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# Assortative Mating in a Polymorphic Salamander

Megan A. Acord<sup>1</sup>, Carl D. Anthony<sup>1</sup>, and Cari-Ann M. Hickerson<sup>1</sup>

**Reproductive isolation due to divergent selection is thought to be one of the mechanisms that promote speciation in sympatry. A key element of reproductive isolation is assortative mating. We examined a polymorphic population of Eastern Red-backed Salamanders (*Plethodon cinereus*) for evidence of reproductive isolation through assortative mating. Our study population was made up of two common color morphs, striped and unstriped. In the field, we turned over natural cover objects to find male–female pairs of *P. cinereus* during peak mating season. We recorded sex, color morphology, and snout–vent length of 112 pairs of salamanders. Estimates of sexual isolation indicated weak assortative pairing in the field with more same-color pairs than expected by chance. Striped females paired with striped males were significantly larger in size than those paired with unstriped males. Intermorph pairs were observed and such pairings, if successful, would interfere with the potential for divergence. Laboratory experiments were conducted to assess the ability of females of each color morph to distinguish between the two phenotypes through fecal odors and male scent. Additionally, mating trials were conducted to examine assortative mating in the laboratory. We found no evidence that scent or natural diet cues (fecal odors) of males contributed to assortative pairing, but females of both phenotypes were more likely to be associated with striped males during mating trials. Our study provides additional evidence that striped males of *P. cinereus* may be more attractive to females and this may contribute to positive assortative mating in the field. Territoriality and diet may be important factors that influence this pattern.**

**A**N important component of ecological divergence within populations is the emergence of assortative mating which can promote speciation in sympatry. Individuals are expected to engage in positive assortative mating if the offspring produced from such pairings are more fit than the offspring resulting from random mating (Bolnick, 2004; Bolnick and Fitzpatrick, 2007; Kopp and Hermisson, 2008). It is thought that positive assortative mating can evolve only when selection for it is stronger than negative selection that results from the costs of females being overly choosy in their mate selection (Bolnick and Fitzpatrick, 2007; Kopp and Hermisson, 2008). A stable polymorphism can form when the environment of an assortatively mating population is heterogeneous, and can be divided into two or more niches, where density-dependent factors operate independently in each niche (Maynard Smith, 1966). Most studies have looked at reproductive isolation in extant species by measuring patterns of evolutionary and ecological separation (Schluter, 2001; Via, 2001), but relatively few studies have looked at populations where speciation has yet to occur but has the potential to do so (e.g., Whiteman and Semlitsch, 2005; Steinfartz et al., 2007; Anthony et al., 2008; Elmer et al., 2009). Focusing on populations undergoing divergent selection may help determine the mechanisms and processes that drive speciation in sympatry (Via, 2001; Whiteman and Semlitsch, 2005).

The role of assortative mating in population divergence has been studied in a variety of taxa (reviewed in Via, 2001). We examined the potential for assortative mating by color in the Eastern Red-backed Salamander, *Plethodon cinereus* (family Plethodontidae). These salamanders, along with eight other members of the genus *Plethodon* (Petranka, 1998), exhibit a color polymorphism that consists of two common color morphs: striped and unstriped (Highton, 1962; Pflingsten and Walker, 1978). The striped phase is characterized by a red dorsal stripe, whereas the dorsum of the unstriped phase is completely black and lacks a dorsal stripe. The proportion of striped and unstriped morphs varies within the range of this species, with some populations being

monomorphic, some having equal proportions of striped and unstriped morphs, or having either the striped or unstriped as the abundant phenotype while the other is rare (Highton, 1959, 1962; Pflingsten and Walker, 1978). Color polymorphism in this species has a genetic basis, with multiple pairs of genes interacting to produce the two phenotypes (Highton, 1975).

