sex of each salamander, snout shape was inspected following Anthony et al. (2008); males have a broad, square snout that is enlarged with visible cirri during the reproductive season (spring and fall), whereas snouts of females appear rounded relative to males. Moreover, if females are gravid, eggs can be seen in the abdominal area. Due to the time of collection, the majority of females used were gravid (visible yolked eggs). One nongravid female was used in this experiment; however, Horne (1988) found that gravid and nongravid females in Virginia displayed similar levels of aggressive behavior. Salamanders were transported in individually labeled Falcon 50 mL conical centrifuge tubes to John Carroll University, University Heights, OH, USA.

Measuring territorial behavior in the laboratory

In the laboratory, environmental conditions were maintained at 15 ± 2 °C under a natural photoperiod. All salamanders were housed in separate 473 mL Pyrex glass containers with damp leaf litter and fed approximately 25-30 *Drosophila melanogaster* per week. To begin experimental set-up, salamanders were placed on circular 15 x 1.5 cm polystyrene petri dishes (i.e., territorial chambers) lined with damp (spring water) filter paper substrate. Animals were held in territorial chambers for five to seven days, depending on the testing day, which has been shown to be sufficient time for *P. cinereus* to establish territorial ownership in a laboratory setting (Nunes and Jaeger 1989). Establishing ownership may entail the production and use of fecal pellets deposited on the substrate to mark territories (Jaeger et al. 1986; Horne and Jaeger 1988; Mathis et al. 2000). Uneaten flies were removed from the chamber approximately 24 h prior to testing.

Each individual was tested three times (once as a resident, once as an intruder, and once as a resident with a control). A resident was paired with a same sex intruder from its own population and with a surrogate control (rolled-and-moistened paper towel similar in size to the resident; Jaeger et al. 1982; Anthony et al. 1997). To reduce the effect of size asymmetry on competition, pairs were size-matched (≤ 2 mm; Mathis et al. 1998) with a random individual from a different population in the same genetic cluster. If possible, in a given pairing, the larger individual was selected to be the intruder to eliminate any possibility of a size advantage to the resident who already has the advantage of prior territorial residency (Wise and Jaeger 2016). A range of 9–19 trials on each of 18 testing days was conducted from 23 May to 16 July 2019, with 40 trials for each of the three populations from the two genetic clusters (240 trials in total). The order of residency status was dispersed evenly among testing days (Hurlbert 1984) and no individual was used more than once in each week. Additionally, all trials were carried out blind by assigning each individual a random alphanumeric code. Trials were run between the hours of 0900 and 1800 over the course of the testing period.

To begin testing, both salamanders were marked with a small spot of DayGlo ECO pigment (Blaze Orange or Signal Green; Day-Glo Color Corp) on the dorsal surface, immediately posterior to the pectoral girdle (Lynn et al. 2019). The pigment color was alternated for each trial, such that a resident in one trial was marked with green pigment and a resident in the following trial was marked with orange pigment. The resident was then lifted and placed under an opaque habituation dish (6 x 1.5 cm) and the assigned intruder was subsequently removed from its home chamber and placed into the resident's chamber on the opposite end of the chamber under a second habituation dish for a five-minute acclimation period. After the acclimation dishes were removed, behavioral interactions were documented for 900s using TrueBasic Event-PC 3.0 data collection software (TrueBasic, Inc.) simultaneously on two laptop computers, one for the resident salamander and one for the intruder.

Several previously documented agonistic behaviors (Jaeger 1984) were recorded including the number of times a behavior was performed and the length for which it occurred. Aggressive behaviors include All Trunk Raised (ATR), Move Toward (MT), Look Toward (LT), and Biting (BITE), and are described as follows: ATR – a salamander lifts its trunk completely off of the substrate; MT – an individual moves directly toward the opponent in a direction that may result in eventual contact (Mathis et al. 2000); LT – a salamander turns its head in the direction of the opponent; BITE – a salamander grasps the opponent with its open mouth. Although ATR can be considered a gradational series of postures (Jaeger and Schwarz 1991) that signal increasing levels of threat, these levels were not distinguished in this study. Move Away (MA), Look Away (LA), and Edging (EDGE) are considered submissive behaviors and can be defined as: MA – a salamander moves away from the opponent to increase its distance; LA – a salamander turns its head away from the opponent to avoid visual contact; and EDGE – a salamander roams the periphery of the territorial chamber and/or may press its snout or a limb to walls or the crevice between the lid and walls of the chamber (Wise and Jaeger 2016). EDGE is recognized as a withdrawal behavior (Jaeger et al. 2016), as well as an escape or hiding behavior (Lynn et al. 2019). Timing of behaviors ended when the focal animal clearly terminated the given behavior, or the animal exhibited a subsequent behavior, such as an individual moving toward its opponent after looking toward (Reiter et al. 2014). The time spent in the aggressive behaviors MT and LT and the time spent in the submissive behaviors MA and LA were combined to calculate an Aggression Index (AI), where AI = (MT + LT) – (MA + LA), an equation used in previous studies looking at aggression in salamanders (Mathis et al. 2000; Reiter et al. 2014).

Statistical Analysis

To determine if salamanders from the Central and Eastern Clusters differed in the average time spent in aggressive and submissive behaviors, statistical analyses were run in R Studio (R Core Team 2016). Based on the distribution of the data, a nonparametric permutation approach was used because it did not require the assumptions of normality, heterogeneity, and equal variance. Data were first converted into Euclidean dissimilarity matrices using the vegdist function and then PERMANOVAs were run using the adonis function in the vegan package, specifically comparing individuals tested as residents. When looking at how individuals within a single cluster differed in behavior when territorial ownership status differed, the strata option was included in the PERMANOVA to account for each individual being used twice (once as a resident, once as an intruder). Sixteen individuals were removed from analysis comparing resident behavior with a control because behaviors were recorded inconsistently. The

Bonferroni-Holm correction for multiple comparisons was used to analyze the time spent in the aggressive behaviors ATR, MT, and LT ($\alpha = 0.01$) and the submissive behaviors MA and LA ($\alpha = 0.025$; Holm 1979). BITE did not occur frequently enough to be included in statistical analysis. Similarly, EDGE did not differ statistically between the two clusters, or between residents and intruders within a cluster, and so was excluded from analysis. Interpopulation differences in the level of aggressive and submissive behaviors were not statistically distinguishable, except for low aggression by females from one population in the Central Cluster; however, overall populations did not appear to be operating independently of one another (Tables 1, 2); therefore, sampling localities within each cluster were pooled for statistical analysis. However, females from one locality in the Central Cluster displayed a significantly lower aggression index (AI) compared to females in the other two localities when paired with a live intruder ($F_{2,21} = 5.32$, $P = 0.02$; Table 1).

Results

Salamanders paired with a conspecific intruder had a higher aggression index (AI) than when they were with a surrogate control in both the Central and Eastern Clusters (Table 3). Specifically, Central Cluster males ($F_{1,44} = 4.59$, $P = 0.02$) and Eastern Cluster females ($F_{1,42} = 6.33$, $P = 0.009$) were significantly more aggressive toward conspecific intruders than they were toward a control, whereas Central Cluster females ($F_{1,46} = 0.52$, $P = 0.50$) and Eastern Cluster males ($F_{1,46} = 0.37$, $P = 0.42$) were not (Table 3).

I compared resident behavior between the clusters and found that Central Cluster residents had a significantly higher AI than Eastern Cluster residents ($F_{1,120} = 9.54$, $P = 0.002$; Table 4; Figure 1). In particular, residents in the Central Cluster spent significantly more time on average in the aggressive behavior MT compared to residents in the Eastern Cluster ($F_{1,120}$ = 9.99, *P-adj* = 0.006; Table 5; Figure 1) and also tended to spend more time in the aggressive behavior LT ($F_{1,120}$ = 3.96, *P-adj* = 0.12; Table 5; Figure 3), although there was not much difference in the mean time spent in the submissive behaviors MA ($F_{1,120}$ = 0.025, *P-adj* = 1.00; Table 6; Figure 1) or LA ($F_{1,120}$ = 0.82, *P-adj* = 0.76; Table 6; Figure 1).

