Differential Responses of Eastern Red-backed Salamanders (Plethodon cinereus) to Conspecifics and Centipedes

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Differential Responses of Eastern Red-backed Salamanders (*Plethodon cinereus*) to Conspecifics and Centipedes

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Abstract: Plethodontid salamanders are known to aggressively interact not only with conspecifics but also with other potential competitors, such as centipedes. Eastern Red-backed Salamanders (*Plethodon cinereus*) are thus expected to respond aggressively toward potential competitors, both conspecifics and heterospecifics. Additionally, the residency status of a salamander can influence aggression levels. Male *P. cinereus* were exposed to one of four substrate chemical cue treatments: control, self, conspecific, or centipede (*Scolopocryptops sexspinosus*) to determine their behavioral reaction to the presence or absence of cues from conspecifics and heterospecifics. Salamanders were additionally paired with a conspecific or a centipede in different "habitats" to determine if behavioral interactions were affected by residency status. Salamanders did not respond aggressively when exposed to chemical cues from conspecifics or heterospecifics. However, *P. cinereus* increased the time spent in aggressive postures when physically paired with a centipede, but showed no increase in aggression when paired with a conspecific. *Plethodon cinereus* in our population did not respond aggressively toward conspecifics in either experimental setting; however, they did respond to centipedes with increased aggression regardless of their residency status, indicating that the decrease in aggression towards conspecifics is not paralleled by a decrease in aggression towards heterospecifics.

Key words: Aggression; Eastern Red-backed Salamander; Intraspecific competition; Plethodontidae; *Scolopocryptops sexspinosus*

INTRODUCTION

Plethodon cinereus often directs aggressive behavior towards conspecifics as well as congenerics (Wrobel et al., 1980; Jaeger, 1981; Jaeger et al., 1982; Townsend and Jaeger, 1998; Deitloff et al., 2008). Aggression levels in *P. cinereus* can vary among individuals and often increase depending on factors such as length of ownership of a territory (Nunes and Jaeger, 1989), threat of competition (Hairston, 1981; Nishikawa, 1985), size of competitor, or residency status (Nunes and Jaeger, 1989). In general, male *P. cinereus* aggressively defend their territory against intruding males and are less aggressive towards intruding females and juveniles (Lang and Jaeger, 2000). An intruding *P. cinereus* is likely to display submissive

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postures when in another salamander's territory (Simons et al., 1997).

In addition to responding to other salamanders (either conspecifics or heterospecifics), *P. cinereus* has been shown to recognize and respond to centipedes and carabid ground beetles with the same aggressive postures as they show towards conspecifics (Gall et al., 2003; Hickerson et al., 2004; Anthony et al., 2007). *Plethodon cinereus* often share very similar habitats with centipedes, *Scolopocryptops sexspinosus* (Petranka, 1998), and carabid ground beetles, *Platynus tenuicollis* (Gall et al., 2003). Although *S. sexspinosus* and *P. tenuicollis* do not pose a physical threat to *P. cinereus*, their diet closely overlaps with *P. cinereus* (Roberts, 1956; Dillion and Dillion, 1961; Jaeger, 1980) making them potential competitors for food as well as space.

