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# Territorial Behavior and Ecological Divergence in a Polymorphic Salamander

Marcie K. Reiter<sup>1</sup>, Carl D. Anthony<sup>1</sup>, and Cari-Ann M. Hickerson<sup>1</sup>

**Polymorphic species provide an opportunity to examine the process of sympatric divergence as it occurs. The Eastern Red-backed Salamander, *Plethodon cinereus*, is a polymorphic species that has served as a model organism in behavioral and ecological studies. Recent work suggests that the two most common color phenotypes (striped and unstriped) exhibit weak assortative mating and are diverging along a number of niche dimensions including temperature optima, diet, and response to predators. Males and females of *P. cinereus* are territorial and this behavior is thought to function in the context of prey and mate acquisition. Striped males have been shown to gain access to larger, and presumably more fecund, females. We posited that this pattern emerges through differential territorial behavior between the two phenotypes. We predicted that striped and unstriped salamanders would differ in their use of cover objects in the field, and in their aggressive responses to intruders in the laboratory. We examined salamander cover use and movement by placing artificial cover objects (ACOs) on the forest floor and monitoring them for 3.5 years. We compared residency time, number of recaptures, and number of ACOs occupied between the two phenotypes. The proportion of striped salamanders that were territorial residents was significantly greater than the proportion of unstriped salamanders that were territorial residents. Striped salamanders also exhibited significantly longer territorial residency, were recaptured more often, and were more often found under multiple, adjacent cover objects than unstriped salamanders. In the laboratory, we examined territorial behavior of the two morphs. As residents, striped salamanders were more aggressive and less submissive than were unstriped residents. When compared to intruders, resident salamanders of both morphs behaved more aggressively, but significant differences between resident and intruder behavior were only detected for the striped phenotype. These differences in aggression and cover object use may help to explain how striped males gain access to larger females and may be important in the interpretation of sympatric niche divergence and assortative mating by color in this species.**

RECENT studies have focused on polymorphic species as models for examining sympatric or parapatric speciation, or the divergence of a species without geographic isolation (reviewed in Schluter, 2009; Feder et al., 2013). In the absence of physical barriers to gene flow between diverging populations, non-random mating becomes a key mechanism by which populations diverge in sympatry. Competition for resources, including food and space, may drive changes in phenotypic expression and can result in reproductive isolation (Rundle and Nosil, 2005). It is presumed that this type of sympatric divergence reduces competition between or within species, thus allowing for coexistence (Adams and Rohlf, 2000). Many studies have looked at polymorphic species that express intraspecific differences in resource use and express some level of reproductive isolation as intermediate steps in speciation (West-Eberhard, 1986; Bush, 1994; Whiteman et al., 2006; Chamberlain et al., 2009; Corl et al., 2010). We chose to examine the potential for sympatric divergence in the red-backed salamander, *Plethodon cinereus*. This salamander is a completely terrestrial, polymorphic species that occurs in moist forest-floor habitats in eastern North America. The behavioral ecology of *P. cinereus* is well studied (reviewed in Jaeger and Forester, 1993 and in Mathis et al., 1995), allowing for a number of interesting hypotheses to be posed in the context of mate acquisition and territoriality. Additionally, because many populations are polymorphic, this species provides an excellent system in which to examine the conditions under which sympatric divergence might emerge.

*Plethodon cinereus* is polymorphic for dorsal color pattern, and most populations are composed primarily of striped and unstriped morphs. The proportion of each color morph

varies geographically, but in most populations the unstriped phenotype makes up less than 20% of individuals (Highton, 1962; Pflingsten and Walker, 1978; Anthony and Pflingsten, 2013). Recent studies have shown that the striped and unstriped morphs of *P. cinereus* differ on a number of niche dimensions including thermal optima, diet, response to predators, and mating behavior (reviewed in Anthony et al., 2008). These studies suggest that, compared to the striped phenotype, the unstriped morph is associated with warmer and drier conditions (Williams et al., 1968; Lotter and Scott, 1977; Moreno, 1989; but see Petrucci et al., 2006), and utilizes a narrower prey base of seemingly lower nutritional value (Anthony et al., 2008). Studies also suggest that unstriped morphs experience more frequent tail breakage, presumably due to interactions with predators (Moreno, 1989; Venesky and Anthony, 2007). Evidence for incipient speciation between the two phenotypes on Long Island, New York suggests that climatic variables and spatial separation play a role in divergence of parapatric pure striped and pure unstriped populations (Fisher-Reid et al., 2013). It is unclear, however, what role social behavior may play in divergence or in the maintenance of distinct monomorphic lineages.