Furthermore, I found a strong residency effect in the Central Cluster, but not the Eastern Cluster, when comparing resident to intruder behavior. That is, Central Cluster residents had a significantly higher mean AI ($F_{1,120}$ = 10.93, P = 0.002; Table 7) than Central Cluster intruders, whereas the difference in mean AI between Eastern Cluster residents and intruders was minimal ($F_{1,114}$ = 1.19, P = 0.23; Table 7). Both males ($F_{1,60}$ = 1.49, P = 0.16; Table 7; Figure 2) and females ($F_{1,58}$ = 13.96, P = 0.002; Table 7; Figure 2) in the Central Cluster exhibited a higher mean AI as residents than as intruders. On average, Central Cluster residents of both sexes spent more time in the aggressive behaviors MT and LT (Table 8) and less time in the submissive behaviors MA and LA (Table 9). In the Eastern Cluster, male residents and intruders tended to exhibit a larger mean difference in AI ($F_{1,54}$ = 2.63, P = 0.09; Table 7; Figure 3), whereas the difference in mean AI for female residents and intruders was very small ($F_{1,58}$ = 0.07, P = 0.73; Table 7; Figure 3). Eastern Cluster male and female residents spent either more time, less time, or about equal time in the aggressive (Table 10; Figure 3) and submissive behaviors making up the AI compared to intruders (Table 11; Figure 3). Therefore, no clear pattern of a residency effect can be confidently attributed to the Eastern Cluster. The time spent in the aggressive behavior ATR was not consistent across either sex, territorial ownership status, or cluster, and the intensity of the behavior was typically not a reliable indicator of aggression. However, Eastern Cluster female intruders did display a noticeably higher mean time spent in ATR than residents ($F_{1,58} = 3.00$, *P-adj* = 0.18; Table 10; Figure 3).

The higher aggression in Central Cluster residents was exhibited in both sexes. Central Cluster males showed a higher mean AI than Eastern Cluster males that tended toward significance ($F_{1,59} = 3.11$, $P = 0.09$; Table 9; Figure 1). However, female resident behavior differed dramatically between the Central and Eastern Clusters. Central Cluster females had a significantly higher AI than Eastern Cluster females ($F_{1,59} = 6.76$, $P = 0.015$; Table 9; Figure 1); therefore, females contributed a much greater degree on average to the higher aggression of residents observed in the Central Cluster, which was particularly influenced by the aggressive behaviors MT ($F_{1,59} = 13.51$, *P-adj* = 0.003; Table 10; Figure 1) and LT ($F_{1,59} = 6.02$, *P-adj* = 0.03; Table 10; Figure 1). Additionally, Central Cluster females spent a significantly greater amount of time in ATR as residents compared to Eastern Cluster females ($F_{1,59} = 10.06$, *P-adj* = 0.003; Table 10; Figure 1). The strong residency effect in the Central Cluster can primarily be attributed to the substantial difference in mean AI of female residents and intruders ($F_{1,58} = 13.96$, $P = 0.002$; Table 7; Figure 2). Specifically, Central Cluster female residents spent significantly more time in MT ($F_{1,58} = 6.07$, *P-adj* = 0.02; Table 8; Figure 2) and significantly less time in LA compared to female intruders ($F_{1,58} = 2.69$, *P-adj* = 0.04; Table 9; Figure 2).

Discussion

Two lines of evidence support the notion that geographic variation in territorial behavior exists in northern Ohio, most likely driven by the strong genetic differentiation between the two groups. The first is that the Central Cluster is more aggressive than the Eastern Cluster, indicated by a higher AI. The second is that while residents of both genetic clusters showed a higher AI as residents than as intruders, the Central Cluster exhibited a much larger difference than the Eastern Cluster, demonstrating a clear residency effect and suggesting they are more likely to secure a territory and defend it against intruders.

Plethodon cinereus is one of the most well-studied salamanders and information on intraspecific territoriality in both males and females is particularly well-known (reviewed in Jaeger et al. 2016). Additionally, variation in territorial behavior would be expected throughout this species' widespread geographic range. Following postglacial expansion, populations would have become established and separated long enough for phenotypic variation to potentially evolve (Radomski et al. 2020). Biotic differences across the range, such as differences in prey availability, moisture on the forest floor, and presence of endo- or ectoparasites, competitors, or predators, could have caused local adaptations that may or may not have necessitated some populations to be territorial. Variation in the degree of territoriality and aggression is known across the range of *P. cinereus*, but the bulk of studies focusing on territorial behavior have been carried out in Virginia, where individuals exhibit very high levels of aggression and territoriality (Jaeger 1981b, Jaeger et al. 1982, 1995; Horne 1988; reviewed in Mathis et al. 1995). Individuals from a population in New York also engage in agonistic behaviors on a level similar to those from Virginia (Jaeger 1984). In contrast, individuals apparently display very little aggression in Michigan, although this inference is based on aggregative space use, suggesting reduced territoriality (Quinn and Graves 1999). Anthony and Pfingsten (2013) summarize seven studies in New York, Virginia, and Ohio that appear to exhibit similar variation in the average time spent (per 900 s trial) in the aggressive behavior ATR by territorial residents. In Ohio, Gall et al. (2003) reported 128 s in ATR by a population located in the Eastern Cluster. By contrast, Hickerson et al. (2004) reported 344 s in ATR by a population in the Central Cluster.

Additionally, Reiter et al. (2014) reported an average of 373 s in ATR by another population in the Central Cluster. In the current study, the average time spent in ATR was 171 s by residents in the Central Cluster and 139 s by the Eastern Cluster (Table 5), which follows the trend of higher aggression in the Central Cluster; however, my results relatively low levels of aggressive behavior in terms of time spent in ATR. Furthermore, the mean AI that I found for Central Cluster residents (AI = 90) is also lower than that reported by Reiter et al. (2014) for residents at her site in the Central Cluster (AI = 160). Perhaps this large difference may be attributed to genetic variation among populations in the Central Cluster, as well as the genetic admixture (Hantak et al. 2019) in the population used by Reiter et al. (2014), which may explain the observed variation in agonistic behavior, while highlighting how geographic variation in intraspecific territorial behavior can occur on a relatively small spatial scale. Contrary to the above comparison, Wise and Jaeger (2016) found that genetically distinct groups of *P. cinereus* occurring over a small geographic area in Virginia did not differ in the time spent in ATR, but did in the submissive/escape behavior EDGE, suggesting that genetic differences may influence territorial behavior, at least for submissive behavior.

Geographic variation in territorial behavior could also result from differences in dispersal behavior and some studies suggest that the two traits may be linked. If an individual is less dispersive, then one might assume that individual would be more territorial, and thus require the ability to defend its territory through some form of agonistic behavior. For example, male water skinks (*Euplamprus heatwolei*) exhibit an inverse relationship between exploratory behavior and territoriality. In a laboratory setting, less territorial floaters spent more time moving and less time in a novel enclosure compared to territorial individuals (Stapley and Keogh 2004). Unstriped morphs of *P. cinereus* are also characterized by a non-territorial floater tactic (Anthony et al.

2008; Reiter et al. 2014). Additionally, they frequently occupy the edges of the species' range (Cosentino et al. 2017), suggesting a greater propensity to disperse. Monomorphic striped individuals in the Eastern Cluster may demonstrate a similar relationship between aggression and dispersal as unstriped morphs in the Central Cluster. According to a recent phylogeographic analysis by Radomski et al. (2020), the Eastern Cluster has dispersed further from its ancestral range since the last glacial maximum compared to the Central Cluster. The Eastern Cluster (called the Pennsylvania Clade in Radomski et al. 2020) expanded into New York, Ohio, Pennsylvania, eastern Maryland, and West Virginia, whereas the Central Cluster (called the Ohio Clade in Radomski et al. 2020) is restricted to Ohio and one geographically isolated population in Kentucky and in West Virginia.

Another possibility for the observed differences in aggressive behavior between the two clusters may be due in part to the presence or absence of interspecific competitors. The Allegheny Mountain Dusky Salamander (*Desmognathus ochrophaeus*), for example, is a potential competitor of small *Plethodon*. The two species often overlap in microhabitat distribution and individuals can occasionally be found under the same cover object (Smith and Pough 1994). However, they may differ in agonistic abilities, such that *D. ochrophaeus* has muscular jaws and a larger mouth gape compared to *P. cinereus*. Using resident-intruder tests, Smith and Pough (1994) found that *D. ochrophaeus* frequently displaced *P. cinereus* and demonstrated aggressive behaviors such as biting, leaving bite marks on several *P. cinereus* individuals. The geographic range of *D. ochrophaeus* coincides with the sampled populations of *P. cinereus* in the Eastern Cluster but does not with those in the Central Cluster (Orr and Davic 2013). As such, interspecific interference competition between *P. cinereus* in the Eastern Cluster and *D. ochrophaeus* could result in variation in aggressive behavior via agonistic character displacement (Grether et al. 2009). A similar example exists in *P. glutinosus* and *P. jordani*, two closely related, ecologically similar plethodontid salamanders. Nishikawa (1985) compared the aggressive behavior of these two species at two localities in Virginia and found evidence of increased aggression via natural selection in *P. glutinosus* at the locality where interspecific competition was more intense (i.e., alpha selection; Gill 1972, 1974). In the Eastern Cluster, aggressive dominance/displacement of *P. cinereus* by *D. ochrophaeus* could lead to the adoption of an alternative territorial tactic in which *P. cinereus* are selected to become less aggressive, a tactic perhaps not used by *P. cinereus* in the Central Cluster where they have not been forced to interact with an aggressively dominant interspecific competitor.