Plethodon cinereus from populations in northwestern Ohio demonstrate aggressive behavior towards conspecifics (Gall et al., 2003; Hickerson et al., 2004; Deitloff et al., 2008), as well as centipedes and beetles (Gall et al., 2003; Hickerson et al., 2004; Anthony et al., 2007). However, preliminary laboratory observations in a central Ohio population of *P. cinereus*, 150 km from the northeastern Ohio populations, suggest that this population may not aggressively respond to or avoid chemical cues of conspecifics (Hurst and Smith, 2006). Similarly, but on a larger geographic scale, Quinn and Graves (1999) observed differences in the extent of aggregation, and thus presumably aggression, between populations of *P. cinereus* in Michigan and Virginia. To gain a better understanding of how aggression varies among populations of *P. cinereus* that are relatively close geographically, we experimentally examined the extent of aggression to conspecifics and centipedes in the central Ohio population of *P. cinereus*. Given the preliminary observations in Hurst and Smith (2006) we expected to find a lack of aggression between conspecifics in our population. If there is an apparent lack of aggression towards conspecifics, we might expect this could be accompanied by a lack of aggression towards centipedes if there is a general decrease in overall aggression in this population of *P. cinereus*. If instead the lack of aggression to conspecifics is due to the specific social or environmental context relevant only to intraspecific interactions then we might expect aggression to be manifested towards the centipedes. To this end, we conducted a series of laboratory experiments to determine the response of *P. cinereus* to chemical cues and physical presence of conspecifics and heterospecifics (centipedes). We conducted behavioral interaction experiments under different residency contexts (e.g., a salamander's own territory, a neutral territory, or another individual's territory) in order to examine potential associations between aggression and territory ownership. Our design allowed us to test the following three hypotheses: (1) *P. cinereus* will demonstrate aggressive postures when exposed to chemical cues of both conspecifics and centipedes, (2) *P. cinereus* will show aggressive behaviors to intruders (salamanders or centipedes), and (3) *P. cinereus* will demonstrate greater aggression when in their resident habitat compared to neutral or conspecific habitats.

Materials and Methods

Substrate recognition

We collected adult male *P. cinereus* (n=55; snout-vent length [SVL] 31 to 52 mm) and adult centipedes (*S. sexspinosus*, n=14; length=8.4 to 43 mm) from the Denison University Biological Reserve, Granville, Licking County, Ohio on 30 September 2005, 12 and 26 October 2005, and 10 November 2005. We housed salamanders and centipedes individually in large Petri dishes (15 cm diameter, 1.6 cm tall) lined with damp filter paper and placed in a laboratory at room temperature (≈19°C), under a normal fall photoperiod. Salamanders and centipedes were misted daily with room temperature aged-tap water. Each individual was kept in their dish for five days in order to allow for the marking of their habitat with chemical cues (see Hickerson et al., 2004).

Each *P. cinereus* was randomly assigned to one of four substrate treatments: control (n=13), self (n=13), centipede (n=13), or salamander (n=13). Each salamander was used in only one treatment. We placed the focal salamander into the assigned treatment and allowed a 5 min acclimation period. For the control treatment, we used damp filter paper lining a clean, non-inhabited Petri dish for five days as in other treatments. The self treatment consisted of lifting the salamander out of its own territory for 10 s and then placing it back in its container. For the salamander treatment, a focal salamander was placed into the dish that another salamander had occupied and marked for the previous five days. In the centipede treatment, the focal salamander was placed into a Petri dish that a centipede had occupied for the previous five days.

After the 5 min acclimation period, behavioral observations began and lasted for 15 min for each individual. We recorded the time each individual salamander spent in several postures or behaviors (see Table 1); each behavior was considered mutually exclusive. The focal salamander was returned to its original home container and allowed at least 24 h recovery period before the behavioral interaction experiments were performed (see below).

Behavioral interactions

The adult male *P. cinereus* used in the substrate recognition experiment above were randomly paired with a centipede or another salamander to observe their behavioral interactions in different territories. The treatments for behavioral interactions included: Resident Salamander (n=11 trials; focal salamander remained in its home container and an intruding salamander was placed with it), Intruder Salamander (n=11 trials; focal salamander introduced into the home container of another salamander), Neutral Salamander (n=20 trials; two salamanders were placed in a clean dish containing only damp filter paper), Resident Centipede (n=12 trials; an intruding centipede was placed into the focal salamander's home container), and Neutral Centipede (n=10 trials; a salamander was paired with a centipede in a neutral container). Resident and intruder observations were made in separate trials. Due to limited numbers of *S. sexpinosa*, and questionable territoriality in this species (Lewis, 1981), a centipede resident territory treatment was not included. Behavioral interaction trials were conducted between 1800 and 2300 h. Animals used in the salamander to salamander and salamander to centipede interactions were randomly chosen, with most salamanders and centipedes being of equal body size (i.e., not including the tail in the salamanders).

