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DIFFERENTIAL TAIL AUTOTOMY BETWEEN TWO GENETIC CLADES OF A WOODLAND SALAMANDER, PLETHODON CINEREUS

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DIFFERENTIAL TAIL AUTOTOMY BETWEEN TWO GENETIC CLADES OF A
WOODLAND SALAMANDER, *PLETHODON CINEREUS*

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Abstract:

Following the retreat of glaciers in North America, a wide diversity of organisms rapidly migrated into previously uninhabited regions, including animals with limited dispersal abilities such as amphibians. I wanted to better understand the strategies that salamanders use to aid in their dispersal abilities and to understand if patterns of post-glacial range expansion affect phenotypic traits such as anti-predator strategies. In this study, I used existing phylogeographic evidence of clade membership as a framework to examine whether red-backed salamanders (*Plethodon cinereus*) differed in tail autotomy behavior at the range edge versus the range core, and across the geographic range of two clades; one of limited range (the Ohio Clade) and one that has dispersed north along the Atlantic coast into Canada, westward, and south into Michigan and Indiana (the Northern Clade). I hypothesized that salamanders belonging to the Northern Clade and salamanders belonging to range-edge groups would exhibit high tail autotomy frequencies and a greater degree of antipredator postautotomy characteristics. I conducted lab experiments and used museum specimens to examine tail autotomy frequencies and other tail autotomy characteristics, including latency to autotomize under predation pressure, duration of tail movement following autotomy, postautotomy tail movement undulations, and tail regrowth. In my lab experiments, I found significantly higher tail autotomy frequencies within range-core populations and significantly greater postautotomy tail movement duration in my sampled populations from the Northern Clade. Further, I found significantly greater frequencies of autotomy in the Northern Clade in my museum specimen analysis. Therefore, I conclude that *Plethodon cinereus* exhibits geographic variation in tail autotomy frequencies, although the observed patterns of tail autotomy in this study did not align with my initial hypotheses and instead appear to be influenced by several other unidentified variables.

Introduction:

Glacial History and Clade Formation

During the Pleistocene Epoch, shifts in climate drastically changed the landscape of North America. Approximately 23,000 years ago, increases in temperature marked the end of an ice age and the beginning of glacial recession in Eastern North America (Ullman et al. 2015). Post-glacial North America has since experienced a radiation of fauna from southern regions of glacial refugia back into the northern regions, which has been the subject of many studies (Highton and Webster 1976; Davis and Shaw 2001; Xu et al. 2020). Phylogenetic analyses of numerous taxa have also revealed the presence of complex geographic and genetic patterns in the northward expansions of some species (Herman and Bouzat 2016). This is especially true of animals with limited dispersal abilities, such as those constrained to specific environments or those with reduced mobility (Baselga et al. 2012).

Salamanders are one group of organisms that have limited dispersal abilities, which is due to their small size as well as environmental constraints such as narrow moisture, soil, and temperature requirements (Heatwole 1962; Grover 1998; Ochs and Siddig 2017). This is particularly true of salamanders of the family Plethodontidae, which lack lungs and respire through their skin. *Plethodon cinereus* is a plethodontid salamander with a limited dispersal ability (Cabe et al. 2007; Cameron et al. 2019; Marsh et al. 2007), yet has a considerably large range across eastern North America (Petranka 1998) and is considered one of the most abundant vertebrates in the region (Burton and Likens 1975). The ecology, behavior, and genetics of this species have been widely studied due to its abundance and wide distribution (Anthony and Pfingsten 2013; Jaeger et al. 2016). Recent phylogeographic analyses by Radomski et al. (2020)

and Waldron et al. (2024) have revealed the existence of distinct genetic clades of *P. cinereus*. The distributions of these clades are located in different regions of North America (Figure 1). The Northern Clade covers the largest range, extending along the East Coast from Virginia to Canada, and further west around the northern regions of the Great Lakes. In comparison, the Ohio Clade has a relatively small distribution, located mostly in Ohio, with single populations identified in West Virginia and Kentucky. Thus, questions arise regarding possible behavioral differences between these two clades that may have facilitated the greater dispersal of the Northern Clade and resulted in the observed differences in distributions between clades.

<center>*Range-core vs. Range-Edge Populations*</center>

The post-glacial northward expansion of the Northern and Ohio clades would have resulted in spatial sorting of range-core and range-edge populations within each clade as animals dispersed into new habitat at the range front. Many behavioral, morphological, and physiological differences between range-core and range-edge populations have been observed in a wide variety of taxa. For example, there is evidence that range-core populations of cane toads (*Rhinella marina*) are less exploratory and take fewer risks in comparison to range-edge populations (Gruber et al. 2017). Range-edge cane toad populations were also found to have faster growth rates compared to range-core populations, as they experience a greater level of *r*-selection due to lower population densities on range edges (Phillips 2009). Cane toads on invasion fronts have also been found to have longer limbs than cane toads from older, core populations, as longer limbs have been linked to greater speed and aid in dispersal (Phillips 2006). Dispersal rates are higher in populations of cane toads on invasion fronts compared to range-core populations due to the presumed high selective pressure on individuals with greater inclinations of dispersal (Phillips 2008). This trend is not limited to amphibians, as it is observed in other taxa as well.

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For example, range-edge bush-winged cricket populations are comprised of greater numbers of long-winged, more dispersive morphs compared to range-core populations (Simmons and Thomas 2004). A similar pattern has also been observed in lacertid lizards, in which parthenogenesis has been suggested as a strategy that facilitated a rapid post-glacial expansion in the Caucasus. This reproductive mode does not come with the restrictions associated with sexual reproduction and appears to have been selected for in populations on the expanding range front (Darevsky 1966). Thus, range-edge and range-core populations can be expected to differ phenotypically. Such differences need not be limited to dispersal proclivities and related traits such as limb length, but may extend to other traits such as those that influence interactions with predators. Therefore, it is possible that similar differences may be present within species between clades of very different dispersal histories (e.g., the Northern and Ohio clades of *P. cinereus*). However, because these clades are not analogous to range-core and range-edge populations, any differences may be less attributable to the spatial selection observed in range-core and range-edge populations, and instead may be reflective of clade-wide differences in dispersal proclivities.

Predation and Salamanders

Salamanders have a wide variety of anti-predator defenses including behavioral, chemical, and physical mechanisms, including the use of toxins (Ducey et al. 1993; Brodie et al. 2002), anti-predator postures (Brodie et al. 1979; Ducey and Brodie 1983), the detection of and response to chemical cues left by predators (Mcdarby et al. 1999; Sullivan et al. 2002), biting (Brodie et al. 1989), and tail loss (Wake and Dresner 1967; Ducey and Brodie 1983). Salamander species differ in their utilization of these strategies. For example, species with greater degrees of toxicity generally exhibit tail autotomy less frequently than those with lower levels of toxins

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(Ducey et al. 1993). The defensive strategies used by salamanders depend on their evolutionary history and the types of predators they frequently encounter (Brodie et al. 2002).

Predators of *P. cinereus* may include shrews (Brodie et al. 1979), birds (Coker 1931; Grant et al. 2018), and snakes such as the Common Eastern Gartersnake (*Thamnophis sirtalis*) (Surface 1906; Hamilton 1951) and the Ringneck Snake (*Diadophis punctatus*) (Lancaster and Wise 1996). Like other salamanders, *P. cinereus* utilizes several different behaviors to avoid predation. For example, in laboratory trials, *P. cinereus* avoids areas that have been treated with predator chemical cue rinses (Sullivan et al. 2002). *Plethodon cinereus* also utilizes distasteful secretions and anti-predator postures to avoid predation by small mammals (Brodie et al. 1979). Lancaster and Wise (1996) determined that the tail of *P. cinereus* is more attractive to *Diadophis punctatus* compared to the body and suggested that attacks directed to the tail increased chances of survival by redirecting attention to a part of the body which can be autotomized.

Tail Autotomy

Tail autotomy is an anti-predator behavior in which an animal detaches its tail from the rest of its body in order to escape a predation attempt (Wake and Dresner 1967). Tail autotomy is well documented in many species of lizards (Downes and Shine 2001; Bateman and Fleming 2009) and salamanders (Ducey et al. 1993). It has been suggested that animals usually autotomize tails only under extreme stress-inducing events such as a predation attempt (Beneski 1989; Wake and Dresner 1967). Therefore, tail autotomy frequencies can be reflective of predation rates on a population (Bateman and Fleming 2009, but see Medel et al. 1988). Lizard populations exhibit increased tail autotomy frequencies in regions where predator diversity, abundance, and pressure are known to be higher as demonstrated by previous studies (e.g. Cooper et al. 2004). Postautotomy tail movement can also make tail autotomy more effective

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against predation attempts (Dial and Fitzpatrick 1983; Labanick 1984). Often, tail movement will continue for a brief period after the tail has been autotomized by the animal, which is used to further distract the predator from the escaping animal. The total amount of time it takes a predator to handle and ingest an autotomized tail increases with the amount of postautotomy tail movement after a predation attempt (Dial and Fitzpatrick 1983). Thus, an autotomized tail that exhibits a greater amount of movement will increase the probability that the prey escapes.

However, there are trade-offs that occur with tail autotomy (Maiorana 1977). Lizards and salamanders frequently use their tails to store energy reserves, and losing a tail is analogous to losing resources (Yurewicz and Wilbur 2004; Vitt et al. 1977). Energetic trade-offs associated with tail autotomy can have profound effects on reproduction as well, with higher tail autotomy frequencies being linked to decreased production of new ova in *P. cinereus* (Yurewicz and Wilbur 2004). Additionally, locomotive abilities may be negatively affected by tail loss, which would decrease chances of survival in future interactions with predators (Arnold 1982). Tail autotomy severely negatively affects different types of movement and mobility, including swimming (Marin et al. 2010), running (Hileman 1995), and jumping (Hessel et al. 2017) in plethodontid salamanders, as well as running (Brown et al. 1995) and jumping (Gillis et al. 2013) in lizards. Tail condition may also be important in agonistic and territorial interactions (Wise and Jaeger 1998; Wise et al. 2004), and a missing tail may decrease future reproductive potential, likely due to loss of territories, resources, and mates associated with autotomy (Jaeger et al. 1981; Mathis 1991). In male *P. cinereus*, the underside of the tail is biofluorescent and may function as a signal in male-male competition for territories and mates (Anthony et al. 2023). Thus, loss of the tail may hinder honest signaling of a male's quality to potential mates or competitors. Therefore, the relationship between tail loss and territoriality may have important

implications for differences between *P. cinereus* clades, as there is evidence that individuals from the Northern Clade are less territorial than individuals from other regions (Quinn and Graves 1999).