Variation in territorial behavior may also differ geographically due to environmental factors including parasites or prey availability. Maksimowich and Mathis (2000) reported that in *P. angusticlavius*, male salamanders with high parasite loads were less aggressive overall than males with low parasite loads. In Michigan, where *P. cinereus* is reportedly less aggressive (Quinn and Graves 1999), three species of endoparasites were found (Muzzall 1990). In Ohio, no parasites were recovered (Odlaug 1954); although more recently, two species of helminth were found in two populations in Pennsylvania, as well as in one Virginia population (Bursey and Schibli 1995). Future research is needed to further surmise how parasitism may be at play among populations and genetic clusters in northern Ohio. Additionally, an inverse relationship may exist between prey abundance and territorial investment, as has been documented in some birds (Stenger 1958) and lizards (Simon 1975). Maerz and Madison (2000) looked at two populations of *P. cinereus* in New York that differed in prey availability and found that individuals from the population with a high abundance of food were less territorial. If the availability of prey is lower in the Central Cluster, individuals may invest in a more aggressive territorial strategy.

Furthermore, the availability of nutrient-rich prey, such as termites (Gabor and Jaeger 1995), is an important indicator of territory quality that females are able to assess via male fecal pellets or pheromones deposited by the territorial owner (Walls et al. 1989). If fewer territories with high quality prey are available, males may need to engage in a greater number of agonistic encounters in order to secure and defend a territory that is attractive to females.

Studies involving plethodontid salamanders have typically focused on the aggressive behavior of males (Hutchison 1959; Cupp 1980; Gabor and Jaeger 1995; Anthony et al. 1997; Townsend and Jaeger 1998; Reiter et al. 2014), although some have looked at aggression in females as well (Horne 1988; Staub 1993; Wiltenmuth 1996; Mathis et al. 2000; Lynn et al. 2019). Mathis et al. (2000) found that female Ozark Zigzag Salamanders (*P. angusticlavius*) had a higher mean AI and bit intruders more often compared to males. In my study, females actually bit less often than males. The large difference in AI between Central and Eastern Cluster female residents can mainly be attributed to the greater amount of time spent in MT by Central Cluster females. Moving toward an opponent could result in eventual contact with the body of the other salamander and is considered an aggressive or preattack behavior (Jaeger et al. 2016). Combined with increases in MT behavior, female residents in the Central Cluster also spent significantly more time in ATR, a “look big” threat posture (Jaeger et al. 2016), compared to female residents in the Eastern Cluster. Clearly, Central Cluster females were more aggressive toward their same-sex opponents. In addition to the strong genetic dissimilarity among Central and Eastern Cluster populations, morph composition may also be playing a role in driving the evolution of female aggressive behavior. The Central Cluster is largely made up of polymorphic populations with varying morph frequencies while the Eastern Cluster is monomorphic striped. In a polymorphic population in the Central Cluster, Anthony et al. (2008) and Acord et al. (2013) found that positive assortative mating by color was occurring, most likely as an outcome of differential access by females to male territories. Striped males are considered more attractive to females than unstriped males, and females of both color morphs are more likely to be associated with them (Acord et al. 2013), possibly due to their higher quality diet (Anthony et al. 2008) and more frequent territory ownership (Reiter et al. 2014). Through intrasexual competition, only the highest quality females gain access to striped males and their territories, and females may be more aggressive as a result. If all males in the Eastern Cluster are striped, and thus presumably considered equally good mates, perhaps there is less of a need for increased aggression among females. Additionally, incomplete assortative mating (Anthony et al. 2008; Acord et al. 2013) may impose evolutionary constraints on the genome of each morph (West-Eberhard 1986). Morph fitness can be affected by the frequency of other morphs in a population and many morph-specific traits arise because they are advantageous for intermorph competition (Corl et al. 2010). The loss of the unstriped morph in the Eastern Cluster may have altered social selection dynamics, whereby alleles that enhance the striped morph's ability to compete with the unstriped morph (i.e., increased aggression and territorial behavior; Reiter et al. 2014) in the Central Cluster are no longer advantageous in the Eastern Cluster (McLean and Stuart-Fox 2014). Increased aggression in the Central Cluster, particularly by females, may be advantageous to compete with unstriped females for access to striped males. Conversely, the change in competitive environment in the Eastern Cluster among striped females may have led to rapid phenotypic selection that favored the release of agonistic behavior (West-Eberhard 1986) because competing for striped males and expending energy on aggression would be less necessary for females in striped monomorphic populations.

In summary, I found a higher degree of aggression and territoriality in the Central Cluster compared to the Eastern Cluster, and this was much more evident in females. The underlying genetic differentiation between the two clusters is the most probable cause of the observed geographic variation in intraspecific territorial behavior, although other possibilities such as interspecific competition, parasite load, and prey availability, as well as the role of morph frequency variation, are worth exploring. Additionally, testing naive neonate *P. cinereus* from the Central and Eastern clusters in controlled, laboratory conditions may be beneficial in demonstrating genetically fixed differences in agonistic behavior (Selby et al. 1996). Moreover, low-order streams have been found to inhibit dispersal and contribute to genetic differentiation among populations of *P. cinereus* in Virginia (Marsh et al. 2007). The Cuyahoga River is a major waterway that coincides with the steep genetic break between the Central and Eastern Clusters and where a strong shift in morph frequency occurs. With such substantial genetic divergence between the two genetic clusters used in my study, it is likely that gene flow is rare across the Cuyahoga River and quite possible that local selection pressures are operating on other behavioral traits aside from agonistic and territorial behavior. Examining geographic variation in behavior may be a valuable avenue of continued research to determine if divergent selection is occurring among populations of *P. cinereus* in northern Ohio and it allows for insight into the adaptive mechanisms of behavior associated with polymorphic species and loss of a morph.

Acknowledgements

Special thanks to my advisors, C. Anthony and C. Hickerson; my labmates, C. Thomas, K. Garner, and T. Evers; and D. Frost and M. Ryan for valuable feedback and support. I thank O. Brooks, A. Eucker, D. Frost, J. Galinis, M. Green, S. Kale, S. Kocheff, C. Lindstrom, E. Palmer,

H. Parson, H. Roberts, and A. Veselka for their assistance in the field with animal collection. M. Hantak provided sampling locality information. T. Evers, K. Garner, C. Lindstrom, H. Roberts, and C. Thomas maintained fly colonies and helped with husbandry upkeep for the duration of the study. J. Watling advised appropriate statistical analysis. The Ohio Department of Natural Resources granted scientific collection permit #ODNR 22-018 to obtain animals for research purposes and the Institutional Animal Care and Use Committees of John Carroll University (Protocol no. 1800) allowed us to work with live animals in the laboratory. DayGlo Color Corporation provided two ECO pigment samples for use in laboratory trials.

Literature Cited

Acord, M.A., C.D. Anthony, and C.A.M. Hickerson. 2013. Assortative mating in a polymorphic salamander. Copeia 2013:676–683.

Anthony, C.D., and R.A. Pfingsten. 2013. Eastern Red-Backed Salamander, *Plethodon cinereus* (Green 1818). Pp. 335–360 in Amphibians of Ohio (R.A. Pfingsten, J.G. Davis, T.O. Matson, G.J. Lipps, D. Wynn, and B.J. Armitage, eds.). Ohio Biological Survey, USA.

Anthony, C.D., M.D. Venesky, and C.A.M. Hickerson. 2008. Ecological separation in a polymorphic terrestrial salamander. Journal of Animal Ecology 77:646–653.

Anthony, C.D., J.A. Wicknick, and R.J. Jaeger. 1997. Social interactions in two sympatric salamanders: Effectiveness of a highly aggressive strategy. Behavior 134:71–88.

Arnold, S.J. 1977. Polymorphism and geographic variation in the feeding behavior of the garter snake, *Thamnophis elegans*. Science 197:676–678.

Brodie, E.D., III. 1993. Homogeneity of the genetic variance–covariance matrix for antipredator traits in two natural populations of the garter snake *Thamnophis ordinoides*. Evolution 47:844–854.

Brown, J.L. 1964. The evolution of diversity in avian territorial systems. The Wilson Bulletin 76:160–169.

Brown, J.L. 1969. Territorial behavior and population regulation in birds: a review and re-evaluation. The Wilson Bulletin 81: 293–329.

Brown, J.L., and G.H. Orians. 1970. Spacing patterns in mobile animals. Annual Review of Ecology and Systematics: 1: 239–262.

Burghardt, G.M., J.M. and Schwartz. 1999. Geographic variations on methodological themes in comparative ethology: a natricine snake perspective. Pp. 69–94 in Geographic Variation in

Behavior: Perspectives on Evolutionary Mechanisms. (Foster, S.A. and J.A. Endler, eds.). Oxford University Press, UK.

