AGGRESSION AFFECTS DISTRIBUTIONS AND HYBRID ZONE EXPANSION IN MONTANE *PLETHODON* SALAMANDERS

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Western Carolina University in partial fulfillment of the
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By

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ABSTRACT

AGGRESSION AFFECTS DISTRIBUTIONS AND HYBRID ZONE EXPANSION IN MONTANE *PLETHODON* SALAMANDERS

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Ecologists have long been interested in the mechanisms that drive the distribution of closely related species. Often overlapping congeneric species do not differ enough in their uses of space and food resources to prevent competition. Over time, insufficient resource partitioning can result in the evolution of an interference mechanisms such as aggression. Aggressive interactions are a well-documented form of competition among *Plethodon* salamanders, and has been shown to affect their distributions. The Nantahala Mountain range of western North Carolina is the site of contact between *P. shermani* and *P. teyahalee* along an elevational gradient. Hybridization occurs between these two species at intermediate elevations. A long-term study suggests that the hybrid zone is expanding at the expense of both parentals' ranges.

I used field enclosures and laboratory behavioral trials to investigate whether elevation-dependent competition and aggressive behavior are influential in delineating the vertical distributions of the parental species, and provide a mechanism driving hybrid zone expansion. I found that *P. shermani* was more aggressive than hybrids or *P. teyahalee*, suggesting that interference competition has maintained the distribution of *P. shermani* at the high elevations. Hybrids exhibited aggressiveness that was intermediate to the parentals, and hybrids may be

competitively superior to *P. teyahalee*. I submit competitive exclusion as the primary mechanism driving the downward hybrid expansion.

INTRODUCTION

Ecologists and evolutionary biologists have investigated the mechanisms that influence the distribution of closely related species for over 150 years (Darwin, 1859; Cunningham et al., 2009). Emphasis has been placed on interspecific interactions, such as competition (Darwin, 1859; McDonald, 2003), population limiting processes such as predation and disturbance, as well as abiotic factors, particularly climate (Hutchinson, 1918; MacArthur, 1958; Gifford and Kozak, 2012). Species distributions are often limited by climate variables related to particular physiological restrictions. Climate can set general boundaries by causing mortality, reducing reproduction, and an inability to complete life cycles outside the physiological tolerance of an organism (Hutchins, 1947; Cunningham et al., 2009). Shifts in climate can affect species distributions and the number of competitors and predators, often resulting in novel interactions (Gross and Price, 2000).

Competition is a biotic driver of species distributions in many ecologically distinct taxa (reviewed in Schluter, 2000). The results of these competitive interactions may influence local adaptation, evolutionary trajectories, and degree of distributional overlap among closely related species (Hairston, 1980a, 1980b; Nishikawa, 1985; Deitloff et al., 2008). Over time, insufficient interspecific and intraspecific resource partitioning can result in the evolution of an interference mechanism such as aggression (Gill, 1974; Nishikawa, 1985).

Aggressive behavior has been extensively studied in many taxa, including: bees (Pearce et al., 2001), ants (Jutsum et al., 1979), fruit flies (Boake and Konigsberg, 1998), birds (Fisher, 1954), mammals (Tannerfeldt et al., 2002), and salamanders (Hairston, 1980a; Jaeger, 1981; Nishikawa, 1985), and may occur in conspecific or heterospecific encounters. The primary purpose of aggressive behavior is to acquire and defend territories, acquire and defend mating opportunities, and inhibit the ability of another individual to acquire resources, reproduce, and survive (Peiman and Robinson, 2010). Aggression may reduce the fitness of the inhibited individual. Based on these fitness influences and the heritability of aggressive behavior, theories and research have been developed that suggest aggressive behavior can influence species occurrence patterns (Hall and Klein, 1942; Fennell, 1945; Deitloff et al., 2008; Peiman and Robinson, 2010). Among these patterns, agonistic behavior may play a role in delineating the distributional range and geographic overlap between two similar species (Hairston, 1980a, 1980b; Nishikawa, 1985).

Competition, aggression, and physiology affect species distributions in montane terrestrial salamanders (Hairston, 1980a; Hairston, 1980b; Nishikawa, 1985, Selby et al., 1996; Gifford and Kozak, 2012; Kozak and Weins, 2006). Within the family Plethodontidae, the genus *Plethodon* consists of fully terrestrial salamanders, many of which are endemic to the southern portion of the Appalachian Mountain range (Highton and Peabody, 2000). There are two major groups in this range, the *P. jordani* and *P. glutinosus* complexes, each of which was previously recognized as a single species (Green, 1818; Blatchley, 1901). The species associated with the two complexes exhibit high similarity, both within and between groups (Kozak and Wiens, 2006). Generally, members of the *P. glutinosus* complex are larger bodied salamanders characterized by white or brass spotting along the back and sides and restricted to the low elevations. Species in the *P. jordani* complex are smaller, characterized by variable red or gray pigment on the cheeks and legs, no white spots, and found in the higher elevations. These complexes are nearly always sympatric to each other at intermediate elevations (Hairston, 1949). This *Plethodon* community structure provides a unique situation in which physiological tolerance (Kozak and Wiens, 2006; Gifford and Kozak, 2012), variation in competitive ability (Hairston, 1980a, 1980b) and aggressive behavior (Nishikawa, 1985), and hybridization (Hairston et al., 1992), are influential in delineating the species' vertical distribution and degree of sympatric overlap. Furthermore, these zones of sympatry vary among populations found in different mountain ranges within the southern Appalachians (Hairston, 1949, 1950).