*Plethodon cinereus* has been used as a model organism for numerous ecological and behavioral studies (Jaeger and Forester, 1993; Mathis et al., 1995). Our understanding of the social system of *P. cinereus* makes it an ideal species with which to ask questions about the factors involved in mate selection. Both males and females are territorial (Horne, 1988; Horne and Jaeger, 1988) and defend areas under cover objects to maintain exclusive access to feeding areas (Jaeger, 1980; Jaeger and Forester, 1993) and potential courtship sites (Mathis, 1991). Glandular secretions and feces are used to mark and to recognize home territories (Horne and Jaeger, 1988). Gravid females squash male and female fecal pellets with their snouts to assess prey quality (Karuzas et al., 2004) and possibly male fitness (Walls et al., 1989; Mathis, 1990a). Because only female *P. cinereus* have been observed fecal squashing, it is thought that males exploit this behavior via strategic placement of fecal pellets within their territories. This may increase encounter rates with potential mates (Karuzas et al., 2004). This species has a mating season that lasts from autumn to early spring, with males breeding annually and females breeding biennially in the northern part of their range (e.g., Ohio; Pflingsten, 1989). This results in a breeding ratio of two males to one female and creates a situation in which males may tolerate non-reproductive females within their territories (Thomas et al., 1989). Additionally, male *P. cinereus* are more aggressive toward intruding male conspecifics than they are toward females (Thomas et al., 1989). Plethodontid salamanders use chemical cues to recognize individuals, determine reproductive condition, and advertise their presence within a territory (Jaeger et al., 1986). Individuals of *P. cinereus* are able to distinguish between the odors of male and female conspecifics (Mathis, 1990a; Dantzer and Jaeger, 2007), and

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females of *P. cinereus* can perceive body size of potential mates in the laboratory when presented with the odors of large versus small male conspecifics (Mathis, 1991).

Because red-backed salamanders are polymorphic, they have been the subject of studies that have focused on possible mechanisms of disruptive selection. Evidence suggests that climate, in particular temperature, plays a role in maintaining disruptive selection on color or associated traits in *P. cinereus*. A number of studies have found that the unstriped morph is associated with warmer and drier microhabitats (Burger, 1935; Lotter and Scott, 1977; Gibbs and Karraker, 2006; but see Petruzzi et al., 2006). In autumn, unstriped individuals retreat from the surface earlier than striped individuals (Lotter and Scott, 1977; Moreno, 1989; Anthony et al., 2008), suggesting that striped salamanders are more tolerant of colder temperatures. Other factors may also aid in maintaining this polymorphism. For example the diet of striped and unstriped salamanders has been found to differ, such that striped *P. cinereus* have a higher quality diet than their unstriped counterparts (Anthony et al., 2008), and this may make striped males and their territories more attractive to females. Differential predation on the two phenotypes may also play a role in maintaining color polymorphism in *P. cinereus* (Fitzpatrick et al., 2009) and could influence mate choice. Studies have suggested that the unstriped morph may experience higher predation risk (as indicated by higher frequency of tail breakage; Moreno, 1989; Venesky and Anthony, 2007), and this may result in increased production of stress hormones (Davis and Milanovich, 2010). Individuals that experience higher mortality, or have elevated levels of stress hormones, may be less attractive as mates.

We investigated the role of color polymorphism in *P. cinereus* as a mechanism for reproductive isolation by examining the potential for positive assortative mating. The field portion of this study involved locating pairs of adult salamanders and determining their pair status (same or mixed phenotype) in an effort to detect non-random pairing. We revisited the hypothesis that striped and unstriped individuals of *P. cinereus* pair non-randomly under natural conditions in the field, based on a study conducted by Anthony et al. (2008) that found evidence for assortative pairing in the field using artificial cover objects. We also tested the hypothesis that striped males, which are thought to have a higher quality diet than unstriped individuals (Anthony et al., 2008), would be found with larger and presumably higher quality females. Red-backed salamanders are considered to be weakly sexually dimorphic for body size, with females being slightly larger than males (Werner, 1971; Nagel, 1977). Female size is expected to be well correlated with female quality in amphibians because larger females typically produce more and/or larger eggs (Salthe, 1969). In addition to the field study, we conducted three laboratory experiments to address questions about the relationship between male odor and diet and female preference of color phenotype. The first experiment used fecal pellets taken from field collected male salamanders and tested the hypothesis that females would exhibit a heightened response to the pellets of striped salamanders compared to those of unstriped salamanders. The second experiment tested the hypothesis that females could determine color morphology by scent in the absence of natural diet cues. The final laboratory experiment was conducted to see if *P. cinereus* would mate assortatively under laboratory conditions.