Bursey, C.R., and D.R. Schibli. 1995. A comparison of the helminth fauna of two *Plethodon cinereus* populations. Journal of the Helminthological Society of Washington 62:232–236.

Cabe, P.R., R.B. Page, T.J. Hanlon, M.E. Aldrich, L. Connors, and D.M. Marsh. 2007. Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. Heredity 98:53–60.

Cameron, A.C., R.B. Page, J.I. Watling, C.A.M. Hickerson, and C.D. Anthony. 2019. Using a comparative approach to investigate the relationship between landscape and genetic connectivity among woodland salamander populations. Conservation Genetics 20:1265–1280.

Corl, A., A.R. Davis, S.R. Kuchta, and B. Sinervo. 2010. Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. Proceedings of the National Academy of Sciences 107:4254–4259.

Cosentino, B.J., J.D. Moore, N.E., Karraker, M. Ouellet, and J.P. Gibbs. 2017. Evolutionary response to global change: Climate and land use interact to shape color polymorphism in a woodland salamander. Ecology and Evolution 7:5426–5434.

Coss, R.G. 1999. Effects of relaxed natural selection on the evolution of behavior. Pp. 180–208 in Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms (Foster, S.A. and J.A. Endler, eds.). Oxford University Press, UK.

Cupp Jr., P.V. 1980. Territoriality in the green salamander, *Aneides aeneus*. Copeia 1980:463–468.

Feder, M.E., and P.L. Londos. 1984. Hydric constraints upon foraging in a terrestrial salamander, *Desmognathus ochrophaeus* (Amphibia: Plethodontidae). Oecologia 64:413–418.

Figler, M.H., and D.M Einhorn. 1983. The territorial prior residence effect in convict cichlids (*Cichlasoma nigrofasciatum* Günther): Temporal aspects of establishment and retention, and proximate mechanisms. Behavior 85:157–183.

Fisher-Reid, M.C., T.N. Engstrom, C.A. Kuczynski, P.R. Stephens, and J.J. Wiens. 2013. Parapatric divergence of sympatric morphs in a salamander: incipient speciation on Long Island? Molecular Ecology 22:4681–4594.

Fisher-Reid, M.C., and J.J. Wiens. 2015. Is geographic variation within species related to macroevolutionary patterns between species? Journal of Evolutionary Biology 28:1502–1515.

Foster, S.A. 1999. The geographic of behavior: An evolutionary perspective. Trends in Ecology and Evolution 14:190–195.

Foster, S.A., and J.A. Endler. 1999. Geographic variation in behavior: Perspectives on evolutionary mechanisms. Oxford University Press, UK.

Gabor, C.R., and R.G. Jaeger. 1995. Resource quality affects the agonistic behavior of territorial salamanders. Animal Behavior 49:71–79.

Gall, S.B., C.D. Anthony, and J.A. Wicknick. 2003. Do behavioral interactions between salamanders and beetles indicate a guild relationship? The American Midland Naturalist 149:363–374.

Gergits, W.F., and R.G. Jaeger. 1990. Site attachment by the red-backed salamander, *Plethodon cinereus*. Journal of Herpetology 24:91–93.

Gill, D.E. 1972. Intrinsic rates of increase, saturation densities, and competitive ability. I. An experiment with *Paramecium*. The American Naturalist 106:461–471.

Gill, D.E. 1974. Intrinsic rate of increase, saturation density, and competitive ability. II. The evolution of competitive ability. The American Naturalist 108:103–116.

Grether, G F., N. Losin, C.N. Anderson, and K. Okamoto. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. Biological Reviews 84: 617–635.

Hantak, M.M., R.B. Page, P.E. Converse, C.D. Anthony, C.A.M. Hickerson, and S.R. Kuchta. 2019. Do genetic structure and landscape heterogeneity impact color morph frequency in a polymorphic salamander? Ecography 42:1383–1394.

Hickerson, C.A.M., C.D. Anthony, and J.A. Wicknick. 2004. Behavioral interactions between salamanders and centipedes: Competition in divergent taxa. Behavioral Ecology 15:679–686.

Highton, R.T. 1975. Geographic variation in genetic dominance of the color morphs of the red-backed salamander, *Plethodon cinereus*. Genetics 80:363–374.

Highton, R.T. 1977. Comparison of microgeographic variation in morphological and electrophoretic traits. Evolutionary Biology 10:397–436.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:65–70.

Horne, E.A. 1988. Aggressive behavior of female Red-backed Salamanders. Herpetologica 44:203–209.

Horne, E.A., and R.G. Jaeger. 1988. Territorial pheromones of female red-backed salamanders. Ethology 78:143–152.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological monographs 54:187–211.

Hutchison, V.H. 1959. Aggressive behavior in *Plethodon jordani*. Copeia 1959:72–73.

Jaeger, R.G. 1972. Food as a limited resource in competition between two species of terrestrial salamanders. Ecology 53:535–546.

Jaeger, R.G. 1979. Seasonal spatial distributions of the terrestrial salamander *Plethodon cinereus*. Herpetologica 35:90–93.

Jaeger, R.G. 1981a. Diet diversity and clutch size of aquatic and terrestrial salamanders. Oecologica 48:190–193.

Jaeger, R.G. 1981b. Dear enemy recognition and the costs of aggression between salamanders. American Naturalist 117:962–974.

Jaeger, R.G. 1984. Agonistic behavior of the Red-backed Salamander. Copeia 1984:309–314.

Jaeger, R.G., and D.C. Forester. 1993. Social behavior of plethodontid salamanders. Herpetologica 49:163–175.

Jaeger, R.G., and W.F. Gergits. 1979. Intra- and interspecific communication in salamanders through chemical signals on the substrate. Animal Behavior 27:150–156.

Jaeger, R.G., and J.K. Schwarz. 1991. Gradational threat postures by the Red-backed Salamander. Journal of Herpetology 25:112–114.

Jaeger, R.G., B. Gollmann, C.D. Anthony, C.R. Gabor, and N.R. Kohn. 2016. Behavioral ecology of the Eastern Red-backed Salamander: 50 years of research. Oxford University Press, UK.

Jaeger, R.G., J.M. Goy, M. Tarver, and C.E. Márquez. 1986. Salamander territoriality: pheromonal markers as advertisement by males. Animal Behavior 34:860–864.

Jaeger, R.G., D. Kalvarsky, and N. Shimizu. 1982. Territorial behavior of the Red-backed Salamander: Expulsion of intruders. Animal Behavior 30:490–496.

Koref-Santibanez, S. 1972. Courtship behavior in the semispecies of the superspecies *Drosophila paulistorum*. Evolution 26:108–115.

Krebs, J.R. 1982. Territorial defense in the great tit (*Parus major*): do residents always win? Behavioral Ecology and Sociobiology 11:185–194.

Kroodsma, D.E., and R.A. Canady. 1985. Differences in repertoire size, singing behavior, and associated neuroanatomy among marsh wren populations have a genetic basis. The Auk 102: 439–446.

Lynn, C.S., B. Dalton, and A. Mathis. 2019. Territorial behavior in Southern Red-backed and Ozark Zigzag Salamanders: Effects of sex, species and ownership. Behavior 156:1017–1037.

Maerz, J.C., and D.M. Madison. 2000. Environmental variation and territorial behavior in a terrestrial salamander. Pp. 395–406 in The Biology of Plethodontid Salamanders (R.C. Bruce, R.G. Jaeger, and L. Houck, eds.). Springer, USA.

Magurran, A.E. 1999. The causes and consequences of geographic variation in antipredator behaviour: perspectives from fish populations. Pp. 139–163 in Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms (Foster, S.A. and J.A. Endler, eds.). Oxford University Press, UK.

Maksimowich, D.S., and A. Mathis. 2000. Parasitized salamanders are inferior competitors for territories and food resources. Ethology 106:319–329.

Marsh, D.M., R.B. Page, T.J. Hanlon, H. Bareke, R. Corritone, N. Jetter, N.G. Beckman, K. Gardner, D.E. Seifert, and P.R. Cabe. 2007. Ecological and genetic evidence that low-order streams inhibit dispersal by Red-backed Salamanders (Plethodon cinereus). Canadian Journal of Zoology 85:319–327.

Mathis, A. 1989. Do seasonal spatial distributions in a terrestrial salamander reflect reproductive behavior or territoriality? Copeia 1989:788–791.

Mathis, A. 1990a. Territoriality in a terrestrial salamander: the influence of resource quality and body size. Behavior 112:162–175.

Mathis, A. 1990b. Territorial salamanders assess sexual and competitive information using chemical signals. Animal Behavior 1990:953–962.

Mathis, A. 1991. Territories of male and female terrestrial salamanders: Costs, benefits, and intersexual spatial associations. Oecologica 86:433–440.