Plethodontid salamanders have been studied extensively in both field and in laboratory contexts (Hairston, 1988). A number of species, including *P. cinereus*, have been shown to be territorial (reviewed in Mathis et al., 1995). Most studies of territoriality in *P. cinereus* have been conducted on populations from New York and Virginia, but populations from northeastern Ohio are also territorial (Gall et al., 2003; Hickerson et al., 2004; Deitloff et al., 2008). Adult males and females of *P. cinereus* defend intersexually overlapping territories under rocks and logs on the forest floor (Mathis,

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1990, 1991) and adults (especially males) rarely co-occur together under the same cover object (Jaeger et al., 1995; Anthony et al., 2008). In numerous vertebrate species, territorial disputes can occur over mates (Hixon, 1987) and often over feeding areas. In *P. cinereus*, location of the territory is thought to influence diet, and a superior diet correlates with a high quality territory (Mathis, 1990; Gabor, 1995). For male salamanders, possession of a superior diet and territory may be attractive to females, who may assess the diet of males and the quality of their territories through fecal squashing (Walls et al., 1989; Mathis, 1990; Karuzas et al., 2004). Although territorial behavior, and its relationship to male/female interactions is well studied in *P. cinereus*, it is unknown if differences in aggressive behavior and cover object use exist between striped and unstriped color phenotypes. Differences in territorial behavior between the two morphs may result in differences in acquiring mates and ultimately in assortative mating. Interestingly, two studies, on a single polymorphic population in Ohio, have shown that the striped and unstriped phenotypes exhibit weak, but statistically significant, positive assortative mating by color (Anthony et al., 2008; Acord et al., 2013).

Anthony et al. (2008) presented a model under which positive assortative mating by color in *P. cinereus* emerges through differential access by females to male territories. They hypothesized that assortative mating may be a result of high-quality diet cues of striped males attracting high-quality females (most of which were striped). Acord et al. (2013) reported similar patterns of non-random mating between the two phenotypes, but found no evidence that females were using diet cues to assess mate quality. However, both studies presented evidence that striped males of *P. cinereus* may be more attractive to female conspecifics. For example, compared to unstriped males, striped males were found paired with significantly larger (and presumably more fecund) females in field samples (Anthony et al., 2008; Acord et al., 2013). Additionally, when presented with males of each phenotype in the laboratory, both striped and unstriped females were found in closer proximity to striped, compared to unstriped, males (Acord et al., 2013). An untested hypothesis of this model is that striped males of *P. cinereus* are in some way more territorial than are males of the unstriped phenotype.

Differences in diet, in response to temperature or moisture, and differences in territorial behavior may interact in ways that affect mate choice. For example, if thermal optima (Lotter and Scott, 1977; Moreno, 1989) and diet (Anthony et al., 2008) differ between the two morphs, differential territorial behavior by phenotype may be expressed. Territorial behavior is thought to be important in gaining access to prey and mates in this species (Jaeger, 1981; Horne and Jaeger, 1988; Nunes, 1988), and most researchers agree that these salamanders defend patches of moisture, and their associated prey, under cover objects on the forest floor (Mathis, 1990, 1991; Jaeger et al., 1995). Adaptations that permit foraging in warmer and/or drier microhabitats could reduce the benefit derived from territorial behavior. Under such a scenario, unstriped phenotypes would exhibit decreased territorial behavior and this might make them less attractive to striped females.

The intention of this study was to determine if the two color morphs of *P. cinereus* have diverged in their expression of territorial behavior, a key component of the social behavior of this species. Based on previous research, we

predicted that the striped morph would be more likely to exhibit territorial and aggressive behavior. This prediction is based on the diet of the striped morph consisting of more profitable prey than the unstriped morph (Anthony et al., 2008) and the ability of the striped morph to attract larger mates (Anthony et al., 2008; Acord et al., 2013). Both of these factors are directly influenced by the quality of territory an individual salamander holds. We used a combination of field and laboratory approaches to examine differences in territorial behavior. In the field portion of our study, we monitored artificial cover objects across multiple years to compare residency status and number of recaptures between the two phenotypes. In the laboratory component of this study, we examined aggressive interactions between the two color morphs of *P. cinereus* in an effort to determine if aggressive behavior differed by phenotype. We also compared behavior of residents to intruders to explore how territorial status affected behavior for each morph. In territorial species, residents are expected to behave more aggressively than intruders (Parker, 1976; Davies, 1978; Anthony et al., 1997), and we predicted that this would be the case for striped, but perhaps not unstriped, salamanders.