## MATERIALS AND METHODS

**Field study.**—We observed and recorded male and female pairs of *Plethodon cinereus* in October and November of 2008 and 2009 at the Cuyahoga Valley National Park (CVNP) near Peninsula, Ohio, USA (41°13'44.15"N, 81°31'6.64"W). Observations took place during the mating season of *P. cinereus* (Blanchard, 1928; Saylor, 1966; Werner, 1969). The study was conducted within a 140-hectare tract of eastern deciduous forest (elevation range 250–275 m). The area supports a large population of *P. cinereus* and is dominated by *Fagus grandifolia* (American beech), *Acer saccharum* (Sugar Maple), *Liriodendron tulipifera* (Tulip Poplar), and *Quercus rubra* (Red Oak). We chose field sites on the basis of topography, amount of riparian cover, and aspect (north-facing slopes) to ensure optimal salamander habitat. We surveyed one site each day for 4–5 hours for a total of 20 sampling days ( $n = 100$  pairs). No site was resampled. Additional observations ( $n = 12$  pairs) from an adjacent site were collected by students enrolled in an ecological field course taught by CDA at John Carroll University in 2008.

We found pairs by flipping over cover objects that included rotting logs, large branches, and bark that were greater than 25 cm<sup>2</sup> in area (Mathis, 1990b). Snout-to-vent length (SVL), color morphology (striped or unstriped), and sex of each individual salamander was recorded for 100 of the pairs. Only color morphology and sex was recorded for the additional 12 pairs observed in 2008. Previous studies have used a maximum intrapair distance of 30 cm to define mated pairs of red-backed salamanders (Gillette et al., 2000; Peterson, 2000; Jaeger et al., 2002) and we followed this convention. Additionally, adults of this species are territorial (Jaeger, 1984; Mathis et al., 1995), including those from northeastern Ohio (Gall et al., 2003; Hickerson et al., 2004; Deitloff et al., 2008) and do not otherwise share cover objects (Mathis, 1990b; but see Quinn and Graves, 1999; Maerz and Madison, 2000). For example, of 518 adult red-backed salamanders observed in Ohio by Anthony et al. (2008), 438 occurred alone and 74 were found paired with another adult. Of the 74 paired individuals, only six were found in same-sex pairs (three female–female pairs). They found no instances of males co-occurring and only two cases where three adults co-occurred. Therefore we assumed that during peak mating season, two adult salamanders of different sexes were a reproductive pair when found within 30 cm of one another under a cover object. 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). Only pairs in which males were obviously in reproductive condition were recorded.

An index of sexual size dimorphism was calculated for each pair (Lovich and Gibbons, 1992) by dividing female SVL by male SVL and multiplying the result by 100. The index is reported as a percentage in which values above 100% indicate cases where the female was larger than the male and values below 100% indicate the opposite condition. We explored differences in sexual size dimorphism among each of the four possible mating combinations with ANOVA. To determine the degree of sexual isolation between the two color phenotypes, we calculated three indices of sexual isolation ( $I_{PSI}$ , Yule's  $V$ , and Levene's  $Y_A$ ) that are thought to be the best estimators of sexual isolation (Rolán-Alvarez and Caballero, 2000; Pérez-Figueroa et al., 2005). For each index, values that significantly deviate from

0 (random mating) suggest either assortative (values  $> 0$ ) or disassortative (values  $< 0$ ) mating among phenotypes within a population. The three index values range from  $-1$  to  $+1$ . We used the program JMATING (Carvajal-Rodriguez and Rolán-Alvarez, 2006) to calculate test statistics for each of the isolation indices. We employed a  $G$  test to compare our observed patterns of monomorphic and mixed pairs to that expected if salamanders were pairing at random.

**Laboratory experiments: Collection and housing.**—Salamanders were collected in late spring 2009 from an area of eastern deciduous forest directly adjacent to and continuous with our field sites in the CVNP. A total of 80 salamanders were collected: 20 striped females; 20 unstriped females; 20 striped males; and 20 unstriped males. All salamanders collected were reproductive adults within the size range of 36–41 mm SVL. In the laboratory, individual salamanders were housed in separate Petri dishes (15 cm diameter) lined with damp paper towel as substrate. Salamanders were maintained under a natural photoperiod at a constant temperature of 16°C. All salamanders were fed a diet of vestigial-winged *Drosophila melanogaster*. Every seven days, salamanders were fed 20–30 flies and their paper towel substrate was replaced.