No salamanders were used in both centipede treatments or more than one salamander treatments, however some salamanders were placed in one of the salamander treatments and one of the centipede treatments, but were given a 24 h recovery period before being exposed to the second treatment. Salamanders were randomly assigned to pairings. However, we avoided pairing salamanders that were originally collected from the same cover board to prevent interactions between salamanders that may already be familiar with one another (e.g., Guffey et al., 1998; Jaeger and Peterson, 2002).

Methods for behavioral interactions followed Hickerson et al. (2004) with the addition of neutral treatments in our experiment. We recorded behavioral interactions for 15 min for both the focal salamander and the intruder. In addition to ATR, FLAT, NT, E, and FTR, we also recorded additional behaviors (WO, WU, NTA, LA, LT, MA, MT, BITE, and C; see Table 1).

After the focal salamander completed its last behavioral trial, it was given a 24 h recovery period and was then weighed (to nearest 0.001 g) and measured (SVL; to nearest 0.001 mm). Mass and length of each centipede was also determined at the end of the experiment. We used a linear regression to determine if the possible confounding effect of size ratio between competitors influenced behavioral

TABLE 1. Behaviors recorded and quantified in the Substrate Recognition Experiment and the Behavioral Interaction Experiment. All behaviors were considered to be mutually exclusive. Behaviors indicated with an * were only recorded in the Behavioral Interaction Experiment. These behaviors and their classification as aggressive, submissive, investigative, or resting were based on Jaeger (1984) and Hickerson et al. (2004). In cases where no classification is given, the nature of the behavior is unclear or undetermined.

Behavior	Description	Count or Duration	Aggressive, Submissive, Investigative, or Resting
All trunk raised (ATR)	The entire trunk of the salamander is lifted off the ground by legs	Duration	Aggressive
*Bite (BITE)	Salamander strikes at the other animal with its mouth open and bites it	Count	Aggressive
*Contact (C)	Salamander continuously touching the other animal without engaging in any other behavior	Duration	
Escape behavior (E)	Salamander circles the perimeter of the dish, lifting its body up along the side of the dish	Duration	
Flattened (FLAT)	Entire ventral side of salamander pressed against substrate	Duration	Submissive
Front trunk raised (FTR)	The head and anterior half of the body raised	Duration	Resting
Head up (HU)	Only the head is raised	Duration	Resting
*Look away (LA)	Salamander turns head away from the other animal	Duration	Submissive
*Look toward (LT)	Salamander turns head towards the other animal	Duration	Aggressive
*Move away (MA)	Salamander moves away from the other animal in a direct path	Duration	Submissive
*Move towards (MT)	Salamander approaches the other animal in a direct path	Duration	Aggressive
Nose tap (NT)	Salamander presses nose down against the substrate	Count	Investigative
*Nose tap on animal (NTA)	Salamander presses nose against the other animal	Count	Investigative
*Walk over (WO)	Salamander makes contact with other animal and walks over it	Duration	
*Walk under (WU)	Salamander makes contact with other animal and walks under it	Duration	

interactions. The ratio of the mass or length of the focal salamander to the other animal (i.e., other animal length/focal salamander length) did not affect the amount of time the salamander spent in any of the aggressive or submissive behaviors or biting (Linear regression: $r^2<0.055$, $P>0.27$) so is not considered further. This finding is similar to that of Jaeger et al. (1982).

Data were square root (x+1) transformed to meet parametric assumptions for normality, however an abundance of *P. cinereus* never performing various behaviors contributed a large quantity of zeros to the dataset, reducing normality after transformation. The robustness of MANOVA tests can account for this lack of normality (Olson, 1974) and thus MANOVA was used to analyze the effect of substrate on each behavior as well as interaction type on behavior. A significant MANOVA was followed by univariate ANOVAs to examine each dependent variable in turn. We used Tukey's HSD post hoc tests to examine differences among treatments for significant ANOVAs. Significance level was set at $\alpha=0.05$.