## MATERIALS AND METHODS

**Artificial cover object use in the field.**—We used artificial cover objects (ACOs; 30 cm<sup>2</sup> ceramic floor tiles) to monitor salamander activity on the forest floor in the Cuyahoga Valley National Park, Summit Co., Ohio, USA (41°13'45.6"N, 81°30'59.6"W). Our forested site was dominated by *Fagus grandifolia* (American Beech), *Acer saccharum* (Sugar Maple), *Liriodendron tulipifera* (Tulip Poplar), and *Quercus rubra* (Red Oak). We arranged ACOs in 3 × 3 plots of nine tiles each (117 ACOs). Tiles within plots were one meter apart, and each plot was separated by two meters. ACOs were lifted every two weeks during the active season from April 2004 to November 2007 and examined for the presence of salamanders. Inclement weather kept us from checking tiles in the months of January and February, and the site was only visited in December and in March when surface temperatures exceeded 6°C. Salamanders were photographed with a Nikon D100 DSLR equipped with a 50 mm macro lens and measured with digital calipers (snout-to-vent length [SVL]). To determine sex of adult salamanders we examined the size and shape of the snout. Adult males of *P. cinereus* exhibit an enlarged snout when in reproductive condition; the snout of females appears blunt relative to a reproductively active male (Anthony et al., 2008). After being measured and photographed, salamanders were immediately released at the point of capture. Salamanders were individually identified based on unique dorsal patterns within each of the 13 nine-tile plots (Anthony et al., 2008). Individual salamander identifications were made by simultaneously comparing enlargements of the dorsal head and pelvic regions on two 22" computer monitors. Both striped and unstriped salamanders have unique constellations of dorsal spots, but unstriped salamanders can be more difficult to identify. Photographic matches were conducted by student observers and by MKR. CDA spot checked 25% of striped identifications and all unstriped identifications. At the end of the study we classified salamanders either as floaters (individuals observed only one time) or residents (individuals observed two or more times during the 3.5-year study; Mathis, 1991). We used Chi-square analysis to examine numbers of floaters versus territorial residents of the color

morphs. We compared residency times, number of times recaptured, and number of tiles occupied between the two color morphs using two-tailed t-tests or non-parametric equivalents.

**Aggressive behavior in the laboratory.**—During May and June 2009 we collected 30 striped and 30 unstriped adult male *P. cinereus* by hand-turning logs and rocks in privately held land adjacent to our field site in the Cuyahoga Valley National Park (CVNP) near Peninsula, Ohio (41°13'37.8"N, 81°31'34.4"W). Only adult males greater than 32 mm SVL (Anthony and Pflingsten, 2013) were used in this study. Individuals were transported in separate 50 mL centrifuge tubes to the laboratory. Males, not females, were used in experiments to reduce the potential confounding effects of courtship behavior, and because males show higher levels of aggressive behavior than do females (Horne and Jaeger, 1988). In the laboratory, individuals were housed separately in 15 cm diameter plastic Petri dishes (hereafter chambers) lined with moist filter paper. Experimental chambers were maintained in a controlled environment with natural photoperiod, and temperature maintained at  $17 \pm 1^\circ\text{C}$ . Salamanders were fed approximately 25 flies (*Drosophila melanogaster*) per week. Territorial residents spent five days in their home chambers on filter paper substrates prior to testing. This allowed residents to mark chambers with pheromones (Jaeger, 1981). Twenty-four hours before testing began, any remaining prey items were removed.

Testing took place in the resident's home chamber on marked filter paper substrates. Immediately prior to testing, an intruder was added to the center of the resident's chamber, and both resident and intruder were placed under separate 5.1 cm opaque acclimation covers for a five-minute habituation period. Once the acclimation covers were removed, behavioral interactions were monitored for 15 minutes. Trials began after the onset of interactive behavior by either the resident or the intruder (Anthony et al., 1997).

Each individual was tested five times. Residents were paired once each with an individual of the same color, a different color, and a control. Each individual was used as an intruder twice; once with a salamander of the same color, and once with one of a different color. In the control treatment the resident was paired with a surrogate intruder, a rolled-and-moistened paper towel the same length and width as the resident (Jaeger et al., 1982).

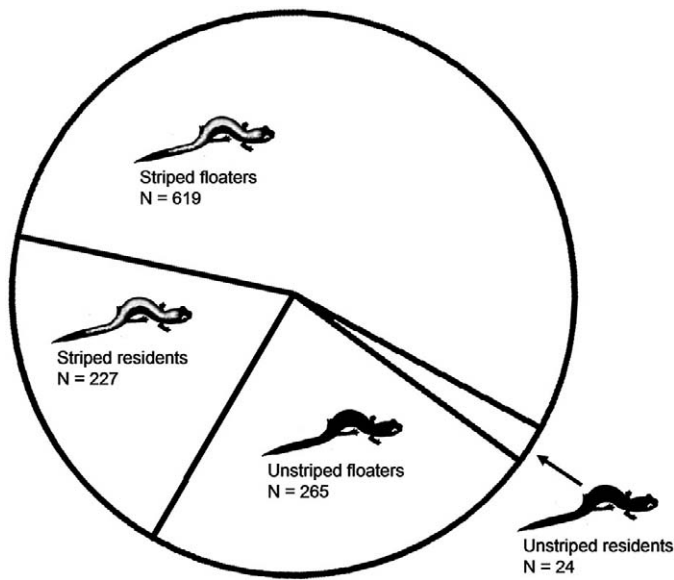
Twelve trials were conducted on each of 15 testing days through June, July, and August 2009 (180 trials in total). Treatments and controls were evenly distributed among trial days (Hurlbert, 1984). Trials were run between 1000 and 1500 hours under indirect fluorescent lighting. One observer (MKR) recorded durations of behaviors using TrueBasic Event-PC 3.0 data collection software (TrueBASIC, Inc.) running simultaneously on two laptop computers (one for the resident and one for the intruding salamander). The following criteria were used to pair residents and intruders: no individual was paired with the same opponent more than once; no salamander was tested more than once per week; size asymmetries were minimized by randomly pairing individuals within size classes (Hickerson et al., 2004) to reduce fighting advantages of larger individuals (Mathis, 1990). The average sizes of the striped and unstriped morphs were 37.2 mm (SE = 0.38) and 37.3 mm (SE = 0.41), respectively, and the mean difference between residents and intruders within each size class was 0.76 mm.