Populations of the *P. jordani* complex in the Balsam, Smoky, and Nantahala mountain ranges are now described as separate species: *P. metcalfi*, *P. jordani*, and *P. shermani* respectively (Highton and Peabody, 2000). It is accepted that the cause of allopatric speciation was vicariance, where warming temperatures impeded gene flow between populations restricted to higher elevations (Kozak and Wiens, 2006). A subsequent study in the Smoky Mountains found that *P. jordani's* vertical range was indeed restricted by climate (Gifford and Kozak, 2012). Conversely, *P. jordani's* low elevation sympatric counterpart, *P. teyahalee*, has the physiological tolerance to persist at high elevations but is excluded from them by competitive interactions with *P. jordani* (Gifford and Kozak, 2012).

Previous studies suggest that the range of overlap between *P. teyahalee* and *P. jordani* in the Smokies is narrow as a result of strong competition (Hairston, 1980a, 1980b). Competitive interactions between *P. teyahalee* and *P. metcalfi* in the Balsam Mountains were thought to be less intense, because these species have a wider range of overlap (Hairston 1980b). Nishikawa (1985) determined that this variation in competition resulted in the evolution of variable levels of aggression in each population and interference competition was an important influence on their overall species distributions.

Hybridization also occurs in this southern Appalachian *Plethodon* system (Hairston et al., 1992). Hybrid zones are characterized by closely related species overlapping and interbreeding to produce individuals of mixed ancestry (Buggs, 2007; Harrison, 1993). The origins of these zones are thought to be the result of secondary contact between two species that formed in allopatry (Mayr, 1942), or a response to variation in selection pressures such as environmental gradients in areas where individuals of mixed ancestry are favored (Harrison, 1993). A zone between the Balsam and Smoky Mountain ranges is the site of contact, and three-way hybridization, between *P. metcafi*, *P. jordani*, and *P. teyahalee* (Hairston et al., 1992). In the Nantahala Mountain range, hybridization occurs along an elevational contact zone between the high elevation, *P. shermani*, and the low elevation, *P. teyahalee* (Hairston et al., 1992). A long term study on the changes in phenotype distribution along this elevational gradient suggests that the hybrid zone is spreading in both directions at the expense of the parentals' ranges (Hairston et al., 1992; Pechmann and Hyman, personal communication).

The spatial dynamics of hybrid zones on an ecological time-scale depend on many factors including genetic and ecological differentiation between parental species, rate of dispersal, and hybrid fitness (Harrison, 1993). It is thought that the expansion of a hybrid zone at the expense of the parental ranges is the result of hybrid fitness that is greater than or equal to both parental species beyond the intermediate ecotonal zone (Buggs, 2007; Harrison, 1993). The inclusion of hybrids makes the behavioral interactions influencing overall species distributions more complex (Hairston, 1980b).

Purpose/Hypotheses

I used laboratory and field experiments to investigate how aggressive behavior and elevation-dependent competition influences the distributions of *P. shermani, P. teyahalee* and their hybrids, as well as hybrid zone expansion. Based on the known community structure and physiological characteristics of these Plethodontids, as well as the relationships previously discussed, I made the following predictions:

a) Despite its smaller body size, *P. shermani* will be more aggressive than its low elevation counterpart, *P. teyahalee*.

b) Hybrid aggressive behavior will be equal or greater than that of the most aggressive parental.

c) *Plethodon teyahalee* individuals will have lower growth rates in cages where they are mixed with hybrids than in cages where they are alone.

d) *Plethodon shermani* individuals will have lower growth rates at the middle elevation, particularly when mixed with hybrids.

e) Growth rates of hybrids will not be affected by elevation or competitive interactions with the parental salamanders.

METHODOLOGY

Laboratory Behavioral Experiment

I collected adult salamanders of both parental species and hybrids along Ball Creek Road in the USDA Forest Service's Coweeta Hydrological Laboratory beginning in April, 2013 and continuing through October, 2014. The color phenotype of each salamander was scored using an established system which assigns a value between zero and three to quantify the degree of red pigment on the legs (*P. shermani* characteristic) and white spots along the sides and back (*P. teyahalee* characteristic; Hairston et al., 1992). Hybrid individuals retain a varying combination of these color patterns. I collected *P. teyahalee* individuals that scored a two or three for white spots along the side (1=few spots; 2=continuous spots along the side; 3=continuous spots along side as well as spots on the dorsum) and zero for red pigment on the legs (0=red pigment absent), and that were captured at altitudes below 685 meters. *Plethodon shermani* individuals collected scored a two or three for red pigment on the legs (1=limited red pigment on the legs that is not continuous from the shoulder to the elbow; 2=continuous line of red pigment that can be traced from the shoulder to the elbow on an anterior leg; 3=continuous line of red from shoulder to the elbow of at least one anterior leg and one posterior leg), a zero for white spots (0=spotting absent), and were captured at altitudes above 1,050 meters. Hybrid salamanders were collected from intermediate altitudes ranging from 813-833 meters and retained a combined color score between negative one and one, when the score for red pigment on the legs was subtracted from the score for white spots.

A minimum of five days prior to experimentation, individual salamanders were placed in separate 28.5x15x7 cm plastic containers lined with bleach free paper towels and sphagnum moss. This allowed each salamander to establish a territory (Jaeger, 1981). During this time salamanders were kept at 22°C, under an artificial 12L:12D photoperiod, and fed two medium sized ants once per week. The substrate and sphagnum moss were moistened with well water three times weekly.

I observed each salamander as a resident under three different conditions. Hybrid residents were tested with a *P. shermani* intruder (N = 9), *P. teyahalee* intruder (N = 9), and a hybrid intruder (N = 12). *Plethodon shermani* residents were tested with a hybrid intruder (N = 10), *P. teyahalee* intruder (N = 8), and conspecific intruder (N = 11). *Plethodon teyahalee* residents were tested with a hybrid intruder (N = 9), *P. shermani* intruder (N = 8), and conspecific intruder (N = 8). To ensure there were no effects from previous encounters, each salamander was used only once, whether as a resident or intruder. Due to the nocturnal nature of the study organisms, trials were performed under dim red lighting a minimum of 30 minutes into the 12 hour dark portion of the photoperiod.