Hypothesis and Predictions

Like many species of salamanders, *P. cinereus* often exhibits tail autotomy as one of many anti-predation behaviors (Wake and Dresner 1967). Tail autotomy in *P. cinereus* has been the subject of a number of studies, although many have focused on tail autotomy within the context of color morph (Moreno 1989; Otaibi et al. 2017; Venesky and Anthony 2007) or territorial interactions (Wise and Jaeger 1998; Wise et al 2004). In the present study, I examined geographic variation in tail autotomy frequencies and postautotomy characteristics within the context of the post-glacial distributions of the Northern and Ohio clades of *P. cinereus*.

I attempted to answer the following questions: 1) Do the Northern and Ohio clades of *P. cinereus* exhibit different relative frequencies of tail-autotomy? 2) Does the duration of applied force needed to induce autotomy differ between the Northern and Ohio clades? 3) Does postautotomy tail movement differ between the Northern and Ohio clades? And 4) Do tail regrowth rates differ between the Northern and Ohio clades? If the Northern Clade of *P. cinereus* is more dispersive and exploratory than the Ohio Clade due to its large geographic range and rapid north and westward expansion from glacial refugia, I predicted that individuals in the Northern Clade experience increased predation pressures from increased dispersal rates (Daly et al. 1990; Dodd and Brodie 1976; Norrdahl and Korpimäki 1998), and thus I also predicted that they would exhibit increased tail autotomy frequencies as a result (Figure 2). Additionally, I expected that intact tails would be more valuable to salamanders from the Ohio Clade where territoriality is presumably more intense compared to within the Northern Clade (Quinn and

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Graves 1998), and where territorial residents with intact tails are at a competitive advantage in agonistic interactions (Wise and Jaeger 1998). I predicted that, in predatory simulations in the laboratory, individuals from the Northern Clade would autotomize tails more quickly than those from the Ohio Clade. I predicted that salamanders from the Northern Clade would increase energy investment in their tails and this investment would be evident in differences in postautotomy tail movement between salamanders from the two clades. Specifically, I predicted that salamanders from the Northern Clade would have a greater duration of postautotomy movement and increased frequencies of postautotomy undulation compared to the Ohio Clade, a difference that would serve to enhance their ability to survive an interaction with a predator. Lastly, I predicted that salamanders from the Northern Clade would exhibit faster postautotomy tail regrowth rates compared to the Ohio Clade, because the presumed increased predation pressures would select for individuals that could rapidly regrow tails and therefore regain the ability to employ autotomy at a quicker rate (Emberts et al. 2019).

Similarly, I also sought to answer the following questions for range-core populations and range-edge populations within each of the two clades: 1) Do within-clade range-core and range-edge populations of *P. cinereus* exhibit different relative frequencies of tail-autotomy? 2) Does the duration of applied force needed to induce autotomy differ between the range-core and range-edge populations? 3) Does postautotomy tail movement differ between range-core and range-edge populations? And lastly, 4) Do tail regrowth rates differ between range-core and range-edge populations of *P. cinereus*? I predicted that members of range-edge populations of *P. cinereus* would exhibit higher tail autotomy frequencies compared to range-core populations as a result of increased dispersal rates, and in turn increased predation pressures. I predicted that range-edge populations would autotomize tails more quickly under a constant force than range-core

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populations. I predicted that postautotomy tail movement would have a greater duration and that postautotomy undulation frequencies would be greater in range-edge populations compared to the range-core populations. Lastly, I predicted that range-edge populations would exhibit faster tail regrowth rates compared to range-core populations. Ultimately, I expected to find increased tail autotomy frequencies, lower latencies to autotomize, a greater degree of postautotomy tail movement, and faster regrowth rates in both range-edge populations and populations in the Northern Clade, as summarized by the "predation hypothesis" outlined in red in Figure 2.

Alternative predictions

Although tail autotomy events mainly occur under life threatening predation attempts, some species may also autotomize tails in territorial and agonistic interactions. Higher tail autotomy frequencies occurred in territorial *P. cinereus* compared to non-territorial individuals (Mathis 1991). Increased territoriality provides the owner access to shelter, mates, and prey resources, which may allow for faster regeneration of autotomized tails (Mathis 1991, Jaeger 1981). Thus, the cost of autotomy may be offset by territory ownership, resulting in an increased propensity to autotomize. Populations associated with higher dispersal rates and less territoriality, such as those in the Northern Clade or at the range edge, may have lower tail autotomy frequencies compared to those with greater resource access or higher levels of intraspecific competition (Mathis 1991). It is possible that populations with higher dispersal rates would not have the resource and energy benefits associated with holding a territory (Jaeger 1981) and may need to maintain tail condition in the event of a predation attempt (Labanick 1984). I present my main predation hypothesis as well as these possible alternative explanations in Figure 2.

Methods:

Preliminary/Control Experiment

I conducted a pilot study and an experiment in August of 2021 to confirm that my laboratory protocol was a valid and reproducible method to induce tail autotomy in *P. cinereus*. Salamanders from prior, unrelated experiments were used in the pilot study experiments. These animals were first used to determine an effective procedure to induce autotomy, including experimental setup, placement of calipers, and applied force on tails. A lab experiment was then conducted in which a treatment group was compared to a control group. For the treatment group, autotomy was induced using an adapted version of the methods described in Cooper et al. (2004), in which calipers were used to hold the animal by the tail, mimicking the behavior of a predator grasping prey. These methods are further described in detail below and are similar to the methodology used for tail removal in genetic studies (Cameron et al. 2019; Waldron et al. 2019). For the control group, no force was applied using the calipers, but all other conditions of the procedure were maintained. A Fisher's exact test was used to test for differences between groups because expected values of count data were less than 5.

Overview of Experimental Design

To examine my research questions, I employed a two-part study design. For the first part of my design, I examined a large sample of field collected preserved museum specimens from core and edge populations within the Ohio and Northern clades. I used these samples to estimate tail autotomy frequencies under natural predation pressures in the field.

For the second part of my design, I collected live individuals from range-edge and range-core localities within the ranges of both the Northern and Ohio clades. I then conducted a controlled lab experiment in which tail autotomy was induced, and frequency of tail loss, latency

to autotomize, duration of postautotomy tail movement, and initial frequency of postautotomy

tail thrashing movements were recorded. This experiment examined the genetic predisposition to

autotomize tails using simulated predation. I employed this laboratory component because

relying solely on museum specimens may not account for different rates of predation and other

environmental variables that could affect tail autotomy frequencies in the field. The laboratory

experiment also allowed me to examine latency and postautotomy movement, measures that are

not possible to collect from preserved specimens.

Collection Localities for Museum Specimens and Laboratory Work

Museum specimens and live salamanders were collected from four localities: A range-

core locality and a range-edge locality for the Northern Clade and a range-core locality and

range-edge locality for the Ohio Clade. Museum specimens from the Cleveland Museum of

Natural History, the University of Michigan Museum of Zoology, and the Carnegie Museum of

Natural History were photographed. For the Northern Clade, localities in western, previously

glaciated Indiana were selected to represent range-edge populations, and localities in

southeastern Pennsylvania represented range-core populations. For the Ohio Clade, localities in

northern, previously glaciated Ohio represented range-edge populations, and localities in

southeastern Ohio were selected to represent range-core populations (Tables 1 and 2). I selected

localities for live specimen collection that were in the general vicinity (within approximately 50

km) of the above museum localities.

Estimation of Autotomy Frequencies from Museum Specimens

Digital photographs of ventral sides of individuals were taken at each museum using a

Panasonic Lumix DMC-FZ150 digital camera. In order to control for differences that may result

from age, sex, and morph (Grant et al. 2018; Meshaka and Wright 2017), only striped adults with

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a snout-vent length (SVL) over 32 mm were included in this study, and approximately an equal number of females and males were used. Individuals that had clear signs of autotomy were identified. Clear signs of autotomy were characterized by an apparent and drastic change in tail width along the length of the tail, followed by either no additional tail material posterior of the autotomy point or by a section of a much thinner tail, signifying regrowth. Individuals with fresh autotomy (no signs of healing or regrowth) were not included in this study to avoid possible autotomy events caused by specimen collection or handling.

Estimation of Autotomy from Simulated Predation

Plethodon cinereus were collected from the above-described localities in the fall of 2021 (specific sites are presented in Table 1). From each site, 30 individuals were collected. However, due to very low autotomy rates in the Ohio range-edge group, an additional 10 salamanders were collected, resulting in 40 individuals collected for that group. These additional 10 salamanders were included in the experiment to increase sample sizes for latency, postautotomy tail movement, and regrowth variables, as only four individuals autotomized tails out of the initial sample size of 30 for the Ohio range-edge group. Due to potential differences in antipredator behavior, agonistic behavior, and diet between striped and unstriped color morphs in *P. cinereus* (Venesky and Anthony 2007; Anthony et al. 2008; Reiter et al. 2014; Stuczka et al. 2016), all animals used were the striped morph and were adults with an SVL greater than 32 mm. Approximately equal numbers of males and females were collected (~15 at each site). After collection, animals were transported to the Anthony/Hickerson laboratory (IACUC #2103) and housed at 17º C on a filter paper substrate and under a natural photoperiod for 48 hours before trials began. Measurements including SVL, tail length, and tail diameter (at 0.5x tail length from

posterior edge of vent) were recorded for each individual. No food was given to animals prior to the trials.

Tail autotomy was induced using calipers similar to the methods described in Cooper et al. (2004), yet was not forced or ensured. Rather, this created a situation for the salamander that encouraged autotomy as described in Beneski (1989) (Figure 3). Moistened Kimwipes® were used to position the animal. Vernier calipers were used to apply force to a salamander's tail and were positioned at 0.5x tail length (from posterior side of vent) and closed to 0.5x tail diameter in order to simulate the pinching/grasping force of a predator. This procedure is also similar to that used for excision of tail tips for genetic analysis (Cameron et al. 2019; Waldron et al. 2019). Trials started when the Kimwipe® was removed from the animal to allow for independent movement. Latency to autotomize was recorded in seconds. If no autotomy occurred after 120 seconds, the calipers were removed. The number of individuals that autotomized their tails was recorded, along with the latency to autotomize for each individual. Duration of movement by autotomized tails was recorded, and the duration ended when the tail did not complete one oscillation for 10 seconds. One oscillation was defined as the movement of the tail from its starting position to the inversion of the tail, and back to the starting position; one undulation refers only to the movement of the tail from its starting phase to the inversion, or one half of an oscillation. Initial frequency of tail undulations was also recorded, in which the number of tail undulations during the first 10 seconds after autotomy were recorded. Postautotomy tail length was recorded for animals that successfully autotomized as the initial length for the regrowth portion of this study.