The aggressive behaviors that were measured for the resident and intruder were modified from Jaeger (1984) and included: look toward (LT) and/or move toward (MT) in which a salamander turns its head toward, or moves in the direction of, the other salamander in a path that if continued would result in contact. The submissive behaviors recorded included: flattened (FLAT) in which the entire body is pressed against the substrate; look away (LA) and/or move away (MA) in which the salamander turns its head away from, or moves in a direction that increases the distance between itself and the other salamander; and escape (ESC) in which one or more limbs of a salamander is pressed against the wall of the experimental chamber. All behaviors were timed in seconds. Timing of behaviors was ended when the focal animal clearly stopped the behavior (e.g., removed its limb from the chamber wall for the behavior ESC) or exhibited a subsequent behavior such as moving the body or head. An index of aggression (AI, modified from Mathis et al., 2000) was calculated using the aggressive behaviors MT and LT and the submissive behaviors MA and LA ( $[\text{MT}+\text{LT}]-[\text{MA}+\text{LA}]$ ).

We used repeated measures MANOVA to examine the aggressive index (AI) and the submissive responses ESC and FLAT exhibited by territorial residents in three scenarios: 1) when paired with an intruder of the same color (intra-morph); 2) when paired with an intruder of the opposite color (intermorph); and 3) when paired with a surrogate "intruder" (control). Treatment was designated as a within-subjects factor because each treatment was applied to each experimental unit (individual). Behaviors were also designated as within-subject factors because each of the behaviors was measured in each of the three treatments. We compared main effects among treatments using Bonferroni confidence interval adjustments. Separate tests were run for striped ( $n = 30$ ) and unstriped ( $n = 30$ ) resident salamanders. We used Mann-Whitney U tests to compare striped ( $n = 30$ ) to unstriped ( $n = 30$ ) territorial residents in intramorph contests. This approach was used to determine if one morph was more or less aggressive than the other. To test for a residency effect we used paired Wilcoxon signed-rank tests (one-tailed) to compare the behaviors of salamanders when tested as residents to their behavior when tested as intruders in intramorph treatments. For these comparisons the color morphs were analyzed separately. For test comparisons,  $\alpha$  was set at 0.05. Repeated measures MANOVAs were run using SPSS version 16.0 (SPSS Inc., Chicago, IL). All other statistical tests were run using NCSS version 9 (NCSS LCC, Kaysville, UT).

## RESULTS

**Artificial cover object use in the field.**—Eastern Red-backed Salamanders (*Plethodon cinereus*) made up over 99% of the salamanders observed at the site, but we also observed *P. glutinosus*, *Eurycea bislineata*, and *Notophthalmus viridescens* under cover objects. We examined 1613 photographs representing 1135 individual red-backed salamanders. Of these salamanders, 289 (34.2%) were unstriped. A significantly larger proportion of striped salamanders (227/846 or 27%) were classified as territorial residents than unstriped salamanders (24/289 or 8.3%;  $\chi^2 = 42.9$ ,  $df = 1$ ,  $P < 0.0001$ ; Fig. 1). For striped salamanders, we observed nearly equal numbers of male ( $n = 98$ ) and female territorial residents ( $n = 96$ ), with the remainder of individuals ( $n = 33$ ) recorded as juveniles or subadults. For unstriped salamanders, twice as



**Fig. 1.** Numbers of territorial and non-territorial striped and unstriped phenotypes of the Eastern Red-backed Salamander, *Plethodon cinereus*. Floaters were those salamanders that were observed only one time during the 3.5-year study; territorial residents were recaptured at least one time. Relative to unstriped salamanders, there were significantly more striped territorial individuals than would be expected by chance alone ( $\chi^2 = 42.9$ ,  $P < 0.0001$ ).

many females ( $n = 13$ ) as males ( $n = 6$ ) were characterized as residents; the remainder of recaptures were subadults ( $n = 5$ ).