Mathis, A., K. Deckard, and C. Duer. 1998. Laboratory evidence for territorial behavior by the Southern Red-backed Salamander, *Plethodon serratus*: Influence of residency status and pheromonal advertisement.

Mathis, A., R.G. Jaeger, W.H. Keen, P.K. Ducey, S.C. Walls, and B.C. Buchanan. 1995. Aggression and territoriality by salamanders and a comparison with the territorial behavior of frogs. Pp. 633–676 in Amphibian Biology, Volume 2, Social Behavior (H. Heatwole and B.K. Sullivan, eds.). Surrey Beatty and Sons, Australia.

Mathis, A., D.W. Schmidt, and K.A. Medley. 2000. The influence of residency status on agonistic behavior of male and female Ozark zigzag salamanders *Plethodon angusticlavius*. American Midland Naturalist 143:245–249.

Maynard Smith, J., and G.A. Parker. 1976. The logic of asymmetric contests. Animal Behavior 24:159–175.

McLean, C.A., and D. Stuart-Fox. 2014. Geographic variation in animal color polymorphisms and its role in speciation. Biological Reviews, 89:860–873.

Moore, J.D., and M. Ouellet. 2014. A review of color phenotypes of the Eastern Red-backed Salamander, *Plethodon cinereus*, in North America. Canadian Field-Naturalist 128:250–259.

Morrison, C., and J.M. Hero. 2003. Geographic variation in life-history characteristics of amphibians: a review. Journal of Animal Ecology 72:270–279.

Muzzall, P.M. 1990. Endoparasites of the Red-backed Salamander, *Plethodon c. cinereus*, from southwestern Michigan. Journal of the Helminthological Society of Washington 57:165–167.

Nishikawa, K.C. 1985. Competition and the evolution of aggressive behavior in two species of terrestrial salamanders. Evolution 39:1282–1294.

Noble, G.K. 1939. The role of dominance in the social life of birds. The Auk 56:263–273.

Nunes, V.S., and R.G. Jaeger. 1989. Salamander aggressiveness increases with length of territorial ownership. Copeia 1989:712–718.

Odlaug, T.O. 1954. Parasites of some Ohio Amphibia. The Ohio Journal of Science 54:126–128.

Orr, L.P., and R.D. Davic. 2013. Allegheny Mountain Dusky Salamander, *Desmognathus ochrophaeus* (Cope 1859). Pp. 245–257 in Amphibians of Ohio (R.A. Pfingsten, J.G. Davis, T.O. Matson, G.J. Lipps, D. Wynn, and B.J. Armitage, eds.). Ohio Biological Survey, USA.

Parker, G.A. 1974. Assessment strategy and the evolution of fighting behavior. Journal of Theoretical Biology 47:223–243.

Petranka, J.W. 1998. Plethodontidae: Plethodontinae: Plethodontini: *Plethodon cinereus* (red-backed salamander). Pp. 335–346 in Salamanders of the United States and Canada (P. Strupp, ed.). Smithsonian Institution Press, USA.

Pfingsten, R.A., and C.F. Walker. 1978. Some nearly all black populations of *Plethodon cinereus* (Amphibia, Urodela, Plethodontidae) in Northern Ohio. Journal of Herpetology 12:163–167.

Pröhl, H., R.A. Koshy, U. Mueller, A.S. Rand, and M.J. Ryan. 2006. Geographic variation of genetic and behavioral traits in northern and southern túngara frogs. Evolution 60:1669–1679.

Quinn, V.S., and B. Graves. 1999. Space use in response to conspecifics by the Red-backed Salamander (*Plethodon cinereus*, Plethodontidae, Caudata). Ethology 105: 993–1002.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radomski, T., M.M. Hantak, A.D. Brown, and S.R. Kuchta. 2020. Multilocus phylogeography of Eastern Red-backed Salamanders (*Plethodon cinereus*): Cryptic Appalachian diversity and postglacial range expansion. Herpetologica 76:61–73.

Reiter, M.K., C.D. Anthony, and C.A.M. Hickerson. 2014. Territorial behavior and ecological divergence in a polymorphic salamander. Copeia 2014:481–488.

Rollinson, N., and D. Hackett. 2015. Experimental evaluation of agonistic behavior, chemical communication, spacing, and intersexual associations of the Red-backed Salamander (*Plethodon cinereus*) near its northern range limit. Canadian Journal of Zoology 93:773–781.

Rosenau, M.L., and J.D. McPhail. 1987. Inherited differences in agonistic behavior between two populations of coho salmon. Transactions of the American Fisheries Society 116:646–654.

Selby, M.F., S.C. Winkel, and J.W. Petranka. 1996. Geographic uniformity in agonistic behaviors of Jordon’s Salamander. Herpetologica 52:108–115.

Simon, C.A. 1975. The influence of food abundance on territory size in the iguanid lizard *Sceloporus jarrovi*. Ecology 56:993–998.

Smith, E., and F. Pough. 1994. Intergeneric aggression among salamanders. Journal of Herpetology 28:41–45.

Soler, J.J., J.G. Martinez, M. Soler, and A.P. Møller. 1999. Genetic and geographic variation in rejection behavior of cuckoo eggs by European magpie populations: An experimental test of rejecter-gene flow. Evolution, 53: 947–956.

Stapley, J., and J.S. Keogh. 2004. Exploratory and antipredator behaviors differ between territorial and nonterritorial male lizards. Animal Behavior 68:841–846.

Staub, N.L. 1993. Intraspecific agonistic behavior of the salamander *Aneides flavipunctatus* (Amphibia: Plethodontidae) with comparisons to other plethodontid species. Herpetologica 49:271–282.

Stenger, J. 1958. Food habits and available food of ovenbirds in relation to territory size. The Auk 75:335–346.

Tinbergen, N. 1957. The functions of territory. Bird study 4: 14–27.

Townsend, V.R., and R.G. Jaeger. 1998. Territorial conflicts over prey: Domination by large male salamanders. Copeia 1998:725–729.

Waldron, B.P., S.R. Kuchta, M.M. Hantak, C.A.M. Hickerson, and C.D. Anthony. 2019. Genetic analysis of a cryptic contact zone between mitochondrial clades of the Eastern Red-Backed Salamander, *Plethodon cinereus*. Journal of Herpetology 53:144–153.

Walls, S.C., A. Mathis, R.G. Jaeger, and C. Martin. 1989. Male salamanders with high quality diets have feces attractive to females. Animal Behavior 38:546–548.

West-Eberhard, M.J. 1986. Alternative adaptations, speciation, and phylogeny (a review). Proceedings of the National Academy of Sciences, 83:1388–1392.

Wilczynski, W., and M.J. Ryan. 1999. Geographic variation in animal communication systems. Pp. 234–261 in Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms (Foster, S.A. and J.A. Endler., eds.). Oxford University Press, UK.

Wiltenmuth, E.B. 1996. Agonistic and sensory behavior of the salamander *Ensatina eschscholtzii* during asymmetrical contests. Animal Behavior 52:841–850.

Wiltenmuth, E.B., and K.C. Nishikawa. 1998. Geographical variation in agonistic behavior in a ring species of salamander, *Ensatina eschscholtzii*. Animal Behavior 55:1595–1606.

Wise, S.E., and R.G. Jaeger. 2016. Seasonal and geographic variation in territorial conflicts by male Red-backed Salamanders. Behavior 153:187–207.

TABLE 1.—Comparison of resident and control aggression indices (AI in s; mean ± SE) among localities[1] within the genetic cluster (GC) known as the Central Cluster (C).[2] Resident males and females from all populations had a higher mean AI with a conspecific intruder than with a surrogate control, except females from RR. Interpopulation differences were not significant among combined sexes or males; however, the AI of RR females was significantly lower when paired with a conspecific than females from BR and WC.

GC	Locality	Sex	*n*	Resident/Control AI	*F, P*	Resident/Intruder AI	*F, P*
C	BR	All	16	62.11 ± 3.67	0.21, 0.88	106.90 ± 8.45	0.21, 0.87
C	RR	All	16	59.84 ± 5.85	"	50.70 ± 7.71	"
C	WC	All	15	44.99 ± 4.32	"	84.37 ± 7.93	"
C	BR	M	7	47.02 ± 5.25	0.33, 0.71	108.13 ± 16.79	1.67, 0.22
C	RR	M	8	35.09 ± 8.36	"	52.67 ± 14.69	"
C	WC	M	8	35.16 ± 6.17	"	58.02 ± 7.18	"
C	BR	F	9	73.85 ± 20.87	0.13, 0.93	105.93 ± 7.70	5.32, 0.02*
C	RR	F	8	84.59 ± 29.70	"	48.73 ± 6.49	"
C	WC	F	7	56.24 ± 10.24	"	114.50 ± 12.13	"

---

[1] Localities: Black River (BR), Rocky River (RR), West Creek (WC).