**Laboratory experiments 1 and 2: Roles of natural diet cues and male odor in phenotype recognition.**—These experiments tested the hypothesis that females would respond most strongly to the odors associated with striped males, either in the form of field-collected fecal pellets (experiment 1) or male odor alone (experiment 2). The studies were conducted over the course of 12 weeks (June–August 2009). For each experiment, we size-matched donor males to females to remove potentially confounding effects of size. The size difference between males and females was no greater than 1 mm, and the mean size difference among treatments was no greater than 0.5 mm. A total of 120 ten-minute trials were conducted per experiment. Each female was exposed to three treatments in each experiment: 1) exposure to a male fecal pellet or male odor of an individual of the same color morphology; 2) exposure to a male fecal pellet or male odor of an individual of the opposite color morphology; and 3) a control. The control for the fecal pellet experiment was a rolled and compressed paper towel of the approximate size and shape of a fecal pellet, whereas the control for the odor experiment was a blank, moistened filter paper substrate. The order in which each female was exposed to treatments was chosen randomly, and each female received a different treatment once per week. Repeated-measures ANOVAs were used to analyze the data from each experiment. Behavior (i.e., number of nose taps) was treated as the dependent variable and phenotype of the treatment (striped male, unstriped male, or control) as independent. Data were transformed ( $\log_{10}(x+1)$ ) to achieve normality.

In the first experiment we utilized natural fecal pellets of striped and unstriped males. The first fecal pellet produced by each field-collected male was frozen and stored in individually labeled plastic vials. This pellet was taken before the salamanders received the laboratory diet of *D. melanogaster* to ensure that the fecal pellet represented the natural diet. Pellets were stored in a freezer at  $-17^{\circ}\text{C}$  for 30 days prior to conducting the laboratory trials. Pellets were thawed to 16°C one hour before running trials and each

pellet was only used once. Eighty fecal pellets were used from 80 different males (40 of the fecal pellets were taken from males being used in other studies that were collected from the same site on the same dates). Immediately prior to collection of behavioral data, females were placed on damp filter paper in a Petri dish and allowed five minutes to acclimate. A fecal pellet was placed in the dish and the behavior of the female was recorded for ten minutes. Measured behaviors included nose taps on the substrate, which involves touching the nasolabial grooves to the substrate in an effort to identify substrate markings (reviewed in Mathis et al., 1995), and fecal squashing, by which females squash fecal pellets with their snouts as a way to assess chemical signals (Walls et al., 1989).

In the second experiment, we assessed the ability of females to distinguish male color via glandular secretions. Males and females were kept in separate containers with a clean dampened paper towel substrate before the trials began. For seven days, males were kept on filter paper and glandular secretions were allowed to accumulate. Males, who had been fed only flies previously, were not fed during this period to avoid the confounding effects of prey odor. At the end of the seven-day period, each male was removed from the container and a female was placed in that container. The female was given a five-minute acclimation period. Female responses (nose taps) were recorded for ten minutes.

**Laboratory experiment 3: Assortative mating in the laboratory.**—This experiment tested the hypothesis that *P. cinereus* would mate assortatively by color in the laboratory. The study was conducted in the last two weeks of October 2009. Eighty trials were run, with all mating trials beginning at 1700 h and ending at 0800 h the following day. Females and males were kept in separate containers on damp paper towel substrate. For the mating trial, males and females were placed together in a clean container with damp paper towel substrate and left together overnight. This methodology has been successful in encouraging mating in other plethodontids (Houck et al., 1988). The following morning we recorded the presence of spermatophores on the substrate or in the cloaca of females. In a previous study (Houck and Verrell, 1993) researchers had difficulty getting *P. cinereus* to mate under laboratory conditions so we also recorded intra-pair distance at the end of the experiment as a proxy for mate choice. Intra-pair distance was measured from mid-body to mid-body of each individual in a pair. For the two treatments, each female was first placed with a male of one color and then a week later, the same female was placed with a male of the other color. The order of the treatments was determined randomly, but equal numbers of each treatment were run on each testing day. To eliminate size as a confounding variable in mate choice, we minimized size differences within pairs. The size difference between males and females was no greater than 1 mm, and the size difference between treatments (differently colored males) was no greater than 0.5 mm. Paired t-tests were used to analyze differences in intra-pair distance. A paired t-test was used to analyze intra-pair male to female distance between pairings with striped and unstriped males.