RESULTS

Substrate recognition experiment

The MANOVA found a significant treatment effect (Wilks' $\lambda=0.186$; df=15, 122, F=6.83, P<0.0001). We therefore followed the significant MANOVA with univariate ANOVAs.

Salamanders spent significantly less time in E when placed on their own substrate than when on other substrate treatments (Fig. 1A; df=3, 48, F=42.17, P<0.0001). Salamanders did not differ in the amount of time spent in E on the centipede, salamander, and control substrates.

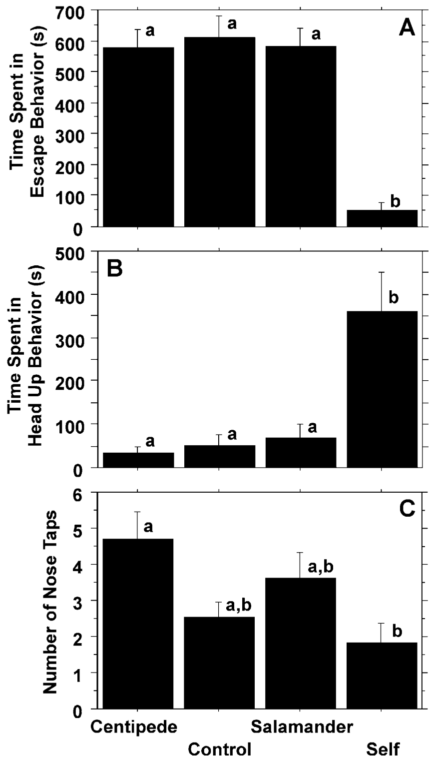

FIG. 1. Time spent by red-backed salamanders (*Plethodon cinereus*) A) in escape behavior, B) head up, and C) nose tapping when placed on different substrates. Means±1 SE are shown. Means sharing letters are not significantly different.

TABLE 2. Time spent in front trunk raised (FTR), flattened (FLAT), and all trunk raised (ATR) by red-backed salamanders (*Plethodon cinereus*) in the Substrate Recognition Experiment. Means±1 SE are given. n=13 for each treatment.

Treatment	FTR (s)	FLAT (s)	ATR (s)
Control	102.8±53.7	132.1±54.2	0
Self	131.4±75.9	353.7±94.6	0
Salamander	92.0±35.7	148.0±40.6	9.5±5.6
Centipede	29.8±26.4	236.2±60.1	19.3±18.6

The substrate a salamander was placed on did not affect the duration of FTR (df=3, 48, F=1.12, P=0.36), FLAT (df=3, 48, F=1.88, P=0.15), or ATR (df=3, 48, F=1.22, P=0.31) behaviors (Table 2). Although treatment had no main effect on ATR, ATR was only seen on the centipede and salamander substrates.

Salamanders spent significantly more time in the resting position of HU when on their own substrate compared to the other substrates (Fig. 1B, df=3, 48, F=6.03, P<0.001). *Plethodon cinereus* showed NT more often on the centipede substrate than on the self substrate, with control and salamander substrates showing no difference in NT from any other treatment (Fig. 1C, df=3, 48, F=4.44, P<0.01).

Behavioral interaction experiments

The MANOVA found a significant treatment effect (Wilks' $\lambda=0.098$; df=44, 186, F=3.53, P<0.0001). We therefore followed the significant MANOVA with univariate ANOVAs.