Trials were initiated by removing the sphagnum moss and placing a randomly selected intruder into the enclosure of an established resident, at which time the resident was picked up and immediately returned so that any effects caused by handling will occur for both contestants. Prior to observations of behavior, salamanders were given a five minute acclimation period, during which behaviors were not recorded (Jaeger, 1982; Nishikawa, 1985; Deitloff et al., 2008). Following the acclimation period, the frequency of aggressive behaviors for both the resident and intruder were recorded for thirty minutes. Upon completing each trial, both salamanders were issued a phenotype score (scoring system described above), sexed, and the SVL (snout-vent length) measured using digital calipers to the nearest 0.01mm.

To quantify aggression, I used a set of behaviors previously used to evaluate agonistic behavior patterns in Plethodontid salamanders (Nishikawa, 1985). Snap behavior describes the action of a salamander opening its jaws and snapping them shut without contacting the opponent. Lunge behavior occurred when an individual rapidly moved, in one continuous motion, directly towards an opponent. Chase behavior occurred when a salamander moved directly towards an opponent who subsequently retreated. Bites, considered a direct attack, occurred when an individual contacted an intruder with an open mouth then closed his mouth on the opponent. Approach behavior involved the aggressor taking three or more steps directly towards a stationary intruder. While not commonly used with *Plethodon* salamanders, approach is a prerequisite for, and is likely to be a reliable predictor of, direct attack (Searcy et al., 2006; Ballentine et al., 2008).

Data Analysis

I compared the relative size (SVL) of each salamander form using a one-way ANOVA with a Tukey's post hoc test. Because some behaviors rarely occurred, I used the sum of aggressive behaviors (approach+snap+lunge+chase+bite) as the response variable. This summation met the assumptions of normality and homogeneity of variance after a square root transformation. For my analysis, I used a multi-factor ANCOVA in R, with snout-vent length as the covariate, to test my predictions on the frequency of aggressive behavior.

Field Enclosure Experiment

From June-August 2013, enclosures were placed at three different elevations along Ball Creek Road at the Coweeta Hydrological Laboratory in the Nantahala National Forest, North Carolina. Enclosures used to investigate the low elevation interactions involving *P. teyahalee* and hybrids were set up at an altitude of 690 meters (N 35˚03.460'; W 083˚25.782') which is at the bottom of the hybrid zone where “pure” *P. teyahalee* dominate (Hairston et al., 1992). Enclosures used to investigate high elevation interactions involving *P. shermani* and hybrids were placed at an altitude of 1,076 meters (N 35˚02.344’; W 083˚27.345’) where “pure” *P. shermani* dominate (Hairston et al., 1992). Near the center of the hybrid zone, at an altitude of 849 meters (N 35˚02.629’; W 083˚26.886’), enclosures were set up to investigate interactions of each parental salamander with the hybrid form in the zone where all, or nearly all individuals, are hybrids (Hairston et al., 1992).

At all three sites, I buried enclosures 12-15 cm deep, on north facing slopes shaded by vegetation consisting of mostly *Rhododendron maximum, Liriodendron tulipifera*, and *Quercus spp*. Each enclosure was 73.5x122x73.5 cm, constructed of screen mesh and PVC pipe, with access through a zipper at the top. The mesh size (diameter=3mm) was sufficient to allow invertebrate prey to enter the enclosure, while preventing immigration and emigration of salamanders. Eight, 10-cm-deep burrows were made in each enclosure and the substrate was covered with a layer of leaf litter that was thoroughly inspected to ensure there were no unwanted salamanders introduced.

I distributed six salamanders in each 0.9 m^2 enclosure, which was 6.7/m^2. *Plethodon* densities in the Nantahala Mountains range from 1.57-7.32 salamanders/m^2(Pursel, 2012), therefore, 6.7 individuals/m^2 facilitated competition without overpopulation. The three treatments at the high and low elevations consisted of the parental species found at that elevation alone, hybrids alone, and a mixed cage containing an equal number of parental species and hybrids (Table 1). Five treatments at the middle elevation consisted of each parental species alone, hybrids alone, and mixed cages containing a parental species and hybrids (Table 1). Each treatment at each elevation was replicated three times, resulting in a total of nine enclosures at the high and low elevations, and 15 enclosures at the center of the hybrid zone. Because the primary interest of the cage experiment was to investigate the hybrid zone expansion, and direct interaction between parental salamanders no longer occurs, I excluded cages containing parental/parental interactions from the treatment design.

Table 1: The treatment design for the field enclosure experiment. Each enclosure at the designated elevations contained (n) number of individuals and was replicated three times.

Treatment	**Low Elevation (690m)**	**Middle Elevation (849m)**	**High Elevation (1,076m)**
1	*P. teyahalee* (n=6)	*P. teyahalee* (n=6)	
2	*P. teyahalee* (n=3)+Hybrids (n=3)	*P. teyahalee* (n=3)+Hybrids (n=3)	
3	Hybrids (n=6)	Hybrids (n=6)	Hybrids (n=6)
4		*P. shermani* (n=3)+Hybrids (n=3)	*P. shermani* (n=3)+Hybrids (n=3)
5		*P. shermani* (n=6)	*P. shermani* (n=6)

Adult salamanders of all three forms were caught along the Ball Creek Road transect at night from May-June 2014. Qualifications for usable *P. teyahalee*, hybrid, and *P. shermani* individuals were the same as for the laboratory experiment described above. I recorded color score and sex, measured snout-vent length to the nearest 0.01mm using digital calipers, and measured mass to the nearest 0.001 on a digital scale for each individual.