[2] $\alpha = 0.05$. Asterisks indicate significant differences; * indicates $P \leq 0.05$.

TABLE 2.— Comparison of resident and control aggression indices (AI in s; mean ± SE) among localities[3] within the genetic cluster (GC) known as the Eastern Cluster (E). Resident males and females from all populations had a higher mean AI with a conspecific intruder than with a surrogate control and interpopulation differences were not significant ($P > 0.05$).

GC	Pop	Sex	*n*	Resident/Control AI	*F, P*	Resident/Intruder AI	*F, P*
E	DB	All	16	37.90 ± 7.77	0.81, 0.49	55.44 ± 6.08	0.71, 0.53
E	SC	All	16	45.96 ± 9.79	"	69.60 ± 10.01	"
E	TC	All	14	22.17 ± 3.54	"	48.04 ± 5.17	"
E	DB	M	9	50.52 ± 13.67	0.11, 0.92	53.31 ± 7.71	0.14, 0.89
E	SC	M	8	49.89 ± 17.18	"	62.59 ± 13.18	"
E	TC	M	7	36.33 ± 4.25	"	50.83 ± 4.73	"
E	DB	F	7	21.67 ± 4.34	2.01, 0.16	58.19 ± 10.28	0.54, 0.56
E	SC	F	8	42.04 ± 10.28	"	76.61 ± 15.95	"
E	TC	F	7	8.01 ± 3.98	"	45.25 ± 9.79	"

---

[3] Localities: Doan Brook (DB), South Chagrin (SC), Tinker's Creek (TC).

TABLE 3.—Comparison of resident aggression indices (AI in s; mean ± SE) with a surrogate control and with a conspecific intruder.[4] Genetic cluster (GC) abbreviated for Central (C) and Eastern (E) Clusters. Male (M) and female (F) salamanders were more aggressive toward intruders compared to controls.

GC	Sex	*n*	Resident/Control AI	Resident/Intruder AI	*F, P*
C	All	47	55.88 ± 11.48	80.58 ± 8.58	2.96, 0.08
C	M	23	38.75 ± 6.58	71.41 ± 13.75	4.59, 0.02*
C	F	24	72.29 ± 21.28	89.36 ± 10.40	0.52, 0.50
E	All	46	35.92 ± 7.60	58.12 ± 7.43	4.36, 0.01*
E	M	24	46.17 ± 12.70	55.68 ± 8.97	0.37, 0.42
E	F	22	24.73 ± 13.26	60.77 ± 9.37	6.33, 0.009**

---

[4] $\alpha = 0.05$. Asterisks indicate significant differences; * indicates $P \leq 0.05$; ** indicates $P \leq 0.01$.

TABLE 4. —Comparison of resident aggression indices (AI in s; mean ± SE) in the Central and Eastern Clusters.[5] Central Cluster residents had a higher mean AI compared to the Eastern Cluster, indicating higher levels of aggression.

Sex	*n*	Central Cluster AI	Eastern Cluster AI	*F*, *P*
All	122	89.65 ± 5.90	56.48 ± 4.72	9.54, 0.002**
M	61	88.21 ± 9.65	60.33 ± 5.33	3.11, 0.09
F	61	91.13 ± 6.92	52.75 ± 7.83	6.76, 0.015*

---

[5] $\alpha = 0.05$. Asterisks indicate significant differences; * indicates $P \leq 0.05$; ** indicates $P \leq 0.01$.

TABLE 5. — Comparison of aggressive behaviors (timed in s; mean ± SE) in the Central and Eastern Clusters by resident salamanders.[6] Behaviors include All Trunk Raised (ATR), MT (Move Toward), and LT (Look Toward).

Behavior	Sex	*n*	Central Cluster	Eastern Cluster	*F*, *P-adj*
ATR	All	122	171.15 ± 16.01	138.71 ± 12.56	2.98, 0.25
ATR	M	61	160.11 ± 22.66	174.23 ± 21.42	0.10, 1.00
ATR	F	61	182.55 ± 22.91	69.99 ± 9.86	10.06, 0.003**
MT	All	122	23.45 ± 1.63	14.54 ± 1.12	9.99, 0.006**
MT	M	61	20.13 ± 2.06	17.45 ± 1.63	0.51, 1.00
MT	F	61	26.88 ± 2.50	11.72 ± 1.48	13.51, 0.003**
LT	All	122	7.40 ± 0.35	6.13 ± 0.27	3.96, 0.12
LT	M	61	7.34 ± 0.45	7.07 ± 0.42	0.10, 1.00
LT	F	61	7.45 ± 0.56	5.23 ± 0.30	6.02, 0.03*

---

[6] $\alpha = 0.01$. Asterisks indicate significant differences; * indicates *P-adj* ≤ 0.05; ** indicates *P-adj* ≤ 0.01.

TABLE 6. — Comparison of submissive behaviors (timed in s; mean ± SE) by Central and Eastern Cluster resident salamanders.[7] Behaviors include Move Away (MA) and Look Away (LA).

Behavior	Sex	*n*	Central Cluster	Eastern Cluster	*F*, *P-adj*
MA	All	122	3.25 ± 0.47	3.40 ± 0.49	0.025, 1.00
MA	M	61	2.46 ± 0.31	3.73 ± 0.48	1.12, 0.60
MA	F	61	4.07 ± 0.81	3.09 ± 0.69	0.42, 1.00
LA	All	122	12.85 ± 1.51	16.84 ± 2.77	0.82, 0.76
LA	M	61	14.51 ± 1.86	12.72 ± 1.63	0.16, 1.00
LA	F	61	11.13 ± 1.94	20.83 ± 5.03	1.64, 0.49

---

[7] $\alpha = 0.025$.

TABLE 7. —Comparison of intruder and resident aggression indices (AI in s; mean ± SE).[8] Genetic cluster (GC) abbreviated for Central (C) and Eastern (E) Clusters. Male (M) and female (F) resident salamanders had a higher mean AI than intruder salamanders, indicating a residency effect that was more pronounced in the Central Cluster.

GC	Sex	*n*	AI as Intruder	AI as Resident	*F, P*
C	All	61	51.93 ± 7.26	87.88 ± 8.10	10.93, 0.002**
C	M	31	63.25 ± 10.38	83.39 ± 12.82	1.49, 0.16
C	F	30	40.23 ± 9.86	92.52 ± 9.93	13.96, 0.002**
E	All	58	48.15 ± 5.06	57.45 ± 6.84	1.19, 0.23
E	M	28	47.27 ± 5.38	62.49 ± 7.69	2.63, 0.09.
E	F	30	48.98 ± 8.48	52.75 ± 11.17	0.07, 0.73

---

[8] $\alpha = 0.05$. Asterisks indicate significant differences; ** indicates $P \leq 0.01$.

TABLE 8. —Differences in the average time spent in aggressive behaviors by male (M) and female (F) salamanders as residents and intruders in the Central Cluster (CC).[9] Behaviors include All Trunk Raised (ATR), MT (Move Toward), and LT (Look Toward).

Behavior	Sex	*n*	CC Intruder	CC Resident	*F*, *P-adj*
ATR	All	61	179.2 ± 21.12	162.64 ± 22.17	0.29, 1.00
ATR	M	31	193.12 ± 27.90	152.25 ± 31.27	0.95, 0.99
ATR	F	30	164.81 ± 32.11	173.37 ± 31.85	0.03, 1.00
MT	All	61	17.03 ± 1.79	23.79 ± 2.33	5.27, 0.03*
MT	M	31	17.52 ± 2.73	20.52 ± 2.91	0.57, 1.00
MT	F	30	16.53 ± 2.36	27.16 ± 3.61	6.07, 0.02*
LT	All	61	5.89 ± 0.40	7.39 ± 0.51	5.41, 0.08
LT	M	31	6.16 ± 0.54	7.42 ± 0.64	2.27, 0.46
LT	F	30	5.60 ± 0.60	7.37 ± 0.80	3.07, 0.27

---

[9] $\alpha = 0.01$. Asterisks indicate significant differences; * indicates *P-adj* ≤ 0.05.

TABLE 9. —Differences in the average time spent in submissive behaviors by male (M) and female (F) salamanders as residents and intruders in the Central Cluster (CC).[10] Behaviors include Move Away (MA) and Look Away (LA).

Behavior	Sex	*n*	CC Intruder	CC Resident	*F*, *P-adj*
MA	All	61	5.74 ± 0.85	3.19 ± 0.67	5.61, 1.00
MA	M	31	6.83 ± 1.40	2.50 ± 0.67	7.70, 0.03*
MA	F	30	4.61 ± 0.90	3.89 ± 1.16	0.24, 1.00
LA	All	61	21.40 ± 3.86	12.71 ± 2.17	3.85, 0.03*
LA	M	31	23.30 ± 6.37	14.44 ± 3.30	1.52, 0.47
LA	F	30	19.44 ± 4.37	10.92 ± 2.81	2.69, 0.04*

---

[10] $\alpha = 0.025$. Asterisks indicate significant differences; * indicates *P-adj* ≤ 0.05.