Duration of territorial residency, defined as the number of days between first and last capture, also differed between the two phenotypes, with striped salamanders exhibiting significantly longer territory residence times (mean of 373.9 versus 236.0 days;  $t = 2.71$ ,  $df = 249$ ,  $P = 0.011$ ; two-tailed; Table 1). This difference was more pronounced when only adult salamanders greater than 32 mm SVL were analyzed (mean of 407.9 versus 220.8 days;  $t = 3.85$ ,  $df = 195$ ,  $P = 0.001$ ; two-tailed; Table 1). The duration of residency ranged from 10 to 1281 days across both morphs, but only two unstriped salamanders exhibited residency across all four

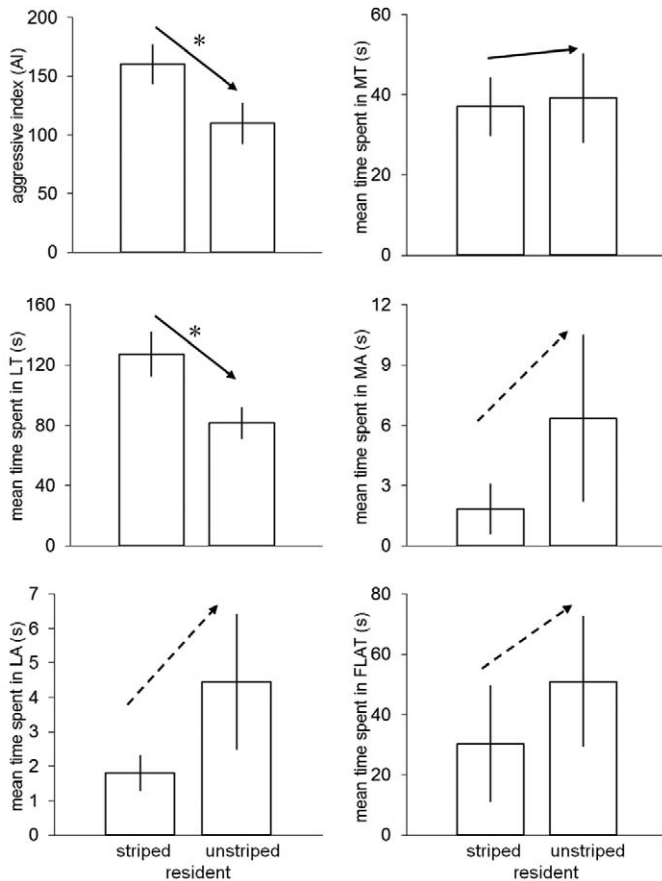
active seasons (over 700 days), compared to 44 striped salamanders. For both morphs, females had longer mean residency times (80.4 days longer for striped salamanders versus 24.5 days for unstriped salamanders), but these differences were not statistically significant for either morph. Floaters, those salamanders that were observed only one time, were not included in the above calculations.

Striped salamanders were recaptured more often than unstriped salamanders (mean of 2.5 versus 1.5 recaptures;  $z = 2.19$ ,  $df = 249$ ,  $P = 0.028$ ; two-tailed; Table 1), but this difference disappeared when residency duration was factored in and mean recaptures per day were compared ( $z = 0.59$ ,  $df = 249$ ,  $P = 0.720$ ; two-tailed; Table 1). Most recaptured salamanders (95.2%) were found under only one or two adjacent cover objects, but striped salamanders tended to be found under adjacent cover more often than did unstriped salamanders (mean of 1.5 versus 1.3 cover objects;  $z = 1.71$ ,  $df = 249$ ,  $P = 0.087$ ; two-tailed; Table 1). Adult striped salamanders in particular were significantly more likely to use more cover objects than unstriped salamanders (mean of 1.5 versus 1.2 objects;  $z = 2.11$ ,  $df = 195$ ,  $P = 0.035$ ; two-tailed; Table 1). We detected no differences between sexes (for either morph) in the number of times captured or in cover object use.

**Aggressive behavior in the laboratory.**—Species typical behaviors, as described by Jaeger (1984), were exhibited by both color morphs. We used behaviors exhibited by residents in intramorph pairs to assess levels of aggression for each morph. Striped salamanders had a significantly higher aggression index ( $z = 2.51$ ,  $df = 29$ ,  $P = 0.012$ ; Fig. 2), while unstriped resident salamanders spent more time in the submissive behavior FLAT, although this result was not statistically significant. To assess whether residency affected behavior, we compared the behavior of salamanders of each morph when they were used as residents to when they were used as intruders. We only detected a residency effect among individuals of the striped phenotype (Fig. 3). For example, striped resident salamanders were significantly more aggressive, and less submissive than when they were tested as intruders. Specifically, striped residents had significantly higher aggression index (AI) values than did striped

**Table 1.** Field observations of territorial behavior of striped and unstriped phenotypes of the Eastern Red-backed Salamander, *Plethodon cinereus*. Mean days of residency refers to the span of days between the first and final recapture under cover objects in the field. Mean number of recaptures indicates how many times salamanders were observed during residency. Mean number of tiles occupied refers to the number of adjacent artificial cover objects salamanders were observed under during residency. Analyses were conducted on all salamander size classes combined and on adults only (salamanders that were at least 32 mm SVL when first observed). Floaters, those salamanders observed only one time, were not included in the calculations. Mean, (SE), and (range) are reported for all values.