Due to the permeability of amphibian skin, water retention can affect the mass of these terrestrial salamanders. To control for variation in water weight, each individual underwent the following hydration protocol before weighing. Salamanders were kept at 20˚C in individual ziplock bags containing bleach-free paper towels saturated with well water. Once an hour, each salamander was moistened with a one part 0.45% saline to one part 2.5% dextrose solution (Hadfield et al., 2006) and weighed. This was repeated until weights remained constant for two consecutive measurements.

Prior to placing salamanders in enclosures, each individual was marked on the ventral side using subcutaneous elastomer dye injections. Using a combination of dye color and mark location (leg adjacent), I was able to accurately identify each individual throughout the experiment. Once marked, each salamander was randomly assigned to an appropriate enclosure based on the treatment design in Table 1.

Salamanders for replicates one, two, and three were placed on May 26th, June, 19th, and June 26th, respectively, and recovered between October 14th and 19th, 2014. Throughout the duration of the experiment, I visited sites (N=17) in randomized order to take a point count of the number of surface active individuals in each enclosure. I characterized a salamander as being surface active when at least half the body was visible. During these nighttime trips I used a Kestrel 2500 (Nielsen-Kellerman; Boothwyn, PA.) to measure temperature and humidity at each elevation. After recovery, each salamander was taken back to the lab at Western Carolina University where SVL and final mass were measured using the same initial protocol stated above.

Data Analysis

To compensate for variation in the amount of time salamanders were residents of each replicate, I used instantaneous growth rates ($\frac{\ln(\mathrm{mass\ (g)_f}) - \ln(\mathrm{mass\ (g)_i})}{\mathrm{number\ of\ days}}$, where mass $(\mathrm{g})_\mathrm{f}$ is the final mass and $(\mathrm{g})_\mathrm{i}$ is the initial mass) as the response variable. Because high elevation *P. shermani* individuals were not used at low elevations, *P. teyahalee* individuals were not used at high elevations, and no treatment involved an interaction between parental species, each salamander form was analyzed separately in order to avoid incomplete blocks. I used mixed linear models (lme4 package in R) with enclosures designated as the error term to avoid pseudoreplication from the individual data to determine influences of elevation and/or treatment on the instantaneous growth rates of each salamander form.

Similarly, I used separate mixed linear models for each salamander form to analyze the influences of pure or mixed treatments, elevation, and humidity on the proportion of salamanders active on the surface each night. An arcsin(log(proportion+1)) transformation of this proportion was performed to meet the assumptions of normality and homogeneity of variance. I used the lme4 package in R and designated enclosures as the error term.

RESULTS

Laboratory Behavioral Experiment

The mean SVL of *P. teyahalee* (x ± SE=64.71 ± 1.24 mm) was significantly higher than hybrids (60.23 ± 0.65 mm; Tukey's Post Hoc test, p=0.0007), and hybrids were significantly larger than *P. shermani* (57.09 ± 0.49 mm; Tukey's Post Hoc test, p=0.0009). Opponent form had no significant effect on the frequency of aggressive behavior and was subsequently removed from the analysis ($F_{2,148}$=0.8993; p=0.409). Whether the salamander was a resident or an intruder had no significant effect on aggression ($F_{1,148}$=0.0702; p=0.7914); therefore the aggressive behaviors of both groups were pooled and used to analyze overall aggression.

Salamander form, SVL, and the interaction between these variables produced a significant effect on aggression (Table 2). The significant interaction between the salamander form and SVL resulted from a larger body size being related to higher aggression in only the most aggressive species (Figure 1). *Plethodon shermani* (mean=3.11; SE=0.71; n=57) was the most aggressive with more than six times the number of aggressive behaviors as *P. teyahalee* (mean=0.42; SE=0.11; n=50) and more than two times the number of aggressive actions as hybrids (mean=1.15; SE=0.18; n=61) (Figure 2). The level of aggression in hybrids was intermediate to that of the parental species (Figure 2). A total of 33 bites were administered by *P. shermani* and 6 by hybrids. No bites were produced by *P. teyahalee*. As previously stated, the frequency of aggressive behavior increased with the SVL of *P. shermani* individuals, however, size did not affect aggression in *P. teyahalee* or hybrid individuals.

Table 2: Two-factor ANCOVA table for the influence of salamander form and snout-vent length on the transformed sum of aggressive behaviors.

	Sum sq	Df	F value	P value
Salamander Form	30.946	2	22.6139	<0.0001
Snout-vent Length	2.892	1	4.2271	0.0414
SVL : Form	9.387	2	6.8595	0.0014
Residuals	110.844	162		