TABLE 10. —Differences in the average time spent in aggressive behaviors by male (M) and female (F) salamanders as residents and intruders in the Eastern Cluster (EC).[11] Behaviors include All Trunk Raised (ATR), MT (Move Toward), and LT (Look Toward).

Behavior	Sex	*n*	EC Intruder	EC Resident	*F*, *P-adj*
ATR	All	58	141.63 ± 18.11	121.63 ± 18.37	0.60, 1.00
ATR	M	28	166.58 ± 26.85	176.97 ± 32.07	0.06, 1.00
ATR	F	30	118.34 ± 24.12	69.99 ± 14.06	3.00, 0.18
MT	All	58	18.85 ± 2.31	14.79 ± 1.62	2.06, 0.31
MT	M	28	19.85 ± 2.97	18.07 ± 2.37	0.22, 1.00
MT	F	30	17.91 ± 3.54	11.72 ± 2.11	2.25, 0.36
LT	All	58	5.69 ± 0.45	6.24 ± 0.38	0.88, 0.92
LT	M	28	5.79 ± 0.59	7.32 ± 0.57	3.48, 0.25
LT	F	30	5.60 ± 0.68	5.23 ± 0.43	0.21, 1.00

---

[11] $\alpha = 0.01$.

TABLE 11. — Differences in the average time spent in submissive behaviors by male (M) and female (F) salamanders as residents and intruders in the Eastern Cluster (EC).[12] Behaviors include Move Away (MA) and Look Away (LA).

Behavior	Sex	*n*	EC Intruder	EC Resident	*F*, *P-adj*
MA	All	58	4.63 ± 1.06	3.46 ± 0.72	0.84, 0.70
MA	M	28	6.42 ± 1.96	3.86 ± 1.06	1.31, 0.54
MA	F	30	2.97 ± 0.84	3.09 ± 0.98	0.008, 1.00
LA	All	58	12.44 ± 3.60	17.13 ± 4.04	0.75, 0.83
LA	M	28	6.07 ± 1.19	13.17 ± 3.30	4.11, 0.11
LA	F	30	18.39 ± 6.74	20.83 ± 7.18	0.06, 1.00

---

[12] $\alpha = 0.025$.

FIG. 1.—Time (s) spent in aggressive and submissive behaviors by female (white) and male (gray) residents in the Central and Eastern Clusters. (A.-E.) Aggression index (AI) with composite behaviors. (F.) All Trunk Raised. $\alpha = 0.05$ for AI; $\alpha = 0.01$ for aggressive behaviors MT, LT, and ATR; $\alpha = 0.025$ for submissive behaviors MA and LA. Asterisks indicate significant differences; * indicates *P* and *P-adj* ≤ 0.05; ** indicates *P* and *P-adj* ≤ 0.01. Data are presented as nested boxplots with the horizontal lines in the boxes representing medians, the "x" symbols representing means, the boxes indicating interquartile range, and the solid circles denoting outliers.

FIG. 2.—Differences in the time spent in resident and intruder behaviors by female (white) and male (gray) salamanders in the Central Cluster. (A.-E.) Aggression index (AI) with composite behaviors. (F.) All Trunk Raised. $\alpha = 0.05$ for AI; $\alpha = 0.01$ for aggressive behaviors MT, LT, and ATR; $\alpha = 0.025$ for submissive behaviors MA and LA. Asterisks indicate significant differences; * indicates *P* and *P-adj* ≤ 0.05; ** indicates *P* and *P-adj* ≤ 0.01. Data are presented as nested boxplots with the horizontal lines in the boxes representing medians, the "x" symbols representing means, the boxes indicating interquartile range, and the solid circles denoting outliers.

FIG. 3.—Differences in the time spent in resident and intruder behaviors by female (white) and male (gray) salamanders in the Eastern Cluster. (A.-E.) Aggression Index with composite behaviors. (F.) All Trunk Raised. $\alpha = 0.05$ for AI; $\alpha = 0.01$ for aggressive behaviors

MT, LT, and ATR; $\alpha = 0.025$ for submissive behaviors MA and LA. Data are presented as nested boxplots with the horizontal lines in the boxes representing medians, the “x” symbols representing means, the boxes indicating interquartile range, and the solid circles denoting outliers.