## RESULTS

**Field study.**—Over two consecutive autumn field seasons, we found a total of 112 presumptive reproductive pairs of adult

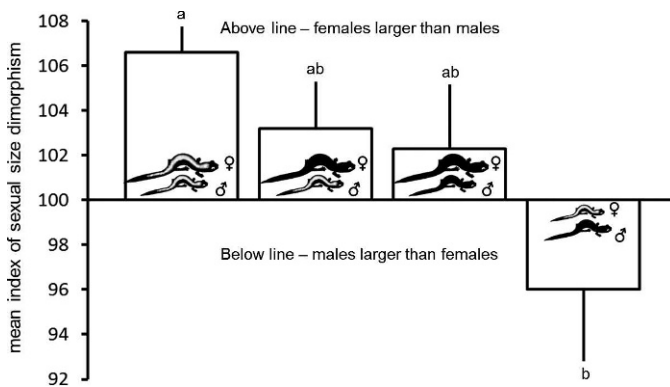
**Table 1.** Indices of Sexual Isolation between Striped and Unstriped Phenotypes of *Plethodon cinereus*. Index values range from  $-1$  to  $+1$ , with values greater than zero indicating assortative pairing by phenotype. SD = standard deviation; df = degrees of freedom. The indices were calculated using the program JMATING (Carvajal-Rodríguez and Rolán-Alvarez, 2006) and are based on field observed pairs of presumptive mated pairs.

| Measure                 | Value | SD   | Test statistic  | df  | <i>P</i> |
|-------------------------|-------|------|-----------------|-----|----------|
| <i>I</i> <sub>PSI</sub> | 0.26  | 0.09 | <i>t</i> = 2.86 | 111 | <0.005   |
| Levene's <i>YA</i>      | 0.25  | 0.12 | <i>t</i> = 2.07 | 111 | <0.05    |
| Yule's <i>V</i>         | 0.19  | 0.10 | <i>t</i> = 1.85 | 111 | <0.1     |
| G-test                  |       |      | <i>G</i> = 3.81 | 1   | 0.052    |

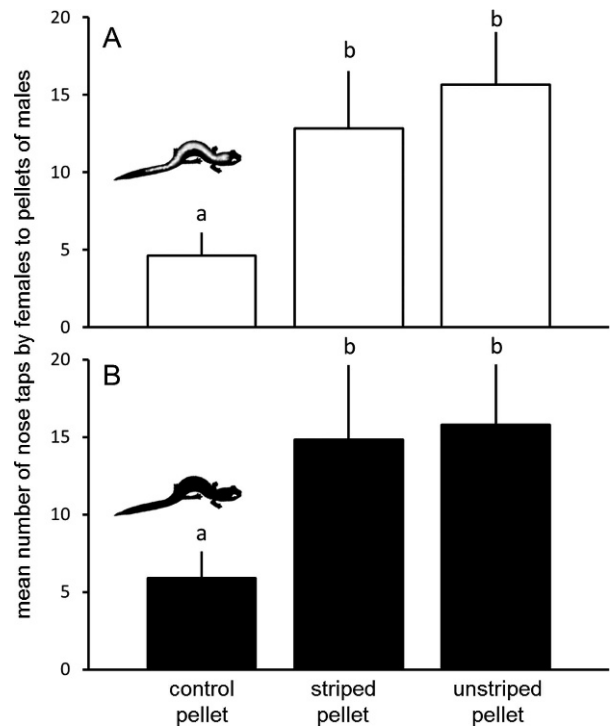
*Plethodon cinereus*. Of the 112 pairs, 80 were found in same color male–female pairs (71 striped–striped pairs, 9 unstriped–unstriped pairs), whereas 32 pairs were males and females of differing color morphology (21 unstriped female–striped male; 11 striped female–unstriped male). We recorded more matched than unmatched phenotype pairs ( $G = 3.81$ ,  $P = 0.052$ ,  $df = 1$ ), a marginally significant result. All three indices of sexual isolation were greater than zero. *I*<sub>PSI</sub> and Levene's *YA* indicated moderate but significant assortative pairing with respect to color phenotype (Table 1).