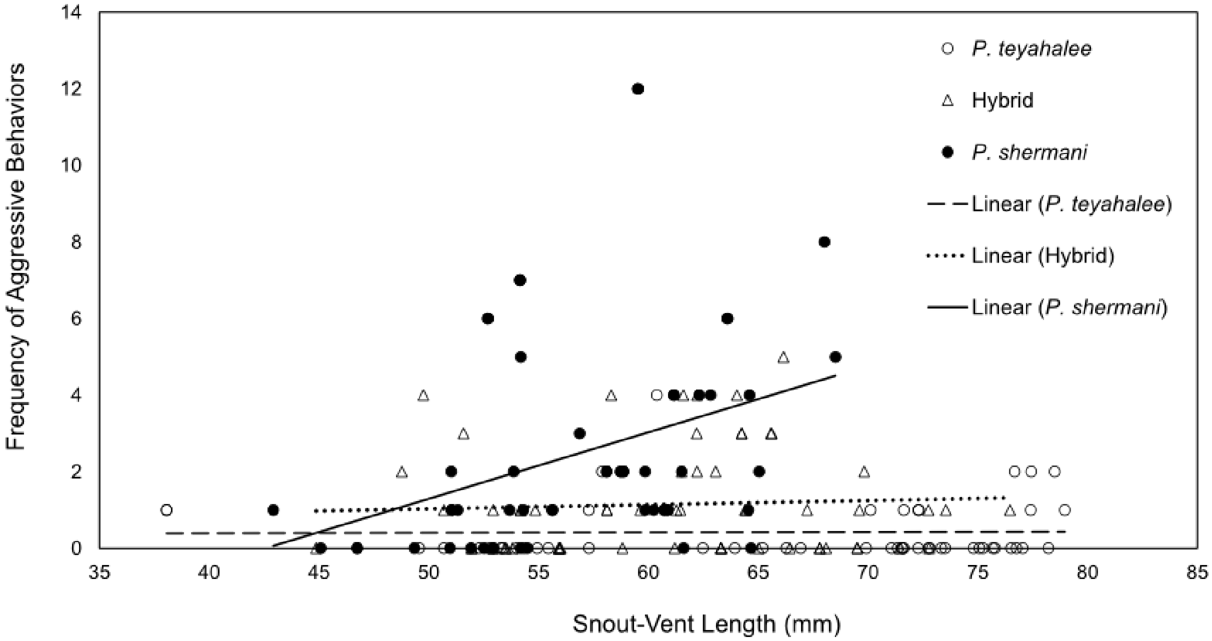

Figure 1: The relationship between frequency of aggressive behaviors and snout-vent length by form. One outlier *P. shermani* individual that showed extreme aggression was excluded from this plot; however, the removal of this data point did not qualitatively change the significance and it was subsequently included in the analysis.

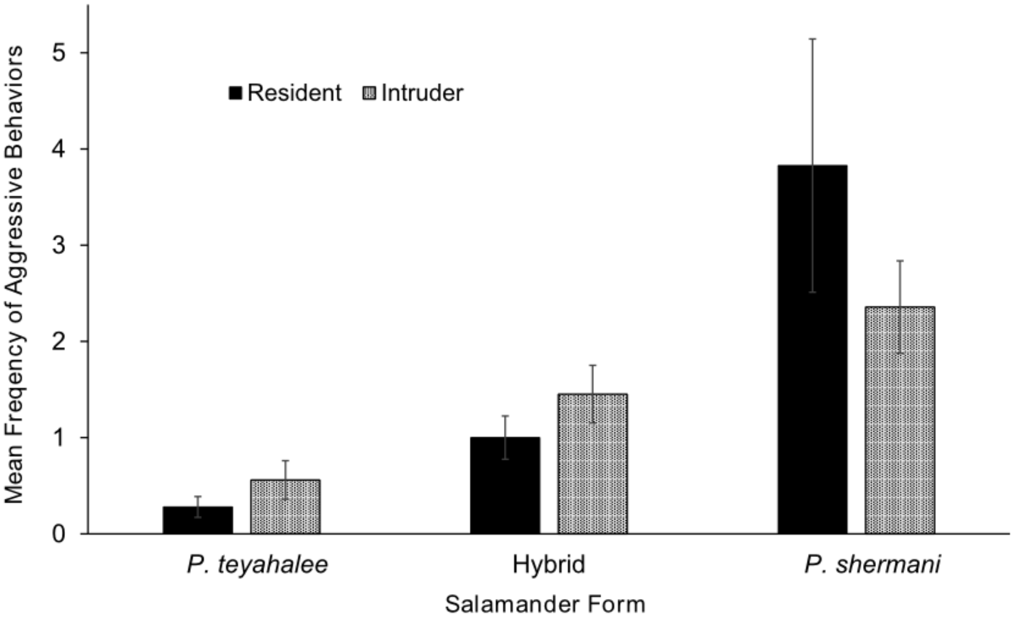

Figure 2: Average frequency (+ 1 SE) of aggressive behavior exhibited by the two parental salamander species and their hybrids. Represented are the true means of aggressive behavior and not the transformed response variable used for the ANCOVA.

Field Enclosure Experiment

Individuals of the hybrid form decreased in mass similarly in all treatments and elevations ($F_{1,7.8448}$=1.2063, p=0.3046; $F_{2,7.7308}$=1.4274, p=0.2968, respectively; Fig. 3). Treatment and elevation also had no influence on the relative mass changes in *P. shermani* ($F_{1,7.4996}$=0.44637, p=0.5241; $F_{1,7.8448}$=0.44615, p=0.5247, respectively; Fig. 4). While not statistically significant, a trend in the instantaneous growth rates of *P. teyahalee* suggested that when mixed with hybrids at middle elevations, *P. teyahalee* experienced a greater decrease in mass (Table 3; Fig. 5).

Independent of treatment or elevation effects, surface activity of *P. teyahalee* (treatment: $F_{1,148}$=0.0.0165, p=0.8980; elevation: $F_{1,148}$=0.2063, p=0.6503) and *P. shermani* (treatment:

$F_{1,117.24}$=0.091, p=0.7634; elevation: $F_{1,117.24}$=2.639, p=0.1070) increased with relative humidity ($F_{1,148}$=24.645, p<0.0001; $F_{1,115.17}$=67.773, p<0.0001, respectively). Analysis of hybrid surface activity resulted in a significant three-way interaction between elevation, treatment, and humidity ($F_{1,128}$=6.176, p=0.0142). Observation of the linear relationship of subsets of the data indicated that the significant interaction was restricted to the high and low elevations, therefore, the middle elevation treatment was removed. The final analysis of surface active hybrids retained the significant interaction between treatment, elevation, and relative humidity at the high and low elevational boundaries of the hybrid zone. At the low elevation site the positive slope of the linear relationship between relative humidity and proportion of surface active hybrids was lower when hybrids were alone compared to cages where hybrids were mixed with *P. teyahalee* (Figure 6). Conversely, at the high elevation, the linear increase was greater when hybrids were alone than when mixed with *P. shermani* (Figure 6). At high humidity levels, hybrids at the low elevation showed similar surface activity whether mixed with *P. teyahalee* or alone, suggesting that the increased numbers of surface active *P. teyahalee* did not inhibit hybrid surface activity. However, at the high elevation and at high humidities, hybrid surface activity was lower when mixed with *P. shermani* than when mixed with other hybrids. This may suggest that in optimal conditions facilitating an increase in the surface activity of more aggressive *P. shermani* individuals, hybrids were more reluctant to forage on the forest floor.