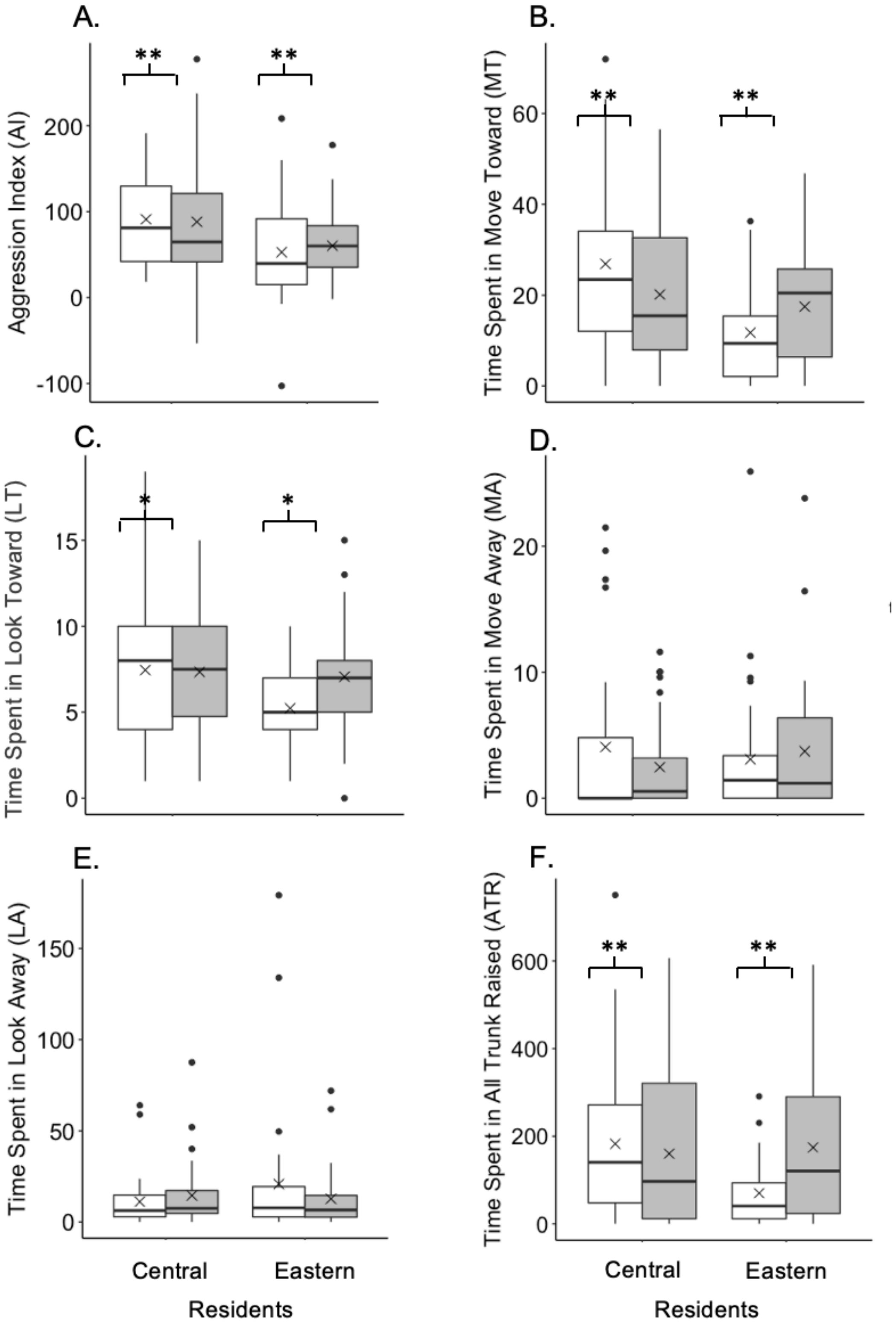
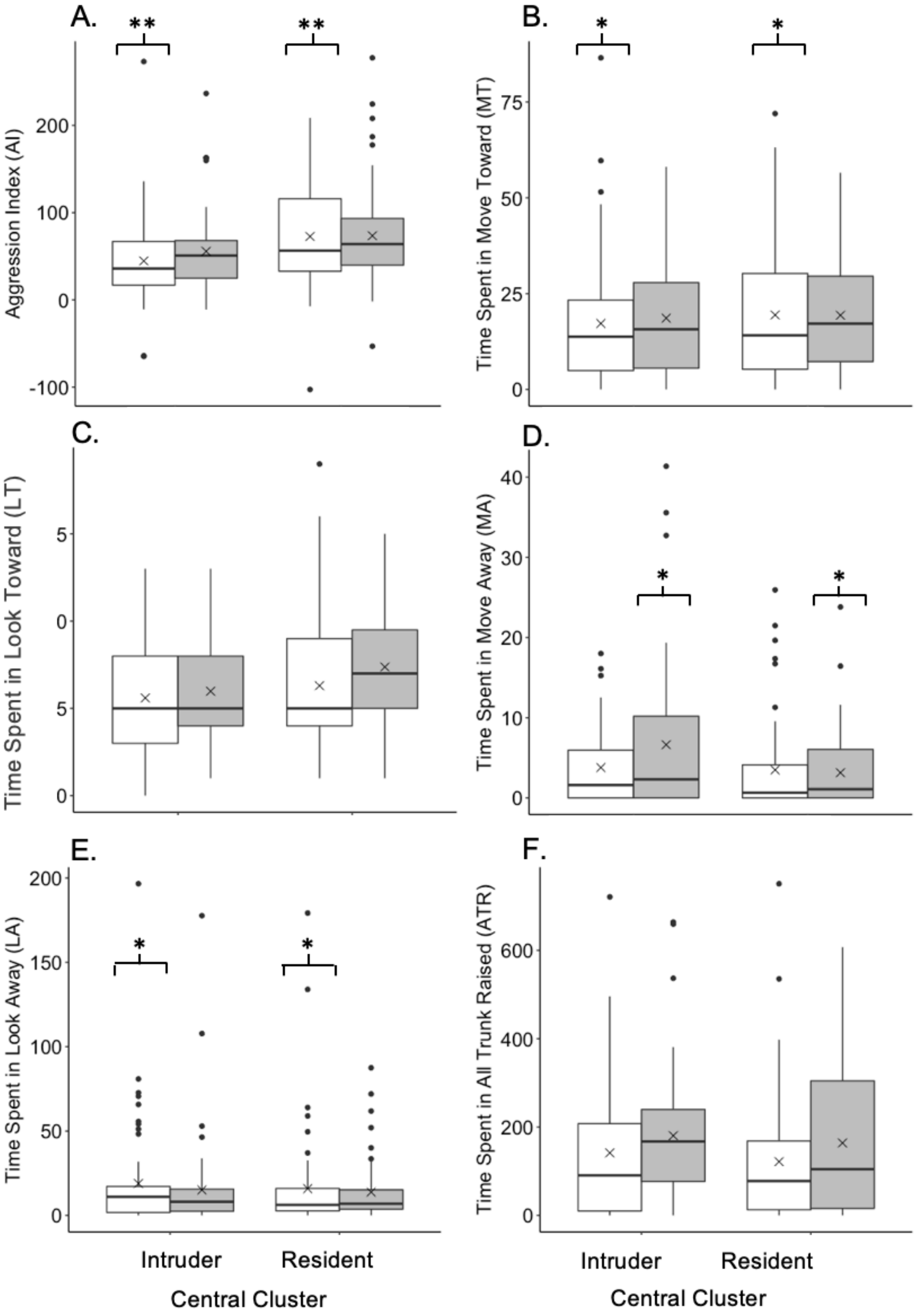
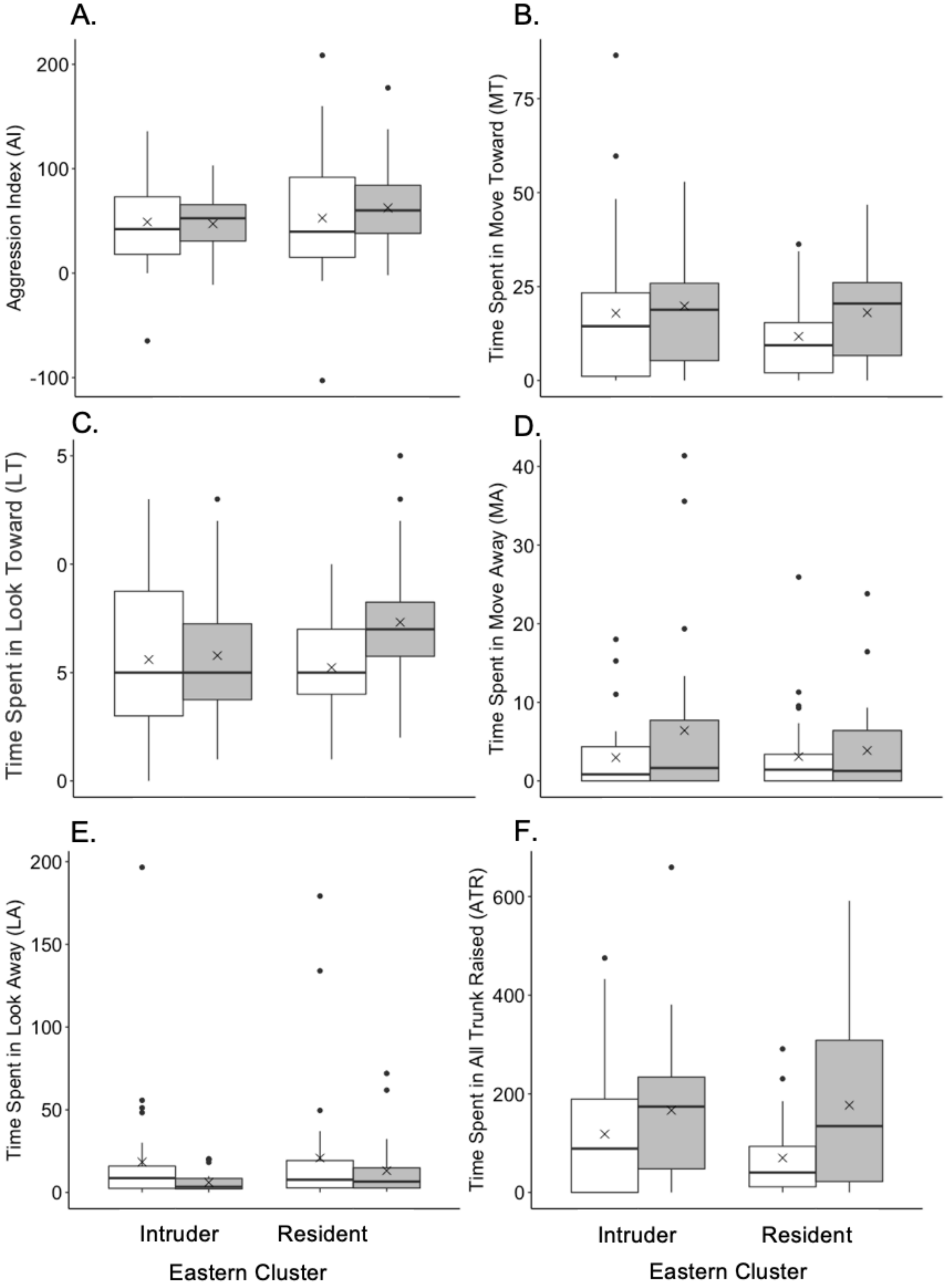

A.
Aggression Index (AI)
200
100
0
-100
**
**
B.
Time Spent in Move Toward (MT)
60
40
20
0
**
**
C.
Time Spent in Look Toward (LT)
15
10
5
0
*
*
D.
Time Spent in Move Away (MA)
20
10
0
E.
Time Spent in Look Away (LA)
150
100
50
0
F.
Time Spent in All Trunk Raised (ATR)
600
400
200
0
**
**
Central
Eastern
Residents
Central
Eastern
Residents

FIG. 1.



A.
**
**
200
100
0
-100
Aggression Index (AI)
B.
*
*
75
50
25
0
Time Spent in Move Toward (MT)
C.
5
0
5
0
Time Spent in Look Toward (LT)
D.
40
30
20
10
0
*
*
Time Spent in Move Away (MA)
E.
200
150
100
50
0
*
*
Time Spent in Look Away (LA)
Intruder
Resident
Central Cluster
F.
600
400
200
0
Time Spent in All Trunk Raised (ATR)
Intruder
Resident
Central Cluster

FIG. 2.



A.
Aggression Index (AI)
200
100
0
-100
B.
Time Spent in Move Toward (MT)
75
50
25
0
C.
Time Spent in Look Toward (LT)
5
0
5
0
D.
Time Spent in Move Away (MA)
40
30
20
10
0
E.
Time Spent in Look Away (LA)
200
150
100
50
0
F.
Time Spent in All Trunk Raised (ATR)
600
400
200
0
Intruder
Resident
Eastern Cluster
Intruder
Resident
Eastern Cluster

FIG. 3.