Estimation of Tail Regrowth Rates

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After autotomy trials, animals were housed in the lab under the same conditions described above, with the exception of a leaf litter substrate instead of the filter paper substrate used for lab trials. Salamanders were provided ~20 *Drosophila melanogaster* flightless fruit flies and water every week for three months following autotomy trials. I calculated the difference between the tail length after a regrowth period of three months and the tail length immediately following autotomy. This difference was used to compare tail regrowth rates between groups. Regrowth measurements were temporally offset according to the time since initial autotomy trials for each group to ensure that regrowth was measured at equal intervals for each group.

Statistical Analysis

Generalized linear models (GLMs) were used to analyze tail autotomy frequencies (in both lab and museum specimen analyses), postautotomy variables, and tail regrowth. I used GLMs due to the binary nature of autotomy data, and because ANCOVA assumptions could not be met for continuous variables, including lack of homogeneity of variance and data departure from normality. For each response variable (proportion autotomized for museum and laboratory data, latency to autotomize (s), autotomized tail movement duration (s), number of undulations, and regrown tail length), I examined four different models; a model including the interaction between clade and range membership as fixed effects (model A), a model containing both clade and range membership as fixed effects (model B), a model containing solely clade membership as the fixed effect (model C), and a model containing solely range membership as the fixed effect (model D). I included the interaction between clade and range membership as a fixed effect in model A to examine whether the effect of clade depended on range membership, and vice versa. I examined the other models that did not include the interaction so that I could rank several different model structures for each variable and select the most explanatory model

accordingly. SVL was also included as a covariate for each model but was not statistically significant in any of the models. Therefore, the effect of SVL was not reported in the results. Sex was not included as an effect in the models because I collected approximately even numbers of males and females from each population and to my knowledge, no studies report a sexual dimorphism of tail autotomy frequencies in *P. cinereus*. Models were selected using AIC scores and Akaike weights (Symonds and Moussalli 2011). All analyses were conducted in R Statistical Programming Software (R Core Team 2022).

Results:

Pilot experiment:

No salamanders in the control group autotomized their tails, and over 50% of the treatment salamanders autotomized ($n = 15$, $P < 0.005$). These results indicate that my methods were in fact effective at causing autotomy, and that autotomy is unlikely to occur without applying a force. Additionally, because nearly 50% of the salamanders that experienced simulated predation retained their tails, the methodology was effective in creating a scenario wherein the salamanders were free to choose to autotomize or not. This is important because I was interested in learning to what degree salamanders from different populations were predisposed to autotomy.

Model Averaging

Due to similar AIC values between linear models, I used full-model averaging according to the procedures presented in Symonds and Moussalli (2011). Models with a delta-AIC of less than 6 were included in the averaged model, and models with a delta-AIC of greater than 6 were excluded. Models with an Akaike weight of less than 0.05 were also excluded from averaged

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models. I employed model averaging for every variable, because there were several models within each variable that had very low delta-AIC values which were included in the model averaging. Unless otherwise stated, all models were included in the averaged model.

Museum Results:

Tail Autotomy Counts

Autotomy counts for museum specimens are presented in Table 3, and corresponding models are described in Table 4. Model 1D was excluded from the averaged model due to having an Akaike weight of less than 0.05. Therefore, only models 1A, B, and C were included in the averaged model. The effect of clade was significant in the averaged model (z = -2.172, P = 0.030, Table 4) and in models 1A, B, and C, in which the Northern Clade exhibited autotomy frequencies 1.4 - 2.2 times Ohio Clade individuals (Figure 4). The effect of range and the interaction between clade and range were not significant in any model.

Lab Results:

Frequency of Tail Autotomy

Autotomy frequencies are presented in Table 5, and the corresponding models are described in Table 6. Model 2C was excluded from the averaged model due to having a delta-AIC of > 20. Therefore, only models 2A, B, and D were included in the averaged model. The effect of range was significant in models 2B, C, and the averaged model (z = -2.100, P = 0.036, Table 6), in which the range-core groups exhibited autotomy frequencies 1.5 – 3.8 times greater than range-edge individuals (Figure 5). There was no effect of clade membership on frequency of tail autotomy in the laboratory study.

The interaction between range and clade in model 2A was significant, wherein range-edge vs range-core within the Ohio Clade differed to a greater degree than in the Northern Clade. Ohio Clade range-core individuals had the highest frequency of autotomy with 73.3% of individuals from that group autotomizing during trials, while Ohio Clade range-edge individuals had the lowest frequency of autotomy with 17.5% of individuals autotomizing tails during trials (Figure 5). However, upon further examination of the data, the effect of the interaction appeared minimal and the interaction was not significant in the averaged model.

Latency to Autotomize

Latency data are presented in Table 7, and corresponding models are described in Table 8. There were no significant fixed effects for latency across all four models (models 3A-D) or in the averaged model for latency (Table 8, Figure 6).

Duration of Post Autotomy Tail Movement

Post autotomy tail movement duration data are presented in Table 9, and corresponding models are described in Table 10. Model 4D was excluded from the averaged model due to a relatively high delta-AIC (~9) and low AIC weight. Therefore, only models 4A, B, and C were included in the averaged model.

For models 4A, C, and the averaged model, there was a significant effect of clade ($z = -3.061$, $P = 0.002$, Table 10), in which mean duration of postautotomy tail movement for the Northern Clade was 1.6x higher than postautotomy duration for the Ohio Clade (Figure 7). The interaction between range and clade in model 3A was significant, in which the range-edge population in the Northern Clade had a ~4% greater duration of postautotomy movement compared to the Northern Clade range-core populations, while the range-edge population in the

Ohio Clade had a ~2% lower duration of postautotomy movement compared to range-core populations in the Ohio Clade. However, this interaction was not significant in the averaged model (Table 10).

Post Autotomy Tail Undulations

Postautotomy tail undulation frequency data are presented in Table 11, and corresponding models are described in Table 12. Range was a significant predictor in model 5A in which range-edge populations exhibited lower undulation rates than sampled range-core populations (Figure 8). However, range was not statistically significant in models 5B and D or in the averaged model (Table 12). No other fixed effects were significant across all models.

Regrowth Results:

Regrowth data are presented in Table 13, and corresponding models are described in Table 14. There were no significant fixed effects for regrowth across all four models (models 6A-D) or in the averaged model (Table 14, Figure 9).

Discussion:

In summary, I observed significant geographic variation in tail autotomy frequencies in both my museum specimen analysis and in my lab experiment, although the results differed between the two studies. The duration of autotomized tail movement was the only postautotomy trait that significantly differed between clades; the Northern Clade exhibited longer postautotomy movement compared to the Ohio Clade. There were no significant differences in latency to autotomize or in tail regrowth between any groups.

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My results show that there are clear differences in tail autotomy frequencies across the geographic range of this widespread species. I observed higher tail autotomy frequencies in the Northern Clade in the museum specimen analysis. My predation hypothesis is one possible explanation for the observed results in museum specimens. I predicted that individuals in the Northern Clade may exhibit a greater propensity to autotomize based on their likelihood of having a greater degree of exploratory, bold, and risk-taking traits, and therefore would be more likely to experience predation attempts. This suite of exploratory traits aligns with their history of movement/range expansion and may explain the high frequencies of tail autotomy observed in these populations. Although the museum results are consistent with my predation hypothesis, they are not clearly consistent with the results from the lab experiments, which may imply a relationship between tail autotomy and seasonality or other untested variables. This study was designed to account for seasonality by studying groups at roughly the same latitude and by conducting the experiment within a short timeframe. However, it is possible that adaptations to harsher climates, as experienced by much of the Northern Clade, may result in seasonal lower tail autotomy frequencies as a tradeoff to avoid losing fat reserves, especially when the active foraging season is relatively short. Other recent research on *P. cinereus* behavior including boldness (Garner et al. 2024) and agonistic behavior (Ryan et al. 2024) have had inconsistent conclusions regarding a clear effect of clade.

I conducted the museum analysis to examine natural autotomy frequencies as they occur in the field. Therefore, autotomy frequencies should reflect the natural predation rates for each locality. Predation rates may vary between these groups and should be examined further to determine their effect on tail autotomy frequencies. Predation rates due to local predator species,

seasonality, overall predator abundance, or salamander exploratory behavior could have driven differences within specimens preserved as collected (Medel et al. 1988; Fox et al. 1994; Cooper et al. 2004), whereas the lab component of this study focused solely on predisposition to autotomize. It is important to consider the effect of predator efficiency on the museum specimen data. Regions with more efficient predators may appear to have lower rates of autotomy because predators are successful in their predation attempts, thus removing the salamander from the sample pool whether it autotomized or not. Conversely, regions with less efficient predators may exhibit higher autotomy rates due to the high predation attempt-success ratio. Regional differences in types of predators may account for such differences in efficiency (Medel et al. 1988; Fox et al. 1994). Different types of predators may also differ in their responses to autotomy; where some predators may consume and handle tails, others may ignore autotomized tails and continue to focus on the prey, making autotomy less effective against predators that can consistently make the decision to ignore tails (Emberts et al. 2019). Biodiversity decreases with increasing latitudes, including biodiversity of predators (Mannion et al. 2014). Additionally, due to dispersal ability, bird and mammal diversity is considerably greater than amphibian and reptile diversity at northern latitudes (Jenkins et al. 2013, Jenkins et al. 2015). The interactions between *P. cinereus* and mobile predators such as birds and mammals may differ profoundly from interactions between *P. cinereus* and more ambush-oriented predator taxa, including amphibians such as *Pseudotriton ruber* (Bock and Fauth 1992; Bruce 2008), reptiles such as *Diadophis punctatus* (Lancaster and Wise 1996) and *Thamnophis sirtalis* (Sullivan et al. 2002), and possibly some invertebrate predators (Jung et al 2000; but see Hickerson et al 2018). It is possible that the Northern Clade in fact experiences greater rates of inefficient predation, thus increasing the observed autotomy frequencies in the museum experiment results. However, for

unknown reasons perhaps not detected due to my methods to induce autotomy, this may not translate into a genetic predisposition to autotomize as expected. This may help explain the disparity between the results of the lab and museum experiments, and therefore, these two components of the study may not be directly comparable.