Phenotype	Mean days of residency	Mean # recaptures	Mean # tiles occupied
Striped	373.9 (22.3) (10–1281)	2.53 (0.17) (1–19)	1.50 (0.04) (1–4)
Unstriped	236.0 (45.8) (13–756)	1.46 (0.18) (1–4)	1.33 (0.14) (1–4)
Test statistic	$t = 2.71$	$z = 2.19$	$z = 1.71$
$P$	0.011 *	0.029 *	0.087 ns
Striped adults only	407.9 (22.3) (10–1281)	2.71 (0.21) (1–19)	1.50 (0.04) (1–4)
Unstriped adults only	220.8 (45.8) (14–494)	1.47 (0.19) (1–4)	1.18 (0.09) (1–2)
Test statistic	$z = 3.85$	$z = 1.84$	$z = 2.11$
$P$	0.001 ***	0.066 ns	0.035 *

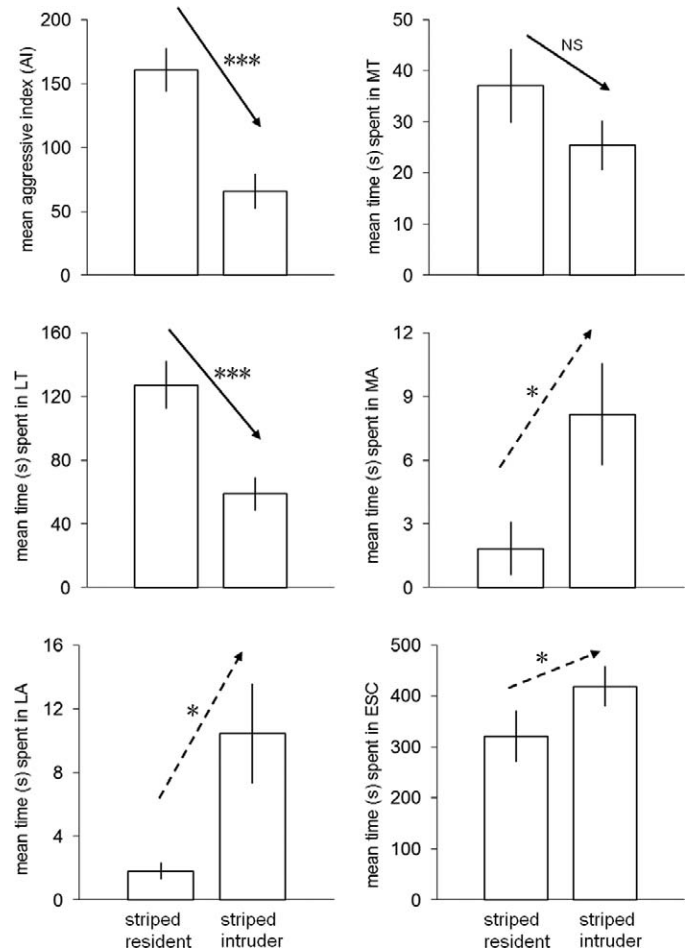


**Fig. 2.** Mean time (seconds) spent in aggressive and submissive behaviors by residents of each color morph when paired with same-color intruders. Solid arrows indicate differences in aggressive behaviors between morphs, and dashed arrows, submissive behaviors. Significant differences between morphs (two-tailed Mann Whitney U tests;  $P < 0.05$ ;  $n = 30$ ) are indicated by asterisks. Abbreviations for behaviors are as follows: aggression index (AI), move toward (MT), look toward (LT), move away (MA), look away (LA), and flattened (FLAT).

intruders ( $t = 4.37$ ,  $df = 29$ ,  $P = 0.0002$ ; one-tailed), and spent significantly less time in escape behavior ( $t = 2.33$ ,  $df = 29$ ,  $P = 0.014$ ; one-tailed). In contrast, residency status had no effect on the aggressive behavior of unstriped salamanders (Fig. 4), though they did spend significantly more time in escape ( $t = 3.04$ ,  $df = 29$ ,  $P = 0.004$ ; one-tailed) as intruders. Residents of both phenotypes behaved similarly in intramorph and intermorph pairings, and intruder phenotype did not affect any of the recorded resident behaviors (Table 2).