Striped females paired with striped males were significantly larger than those paired with unstriped males (mean difference = 2.39 mm,  $t = 2.93$ ,  $P = 0.005$ ,  $df = 71$ , two-tailed test). There was a statistically significant effect of pair category on the index of sexual dimorphism ( $F_{3,96} = 4.31$ ,  $P = 0.007$ , Fig. 1), such that only unstriped males paired with striped females were, on average, larger than their mates. In each of the other pair types, males were on average smaller than their mates. When sexes were combined, striped and unstriped individuals did not differ in size, but females were significantly larger than males (mean female SVL = 38.05 mm, mean male SVL = 36.96,  $t = 2.52$ ,  $P = 0.006$ ,  $df = 99$ , one-tailed).

**Laboratory experiments 1 and 2: Roles of natural diet cues and male odor in phenotype recognition.**—Field collected males all produced fecal pellets within 24 hours of collection, and



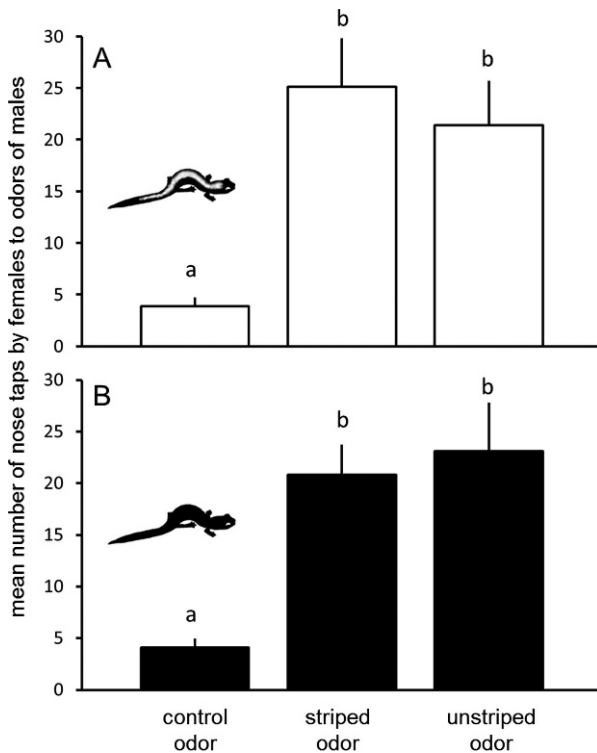
**Fig. 1.** Size differences between males and females that shared natural cover on the forest floor ( $n = 100$  pairs). Mean index of sexual size dimorphism in the four pair categories is shown. Female salamanders are depicted above males and lines represent standard errors. Different letters associated with bars represent statistically significant differences at  $\alpha < 0.05$ . Index values above 100% indicate that, on average, males were smaller than their presumptive mates.



**Fig. 2.** Laboratory experiment 1. Mean nose taps performed by females of *Plethodon cinereus* when exposed to control fecal pellets, pellets from striped males, and pellets from unstriped males. (A) Responses of striped females. (B) Responses of unstriped females. Different letters associated with bars represent statistically significant differences at  $\alpha < 0.05$ . Lines represent standard errors. Females exhibited significantly elevated responses to male fecal pellets, but they failed to distinguish between those of striped and unstriped males.