I observed greater tail loss in range-core populations compared to the range-edge populations in the laboratory experiment, which was contrary to my predation hypothesis. Further research is necessary to better understand differences in autotomy frequencies between these groups and my results highlight the importance of understanding geographic variation in both predation rates on *P. cinereus* and resource use. Possible differences in territoriality between range-core and range-edge groups within each clade may result in disparities in resource accessibility, which may influence tail autotomy rates (Mathis 1990; Quinn and Graves 1999). The resource access associated with controlling a territory may provide individuals with consistent food sources and quality habitat, which in turn may reduce the consequences of an autotomy event by offsetting the costs associated with autotomy (Mathis 1990). Tail loss resulting from interactions with other *P. cinereus* may also affect total autotomy rates in populations. It seems unlikely, however, that a genetic predisposition to autotomy would arise in the context of territorial disputes, especially when maintaining an intact tail is important in mating interactions (Dyal 2006; Anthony et al. 2023) and future territorial disputes (Martin and Salvador 1993; Wise and Jaeger 1998). Thus, it does not appear to be a valuable strategy to autotomize a tail during territorial encounters when there is a greater risk of predation, especially if there are low chances of mortality within resident/intruder interactions (however, see Itescu et al. 2017 for an example of intraspecific aggression driving tail loss in lizards). While tail autotomy as a result of territorial interactions has been observed (Jaeger 1981), research has

indicated that tail autotomy only occurs under extreme stress and with applied force that allows

salamanders to twist their tails off (Wake and Dresner 1967, Beneski 1989). Future research

should explore the frequency of mortality during these interactions, which may provide insight

into mechanics of inducing autotomy in salamanders. It is possible that tail autotomy could be an

example of a fixed action pattern (Waters 2018), where autotomy frequently occurs when a few

conditions or key stimuli are met regardless of the contextual origin of those stressors (such as a

bite from a conspecific compared to a bite from a predator).

Postautotomy Tail Movement

The lab experiments showed that postautotomy duration of tail movement was

significantly higher in the Northern Clade. This aligns with my findings of increased autotomy

frequencies in the Northern Clade in the museum specimen analysis. I predicted that

postautotomy tail movement would last longer in the Northern Clade as part of my predation

hypothesis. If the Northern Clade of *P. cinereus* truly disperses at a greater rate or exhibits higher

activity levels, it is possible that the associated increase in risk of predation could drive the

duration of postautotomy tail movement. However, it is important to consider why variation

might exist within the trait of postautotomy movement duration at all. Increased duration of

postautotomy tail movement should be a beneficial trait in surviving predation attempts by

diverting the predator's attention to the detached tail (Labanick 1984; Otaibi et al. 2017).

Therefore, I would expect that it would be beneficial for all groups to exhibit long durations of

postautotomy movement due to the presumed increase in fitness from expressing such a trait

(increased postautotomy movement should increase survivorship from a predation event, and

thus increase chances of reproduction). Yet it is likely that there are tradeoffs that drive variation

in postautotomy tail movement, and it is possible that *P. cinereus* has evolved postautotomy

traits that are region specific and will increase overall fitness based on a suite of local challenges (Maiorana 1977; Cooper et al. 2004; Pierce and Gonzalez 2019). For example, a larger tail that supports greater musculature may be advantageous to have in a predation attempt, as it may be a larger, more visible target and may allow for faster, more sustained postautotomy movement (Daniels et al. 1986; Jamison and Harris 1992; Fox et al. 1994). However, growing and maintaining a large tail is heavily resource intensive (Fernández-Rodríguez and Braña 2022), which may not be viable for certain individuals in all environments. Allocating resources to a larger or longer tail may decrease the amount of resources being allocated to grow overall body size, which in turn may have adverse effects in agonistic interactions (Mathis 1990; Jamison and Harris 1992; Russel et al. 2014; Pierce and Gonzalez 2019; Fernández-Rodríguez and Braña 2022). Additionally, it is possible that larger tails are more difficult to detach from the body in a predation attempt, which may nullify any postautotomy advantages of growing a large tail (Fox et al. 1994). Dial and Fitzpatrick (1983) demonstrated that lactate concentrations in thrashing, autotomized *Scincella lateralis* tails were eight times greater than intact tails, and thrashing tails were more successful in directing predator attacks compared to exhausted tails. This was observed to a lesser degree in *Anolis caroliniensis* tails, suggesting that different species of lizards may invest more heavily in anaerobic metabolism in the tail compared to other species, and that extended tail thrashing may be limited by energy and resource demands. Allocation of energy into glycogen stores in the tail and the consequent metabolization into lactate (Dial and Fitzpatrick 1983) is likely a costly investment that may detract from other energy-intensive life history traits such as reproduction, growth, territory defense, and other antipredator behaviors. Infrequent predation on populations or the employment of other anti-predator strategies may

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decrease the value of investing energy resources into such postautotomic traits (Otaibi et al. 2017).

I predicted that latency to autotomize would be shorter in the Northern Clade and in range-edge populations as part of my predation hypothesis; however, my results did not indicate any relationship between clade membership or range membership and latency to autotomize. Interestingly, other studies have found a clear relationship between population predation pressure and genetic predisposition of greater latency or force required to autotomize in lizards (Fox et al. 1994; Cooper et al. 2004). It is possible that this does not directly translate to *P. cinereus*, and the extent to which the resource availability tradeoff plays a role in latency to autotomize should be examined further. It may be a greater risk to have a shorter latency to autotomize for individuals that have low access to food resources (for example, individuals that have a shorter foraging season), because they are sacrificing fat stored in their tail for antipredator defense. Aside from losing stored resources, tail autotomy reduces chances of survival (Arnold 1982; Ducey and Brodie 1983), as animals can no longer rely on autotomy to escape future predation events. This tradeoff has been demonstrated in brown anoles (Kuo et al. 2015), in which bolder individuals required less force to autotomize tails than more reserved individuals, but only with unrestricted access to food.

I predicted that individuals from the Northern Clade would exhibit greater postautotomy undulations as part of my predation hypothesis. Increased frequencies of tail movement should attract the attention of a predator more than a tail that exhibits less movement (Cooper et al. 2004), similar to my prediction that postautotomy tail movement would be more effective at attracting a predator's attention. However, I did not observe any significant relationship between clade or range membership and postautotomy undulation frequencies. I would expect the

mechanism of postautotomy undulation frequencies to be similar for postautotomy tail movement duration—as an effect of musculature, chemistry, and stored energy resources (Dial and Fitzpatrick 1983; Daniels et al. 1986; Jamison and Harris 1992; Fox et al. 1994).

Tail Regrowth

I observed no significant relationship between the amount of tail regrowth over a three-month period and clade or range membership. I predicted that animals with territory ownership would have the resource availability to regrow tails at a quicker rate than less territorial individuals. Therefore, under a feeding regime that provided standardized food access across all individuals, I predicted that less territorial, more exploratory individuals such as those from the Northern Clade (see Quinn and Graves 1999; Stapley and Keogh 2004) would allocate an increased proportion of energy to more rapid tail regrowth in order to maintain tail condition in the event of a predation attempt. Pierce and Gonzalez (2019) examined tail autotomy frequencies in two populations of *Eurycea* salamanders and found that relative tail width (as a measure of body condition) was only negatively affected by tail loss in one of the two populations studied, suggesting that populations may regrow tails differently depending on energy allocation. Although I did not observe any significant differences in tail regrowth between groups, I did not account for body condition in the tail regrowth measurements. Species that utilize tail autotomy as an anti-predator strategy prioritize the growth of the tail even when such prioritization incurs a loss of fat reserves (Jamison and Harris 1992; Fernández-Rodríguez and Braña 2022). Future studies should examine the effects of tail loss and body condition on each other and examine differences in trends of regrowth between populations with respect to morphometry.

Several factors can influence the rate at which salamanders regrow their tails. Marvin (2010) reported that regeneration time of the tail increased with body size in *Desmognathus*

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quadramaculatus. This suggests that the cost of autotomy may be greater for larger individuals compared to smaller individuals, as smaller individuals take significantly less time to regenerate. Salamanders receiving corticosterone treatments exhibited significantly less regrowth compared to salamanders receiving a control (Lewis and Sullivan 2020). Similarly, caudal regeneration in *Desmognathus ochrophaeus* was lower in individuals that had been exposed to predator kairomones compared to individuals that were not exposed (Payette and Sullivan 2019). Therefore, stress appears to have adverse effects on salamander tail regeneration across several species. Additionally, several other factors including temperature and food availability have been shown to affect the rate at which autotomized individuals can regenerate tails (Marvin and Lewis 2013). The salamanders in this study were all of adult size, were fed in the same manner, and presumably experienced similar levels of stress, so it may not be surprising that I did not detect differences in tail regrowth after autotomy. The lack of significant clade or range effects on regrowth rates in the laboratory does suggest, however, that this ability may not respond to predation risk or be related to other dispersal linked traits.

Conclusions

My results did not clearly support either my predation or territorial hypotheses. I expected to observe increased tail autotomy frequencies in the Northern Clade and in range-edge populations, because I predicted that a greater genetic predisposition to autotomize would be beneficial in more exploratory and mobile populations. In my lab trials, I did observe increased postautotomy movement duration in the Northern Clade, but I simultaneously and unexpectedly observed increased autotomy frequencies in range-core populations. As previously discussed, morphological differences between range-edge and range-core populations have been observed in a variety of taxa. However, Simmons and Thomas (2004) found that this predisposition to

dispersal was relatively temporary in wing-dimorphic bush crickets, and after 5-10 years there were similar numbers of long-winged morphs between both range-edge and range-core populations. This is consistent with other invasions as well; after a population has been established, high reproductive output is prioritized over dispersal tendency by natural selection (Travis and Dytham 2002). It is possible that the period of time following glacial recession was sufficient to remove genetic range-selective disparities between range-core and range-edge populations in *P. cinereus*. To more clearly understand the role that tail autotomy plays in dispersal, future studies should examine a species that has recently been introduced to a new region, such as introduced wall lizards (*Podarcis* spp.) in the United States. These populations of lizards are the result of relatively recent introductions (Oskyrko et al. 2022), and range-edge populations may be more likely to exhibit the suite of tail autotomy traits as hypothesized in this study, as the range-edge populations would likely still be expanding and not yet in the process of being selected for high reproductive output.