## DISCUSSION

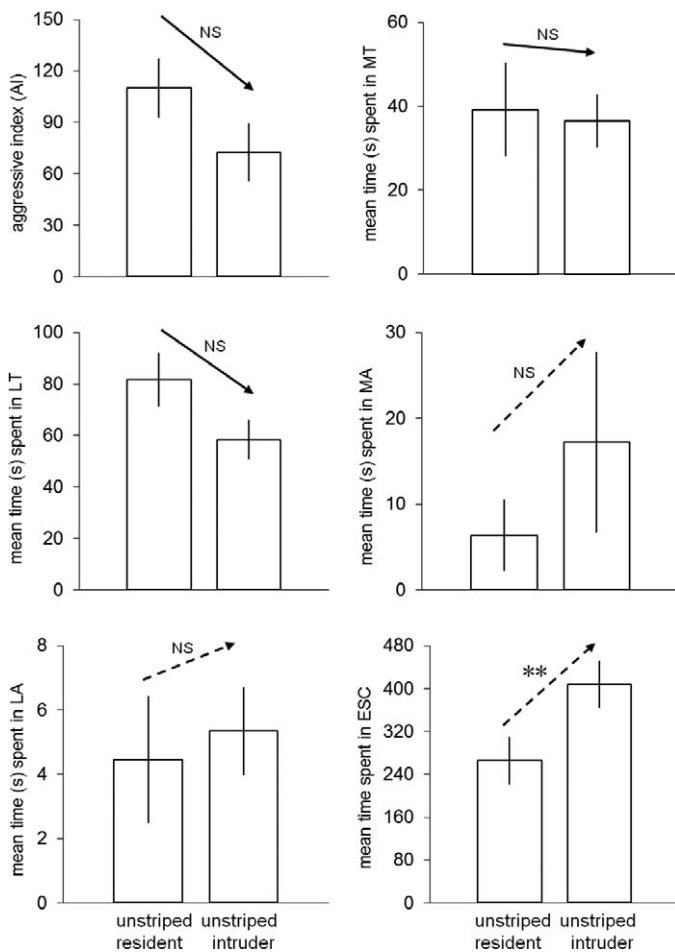
Polymorphic species provide an opportunity to examine the mechanisms involved in the initial stages of ecological divergence (West-Eberhard, 1986; Nosil et al., 2009). We examined a polymorphic population of Eastern Red-backed Salamanders to investigate the role of social behavior in assortative mating, a key step in the divergence process. Red-backed salamanders provide an excellent system to test hypotheses regarding social behavior as they have been well studied for decades (Jaeger and Forester, 1993; reviewed in Mathis et al., 1995), and much is known regarding factors involved in mate acquisition. In a model presented by Anthony et al. (2008), positive assortative mating by color in



**Fig. 3.** Differences in resident and intruder behavior (i.e., residency effect) of striped salamanders in intramorph trials. Durations of behaviors per 15-minute trial are indicated. Solid arrows indicate differences in aggressive behaviors between residents and intruders, and dashed arrows, submissive behaviors. Significant differences between striped residents and striped intruders (Wilcoxon signed ranks tests or paired t-tests; one-tailed; mean  $\pm$  SE,  $n = 30$ ) are indicated by asterisks ( $* = P < 0.05$ ;  $*** = P < 0.001$ ). Abbreviations for behaviors are as in Figure 2 with the addition of escape (ESC).

*Plethodon cinereus* was postulated to be an outcome of differential access by females to male territories. Under this model, females may compete for access to high quality males and their territories, and as a result only the highest quality females (most of which are striped) gain access to these territories. An untested hypothesis of this model is that striped males of *P. cinereus* are more aggressive, or more territorial, than are unstriped males. In the field portion of this study, we found significant differences between the two color morphs in the way in which territorial cover was used. Striped morphs exhibited longer duration of residency, were recaptured more often, and used more cover objects than did unstriped morphs. Our laboratory results indicated significant differences in territorial behaviors between striped and unstriped *P. cinereus*. Striped salamanders were generally more aggressive, and unstriped salamanders were generally more submissive. Our results are consistent with the predictions of Anthony et al. (2008), and provide further evidence that ecological divergence in this species may be mediated by social interactions.