In general, salamanders demonstrated significantly different responses to conspecifics than to heterospecifics. When exposed to centipedes, *P. cinereus* spent significantly more time in ATR (Fig. 2A, df=4, 58, F=8.89, < 0.0001) and MT (Fig. 2B, df=4, 58, F=3.10, P=0.022) compared to when they were exposed

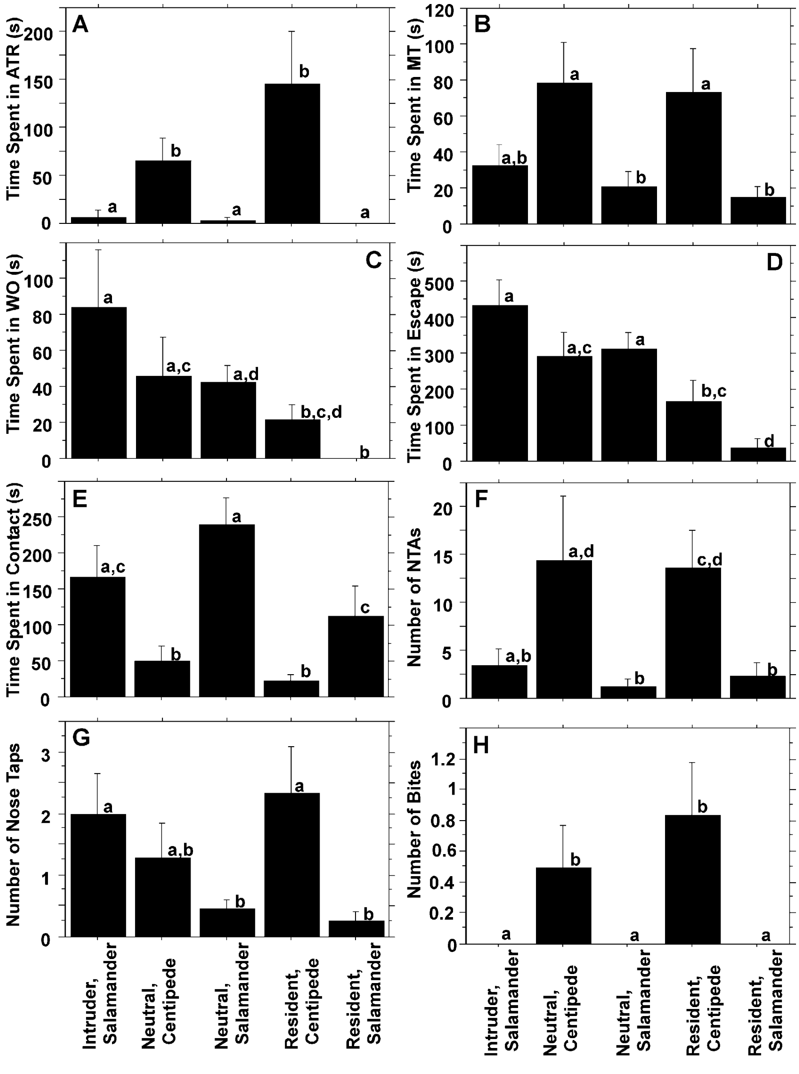

FIG. 2. Time or number of times that focal red-backed salamanders (*Plethodon cinereus*) engaged in several behaviors when interacting with conspecifics or with centipedes in different contexts. Means±1 SE are shown. Means sharing letters are not significantly different.

to conspecifics.

Plethodon cinereus performed WO less frequently when in its own dish (substrate marked by itself) than when in a conspecific's dish (Fig. 2C; df=4, 58, $F=4.37$, $P=0.0037$). While there was a significant difference in WU among the different treatments (df=4, 58, $F=2.69$, $P=0.04$: Intruder, salamander, 0±0 s; Neutral, centipede, 3.8±2.9 s; Neutral, salamander, 20.8±8.8 s; Resident, centipede, 0±0 s; Resident, salamander, 4.9±4.9 s), the Tukey's HSD post-hoc test revealed no significant pairwise differences among means. *Plethodon cinereus* in its own habitat spent less time in E than when in a novel or conspecific's habitat (Fig. 2D, df=4, 58, $F=10.82$, $P<0.0001$).