Although my results did not clearly align with either of my original hypotheses, I did observe clear geographic variation in tail autotomy frequencies and traits between populations. Geographic variation in tail autotomy frequencies has also been observed in the Georgetown salamander, *Eurycea naufragia*. Pierce and Gonzalez (2019) found that two populations of *E. naufragia* differed significantly in their frequencies of tail autotomy, and that other factors contributed to patterns of tail autotomy, including seasonality and body condition. Geographic variation in tail autotomy should be further examined for *P. cinereus* and other species to determine the extent of within species variation, and the factors that influence tail autotomy rates should be further explored to identify drivers of variation.

The link between tail autotomy and animal behavior should also be examined further, as there are profound implications of this relationship on salamander ecology when considering spatial variation in tail autotomy traits. Under simulated predation attempts, plethodontid salamanders that had previously autotomized altered antipredator behaviors, such as exploratory movement, escape distance, and temperature preference (Bliss and Cecala 2017). Previously autotomized plethodontid salamanders also spent less time on substrates treated with predator chemical cues, which may stem from the higher risk associated with being tailless in the presence of a predator (Johnson 2011). Shifts in behavior following autotomy may have the capacity to differentially affect reproductive, feeding, and exploratory behavior at the population level depending on variation of tail autotomy frequencies and predation pressures.

Implications of Research

This study presents viable methodology for inducing tail autotomy in plethodontid salamanders for future research. The methods used in this study were adapted from Cooper et al. (2004). My methods were effective in causing ~48% of all animals to autotomize, although I acknowledge that only *P. cinereus* was studied. Beneski et al. (1989) previously attempted to induce autotomy in *Ensatina* salamanders but observed low frequencies of autotomy when using pins or alligator clips. Although I was able to effectively induce autotomy in approximately one half of the salamanders in my trials, these rates are likely not reflective of the rates that would be observed in a field setting because successful predation would remove many of the autotomized salamanders from the possible sampling pool, and predation attempts would likely not be as effective as my simulated trials. This is consistent with the autotomy rates observed in the museum specimen data, in which approximately only 19% of all observed specimens exhibited clear signs of autotomy.

Given the diverse functionality of the tail, it is possible that the use of tail autotomy is a highly adaptive behavior that can vary geographically, dependent on predation pressures, territoriality, and resource abundance. Fully understanding how tail autotomy ties into dispersal behavior may provide a better understanding of the strategies animals may use to avoid impacts of climate change and habitat loss. For example, if autotomic traits relate to dispersal ability, I may be able to use these traits to estimate how certain organisms will fare in their ability to disperse to mitigate the effects of climate change. This is particularly important within amphibians specifically; as a taxonomic group, they are under high risk of extinction. Thus far, salamanders have shown resilience to the effects of a warming climate. Riddell et al. (2018) found that gray-cheeked salamanders (*Plethodon metcalfi*) were able to acclimate quickly to changing climate by reducing cutaneous water loss and slowing their metabolisms. However, it is important to understand the strategies that salamanders may use to minimize the effects of a changing climate, including dispersal abilities. Autotomic traits may comprise a portion of a larger suite of traits that influence dispersal abilities. The ability to disperse to cooler and wetter microclimates will undoubtedly be beneficial within the context of a warming and drying climate – and is of special interest regarding some of the many plethodontid species in the Appalachian Mountain region of the eastern United States, where many species are constrained to considerably small ranges.

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Tables and Figures:

Table 1. Collection localities and sample sizes for lab experiments. For the Ohio Edge group, 30 salamanders were initially collected on October 17, 2021, and an additional 10 (40 total) were collected on November 13, 2021.

Clade	Range	n	State	County	Collection Date
Northern	Core	30	PA	Lancaster	9 Oct. 2021
Northern	Edge	30	IN	Parke	25-26 Sept. 2021
Ohio	Core	30	OH	Hocking	30 Oct. 2021
Ohio	Edge	40	OH	Lorain	17 Oct. and 13 Nov. 2021

Table 2. Localities and sample sizes for museum specimen experiments.

Clade	Range	n	State	Counties
Northern	Core	98	PA	Berks, Bucks, Chester, Lancaster, Montgomery, and adjacent counties
Northern	Edge	131	IN	Fountain, Montgomery, Parke, Vermillion, and adjacent counties
Ohio	Core	89	OH	Athens, Hocking, Vinton and adjacent counties
Ohio	Edge	109	OH	Erie, Huron, Lorain, Sandusky and adjacent counties

Table 3. Tail autotomy frequencies and proportion of tails autotomized for museum specimen data of each group.

Clade	Range	n	# Autotomized	# Not Autotomized	Proportion Autotomized
Northern	Core	98	29	69	0.296
Northern	Edge	131	25	106	0.191
Ohio	Core	89	12	77	0.135
Ohio	Edge	109	15	94	0.138

Table 4. AIC values and predictors for each museum specimen autotomy frequency model with associated z-values and p-values. Interactions are denoted by a colon (:). Supported models are indicated with an asterisk(*) and are bolded. SVL was also included in each model as a covariate, but the statistics were not included in this table. Degrees of freedom are residual degrees of freedom.

Model	AIC	df	Predictor	z-value	P (>|z|)
1A	414.2	422	Clade	-2.457	0.014
			Range	-1.754	0.080
			Clade:Range	1.116	0.265
1B	413.5	423	Clade	-2.439	0.015
			Range	-1.357	0.175
1C	413.3	424	Clade	-2.388	0.017
1D	417.6	424	Range	-1.257	0.209
1 Full-Average (1A, 1B, 1C)*			**Clade**	**-2.172**	**0.030**
			Range	**-0.824**	**0.410**
			Clade:Range	**0.393**	**0.694**

Table 5. Tail autotomy frequencies and proportion of tails autotomized for lab trials of each group.

Clade	Range	n	# Autotomized	# Not Autotomized	Proportion Autotomized
Northern	Core	30	20	10	0.667
Northern	Edge	30	13	17	0.433
Ohio	Core	30	22	8	0.733
Ohio	Edge	40	7	33	0.175

Table 6. AIC values and predictors included in each lab trial tail autotomy count model with associated z-values and p-values. Interactions are denoted by a colon (:). Supported models are indicated with an asterisk(*) and are bolded. SVL was also included in each model as a covariate, but the statistics were not included in this table. Degrees of freedom are residual degrees of freedom.

Model	AIC	df	Predictor	z-value	$P\,(>|z|)$
2A	161.5	125	Clade	0.570	0.569
			Range	-1.797	0.072
			Clade:Range	-2.038	0.042
2B	163.6	126	Clade	-1.323	0.186
			Range	-4.478	< 0.0005
2C	183.5	127	Clade	-1.540	0.124
2D	163.2	127	Range	-4.523	< 0.0005
2 Full-Average (2A, 2B, 2D)*			**Clade**	**0.140**	**0.889**
			Range	**-2.100**	**0.036**
			Clade:Range	**-0.909**	**0.363**

Table 7. Latency (s) data for each group, including sample size, mean latency to autotomize, standard deviation, minimum and maximum latencies, and standard error. The minimum latencies of 0 seconds for the Ohio core and edge groups were recorded when autotomy was virtually instantaneous.

Clade	Range	n	Mean	Stdev	Min	Max	SE
Northern	Core	20	38.5	19.4	3.5	76.7	4.3
Northern	Edge	13	47.6	24.2	11.3	102.5	6.7
Ohio	Core	22	37.3	28.0	0	108.7	6.0
Ohio	Edge	7	51.5	52.0	0	118.7	19.7

Table 8. AIC values and predictors for each autotomy latency model with associated z-values and p-values. Interactions are denoted by a colon (:). Supported models are indicated with an asterisk(*) and are bolded. SVL was also included in each model as a covariate, but the statistics were not included in this table. Degrees of freedom are residual degrees of freedom.

Model	AIC	df	Predictor	z-value	P (>|z|)
3A	592.9	57	Clade	-0.501	0.616
			Range	0.476	0.634
			Clade:Range	0.884	0.377
3B	591.2	58	Clade	0.018	0.986
			Range	1.339	0.181
3C	590.6	59	Clade	-0.113	0.910
3D	588.8	59	Range	1.357	0.175
3 Full-Average (3A, 3B, 3C, 3D)*			**Clade**	**-0.088**	**0.930**
			Range	**0.927**	**0.354**
			Clade:Range	**0.177**	**0.860**

Table 9. Postautotomy movement data for each group, including sample size, mean duration of postautotomy tail movement (s), standard deviation, minimum and maximum durations, and standard error.

Clade	Range	n	Mean	Stdev	Min	Max	SE
Northern	Core	20	261.3	144.5	51.3	496.1	32.3
Northern	Edge	13	272.1	148.7	54.6	484.8	41.2
Ohio	Core	22	165.2	86.1	26.5	342.1	18.4
Ohio	Edge	7	162.3	106.0	50.4	370.7	40.1

Table 10. AIC values and predictors for each postautotomy tail movement duration model with associated z-values and p-values. Interactions are denoted by a colon (:). Supported models are indicated with an asterisk(*) and are bolded. SVL was also included in each model as a covariate, but the statistics were not included in this table. Degrees of freedom are residual degrees of freedom.

Model	AIC	df	Predictor	z-value	P (>|z|)
4A	773.7	57	Clade	-2.663	0.008
			Range	0.124	0.902
			Clade:Range	0.151	0.880
4B	771.2	58	Clade	-3.108	0.002
			Range	0.277	0.782
4C	768.9	59	Clade	-3.192	0.001
4D	777.8	59	Range	0.681	0.496
4 Full-Average (4A, 4B, 4C)*			**Clade**	**-3.061**	**0.002**
			Range	**0.121**	**0.904**
			Clade:Range	**0.038**	**0.970**

Table 11. Postautotomy tail undulation frequency (undulations/10 sec) data for each locality, including sample size, mean number of undulations, standard deviation, minimum and maximum durations, and standard error.

Clade	Range	n	Mean	Stdev	Min	Max	SE
Northern	Core	20	32.6	9.6	9	49	2.2
Northern	Edge	13	26.1	9.1	3	37	2.5
Ohio	Core	22	29.4	5.7	18	39	1.2
Ohio	Edge	7	28.6	3.6	24	32	1.4

Table 12. AIC values and predictors for each postautotomy undulation frequency model with associated z-values and p-values. Interactions are denoted by a colon (:). Supported models are indicated with an asterisk(*) and are bolded. SVL was also included in each model as a covariate, but the statistics were not included in this table. Degrees of freedom are residual degrees of freedom.