Table 3: ANOVA table of the mixed linear model analyzing the influence of treatment and elevation on *P. teyahalee* instantaneous growth rates.

	Sum sq	**Df**	**DenDf**	**F value**	**P value**
Treatment	0.000001341	1	50	1.1753	0.28352
Elevation	0.000003687	1	50	3.2316	0.07827
Treatment:Elevation	0.000003417	1	50	2.9944	0.08972

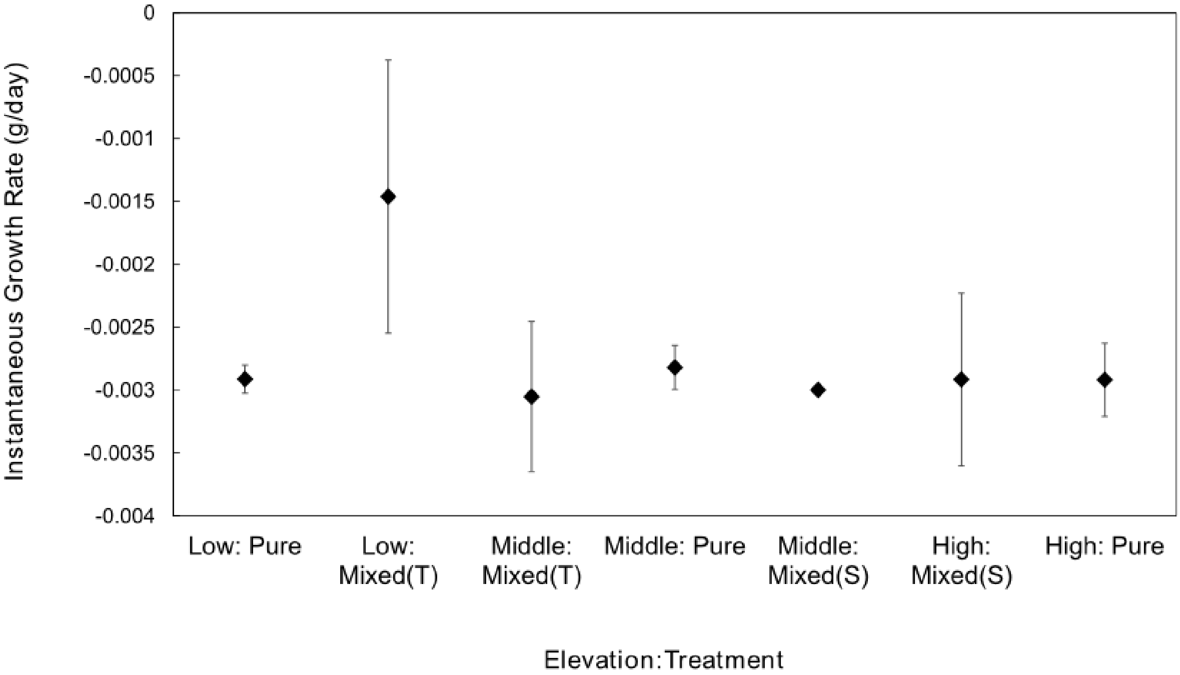

Figure 3: Plot of mean hybrid instantaneous growth rates as a function of treatment and elevation. “T” represents cages in which hybrids were mixed with *P. teyahalee* and “S” designates cages in which hybrids were mixed with *P. shermani*.

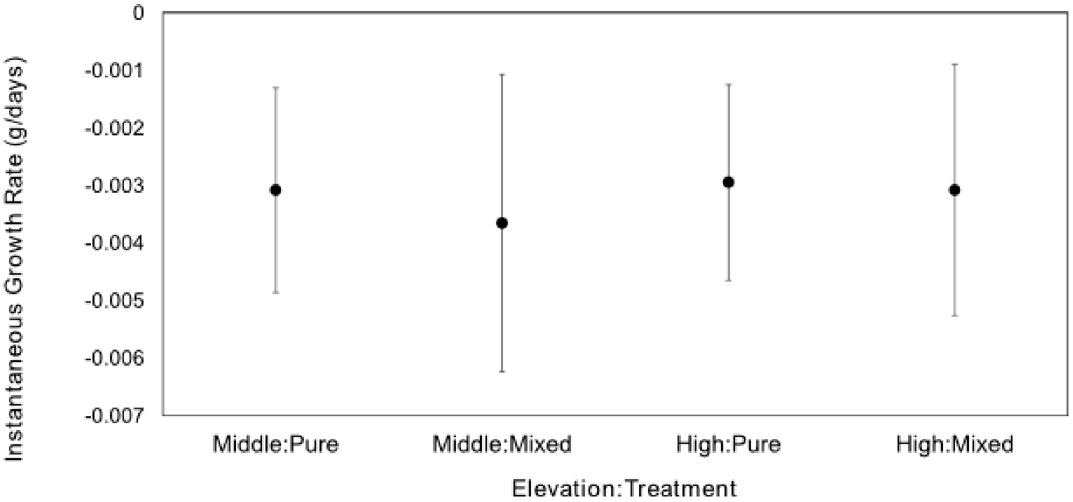

Figure 4: Plot of mean instantaneous growth rate for *P. shermani* as a function of treatment and elevation. *Plethodon shermani* was mixed with hybrids in mixed cages. Pure cages enclosed only *P. shermani* individuals.