these pellets were used to represent natural diet odor cues of the two color morphs. In both experiments, females displayed significantly more interest in odor treatments compared to controls (Figs. 2, 3). Females of both phenotypes exhibited more nose taps when presented with male fecal pellets compared to controls (100% of striped females;  $F_{2,38} = 9.38$ ,  $P < 0.0001$  and 90% of unstriped females;  $F_{2,38} = 2.91$ ,  $P = 0.067$ ). Few females in experiment 1 displayed fecal squashing behavior, but 35% of females nose tapped directly to pellets. In experiment 2, females were exposed only to the odors of males (no natural diet cues present), and both striped ( $F_{2,38} = 31.45$ ,  $P < 0.001$ ) and unstriped ( $F_{2,38} = 26.44$ ,  $P < 0.001$ ) females showed increased interest in male odors over controls. However, in neither experiment were females able to distinguish between striped and unstriped treatments (Figs. 2, 3). We examined how consistent females were in their preference for striped or unstriped treatments across both experiments. For example, if a female exhibited preference for striped males in both experiment 1 and 2, we scored that as a consistent response. We found no evidence of consistency (binomial test,  $n = 34$  [ties excluded],  $P = 0.39$ ), again suggesting that females could not distinguish male phenotype by odor or by natural diet cues.

**Laboratory experiment 3: Assortative mating in the laboratory.**—Pairs of *Plethodon cinereus* did not mate under laboratory conditions and the males did not produce spermatophores. Paired t-tests did not detect a significant effect of pair status (i.e., same colored pairs or opposite colored pairs) on intra-pair distance ( $t = 0.78$ ,  $P = 0.45$ , two-tailed for striped



**Fig. 3.** Laboratory experiment 2. Mean nose taps performed by females of *P. cinereus* when exposed to controls (no odor), odors of striped males, and odors of unstriped males. (A) Responses of striped females. (B) Responses of unstriped females. Different letters associated with bars represent statistically significant differences at  $\alpha < 0.05$ . Lines represent standard errors. Females exhibited significantly elevated responses to male odors, but they failed to distinguish between those of striped and unstriped males.

females;  $t = 1.77$ ,  $P = 0.09$ , two-tailed for unstriped females), but females tended to be found closer to striped males, regardless of the phenotype of the female (mean difference 1.31 cm;  $t = 1.84$ ,  $P = 0.07$ ,  $df = 19$ ,  $n = 20$ , two-tailed).

## DISCUSSION

When natural selection favors alternate phenotypes within a polymorphic population, reproductive isolation may arise if there is potential for assortative mating. We monitored a polymorphic population of terrestrial salamanders for evidence of assortative mating, and our results add to a growing body of evidence suggesting that the two common color morphs of *P. cinereus* may have the potential for environmental and genetic isolation. We found evidence that the two phenotypes assortatively pair in the field and some evidence for a striped male advantage when paired with females in the laboratory. Although females did not differentiate between phenotypes in fecal pellet (natural diet) and substrate odor studies, they did respond significantly to chemical odors relative to the control treatments.

When we controlled for potential diet differences between the two phenotypes, females did not distinguish between striped and unstriped males based on odor alone. Red-backed salamanders use odor to determine sex, species identity, and body size of potential mates and competitors (Jaeger and Gergits, 1979; Mathis, 1990a, 1991; Dantzer and Jaeger, 2007), but our data suggest that females do not use odor alone to detect male color morphology. This result may

be a consequence of our experimental design where females were not forced to choose between two male odors (i.e., Y-Tube choice tests [Dawley, 1986]). A disadvantage of our design may be that females show a similar level of interest across treatments in the absence of a choice. However, females in our mating trials tended to be found closer to striped versus unstriped males, suggesting that they are using some cue to distinguish between the two phenotypes. Recent work (Kohn and Jaeger, 2009) suggests that red-backed salamanders use multiple cues in the recognition of individuals, including vision, and future work on mate choice in this species should incorporate a visual component into the design.

Previous studies have indicated that red-backed salamanders use fecal pellets in territorial and reproductive contexts. Specifically, it is thought that female salamanders derive information about male diet and/or territory quality from male fecal pellets (Walls et al., 1989). Despite the suggestion that striped males in nature may have a superior diet that could be attractive to females (Anthony et al., 2008), females in our study did not behave differently toward the fecal pellets of striped males compared to those of unstriped males, and though females were observed nose-tapping the substrate and fecal pellets, few squashed fecal pellets containing remains of their natural diet (i.e., the first fecal pellets after salamanders were collected and before they received laboratory raised prey). Whereas chemical signals such as scent marks are often non-volatile due to their heavy molecular weight (Dantzer and Jaeger, 2007), it is unknown how volatile the chemical cues of fecal pellets are, and because the pellets had been frozen and thawed, some of the important odors may have been lost. Another possibility is that the diets of the two phenotypes did not differ immediately prior to pellet collection, and differences in diet between color morphs may exist only during certain seasons. For example, we collected fecal pellets in the spring; Anthony et al. (2008) reported diet differences between the two phenotypes in autumn. The effect of seasonality on differential prey use between the phenotypes is currently unknown.