Model	AIC	df	Predictor	z-value	P (>|z|)
5A	452.7	57	Clade	-1.316	0.188
			Range	-2.322	0.020
			Clade:Range	1.348	0.177
5B	452.1	58	Clade	-0.664	0.506
			Range	-1.856	0.063
5C	453.1	59	Clade	-0.402	0.688
5D	450.1	59	Range	-1.780	0.075
5 Full-Average (5A, 5B, 5C 5D)*			**Clade**	**-0.445**	**0.656**
			Range	**-1.382**	**0.167**
			Clade:Range	**0.318**	**0.751**

Table 13. Regrowth data for each locality, including sample size, mean regrowth length, standard deviation, minimum and maximum durations, and standard error. Regrowth measurement units were mm.

Clade	Range	n	Mean	Stdev	Min	Max	SE
Northern	Core	20	9.54	2.21	5.04	13.15	0.49
Northern	Edge	13	9.05	1.79	6.5	12.4	0.50
Ohio	Core	21	9.30	3.40	3.88	14.78	0.74
Ohio	Edge	6	8.14	3.99	2.66	12.94	1.63

Table 14. AIC values and predictors for each tail regrowth model with associated z-values and p-values. Interactions are denoted by a colon (:). Supported models are indicated with an asterisk(*) and are bolded. SVL was also included in each model as a covariate, but the statistics were not included in this table. Degrees of freedom are residual degrees of freedom.

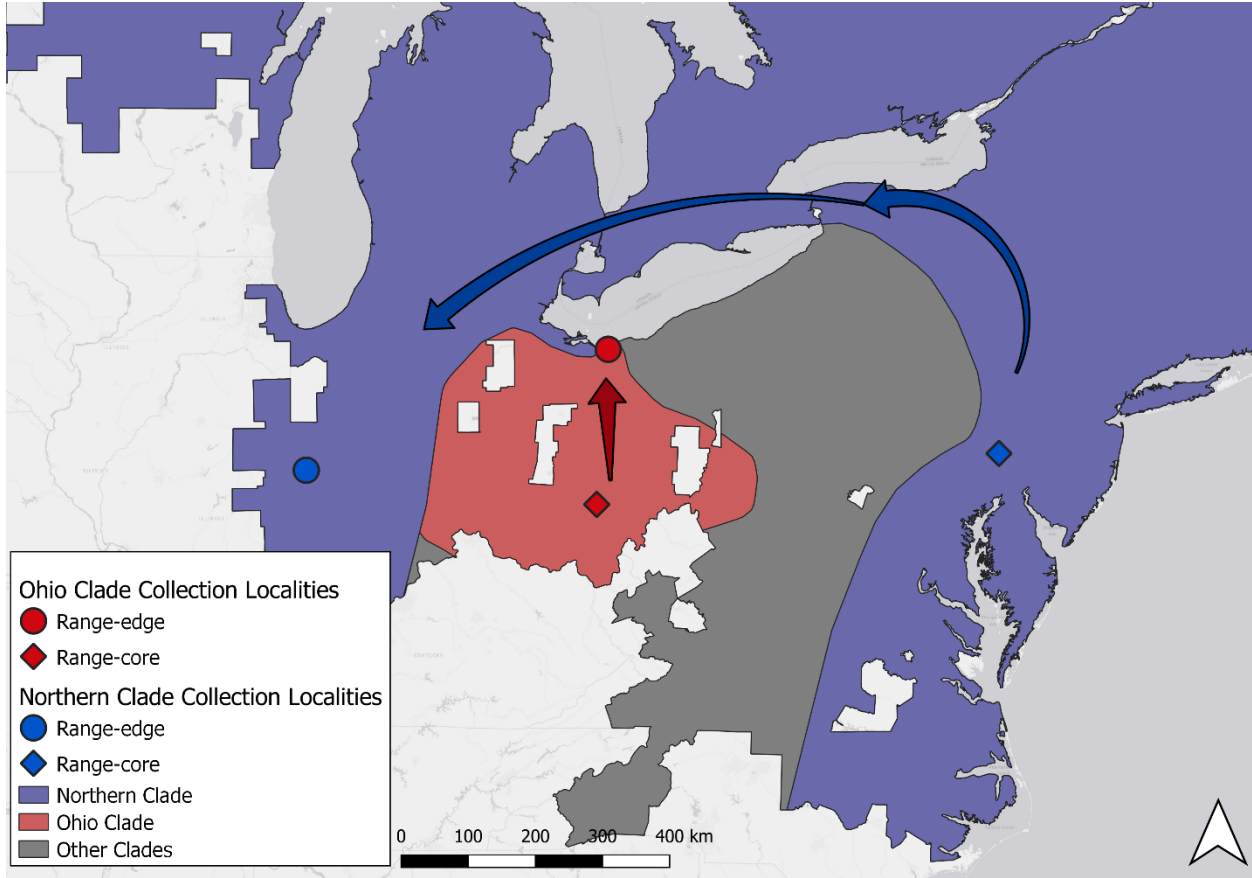| Model	AIC	df	Predictor	z-value	P (>|z|)
6A	305.1	55	Clade	-0.109	0.913
			Range	-0.383	0.702
			Clade:Range	-0.610	0.542
6B	303.0	56	Clade	-0.516	0.606
			Range	-0.934	0.350
6C	301.5	57	Clade	-0.360	0.719
6D	300.9	57	Range	-0.858	0.391
6 Full-Average (6A, 6B, 6C 6D)*			**Clade**	**-0.262**	**0.794**
			Range	**-0.595**	**0.552**
			Clade:Range	**-0.121**	**0.903**

Figures

Figure 1. The distributions of the Northern and Ohio clades of *P. cinereus* as identified by Radomski et al. (2020). The Northern Clade is represented in blue, the Ohio Clade is represented in red, and other clades are represented in gray. The collection localities in Indiana and Pennsylvania are representative of the Northern Clade, whereas localities in northern and southern Ohio represented the Ohio Clade. It is presumed that the Northern Clade expanded into present day Indiana following glaciation as shown in the figure above (Waldron et al. 2024). Made using QGIS (QGIS.org 2024).
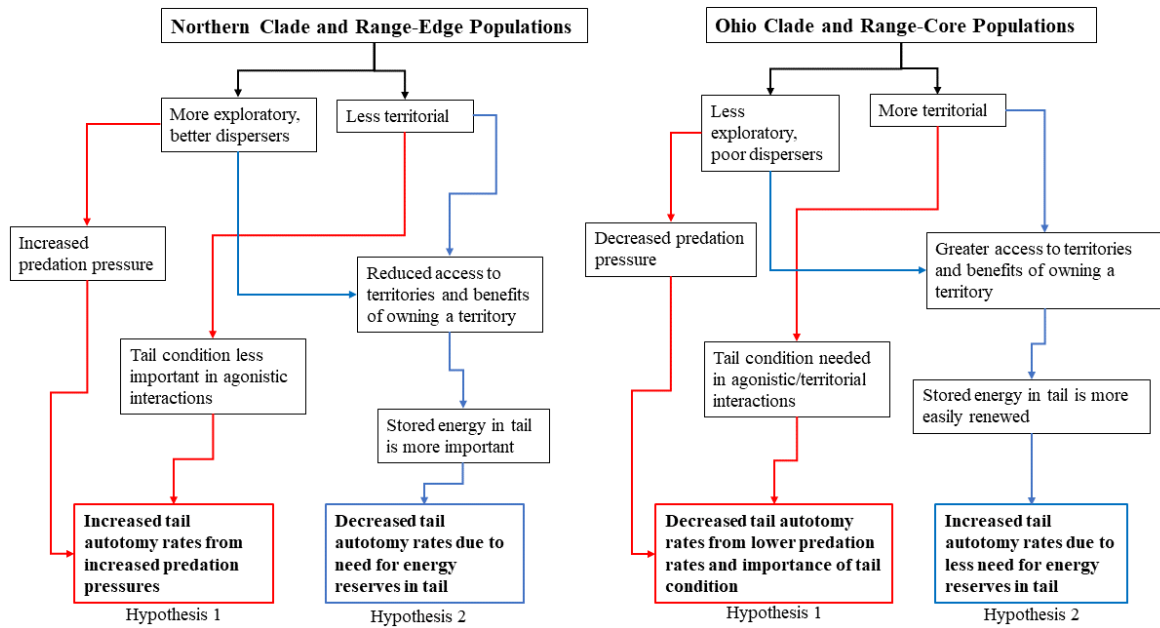
Figure 2. Hypothesis flowchart with conditions for each possible outcome. Red lines outline the mechanism that would result in the Northern Clade/range-edge populations exhibiting higher tail autotomy frequencies compared to the Ohio Clade/range-core populations (expected). Blue lines outline the converse mechanism in which the Ohio Clade/range-core populations would exhibit higher tail autotomy frequencies compared to the Northern Clade/range-edge populations (not expected).