Treatments had a significant effect on the time spent in LT for the focal salamanders, with the only significant pairwise difference being between the Neutral, centipede and the Neutral, salamander treatments (df=4, 58, F=2.82, P=0.033: Intruder, salamander, 1.1±0.1 s; Neutral, centipede, 1.9±0.3 s; Neutral, salamander, 1.1±0.05 s; Resident, centipede, 1.5±0.4 s; Resident, salamander, 1.4±0.2 s). There was no significant effect of treatments on the time spent by the focal salamanders in LA, which was rarely observed (df=4, 58, F=1.33, P=0.27: Intruder, salamander, 0.2±0.2 s; Neutral, centipede, 0.2±0.1 s; Neutral, salamander, 0±0 s; Resident, centipede, 0±0 s; Resident, salamander, 0.3±0.1 s).

The pairing of salamanders with a conspecific or heterospecific had no effect on the time spent in the submissive behaviors of FLAT and MA, or in the resting behavior of FTR (df=4, 58, F<2.12, P>0.09 in all cases). However, the amount of time focal *P. cinereus* spent in C with a conspecific was significantly higher than when paired with a heterospecific (Fig. 2E, df=4, 58, F=9.34, P<0.0001). *Plethodon cinereus* performed the investigative behavior of NTA more often when paired with a centipede as opposed to another salamander (Fig. 2F, df=4, 58, F=4.79, P=0.0021). Resident *P. cinereus* paired with a centipede, and those that intruded in a conspecific's territory showed NT more than other treatments (Fig. 2G, df=4, 58, F=4.51, P=0.0031). *Plethodon cinereus* never attempted to BITE a conspecific; however, they did attempt to BITE centipedes during numerous pairings in both a neutral environment and when they were a resident of the habitat (Fig. 2H, df=4, 58, F=5.51, P=0.0008).

Discussion

Plethodon cinereus in our experiments showed no significant aggressive behaviors towards chemical cues of either conspecifics or heterospecifics. Jaeger et al. (1986) indicated that *P. cinereus* showed no aggressive response towards the chemical cues of conspecifics in laboratory trials, consistent with our findings and concluded that a visual display was necessary to elicit a threat posture from *P. cinereus*. However, Hickerson et al. (2004) found that *P. cinereus* responded to chemical cues of both conspecifics and centipedes with higher levels of aggressive postures than they did towards a control, as did Martin et al. (2005) when *P. cinereus* were placed on a substrate marked with chemical cues from an intruder male. Our population of *P. cinereus* reacted to the substrates of control, a conspecific, and a centipede with similar escape behaviors, indicating that only the familiar substrate of self was recognized as non-threatening. Jaeger et al. (1982) also found an increase in escape behaviors in *P. cinereus* introduced into the home container of another *P. cinereus*. Interestingly, *P. cinereus* did perform the investigative behavior of NT more often on the centipede substrate in our experiment.

Consistent with the results of the Recognition Experiment, behavioral pairings with conspecifics occurring in resident or neutral territories did not alter the time *P. cinereus* spent in aggressive or submissive postures, suggesting residency did not predict aggression levels in this study. Our results indicate an overall lower level of aggression towards conspecifics in this population of *P. cinereus*; however, behavioral pairings indicated that *P. cinereus* reacted aggressively to centipedes, regardless of substrate. Indeed, *P. cinereus* in our experiments never attempted to strike at or bite conspecifics, but these behaviors occurred fairly frequently in pairings with centipedes. Thus, our results indicate that there is not a generalized lowering of aggression of *P. cinereus* in this population. Rather, there appears to be a specific reduction in aggression directed at conspecifics. This contrasts with Hickerson et al. (2004) who found that *P. cinereus* responded with similar levels of aggression to centipedes as they did to male conspecifics.

Our results indicate that the aggressive behavior of *P. cinereus* varies more among populations than has previously been understood (see also Maerz and Madison, 2000 for field evidence of variation in territoriality in *P.*

cinereus). In particular, our results suggest that variation in aggressive behavior can differ between populations that are not isolated by large geographic distances. The populations in northeastern Ohio studied by Hickerson et al. (2004) and Deitloff et al. (2008) are approximately 150 km from our population. In addition, our results suggest that aggression towards conspecifics can vary independently from aggression towards heterospecifics, such as centipedes.

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