When we paired males and females in the laboratory, we did not observe spermatophores in the cloacae of females, nor did we observe spermatophore deposition on the substrates of experimental chambers. Therefore, there was no evidence that individuals of *P. cinereus* mated in laboratory pairings. Despite the fact that *P. cinereus* acclimate readily under laboratory conditions, other researchers have not been successful in observing courtship or mating in the laboratory (Houck and Verrell, 1993). In our laboratory tests, the distance between pairs of the same color morphology was not less than that of mixed pairs; however, females tended to be found closer to striped males (mean distance = 4.55 cm) than to unstriped males (mean distance = 5.86 cm), a difference of about 23%. Though this does not directly support our hypothesis that *P. cinereus* mates assortatively, it is consistent with our field data, where we found striped males paired with the largest females. This suggests that striped males may have some advantage over unstriped males.

Our field observations indicate that striped males were paired with the largest females (mean female SVL 38.63 mm), whereas unstriped males were paired with smaller females (mean female SVL 36.76 mm), a result similar to that reported by Anthony et al. (2008). One way in which striped

males might be able to attract larger, and presumably more fecund, females is through territorial advertisement of a high-quality diet. Striped individuals in this population (Anthony et al., 2008) have been shown to have a higher quality diet made up of easily digestible soft-bodied prey. One benefit thought to offset the cost of territorial behavior is access to prey populations, especially during periods of leaf litter drying (Jaeger et al., 1995). Thus a high quality diet may indicate more effective defense by striped territorial residents. Males and females of *Plethodon cinereus* are territorial (Lang and Jaeger, 2000), so one possibility is that co-defense (Jaeger et al., 2000) by both striped males and females results in assortative mating. For example, if a striped female is able to exclude an unstriped female from a high quality territory, the unstriped female may have no alternative but to mate with a male of her same phenotype, thus avoiding the cost of interacting with large, territorial females. These ideas are untested in polymorphic *P. cinereus*, but studies on other taxa have shown that individuals may pair along a mate-quality gradient. In Zebra Finches (*Taeniopygia guttata*), high quality females are attracted to the songs of high-quality males, and low-quality females are attracted to the songs of low-quality males (Holbeck and Riebel, 2010). The authors hypothesized that low-quality females preferred low quality males because they could not afford to invest time and energy searching for a high quality male. The likelihood of rejection by high quality males and increased competition from high quality females adds to this energetic cost.

At our field site, 71% of the mated pairs were of the same color morphology. This is similar to the findings of Anthony et al. (2008), in which 72.3% of the population paired assortatively. Most male/female pairs observed were of the same phenotype and this pattern deviated significantly from random ( $I_{psi}$ , Levene's *YA*; Table 1). However, the magnitude of these indices of sexual isolation were relatively small and our G-test revealed marginally significant evidence of non-random pairing ( $P = 0.052$ ). At this rate of gene flow (i.e., 29% of pairs are mismatched), genetic divergence is unlikely. It is interesting to note, however, that the fecundity of females in mismatched pairs should be lower than that of females in matched pairs due to their smaller size (Nagel, 1977; Lotter, 1978; Fig. 1). This could offset the contribution of those females to recruitment into the next generation and effectively reduce the rate of gene flow between the morphs. Additionally, over time there may be a potential for further divergence between the color morphs as global climate change continues to affect the deciduous forest ecosystem (Gibbs and Karraker, 2006). As the climate continues to warm and forests continue to dry, thermal and diet niches may be created. Selection may favor the unstriped morph in drier microhabitats, and may consequently isolate the striped morph into areas that retain moisture, thereby facilitating reproductive isolation. Future studies involving mechanisms of assortative mating within polymorphic populations, as well as genetic studies that focus on the rate of gene flow between morphs, would be valuable in determining the probability of future divergence in polymorphic populations of *P. cinereus*.

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