Figure 3. Lab experiment methods using calipers, adapted from Beneski (1989). Animals were positioned using a moist kimwipe, and the calipers were closed around the tail of the animal. Trials began when the kimwipe was fully removed from the animal, allowing free movement with the exception of the held tail.
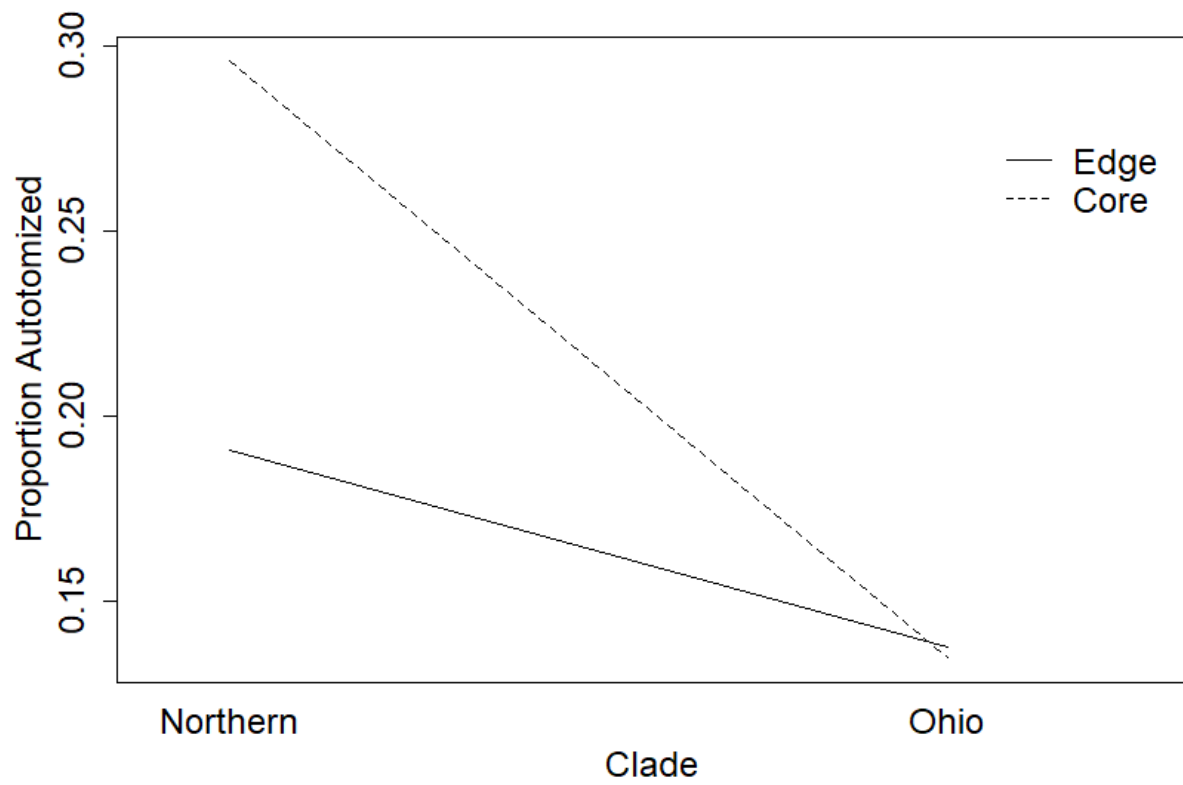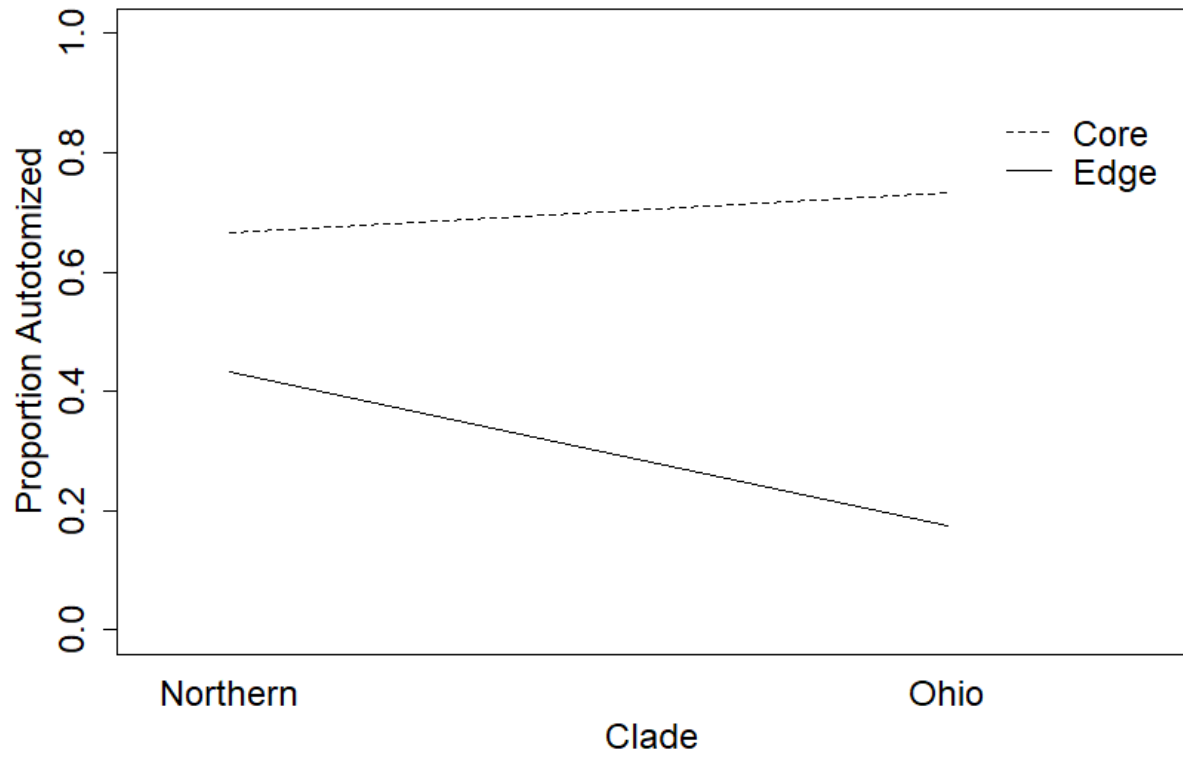
Figure 4. Interaction plot of autotomy frequencies shown as the proportion of each sample that autotomized for museum specimen data. Tail autotomy proportions were significantly higher in the Northern Clade compared to the Ohio Clade (Northern Clade n = 229, Ohio Clade n = 198, P = 0.030), contrary to my lab experiment results. The effect of range membership was not statistically significant.