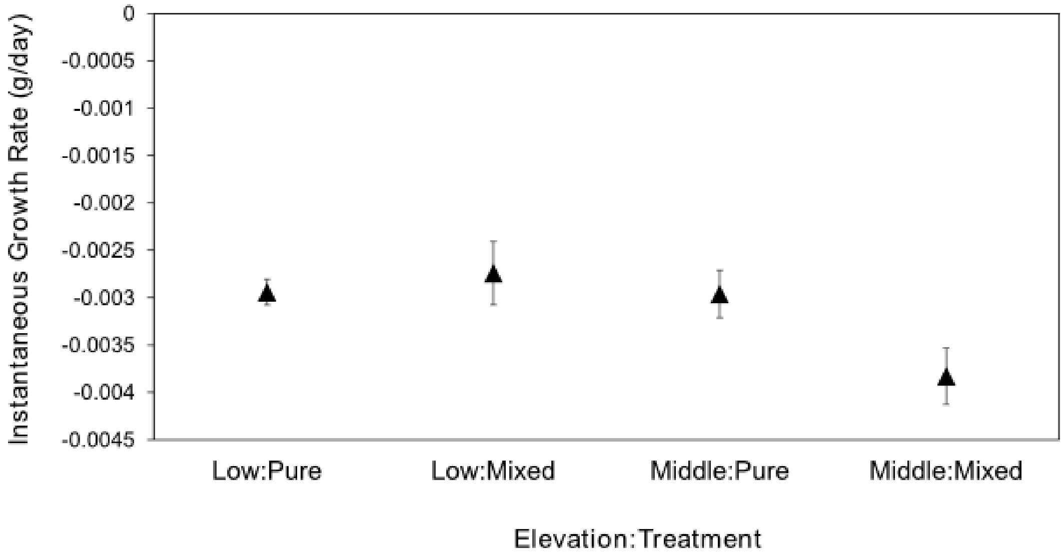

Figure 5: Plot of mean instantaneous growth rate for *P. teyahalee* as a function of treatment and elevation. *Plethodon teyahalee* was mixed with hybrids in mixed cages. Pure cages enclosed only *P. teyahalee* individuals.

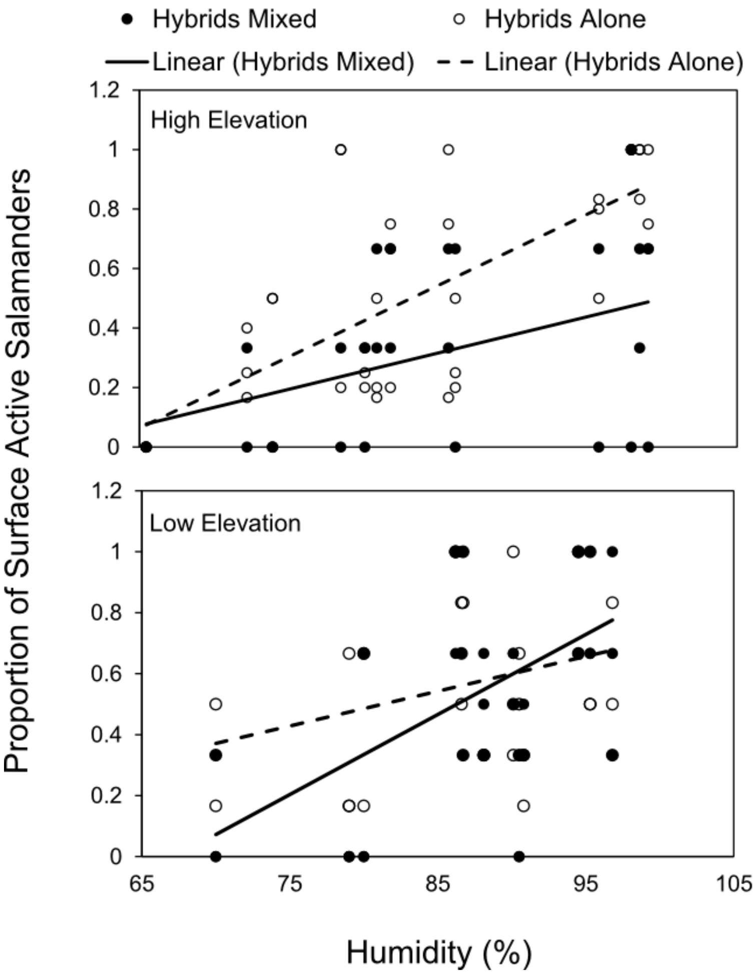

Figure 6. Relationship between the proportion of surface active hybrid salamanders and relative humidity and treatment at the upper and lower bounds of the hybrid zone. At the high elevation, hybrids were alone or mixed with *P. shermani*. At the low elevation, hybrids were alone or mixed with *P. teyahalee*.

DISCUSSION

Parental Distributions

Despite a smaller body size, *P. shermani* exhibited the highest frequency of aggressive behavior, which did not differ whether the opponent was *P. teyahalee*, hybrid, or other *P. shermani*. Conversely, the larger bodied salamander, *P. teyahalee* showed very little aggression in all encounters. The distribution of males and females in my samples did not allow for an analysis on the effects of sex on aggression, but previous studies on *Plethodon* found that sex did not influence aggressive behavior (Nishikawa, 1985; Selby et al., 2006). Given that *P. teyahalee* has the physiological tolerance to live at the higher elevations (Gifford and Kozak, 2012), my data suggest that interference competition is the mechanism which has maintained *P. shermani's* distribution at the high elevation. These findings are similar to studies which found that *P. jordani*, a sister taxa to *P. shermani*, was more aggressive than its sympatric counterpart, *P. teyahalee* (Nishikawa, 1985), and that *P. jordani's* competitive superiority limited the distribution of *P. teyahalee* to low elevations (Gifford and Kozak, 2012).