Acord et al. (2013) suggested that territorial behavior by striped females may play a role in driving assortative



**Fig. 4.** Differences in resident and intruder behavior (i.e., residency effect) of unstriped salamanders in intramorph trials. Durations of behaviors per 15-minute trial are indicated. Solid arrows indicate differences in aggressive behaviors between residents and intruders, and dashed arrows indicate submissive behaviors. Significant differences between residents and intruders (Wilcoxon signed ranks test or paired t-tests; one-tailed; mean  $\pm$  SE,  $n = 30$ ) are indicated by asterisks (\*\* =  $P < 0.01$ ). Abbreviations for behaviors are as in Figure 2 with the addition of escape (ESC).

pairings, and our work provides support for these ideas. Males and females of this species are territorial (Lang and Jaeger, 2000) and cooperate in co-defense of high quality territories. Our data suggest that the males of the striped morph exhibit higher levels of aggression and territorial behavior. Thus co-defense of territories is more likely to

occur by striped salamander pairs, and this may exclude all but the largest unstriped females from access to co-defended striped territories. Indeed, only the largest unstriped females associated with striped males in the field (Anthony et al., 2008). Smaller unstriped females may then select down a male quality gradient and pair with non-territorial unstriped males. Our field results corroborate these predictions in that striped salamanders of both sexes maintained territory residency for the longest periods. For the unstriped phenotype, it was females rather than males that were more likely to be residents, suggesting that unstriped male salamanders rarely hold territories.

Our laboratory tests of aggressive behavior in the two phenotypes are also consistent with a striped male advantage in territory and mate acquisition. Striped males were more aggressive and exhibited a clear residency effect as predicted by territoriality theory (Parker, 1976; Davies, 1978; Figler and Einhorn, 1983). Theory predicts that residents should be more aggressive than intruders, but unstriped males showed no such effect even when allowed to establish residency in laboratory trials. This result is consistent with our field observations of unstriped salamanders being more likely to behave as floaters. These two lines of evidence suggest that the unstriped morph is less likely to exhibit territorial behavior which has been shown to be important in both mate and prey acquisition in this species (Jaeger, 1981; Horne and Jaeger, 1988; Nunes, 1988). Differential aggression by phenotype reinforces the notion that unstriped females may be forced to accept lower quality mates (either unstriped males or small striped males). Field data of presumptive mated pairs show this very pattern of size dimorphism within pairs (Anthony et al., 2008; Acord et al., 2013) where striped males are found with large females.

Numerous researchers have suggested that the unstriped phenotype is adapted to warmer and drier conditions (Burger, 1935; Test, 1952; Williams et al., 1968; Lotter and Scott, 1977), but more recent studies have found that the relationships between climate and morph frequency are more complex than previously thought (Gibbs and Karraker, 2006; Petrucci et al., 2006; Fisher-Reid et al., 2013). For example, forest drying was implicated in a historical increase in the numbers of unstriped salamanders across the range of *P. cinereus* (Gibbs and Karraker, 2006), and dry conditions, not warm temperatures, were found to be more important in predicting the distribution of the unstriped morph on Long Island (Fisher-Reid et al., 2013). Because they lack lungs, plethodontid salamanders are limited to foraging in moist microhabitats (Feder, 1983). The territorial behavior of *P. cinereus* involves the defense of a cover object

**Table 2.** Behaviors (in seconds) of resident *Plethodon cinereus* in intramorph, intermorph, and control trials. Both morphs responded similarly to same color and opposite color intruders. Mean, standard error, and range (in parentheses) are reported for all behaviors.

Behaviors	Same color intruder	Opposite color intruder	Control	<i>P</i>
Striped resident				
AI	160, 16.9 (0–124)	155, 28.0 (0–637)	52.6, 8.5 (0–188)	0.002
FLAT	30.3, 19.3 (0–524)	24, 14.4 (0–364)	2.56, 2.4 (0–72)	0.187
ESC	320, 50.6 (0–845)	228, 42.9 (0–753)	287.6, 40.1 (0–737)	0.212
Unstriped resident				
AI	110, 17 (0–506)	134, 20 (0–596)	51.1, 9.8 (0–261)	0.003
FLAT	50.9, 21 (0–470)	16.5, 10.5 (0–266)	9.8, 9.8 (0–295)	0.154
ESC	265, 44.4 (0–680)	318, 46 (0–856)	335, 41.3 (0–820)	0.335

that provides cool and moist microhabitat when the surrounding habitat may be inhospitable. Territorial defense is costly (Brown and Orians, 1970; Jaeger et al., 1983; Mathis, 1991), and so the ability to forage without having to defend a territory may represent an evolutionary trade-off that helps to explain how the unstriped morph is maintained in polymorphic populations despite their reduced access to high quality mates. Our data suggest that unstriped males are more likely to play the role of “floaters” (i.e., those that are unable to hold territories [Mathis, 1991]), partially because they are less aggressive, and also because they may be able to tolerate drier leaf litter microhabitats relative to striped individuals. As a result, unstriped floater males may only acquire small female mates that are unable to access co-defended striped territories; many of these small females would likely be unstriped (Anthony et al., 2008; Acord et al., 2013). Differences in territorial behavior between the two phenotypes may, then, be one mechanism underlying the occurrence of positive assortative mating by color in dimorphic populations of *P. cinereus*.

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