Figure 5. Interaction plot of autotomy frequencies shown as the proportion of each sample that autotomized for lab trial data. Tail autotomy proportions were much greater in range-core populations compared to range-edge populations (range-core n = 60, range-edge n = 70, P = 0.036), whereas the effect of clade was not statistically significant.
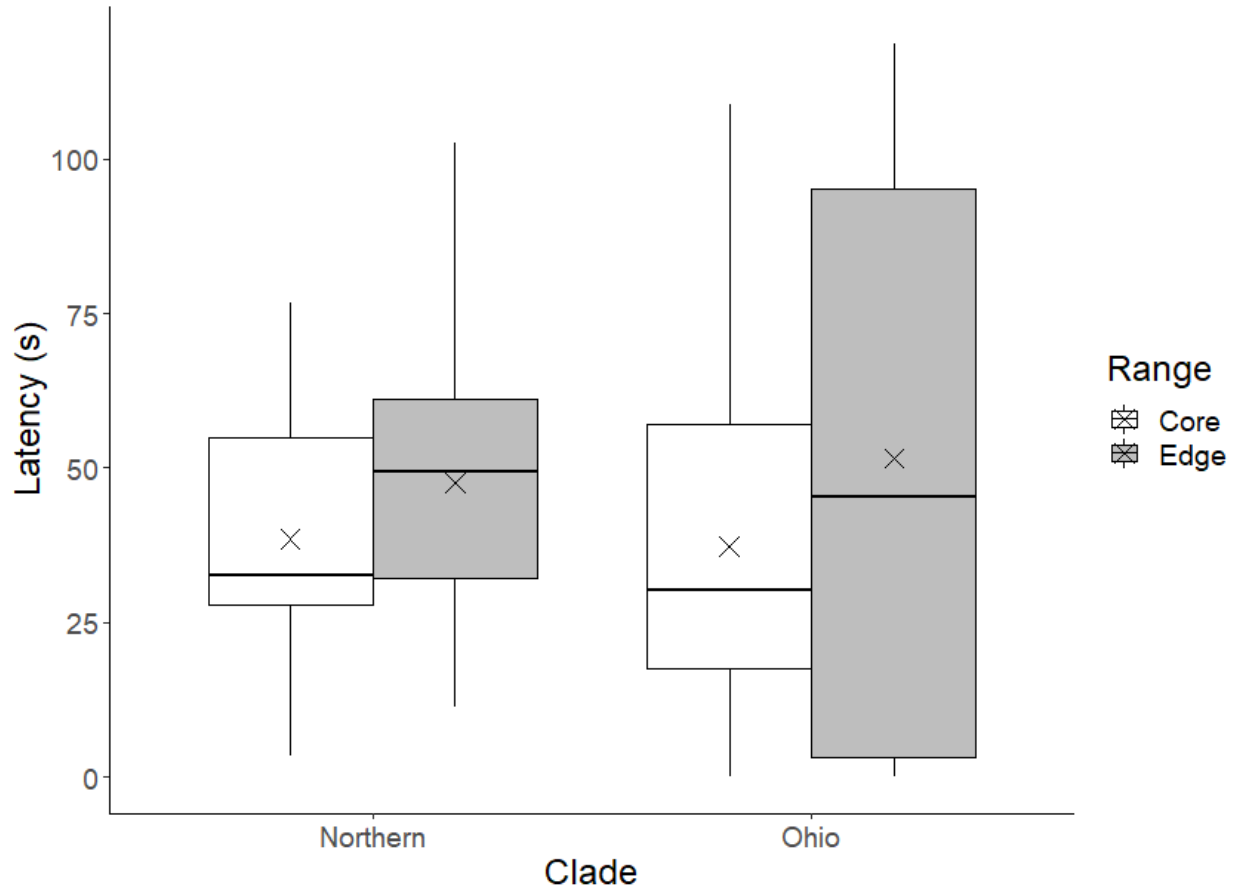
Figure 6. Boxplot of tail autotomy latencies for each population. Boxes and whiskers represent quartiles of data for each population. Mean latencies are denoted with an "X", and median latencies are represented by the horizontal black lines. Neither range-membership nor clade had a significant effect on latency to autotomize.
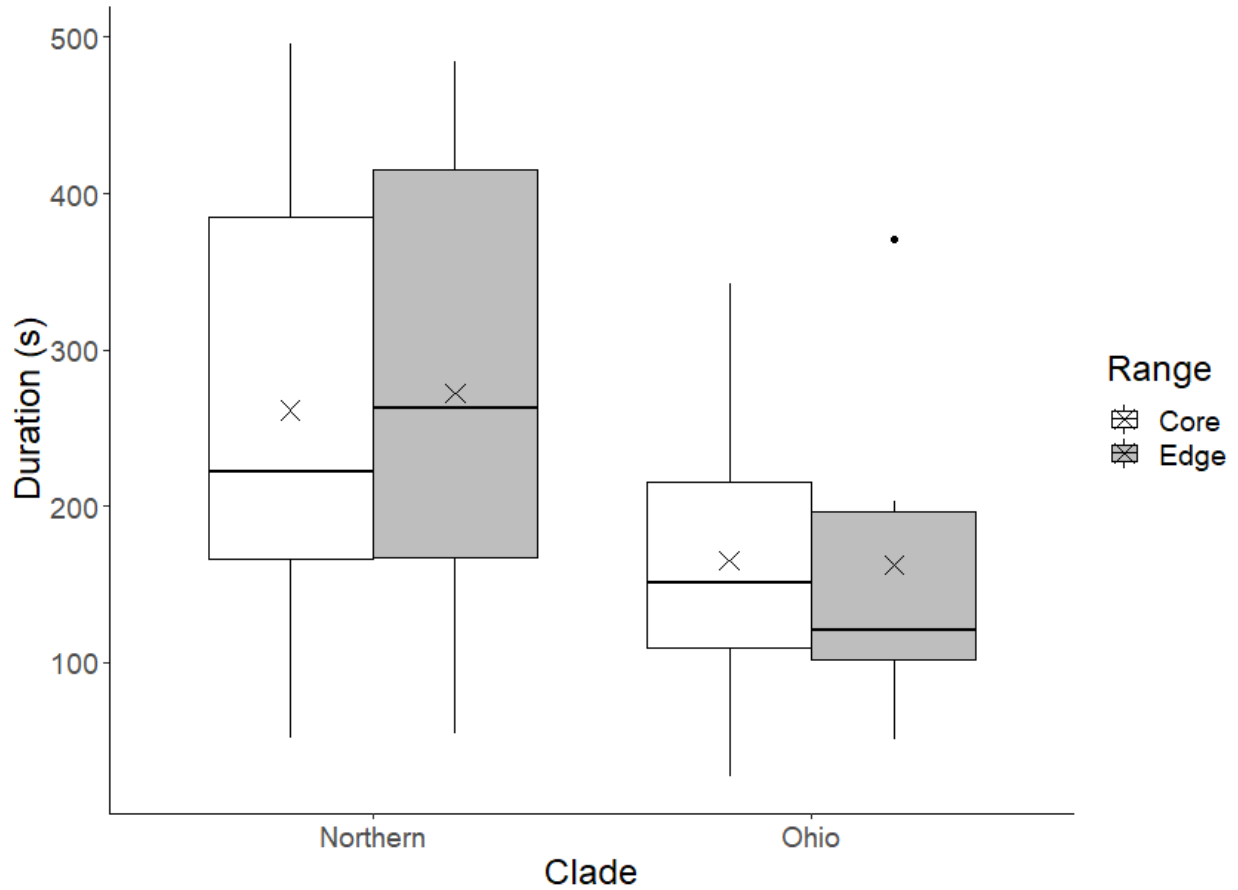
Figure 7. Boxplot showing duration of postautotomy tail movement for each population. Boxes and whiskers represent quartiles of data for each population. Mean postautotomy tail movement durations are denoted with an "X", and median durations are represented by the horizontal black lines. Black dots represent outliers in the data. The effect of clade was significant, in which the duration of postautotomy tail movement in the Northern Clade was greater than in the Ohio Clade (Northern Clade n = 33, Ohio Clade n = 29, $P = 0.002$). The effect of range membership was not statistically significant.
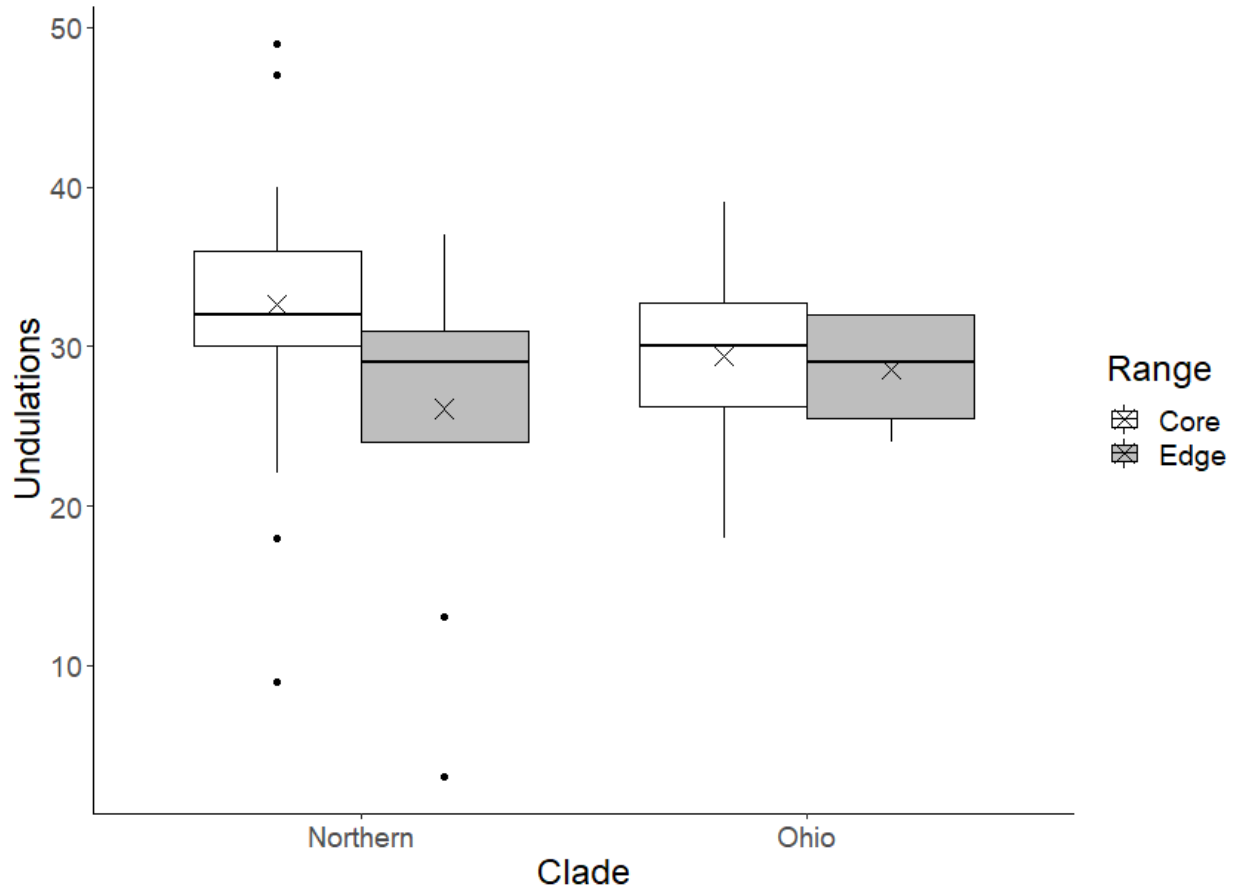
Figure 8. Boxplot showing frequencies of postautotomy tail undulation for each population. Boxes and whiskers represent quartiles of data for each population. Mean postautotomy undulations are denoted with an "X", and median undulations are represented by the horizontal black lines. Black dots represent outliers in the data. Neither range-membership nor clade had a significant effect on number of autotomized tail undulations.
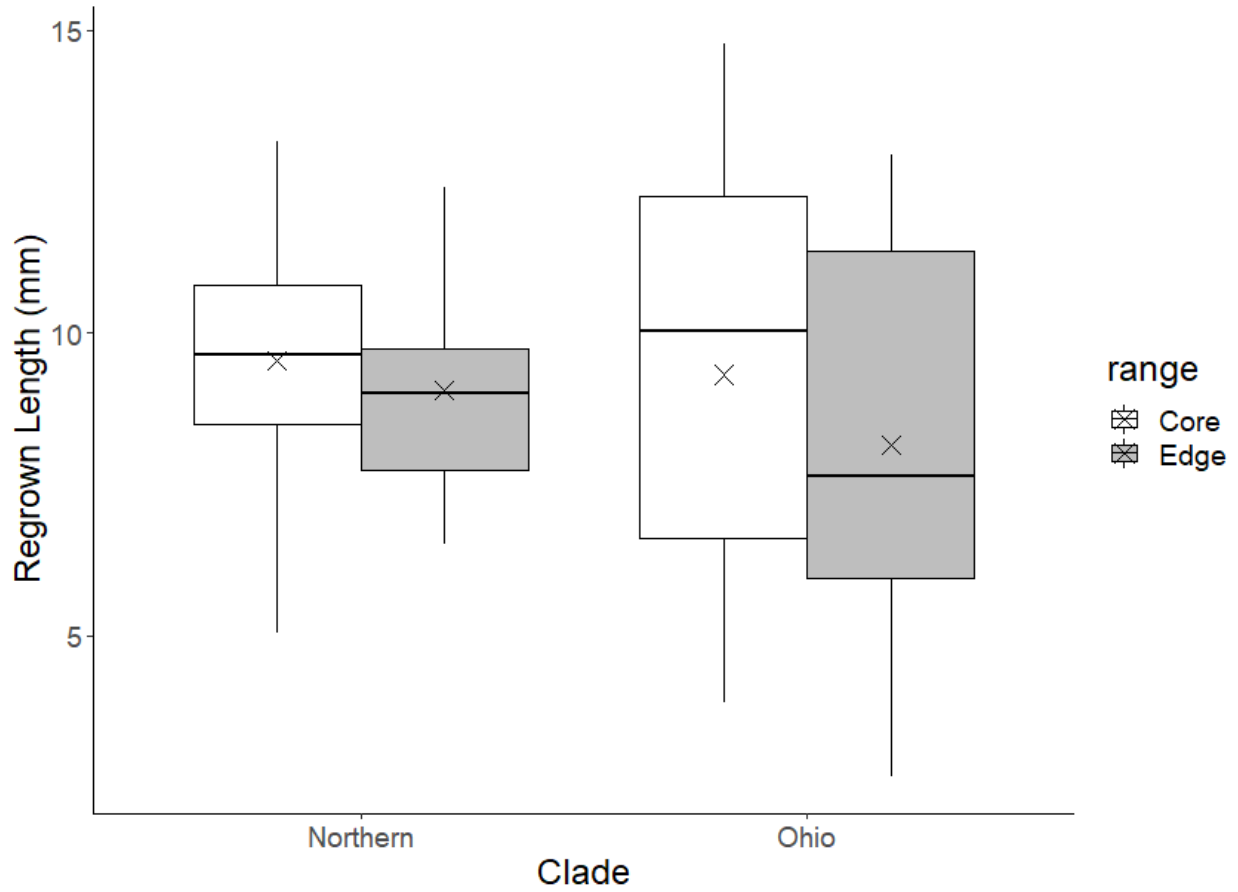
Figure 9. Boxplot of regrown tail lengths for each population after a period of three months following autotomy. Boxes and whiskers represent quartiles of data for each population. Mean regrown tail lengths are denoted with an "X", and median tail lengths are represented by the horizontal black lines. Neither range-membership nor clade had a significant effect on tail regrowth.