The literature presumes that, despite its competitive advantage, the distribution of *P. jordani* is restricted to high elevations by physiological tolerance (Kozak and Wiens, 2006; Gifford and Kozak, 2012). The research presented here was unable to find reduced growth rates of *P. shermani* in cages at the microclimate associated with the middle elevation. However, the study was conducted over a single summer season and physiological restrictions may become more apparent on an increased temporal scale or at lower elevations.

Hybrid Zone Expansion

Direct interactions between the parental species have been limited by the presence of a hybrid intermediate that occurs at the middle elevations (Hairston et al., 1992). Hybridization increases the complexity of behavioral interactions affecting species and overall species distributions (Hairston, 1980b). This complexity increases further as a result of hybrid zone expansion in both directions at the expense of the parentals' ranges (Hairston et al., 1992; Pechmann and Hyman, personal communication). Nonetheless, the competitive rankings of hybrids relative to parentals should indicate mechanisms driving the hybrid zone expansion (Pearson and Rohwer, 2000).

By my measures, hybrids were intermediate to the parentals in overall aggressiveness. Specifically, the number of aggressive behaviors exhibited by hybrids was higher than for *P. teyahalee* and lower than for *P. shermani*. The interaction between body size and aggressive behavior for hybrids may indicate the heritability of size and aggressive behavior. If larger hybrids are more *P. teyahalee* "like," then aggression may also be similar to that exhibited by *P. teyahalee*, which could result in larger individuals also showing little aggression. Studies on other types of territorial taxa have shown that hybrid individuals can retain an intermediate level of aggression when the aggressiveness varies greatly between the parental taxa (Pearson and Rohwer, 2000). More aggressive species have been shown to limit access to resources and exclude subordinate taxa from their range, which influences community structure and species distributions (Miller, 1964). Within a hybrid zone, if a parental species and hybrids overlap in requirements, the more aggressive form should have a selective advantage (Pearson and Rohwer, 2000). Therefore, at the bottom of the hybrid zone, where hybrids and *P. teyahalee* overlap, the fitness of the parental salamander may be reduced by the aggressively superior hybrids.

This conclusion was partially supported by my cage experiment where a trend suggested that *P. teyahalee* lost the most mass in cages mixed with hybrids at the middle elevations. The instantaneous growth rates of hybrids were not influenced by elevation or competition with *P. teyahalee* which suggests that hybrids were not at a disadvantage, and in fact, may retain an advantage over the low elevation parental. These findings support competitive exclusion as the primary mechanism driving hybrid expansion down the elevational gradient.

As it pertains to aggression, competitive exclusion occurs when one species interferes with the ability of another species to exploit a resource shared by both species, ultimately displacing the competitively inferior species (Crombie, 1947). Competitive exclusion has been shown in other species of *Plethodon*. A study by Jaeger (1971) suggested that *P. cinerus* restricted *P. shenandoah* to suboptimal talus habitats. My study indicates a similar paradigm where *P. shermani*, and hybrids, restrict *P. teyahalee* from the optimal moist climates associated with the middle and high elevations.

However, by my measurements, *P. shermani* is aggressively superior to hybrids. My analysis of surface activity indicates that in the presence of more *P. shermani* individuals, fewer hybrids remained active on the surface, suggesting that the aggressive superiority of *P. shermani* affects hybrid behavior in the field. My analyses of instantaneous growth rates in the cage experiment indicate that there is not a competitive advantage or disadvantage for hybrids or *P. shermani* during interactions at the middle or high elevational boundary of the hybrid zone. If the mechanism driving the downward expansion of the hybrid zone applies to the upper boundary, I would expect asymmetric hybrid zone movement in favor of *P. shermani*. This suggests that a different mechanism is facilitating this upward movement of *P. teyahalee* traits through a hybrid intermediate.

Aggression may still play a role in the upward hybrid zone expansion, but the mechanism may be sexual selection rather than competitive exclusion. Specifically, hybrid females may be choosing the more aggressive *P. shermani* males over hybrid males, in a system where aggression appears to provide a competitive advantage. Along my study transect, *Plethodon* densities increases with elevation (Maerz, J., personal communication; personal observations). This should result in a density-dependent increase in competitive interactions for limited space and food resources, perpetuating aggression as an advantageous behavior. Hybrid choice for the more aggressive parental has been documented in previous studies (McDonald et al., 2001; Bronson et al., 2003; Grava et al., 2012), and often leads to asymmetric introgression.

My results suggest that larger *P. shermani* individuals are more aggressive than smaller individuals. Body size has been identified as one of the most important factors influencing the outcome of an aggressive encounter (Archer, 1987). Also, male preference for larger females has been described in other Plethodontid salamanders (Verrell, 1995). Therefore, given the opportunity as a result of *P. shermani* male-male competition, aggressively superior *P. shermani* males may prefer hybrid females, which are larger than *P. shermani* females. This suggests, not only that hybrid females may be choosing the more aggressive *P. shermani* males, but that *P. shermani* males may have a preference for the larger-bodied hybrid females.

In this study I found that aggression was higher in *P. shermani* and lower in *P. teyahalee*, with the hybrids intermediate to the parental salamanders. Although this study did not detect effects on the competitive abilities of *P. shermani* at the middle elevations, the literature supports physiological restriction for the high elevation endemic and plasticity for the low elevation species (Gifford and Kozak, 2012). If the hybrids are also intermediate in physiological tolerance, this would give them an advantage over *P. shermani* at middle elevations, particularly in the light of a changing climate.

It is thought that hybrids can acquire the most adaptive traits retained by the parentals, a mixture of which may provide an overall hybrid advantage (Robbins et al., 2014). In another salamander hybrid zone, researchers have found hybrid vigor in survival rates due to resulting genetic heterozygosity (Fitzpatrick and Shaffer, 2007). There are many possible explanations for hybrid advantage, here I was able to support one and suggest others for future research. Understanding how behavioral interactions among hybrids and parentals affect spatial dynamics can provide valuable information for understanding evolution as well as processes associated with conservation efforts.

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